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C O N T E N T S

EFFECTS OF TILLAGE PRACTICES AND NUTRIENT MANAGEMENT ON CROP PRODUCTIVITY AND PROFITABILITY IN JUTE-T. AMAN RICE- ONION CROPPING SYSTEM

N. Salahin[1], M. K. Alam[2], N. C. Shil[3]
A. T. M. A. I. Mondol[4] and M. J. Alam[5]

Abstract

Conservation agriculture (CA) with appropriate nutrient management can help increase crop production, improve soil health and bring economic return. An experiment was conducted at farmer's field of Rajbari district under Low Ganges River Floodplain (AEZ-12) during 2017-2018 to evaluate the effects of tillage and nutrient management on the system productivity and profitability in the jute - T. Aman rice- onion cropping system. Strip tillage (ST) for jute, non-puddling for T. Aman rice and one pass minimum tillage (MT) for onion was contrasted with conventional crop establishment practice (CT). Four different nutrient management practices were NM_1: farmer's practice (FP), NM_2: soil test based (STB) fertilizer, NM_3:25% extra of STB and NM_4: organic amendments (20 % nutrients from cowdung). The experiment was laid out in a split-plot design with three replications by assigning tillage practices in main plots and nutrient management in sub-plots. The yield attributes of jute, T. Aman rice and onion responded similarly to tillage practices. On the other hand, the system productivity and yield of component crops varied due to different nutrient management practices ($p<0.05$). The highest fibre yield of jute (3.64 t ha^{-1}) was obtained in 25% extra of STB fertilizer dose; the highest T. Aman yield (6.02 t ha^{-1}) and the highest onion bulb yield (16.0 t ha^{-1}) in 20 % organic nutrient management, followed by25 % extra of STB fertilizer dose. The highest system productivity was also produced by 20 % organic amendments (33.6 t ha^{-1}), followed by 25 % extra of STB fertilizer dose (32.3 t ha^{-1}). The lowest yield of crops and system productivity was recorded in FP (28.6 t ha^{-1}).The increased yield of T. Aman rice and onion in 20 % organic amendment by cowdung and ST resulted in the highest net return (NR) and benefit-cost ratio (BCR), while 25% extra of STB and FP under CT gave the lowest NR and BCR. Residual nutrient from successive use of cowdung combined with chemical fertilizers outperformed other management practices in terms of crop yield and economic return.

Keywords: Benefit-cost ratio; cropping system productivity; minimal soil disturbance; nutrient management; strip planting

Introduction

In general, agriculture in Bangladesh depicts excessive tillage, crop residue removal, imbalance fertilization, etc. that degraded soil health with accelerated

[1-5]Soil Science Division, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, Bangladesh.

decomposition of soil organic matter (SOM).Cropping intensity has increasedover time by increasing puddled rice production (BBS, 2012). Consequently, most of the soils contain less than 1.5% SOM, and some soils have even less than 1% SOM (BARC, 2018). The sub-tropical humid climate causes rapid breakdown of SOM by heterotrophic microbes; consequently, nutrients loss occurs through different processes, viz. leaching, volatilization, runoff etc. The low SOM is a cause of low productivity and is considered as a serious threat to the sustainability of agriculture in Bangladesh (Jahiruddin and Satter, 2010). Organic matter (OM) depletion occurs in high land and medium high land soils (WRC, 2008-2009), while OM content has little increased in low land soils with rice-rice cropping pattern (BRRI, 2008). Intensification of agricultural land use with high expansion of modern crop varieties has increased remarkably which has exhausted nutrients from soil. Thus, with advancement of time, soil fertility has declined and chronologically the deficiency of nitrogen, phosphorus, potassium, sulphur, zinc and boron has arisen in the soils (Jahiruddin and Satter, 2010).

Most agricultural soils have become vulnerable to tillage-stimulated rapid loss of SOM in the coarse texture soils (Stewart *et al.*, 2007). As the delta country has most of the cropping systems based on rice (Alam *et al.*, 2016b), the puddling of soil for rice crop establishment causes heavy havoc on soil properties by destroying structure of soil. In addition, puddling of soil accelerates decomposition of SOM, increases greenhouse gas emissions (GHGs)and nitrogen loss (Alam *et al.*, 2019b) and inflicts increased costs for upland crop establishment (Bell *et al.*, 2019).In contrary, minimum or zero tillage practice increases SOM levels (Busari and Salako, 2013),enriches nutrients in soils (Alam, 2018) and water retention capacity (Aziz *et al.*, 2013), and decreases production costs (Salahin, 2017) and GHGs (Alam *et al.*, 2019b) by reducing fuel use for intensive tillage and irrigation requirements (Johansen *et al.*, 2012). There are many researches on zero and minimum tillage that has been proved as a greater technique to increase SOM, microbial activity, total N, and extractable P, S, Zn and B at the soil surface compared to conventional tillage (Alam *et al.*, 2016a; Vu *et al.*, 2009). Besides, non-puddled rice crop establishment has been recently developed and performed well in yield, soil health, economics and climate change mitigation (Bell *et al.*, 2019; Salahin, 2017; Alam, 2018; Alam *et al.*, 2019a). In these situations, the idea of conservation agriculture (CA) in rice-based intensive cropping systems through employing minimal soil disturbance, crop residue retention with suitable crop rotations (Kassam *et. al.,* 2009) has emergedas a holistic alternative agricultural system for its production sustainability, economic viability, environment friendly approach. However, conservation agriculture (CA) is inadequately developed for intensive upland-rice cropping system widely practiced in Bangladesh (Alam *et al.*, 2016b; Salahin, 2017) and the experimentation of CA with different nutrient management practices on soil properties and crop yields under jute-T. Aman rice- onion

cropping systems has not been examined in the poorly fertile soils of Bangladesh. With a view to address such issues, the present study was undertaken with the following objectives:

i). to find out the suitable combination of tillage practices and nutrient management for maximizing the crop yield in the jute- T. Aman- onion cropping system, and

iii). to evaluate the system productivity and economic profitability in this system.

Materials and Methods

Description of experimental site

The experiment was done at farmer's field of Rajbari district during 2017-2018.It belongs to the agro-ecological zone "Low Ganges River Floodplain" (AEZ-12; located at 23°48"35" N and 89°25'55" E, at 11.0 m above the sea level. Initial soil characteristics of the experimental field were determined and information of soil texture, bulk density, soil pH, organic matter, total N, available P, K, S, Zn, and B contents at 0-15 cm soil depth are presented in Table 1.

Table 1. Initial soil characteristics of the experimental field, Rajbari district (0-15 cm)

pH	SOM	TN	Available nutrients						Textural class	Bulk density
	%		K	P	S	Zn	B			
			meq 100 g soil^{-1}	mg kg^{-1}						(g cm^{-3})
8.1	1.09	0.060	0.15	7.2	13	0.25	0.15			
Slightly alkaline	Low	Very Low	Medium	Low	Medium	Very Low	Low		Sandy loam	1.53

Treatments and design

The unit experimental plot area was 7.2 m × 4.5 m and the design of the experiment was split-plot with three replications. Tillage practices such as, T_1: conventional tillage (CT) and T_2: minimum tillage (MT)/strip tillage (ST)/non-puddling was assigned in main plots and four nutrient management practices such as NM_1: farmer's practice (FP), NM_2: soil test based recommended fertilizer (100% STB as per Fertilizer Recommendation Guide-FRG, all from chemical fertilizers), NM_3: 25% extra of STB as per FRG, all from chemical fertilizers and NM_4: 80% from chemical fertilizer and the 20% nutrients supplemented with cowdung manure were allotted in sub-plots. Details of the treatments are given in Table 2.

Sowing/transplanting

Jute seed (cv. Nabin) was sown on 11 May, 2017 maintaining 25 cm × 7-10 cm spacing, T. Aman rice seedlings (cv. BRRIdhan 72) were transplanted on 10 August 2017 maintaining 20 cm × 20 cm spacing and onion seedlings (cv. King) were transplanted on 23 January 2018 at 15 cm apart from row to row and 10 cm apart from plant to plant.

Table 2. Different tillage and nutrient management practices along with fertilizer application method for component crops of jute-T. Aman- onion cropping system

Treatments		Crops		
		Jute	T. Aman	Onion
Tillage practices	Conventional tillage/puddling	4-5 pass of power tiller machine followed by 2-3 laddering	Puddling was done by 4-5 wet tillage operations using power tiller machine followed by 2-3 laddering	4-5 pass of power tiller machine followed by 2-3 laddering
	Minimum tillage (MT)/ Strip tillage (ST)	ST was done by one pass with PTOS using rotating blades maintaining 25 cm spacing from row to row	Non-puddling; Strip was done by one pass with PTOS using rotating blades maintaining 20 cm spacing from row to row around 18-24 hours before transplanting rice; Full puddling was not done	MT was done by one pass with power tiller operated seeder (PTOS
Nutrient Management practices	NM_1:Farmers' practice (FP)	Fertilizer dose: N_{180}, P_{10}, K_{45} and S_8 kg ha^{-1} *Application method:* ½ urea was applied with all other fertilizers during final land preparation and ½ urea was at 30 DAS	Fertilizer dose: N_{120}, P_{10}, K_{40}, S_6 and Zn_1 kg ha^{-1} *Application method:* ½ urea was applied with all other fertilizers during final land preparation and ½ urea was at 36 DAT	Fertilizer dose: N_{140}, P_{30}, K_{60} and S_{16} kg ha^{-1} *Application method:* ½ urea was applied with all other fertilizers during final land preparation and ½ urea was at 35 DAT

Treatments		Crops		
		Jute	T. Aman	Onion
	NM$_2$: Soil test based recommended fertilizer (100% STB as per FRG-2012, all nutrients from chemical fertilizers)	STB dose: N$_{153}$, P$_{13}$, K$_{72}$, S$_{12}$, Zn$_{1.0}$ and B$_{0.5}$ kg ha^{-1} *Application method:* Urea was applied in two equal splits at 25 and 50 days after sowing (DAS)	STB dose: N$_{100}$, P$_{13}$, K$_{47}$, S$_9$, Zn$_2$ and B$_{0.5}$ kg ha^{-1} *Application method:* all other fertilizers except urea were applied during final land preparation. Urea in 3 equal splits- the 1/3rd at 4 DAT, the second split at 35 DAT and the third split at 48 DAT	STB dose: N$_{100}$, P$_{46}$, K$_{94}$, S$_{23}$, Zn$_4$ and B$_{1.5}$ kg ha^{-1} *Application method:* all required fertilizers in full dose and ½ urea and MoP was applied as basal dose whereas the rest urea and MoP applied in 2 equal splits at 25 and 50 days after transplanting
	NM$_3$: 25 % extra of STB as per FRG-2012, all nutrients from chemical fertilizers	Fertilizer dose: N$_{191}$, P$_{16}$, K$_{90}$, S$_{15}$, Zn$_{1.3}$ and B$_{0.63}$ kg ha^{-1} *Application method:* Same as NM$_2$	Fertilizer dose: N$_{125}$, P$_{16}$, K$_{59}$, S$_{11}$, Zn$_{2.5}$ and B$_{0.63}$ kg ha^{-1} *Application method:* Same as NM$_2$	Fertilizer dose: N$_{125}$, P$_{58}$, K$_{118}$, S$_{29}$, Zn$_5$ and B$_{1.9}$ kg ha^{-1} *Application method:* Same as NM$_2$
	NM$_4$: 80% from chemical fertilizer and the 20 % nutrients supplemented with cowdung manure @ 5 t ha^{-1}	Fertilizer dose: N$_{122}$, P$_{10}$, K$_{58}$, S$_{10}$, Zn$_{0.8}$ and B$_{0.4}$ kg ha^{-1} from CF & N$_{31}$, P$_3$, K$_{14}$, S$_2$, Zn$_{0.2}$ and B$_{0.2}$ kg ha^{-1} from cowdung *Application method:* chemical fertilizers were applied as same as NM$_2$ whereas organic manure (cowdung) was applied before land preparation	Fertilizer dose: N$_{80}$, P$_{10}$, K$_{38}$, S$_7$, Zn$_{1.6}$ and B$_{0.4}$ kg ha^{-1}kg ha^{-1} from CF & N$_{20}$, P$_3$, K$_9$, S$_2$, Zn$_{0.5}$ and B$_{0.2}$ kg ha^{-1} from cowdung *Application method:* chemical fertilizers were applied as same as NM$_2$ whereas organic manure (cowdung) was applied before land preparation	Fertilizer dose: N$_{80}$, P$_{37}$, K$_{75}$, S$_{18}$, Zn$_3$ and B$_{1.2}$ kg ha^{-1} from CF & N$_{20}$, P$_{10}$, K$_{20}$, S$_5$, Zn$_1$ and B$_{0.3}$ kg ha^{-1} from cowdung *Application method:* chemical fertilizers (CF) were applied as same as NM$_2$ whereas organic manure (cowdung) was applied before land preparation

Crop harvesting and data collection

Jute was harvested on 31 July 2017, T. Aman rice on 03 December 2017 and onion was harvested on 08 April 2018. Two (2) m^2 area from each plot was selected immediately after sowing/transplanting for data collection. Ten plants of each plot were selected for yield attributes. Thousand grain weight of T. Aman was measured plot-wise. The plants were cut, then threshed, cleaned, sun-dried and weighed of grain and straw separately from 2 m^2 area of each plot. Finally, the grain and straw were converted into t ha^{-1}.

Statistical analysis

The effects of tillage practices and nutrient management approaches on yield and economics were assessed using a two-factor analysis of variance. All data were statistically analysed with SPSS (Statistical Package for the Social Sciences) software package version 21 (SPSS Inc., Chicago, IL, USA). Means were compared by using least significant difference (LSD) at $p < 0.05$.

Results and Discussion

1st Crop: Jute

Effects of tillage and nutrient management on plant population, plant height and fibre yield of jute

Regardless of tillage methods, different nutrient management showed significant variations on the plant height as well as fibre yield of jute (Table 3). The highest plant height (229 cm) was recorded in 25 % extra of STB which was statistically identical to 20 % organic amended with 80% CF but different from FP and STB. The highest fibre yield was obtained from 25 % extra of STB treatment (NM_3, 3.64 t ha^{-1}) which was different from FP (NM_1,2.96 t ha^{-1}), 100% STB (NM_2,3.16 t ha^{-1}) and 20 % organic amended with 80% CF (NM_4, 3.23 t ha^{-1}), shown in Table 2. Bell *et al.* (2019) and Salahin (2017) recorded increased yield of jute fibre in rice-based cropping system in strip tillage and residue retention practice. Higher yields with ST were associated with better crop establishment that was possibly due to better placement of seed and fertilizers.

2nd Crop: T. Aman rice

Effects of tillage and nutrient management on grain and straw yield of T. Aman rice

Different nutrient management showed significant variations in the grain and straw yields of T. Aman rice. No variations in the rice yields were recorded under tillage methods. The highest grain yield was produced in 20 % organic amended with 80% CF (6.02 t ha^{-1}) which was statistically similar with 25 % extra of STB (5.93 t ha^{-1}) and STB recommended fertilizer (5.72 t ha^{-1}). The lowest grain yield (5.43 t

ha^{-1})was obtained in farmers' practice, FP. Similar pattern was noticed in case of straw yield of T. Aman rice, showing the sequence as, 20 % organic amended with 80% CF>25 % extra of STB >STB recommended fertilizer>farmers' practice (Table 4). The increase in rice yield in the study may not only be due to tillage, but also due to the combined effect of conservation tillage and nutrient management practices. Many studies have reported increased productivity of intensive rice-based systems under conservation tillage (reduced/no- tillage) with integrated nutrient management (Munda *et al.*, 2009), but effects vary with regions due to differences in climatic and edaphic factors (Sun *et al.*, 2010).

Table 3. Effects of tillage and nutrient management on plant height and fibre yield of jute

Treatments	Plant height (cm)	Fibre yield (t ha^{-1})
Tillage methods		
Conventional tillage (CT)	216	3.21
Strip tillage (ST)	218	3.29
LSD$_{0.05}$ value	5^{ns}	1.10^{ns}
Nutrient management practices		
NM$_1$ (FP)	203 b	2.96 b
NM$_2$ (STB)	210 b	3.16 b
NM$_3$ (25% extra of STB)	229 a	3.64 a
NM$_4$ (80% CF + 20% CD)	225 a	3.23 b
LSD$_{0.05}$ value	12**	0.39*
CV (%)	4.34	9.58

NM= nutrient management, CF = chemical fertilizers, STB = soil test basis, CD = cow dung, LSD = least significant difference at P>0.05, ns= non-significant, * = P<0.05, ** = P<0.01

Table 4. Effects of tillage and nutrient management on the grain and straw yields of T. Amanrice

Treatments	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Tillage methods		
Conventional tillage (CT)	5.59	6.21
Strip tillage (ST)	5.96	6.94
LSD$_{0.05}$ value	1.34^{ns}	0.28^{ns}
Nutrient management practices		
NM$_1$ (FP)	5.43 b	5.79 c
NM$_2$ (STB)	5.72 ab	6.12 bc
NM$_3$ (25% extra of STB)	5.93 a	6.46 ab
NM$_4$ (80% CF + 20% CD)	6.02 a	6.71 a
LSD$_{0.05}$ value	0.42*	0.39**
CV (%)	5.80	4.91

NM= nutrient management, CF = chemical fertilizers, STB = soil test basis, CD = cow dung, LSD = least significant difference at P>0.05, ns= non-significant, * = P<0.05, ** = P<0.01.

3rd Crop: Onion

Effects of tillage methods and nutrient management on yield and yield attributes of onion

Tillage methods did not show any significant difference in the bulb diameter and bulb yield of onion (Table 5). However, numerically higher bulb diameter and fresh as well as sundry bulb yield of onion was recorded in minimum tillage (MT) than in conventional tillage (CT).

Different nutrient managements had significant effect on the bulb diameter and fresh as well as sundry bulb yield of onion. The highest bulb diameter of onion (3.88 cm) was found in 20 % organic amended with 80% CF which was statistically identical to 25 % extra of STB (3.87 cm) and the lowest bulb diameter was found in farmers' practice (3.67 cm). Similarly, the highest bulb yield (16.0 t ha^{-1} fresh and 13.9 t ha^{-1} sundry) was recorded in 20 % organic with 80% CF followed by 25 % extra of STB(14.6 t ha^{-1} fresh and 12.5 t ha^{-1} sundry), STB recommended fertilizer(14.2 t ha^{-1} fresh and 12.0 t ha^{-1} sundry) and the lowest bulb yield (12.7 t ha^{-1} fresh and 10.6 t ha^{-1} sundry) was found in farmers' practice (Table 5). No research has been done before on combined effect of tillage methods and nutrient management practices. However, Tayel *et al.* (2017) recorded increased bulb volume and yield under minimum tillage practices, while under conventional tillage; increased moisture stress resulted in decreased onion volume and yield.

Table 5. Effects of tillage and nutrient management on the bulb diameter, fresh and sundry bulb yield of onion at farmer's field of Rajbari

Treatments	Bulb diameter (cm)	Fresh bulb yield (t ha^{-1})	Sundry bulb yield (t ha^{-1})
Tillage methods			
Conventional tillage (CT)	3.74	13.6	11.4
Minimum tillage (MT)	3.84	15.1	13.1
LSD$_{0.05}$ value	0.11^{ns}	2.8^{ns}	2.0^{ns}
Nutrient management			
NM$_1$ (FP)	3.67 b	12.7 c	10.6 c
NM$_2$ (STB)	3.75 b	14.2 b	12.0 b
NM$_3$ (25% extra of STB)	3.87 a	14.6 b	12.5 b
NM$_4$ (80% of CF + 20% CD)	3.88 a	16.0 a	13.9 a
LSD$_{0.05}$ value	0.09**	1.1**	1.1**
CV (%)	1.97	5.88	7.36

NM= Nutrient management, CF = Chemical fertilizers, STB = soil test basis, LSD = least significant difference at P>0.05, ns = non-significant, ** = P<0.0.

Integrated nutrient management is one of the most important factors that greatly influence the quality and yield of onion (Mahanthesh *et al.*, 2009 and Bagali *et al.*, 2012). Singh *et al.* (1997) reported that combined doses of organic and inorganic fertilizer (FYM 25 t ha^{-1} + NPK 100:25:25 kg ha^{-1}) increased marketable yield. Bagali *et al.* (2012) noted that the combination of higher levels of inorganic with higher levels of organic fertilizers recorded higher bulb yield and the market value.

Effects of tillage methods and nutrient management on cropping system productivity

Cropping system productivity refers to the total yields of the crops (main produce) grown in sequence in the same piece of land in a year, expressed as, rice equivalent yield (REY). Between the tillage methods, strip/minimum tillage(ST/MT) numerically gave higher REY over conventional tillage (CT).

Among the different nutrient managements, the maximum REY (33.6 t ha^{-1}) was recorded in NM$_4$treatment, where 80% of chemical fertilizers and 20% cowdung was applied which was statistically identical to NM$_3$ treatment (32.3 t ha^{-1}), where 25% extra CF of STB applied but different from NM$_2$ (STB, 30.6 t ha^{-1}) and NM$_1$ (FP), whereas the minimum REY (28.6 t ha^{-1}) was recorded in NM$_1$ (FP), as presented in Table 6.

Table 6. Effects of tillage and nutrient management on rice equivalent yield (t ha^{-1})

Treatments	Rice equivalent yield (t/ha)
Tillage methods	
Conventional tillage (CT)	30.2
Strip/minimum tillage (ST/MT)	32.3
LSD$_{0.05}$ value	2.8^{ns}
Nutrient management	
NM$_1$ (FP)	28.6 c
NM$_2$ (STB)	30.6 b
NM$_3$ (25% extra of STB)	32.3 a
NM$_4$ (80% CF + 20% CD)	33.6 a
LSD$_{0.05}$ value	1.4**
CV (%)	3.66

NM= nutrient management, CF = chemical fertilizers, STB = soil test basis, LSD = least significant difference at $P>0.05$, ns= non-significant, ** = $P<0.01$.

Note: Market prices of the crops were: Tk. 20.0 kg^{-1} for rice, Tk. 25.0 kg^{-1} for onion (fresh) and Tk. 45.0 kg^{-1} for jute fibre.

Yadav *et al.* (2017) put forward that adoption of conservation tillage and nutrient management practice involving no-tillage and integrated nutrient management along with residue retention can enhance the system productivity of the rice-based cropping system in Indo-Gangetic plains. Yadav *et al.* (2017) concluded that modifications of farmers' practice with inclusions of minimal disturbance of soil improved soil health and increased the system productivity by an average of 30.6%.

Profitability of growing crops under different tillage methods and nutrient management in the jute-T. Aman- onion cropping system

From the first cop, jute cultivation, the highest net return (64,306 taka ha^{-1}) and BCR (1.83) was obtained from strip tillage and 25% extra of STB and the lowest net return (54,129 taka ha^{-1}) and BCR (1.72) was in CT and FP combination (Table 7).

Table 7. Profitability of component crop production under different tillage methods and nutrient management at Rajbari

Particulars	Strip tillage (ST)/Minimum tillage (MT)				Conventional tillage (CT)			
	NM$_1$ (FP)	NM$_2$ (STB)	NM$_3$ (25% extra of STB)	NM$_4$ (80% CF + 20% CD)	NM$_1$ (FP)	NM$_2$ (STB)	NM$_3$ (25% extra of STB)	NM$_4$ (80% CF + 20% CD)
Jute								
Gross return (Tk. ha^{-1})	127120	131620	141450	132750	128910	133010	142820	134420
Total input cost (Tk. ha^{-1})	72989	75218	77144	75190	74781	77010	78936	76982
Net return (Tk. ha^{-1})	54131	56402	64306	57560	54129	56000	63884	57438
BCR	1.74	1.75	1.83	1.77	1.72	1.73	1.81	1.75
T. Aman rice								
Gross return (Tk. ha^{-1})	120370	123330	125700	126630	116200	119370	121540	122660
Total input cost (Tk. ha^{-1})	75227	76869	79686	77416	78699	80341	83158	80888
Net return (Tk. ha^{-1})	45143	46461	46014	49214	37501	39029	38382	41772
BCR	1.60	1.60	1.58	1.64	1.48	1.49	1.46	1.52

Particulars	Strip tillage (ST)/Minimum tillage (MT)				Conventional tillage (CT)			
	NM$_1$ (FP)	NM$_2$ (STB)	NM$_3$ (25% extra of STB)	NM$_4$ (80% CF + 20% CD)	NM$_1$ (FP)	NM$_2$ (STB)	NM$_3$ (25% extra of STB)	NM$_4$ (80% CF + 20% CD)
Onion								
Gross return (Tk. ha^{-1})	266000	298000	308000	340000	242000	270000	276000	300000
Total input cost (Tk. ha^{-1})	114802	122492	128455	122762	109874	117004	123527	116714
Net return (Tk. ha^{-1})	151198	175508	179545	217238	132126	152996	152473	183286
BCR	2.32	2.43	2.40	2.77	2.20	2.31	2.23	2.57

In case of T. Aman rice cultivation, the highest net return (49,214 taka ha^{-1}) and BCR (1.64) was found in 20 % organic amended with 80% CF + 20% CD) with non-puddling treatment (ST), while CT and 25% extra of STB combination gave the lowest BCR (1.46), as presented in Table 7. It was due to the use of higher rate of costly chemical fertilizers requirements.

In case of onion cultivation, the highest net return (2,17,238 taka ha^{-1}) and BCR (2.77) was found in minimum tillage and 20 % organic amended with 80% CF, whereas farmers' tillage and nutrient practice gave the lowest net return (1,32,126 taka ha^{-1}) and BCR (2.20), as shown in Table 7.It was due to lower yield obtained with the farmers' practice.

Conclusions

Different nutrient management showed significant variations on fibre yield of jute; grain yield of T. Aman rice as well as the bulb yield of onion at the farmer's field of Rajbari district. The yield and yield attributes of jute, T. Aman rice and onion responded similarly to tillage methods (p>0.05). The highest fibre yield (3.64 t ha^{-1}) of jute was obtained from 25 % extra of STB and20 % organic amended with 80% CF (3.23 t ha^{-1}) compared to STB dose(3.16 t ha^{-1}) and farmers' practice (2.96 t ha^{-1}). The highest grain yield of rice was produced in 20% organic amended with 80% CF (6.02 t ha^{-1}), which was statistically similar to 25 % extra of STB(5.93 t ha^{-1}) and STB dose(5.72 t ha^{-1}). In case of onion, 20% organic amended with 80% CF outperformed yields of 16.0 t ha^{-1} fresh and 13.9 t ha^{-1} sundry than other nutrient management practices. Supplementing 20% of RFD by CD along with 80% chemical fertilizer exhibited the best performance over other treatments (25% extra of STB dose, STB dose and farmers' practice) in terms of crop yield and economic return.

Acknowledgement

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SOCIO ECONOMIC ASPECT OF ORGANIC FARMING PRACTICES FOR IMPROVING FARMER'S INCOME IN SOME LOCATIONS OF KERALA, INDIA

N. KARUNAKARAN[1] AND M. S. SADIQ[2]

Abstract

Agriculture is the major source of livelihood and is under the big threat of liberalization and modernization. Organic farming is eco-friendly, promotes sustainable development, protects the fertility of the soil and ensures long term crop income to the farmer. In 2018, total area under organic certification process is **3.56 million hectare and** produced around **1.70 million MT** of certified organic products. In Kerala, the total area under organic farming stands at 15790.49 hectare. Organic farmers fail to capture the market for selling their products and have less capacity in the competing world, leads to worse financial situation of farmers. The total volume of export during 2017-18 was **4.58 lakh MT.** Fair trade has flourished as an initiative for lifting poor organic farmers by providing higher price, credit and improved community life. It is also a market for high value products in the global trade policies. Fair Trade Alliance Kerala (FTAK) is a small farmers' organisation to access global market on fair trade in an equitable trading terms and improved income. The system provides better price to products compared to open market and benefited fair trade exporting. This paper by studying FTAK focused the impacts of it on income and highlights the increased production of organic crops, better prices, premium and schemes to farmers. The study revealed that fair trade farmers earned higher price (20 to 50 percent) for commodities and marketing of products to foreign countries without intermediaries and organic farming is a better option for increasing farmer's income in India.

Keywords: farmers' income; Fair Trade Alliance Kerala; organic Farming: India

JEL Classification: Q7; Q10; Q11; Q16.

1. Introduction

Organic farming in India over the years is growing and is important in the present day economy. The area under different organic practices across the country is very high and different agencies involved directly and indirectly in promoting organic farming. It includes: government agencies, non-governmental organizations and farmer's groups. In addition, there are many farmers isolated from formal groups and agencies. Major government agencies involved in the promotion of organic farming are the Department of Agriculture, Panchayath Raj

[1]Head of the Post-graduate Department of Economics, EKNM Government College Elerithattu, 671314, Kasaragod, Kerala, India, [2]Department of Agricultural Economics and Extension Technology, FUT, Minna, Nigeria.

Institutes, Agricultural Universities, State Horticultural Missions, State level offices of the Commodity Boards (like Spices Board, Tea Board and Coffee Board), and NABARD. Non-governmental organizations were active for promoting poison-free agriculture and used different concepts, approaches and methods (Karunakaran and Silna Thomas, 2017). There is an increasing tendency among farmers for ruthless conversion of land for cash crops, indiscriminate use of insecticides and chemical fertilisers. In the light of existing problems, farmers have to provide access to the required inputs and knowhow for alternate organic methods of farming.

Fair Trade is an organised social movement aims to help producers in developing countries for better trading condition and sustainable agriculture. This implies certification that advocates higher price and better social and environmental standards (Benjamin, 2000). It focused particularly on exports from developing countries to developed countries most notably, handicrafts, coffee, cocoa, sugar, cashewnut, tea, bananas, honey, cotton, wine, fresh fruits and gold. There are a number of fair trade and ethical marketing organisations with different marketing strategies over the last twenty years (Kristen, 2011). Most fair trade organisations are members certified by several national and international federations. Fair Trade Labelling Organisation International (FLO) is named as Fair Trade International in 2009 (Reynolds, 2012). In India, fair trade movement opened huge markets for farmers.

Fair Trade Alliance Kerala (FTAK) was formed in 2005 in the Malabar Coast of India. It draws its membership and charter from mass movement of farm houses, struggled against rural appropriation and indebtedness (John, 2012). Among the farmers, 50 percent are certified organic and the aim is 100 percent. FTAK farmers grow a host of crops like cashew-nut, coconut, coffee, cocoa, pepper, vanilla and other spices. In mid-2000s, particularly in south-western India, FTAK addressed the devastating impact of fluctuating market prices on the ability of small farmers to earn a stable income. The prices in the global market fluctuated dramatically and the farmer's ability to plan and make investment affected. FTAK members are participating in fair trade system, exports, assure minimum price above market price and plan more confidently their future and reinvest in their farms for social projects (Karunakaran and Libin, 2014). Fair trade thus is a social movement with stated goal to help farmers to achieve better trade, income and promote sustainability (Kristen, 2011) in organic farming. In this context, this paper made an attempt to examine organic farming as an option for increasing farmer's income in India by analysing Fair Trade Alliance Kerala as a case in terms of price of products, socio-economic condition of farmers, living standard, sustainability of organic farming and schemes adopted by fair trade. More specifically, the main objectives of the study include:

   (i)   To examine organic farming as an option for increasing farmer's income in India, and

(ii) To analyse Fair Trade Alliance Kerala as a case by examine the price of products, socio-economic conditions, living standards, sustainability of farming and schemes in fair trade.

2. Materials and Methods

FTAK in Kerala was taken as a case and is mainly concentrated in the districts of Kasaragod, Kannur, Kozhikode and Wayanad. Among these districts, primary data were collected from 120 farmers from three panchayaths of Kasaragod, Kannur and Wayanad districts, taking one panchayath from each district. Data were collected from these 120 farmers taking 40 farmers from each panchayath for pepper, ginger, turmeric, cocoa, coconut and cashew-nut crops during the period of 2018. Primary data is obtained directly from the farmers of these panchayaths through questionnaire, interview schedule and focus group discussions. Secondary data were collected from published reports, personal and FTAK records and fair trade alliance branches in Kerala. Statistical tools like simple growth rate, percentages and diagrams were used for analysis.

3. Results and discussion

3.1. Fair Trade Alliance Kerala (FTAK), origin and growth: Fair trade means a trading partnership based on dialogue, transparency and respect that seeks greater equity in the international trade (Karunakaran and Silna Thomas, 2017). Fair Trade Alliances Kerala was founded in 2005 by Kerala's first organic store elements in Kozhikode, which was seeking to offer local market access to the growing number of organic farmers in Kerala. It is a mass based, farmers led movement in Kerala focusing solely on justice and concern in trade, positioned to offer fair trade market for high value products (Karunakaran and Libin Thomas, 2014).

Initially, Fair Trade Alliance Kerala was formed with 4500 farmers of Kannur, Kasaragod, Wayanad and Kozhikode (Karunakaran and Silna Thomas, 2017). It is uniquely positioned to offer fair trade market to products including cocoa, coffee, cinnamon, turmeric, cashewnut, ginger, pepper, cardamom and coconut. FTAK also aimed to enable farmers the access to global market and improved income through fair trade. The basic idea behind this is to promote sustainable development through trade and works according to a range of fair trade principles like fair price, fair labour condition, direct trade, commodity development and environmental sustainability (Chamorro, 2005). Organic marketing and fair trade are quite different from that of regular marketing and free trade (Benjamin, 2000). In Kerala among Kasaragod, Kannur, Wayanad and Kozhikode districts, around 34500 farmers have memberships in FTAK. Kannur district have the highest farmers membership (1870) and highest total area (3008 hectare) compared to other districts. Among 5804 hectare land existed, 1572 hectare is organic and 2255 hectare land is in-conversion (Table 1).

Table 1: Organic farmers and area cultivated in Fair Trade Alliance Kerala

Sl. No.	Districts	Number of Farmers	Total Area (in Hectare)	Organic Area (in Hectare)	In-conversion Area (in Hectare)
1	Kasaragod	923	1879	510	620
2	Kannur	1870	3008	644	1202
3	Wayanad	513	616	298	259
4	Kozhikode	144	301	120	174
	Total	34500	5804	1572	2255

Source: Karunakaran and Libin Thomas (2014)

FTAK aims to ensure small scale farming a sustainable occupation. The main objectives of FTAK include: (i) to promote the overall welfare of farmers by enhancing unity among farmers, introducing agricultural operations for nature and human community and ensure participation of farmers in production, processing and distribution of agricultural products; (ii) to introduce plans and programmes for farmers to market their products at a reasonable price; (iii) to promote ancillary activities for making agriculture profitable; (iv) to promote the quality of agricultural products; (v) to encourage self confidence, mutual understanding and unity among farmers; and (vi) to constitute cells of farmers at panchayath, district and state levels.

FTAK followed organic and poison-free farming, and productivity increased (Table 2); compared to 2009 it increased to 180 percent in 2017, showing continuous increase in productivity.

Table 2. Productivity growth under organic farming in FTAK

Sl. No	Year	Productivity growth (in %)
1	2009	100
2	2010	106
3	2011	127
4	2012	152
5	2013	161
6	2014	162
7	2015	165
8	2016	171
9	2017	180

Source: Calculated using primary data taking 2009 as base year

FTAK is democratic in nature and the entire activity is carried out by elected members from the grass root to state level. It provides a platform to sit together and decide the course of action. Since its inception, FTAK has been active in the

international fair trade commodity market. The flagship products of the organization are cashew, coffee and spices. About 2100 metric tonne of fair trade cashew has been sourced from Kannur and Kasaragod districts under fair trade valued around Rs 200 million (Govt. of Kerala, 2016). FTAK farmers have shifted to organic farming; now all are either organic or working towards it.

3.2. FTAK and price of organic products: Figure 1 show that 47 percent and more depend on fair trade for selling their organic products. From table 3 it is revealed that fair trade alliance offered higher prices to the organic products, and the difference is the highest for pepper, turmeric, cocoa and ginger.

Table 3. Price variation in FTAK and open market for organic products

Sl. No	Products	Price (Rs per Kg)		
		FTAK	Other markets	Difference (in %)
1	Pepper	400	300	25
2	Cashewnut	140	110	21
3	Turmeric	30	15	50
4	Coconut	45	35	22
5	Ginger	42	32	24
6	Cocoa	155	105	33

Source: Field survey, 2018
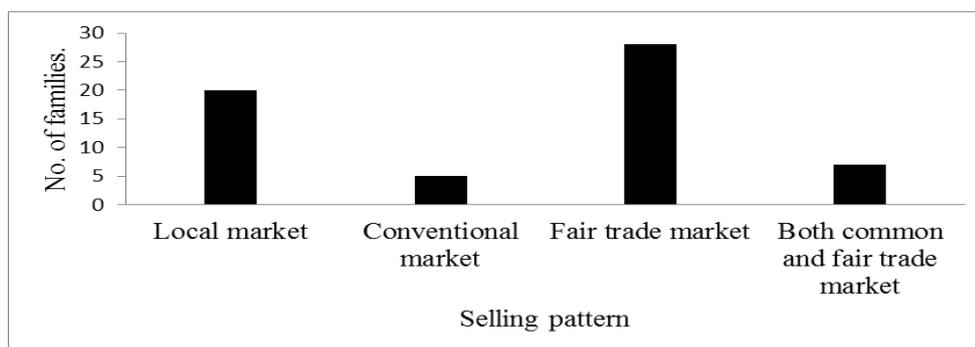
Figure 1: Selling pattern of organic commodities

Table 4: Income from organic farming

Sl. No	Land of households		Annual income of households	
	Size of Land (in hectare)	%	Income (in Rs)	%
1	Below 0.2	32	Below 25000	17
2	0.2 – 0.4	10	25000 – 50000	25
3	0.4 – 1.6	42	50000 - 1 lakh	50
4	Above 1.6	16	1 lakh - 4 lakh	8

Source: Field survey, 2018   Note: (1 USD = 66 Rs)

3.3. FTAK and organic farming: Table 4 shows the income generated by selling products and majority earned more than 50 thousand rupees per year. Ninety five percent of coconut farmers, 92 percent of cocoa cultivators, and majority of others are following organic farming practices to earn income (Table 5).

Table 5: Main organic crops under cultivation

Sl. No	Crops	Families cultivated (in %)
1	Ginger	72
2	Pepper	55
3	Coconut	95
4	Cashewnut	20
5	Turmeric	42
6	Cocoa	92

Source: Field survey, 2018

3.4. FTAK and socio-economic conditions of organic farmers: FTAK provided supportive measures for bringing women to the forefront. The aged persons are promoting organic farming compared to others; 75 percent of the farmers belong to the age group of 55 to 75 years in the study area (Table 6).

Table 6: Organic farmers age under FTAK

Sl. No	Age of farmers	Percent of farmers
1	35 – 45	10
2	45- 55	12
3	55 – 65	42
4	65 – 75	33
5	75 – 80	3
	Total	100

Source: Field survey, 2018

3.5. FTAK and various schemes to organic farmers: FTAK also provided different schemes for increasing farming which include: (i) organic agriculture promotion; (ii) distribution of tools; (iii) training; (iv) community kitchen for school children; (v) solar fencing for farmers; (vi) cash awards to farmers; (vii) community water supply; and (viii) women empowerment programme. Table 7 shows the beneficiaries under various schemes and shows that more than 75 percent were benefited from different schemes like animal husbandry, subsidy and medical facilities,

Table 7: Organic farmer's implemented programmes by FTAK and its benefits

Sl. No	Schemes	Benefited Farmers (in %)
1	Subsidy	25
2	Pension	8
3	Grants	13
4	Animal Husbandry	34
5	Medical Facility	20

Source: Field survey, 2018

3.6. FTAK and sustainability of the organic sector: FTAK is based on dialogue transparency and respect which provides greater equity in international trade, contributed to sustainable development. Better price, decent working conditions, local sustainability, and fair terms of trade in fair trade enables to improve their position. It also awarded to products to meet the standards and certified.

To FTAK, organic agriculture is the only sustainable agricultural development process to get safe, non toxic food and protect the environment in the farming process. Organic foods are those that are produced using environmentally sound methods that do not involve modern synthetic inputs such as pesticides and chemical fertilizers, do not contain genetically modified organisms, and are not processed using irradiation, industrial solvents and chemical food additives.

Organic farmers earned income from the products based on the quality and quantity of products. The prices of crops are different and income earning capacity also related to the product type. Table 8 shows that 8 percent of the farmers earned below 10000 rupees and 63 percent between 15000-25000 rupees; 12 percent attain more than 25000 rupees from their crops.

Table 8: Organic farmer's income earning capacity under FTAK

Sl. No	Income	Number of farmers	%
1	Below 10000	10	8
2	10000 – 15000	20	17
3	15001 – 20000	40	33
4	20001 – 25000	36	30
5	Above 25000	14	12
	Total	120	100

Source: Field survey, 2018

4. Conclusion and recommendations

Organic farming is eco-friendly, promotes sustainable development in the environment and protects fertility of soil. Fair Trade Alliance Kerala helps

organic farming to maintain food security, food safety, fair prices and better socio-economic conditions to farmers. It is a small farmers' organisation created to access the global market on fair trade and equitable trading to improve farmer's income through fair trade minimum price and premium.

From the results of the study it is concluded that fair trade alliance in Kerala has provided for the social and economic developments of farmers. The quality of crops is very high in the study area because the farmers were not using any type of chemical pesticides and fertilizers for the crop. Fair trade not only aimed at the financial uplift of farmers but also the social developments like, women empowerment, environment protection, and sustainable development.

Organic products gained significant social and economic importance as a major foreign exchange earner. It also provided different schemes to organic farmers for increasing their production. From the analysis it is revealed that the programmes of FTAK encouraged agricultural production and productivity. The organic products like pepper, cashewnut, coconut, ginger, turmeric and cocoa have reached high quality from the cultivation and the farmers are benefited in social development, women empowerment and environmental protection. FTAK provided awareness classes to organic farmers and encouraged them by ensuring high prices to the crops. FTAK also helped the farmers for marketing their products in international market and attain better price and increased income. Hence the initiative of the government to increase farmer's income through organic farming practice is essential and authority should develop innovative programmes initiated by FTAK for the promotion of agricultural sector and thereby increase farmer's income for reducing poverty.

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IMPACT OF MULTI-CROP PLANTER BUSINESS ON SERVICE PROVIDERS' LIVELIHOOD IMPROVEMENT IN SOME SELECTED AREAS OF BANGLADESH[1]

M. A. M. MIAH[1], M. E. HAQUE[2], AND R. W. BELL[3]

Abstract

The custom hiring of the Versatile Multi-crop Planter (VMP) is a profitable enterprise for Local Service Providers (LSP) that could improve their household livelihood but the impacts have not yet been assessed in Bangladesh. In this study, we assessed the use pattern and profitability of custom-hiring the VMP, and its impacts on LSPs' livelihood. A total of 18 LSP were purposively selected from Rajshahi, Thakurgaon, Mymensingh, and Rajbari districts for this study. The study revealed that LSPs effectively utilized VMP and 2-wheel tractor (2WT) for 4-6 months. They earned Tk. 1,42,434 (with incentive on VMP) and Tk. 1,36,134 (without incentive) per year as net income. The average payback periods were 0.72 and 0.98 years with and without incentive, respectively. The annual break-even of a VMP use was 7.79 ha. The LSPs reported a considerable cash (annual income increased by 34.9 %), and capital enhancement, e.g., land holdings (8.3 %), value of livestock (11.2 %), dwelling houses (36.6 %), household furniture (19 %), and modern amenities (45.5 %). The increased incomes from VMP custom hiring were mostly spent on nutritious food, land mortgage-in, and dwellings. The LSPs reported minor challenges such as being unable to use this machine in the wetland condition (61 %), no seating arrangement on the machine during operation in small plots (56 %), long time taken to complete sowing due to narrow planting width per pass (50 %), farmers' skepticism about the planting machine as it is still new technology (44 %), and lack of skilled drivers (17 %). Financial support and technical assistance should be made available by the government for LSPs and local manufacturers to accelerate greater adoption of the VMP.

Keywords: break-even point, net return, payback period, planting machine, planter custom hire

Introduction

Mechanization of tillage by 2-wheel tractor (2WT, or power tiller) spread in Bangladesh since the mid-1990s replacing manual and animal-draught tillage. Small farm sizes restrict the purchase and utility of 4-wheel tractors here and in many parts of Asia and Africa. Hence the 4-wheel tractor options for mechanized planting are not available in the regions where 2WT are the predominant form of

mechanized tillage (Haque *et al.,* 2017). In Bangladesh, shortage of hired-labour and substantial increases of crop production cost are leading to greater interest in mechanization in smallholder agriculture (Bell *et al.,* 2017).

Presently, the 2WT are mostly operated in fully rotary tillage mode (Haque *et al.*, 2016). However, there are promising developments in minimum soil disturbance planters for 2WT (Johansen *et al.*, 2012; Haque *et al.,* 2016). The one widely-used option in Bangladesh to date, the Single Pass Shallow Tillage planter (SPST) (also known as the Power Tiller Operated Seeder, PTOS), involves shallow full rotary tillage of the top soil (up to 0.04 m) (Wohab *et al.,* 2007; Roy *et al.*, 2009). Full tillage such as this can be harmful to soil structure and soil health (Johansen *et al.,* 2012).

Bell *et al.* (2017) and Sarker *et al.* (2012) concluded that the field performances of minimum soil disturbance planters were much better than conventional full tillage systems. Different crops can be established and grown successfully through planting with minimum soil disturbance (Haque *et al.*, 2018, Bell *et al.*, 2017, Barma *et al.,* 2014; Alamgir *et al.,* 2015). Crop establishment using minimum soil disturbance planters reduces operational costs; including machinery, labour, and fuel, while increasing yields and efficiency of natural resource use (Haque *et al.,* 2018, Bell *et al.*, 2017, Roy *et al*., 2009). Islam *et al.* (2010) found that 41-43% less irrigation water was used for crop established by VMP planting as compared to a traditional full tillage by 2WT. In this context, minimum soil disturbance is becoming increasingly important in decreasing crop production cost, soil health improvement, and overcoming the problems of declining agricultural productivity in developing countries (Johansen *et al.* 2012).

The cropping intensity of Bangladesh is increasing progressively over time and most small holders are now growing two or more crops per field in a year (BBS, 2018; BER, 2018). Over a 5-year cycle due to changing profitability of crops, farmers cultivate 4-6 crops with diverse seed sizes, seed rate, row spacing, fertilizer rates, and seed depth. Hence a planter for such diverse cropping systems needs to handle such variations in settings as well providing for different crop establishment options according to farmer demand, i.e. strip planting, zero planting, bed planting, single pass shallow tillage and planting, even conventional full tillage. The LSP of the planter also need to be able to hire out their planter for business as much of the year as possible to justify the investment cost. There are a number of other criteria and challenges that would need to be satisfied by potential purchasers of a planter. The VMP was developed in Bangladesh in 2008 to meet the above criteria and successfully establish a diverse range of crops (Haque *et al.,* 2017). It has capability for seeding and fertilizing with fluted roller or vertical plate meters in lines for single-pass shallow-tillage, strip planting, zero tillage and bed planting. Most of the grain seeds including wheat, paddy, maize, jute, pulses, oilseeds etc. can be sown in lines by the VMP (Bell *et al.*, 2017).

The ACIAR funded LWR-2010-080 Project invented new methods to create demand and accelerate farmer testing of planting services by VMP that attract new LSPs and farmers while the technology was still unfamiliar to them. Each and every new VMP owner received a once-only planting incentive @Tk. 300/- per *bigha* for up to 30 *bigha* of planting. This model was applicable for the purchase of VMP alone. The Project conducted audits to verify that planting was completed by the LSP before the incentive was approved. The audits were also used to identify cases of poor planting performance or planter faults so that these could be corrected. Previous experience with LWR/2010/080 suggests that incentive support helps the LSP to quickly build a client base for future business, and reduces the risk for first time users of the VMP planting service so that they can gain confidence in its reliability and cost effectiveness. These plantings also serve as demonstrations to advertise to local farmers the effectiveness of the planting.

The LSPs are conducting business with smallholders on a custom hire basis with different implements. The custom hiring of PTOS was highly profitable at farm level (Miah *et al.*, 2010) and improved LSPs livelihood (Miah and Haque, 2015). However, the impact on LSPs livelihood using VMP was not assessed yet. In this paper, we will report the outcomes of a study to assess the livelihood of LSPs using the VMP in 2016.

Objectives of the study

The specific objectives of the study were:

   (i) To estimate the profitability of the custom hiring of the VMP for seeding operations on farms;

   (ii) To assess the impacts of VMP on LSP's livelihood improvement; and

   (iii) To determine the problems that LSPs reported during VMP operation.

Materials and Methods

Study Area Selection

The project "Overcoming agronomic and mechanization constraints to development and adoption of conservation agriculture in diversified rice-based cropping in Bangladesh" was funded by ACIAR since April 2012 to March 2017. In this project, a total of 47 VMPs were purchased by interested LSP of Rajshahi, Thakurgaon, Mymensingh and Rajbari districts during 2012-16. Therefore, the above mentioned districts were purposively selected for the present study.

Sampling Technique and Period of Study

In order to evaluate the impact of VMP operations at farm level, a total of 18 LSP of VMP who provided at least 2-years of planting services to the farmers

were purposefully selected from the aforesaid districts and interviewed for this study. Data and information were gathered from selected LSPs of VMP through conducting household survey using pre-tested questionnaire and the interview was conducted during December, 2016.

Analytical Technique

*Calculation of costs and return***:** In estimating the profitability of VMP operation at farm level, different cost items such as fuel and oil, wages for a driver, repair and maintenance, spare parts, depreciation, and interest on investment were calculated based on field level data. The costs of depreciation for 2WT or power tiller (add the % used), VMP and machinery shed were assumed to be linear over time. In the study area, the 2WT was not used throughout the year with VMP. However, 12 months were considered in estimating the costs of depreciation and interest on investment for both VMP and 2WT in this study.

The profitability of VMP for planting operation at farm level was estimated both with and without planting incentive. We calculated the breakeven point of VMP operation for the LSP based on fixed cost, variable cost and gross income which were calculated from farm level data. At break-even point, the revenues of the business are equal its total costs and its contribution margin equals its total fixed costs. The break-even on the basis of land area (ha) planted by VMP was estimated using the following equation.

$$\text{Breakeven land area (ha) planted by VMP} = \frac{FC}{P - VC}$$

Where, FC is total fixed cost, P is the gross income, and VC is total variable cost

*Calculation of impacts***:** The impacts of VMP on the livelihoods of LSPs were assessed through analyzing *'Before'* and *'After'* socio-economic standings of the LSPs. It means that we asked the respondent to report what was their situation before and after operating the VMP. Data regarding land holdings, livestock resources, yearly household income, ownership of farm equipment, household assets, liability status, and food intake were analyzed and compared for measuring the impacts of VMP service on its LPS's livelihoods. The values of different household assets were collected based on present value. For example, the house of a service provider was built five years back with Tk.50000 but due to price hiking; the present value of this house is Tk.70000 which was used for reporting. Besides, if that farmer invests extra money for renovation and/or extension of the house that amount was also added to the present value in this report. Two-tail t-test was employed to determine significant difference between two periods.

Results and Discussion

Socioeconomic Profile of VMP Local Service Providers

There are numerous interrelated and constituent attributes that characterize a person and these profoundly influence development behavior. Some related socioeconomic characteristics of the LSPs of VMP are shown in Table 1.

Age is an important factor that may influence an entrepreneur's decision to operate the VMP as a custom hiring business. The average age of the respondents was 34 years ranging from 22 to 58 years. They were grouped into four categories based on their level of education. About 56 % of them completed primary level of education, followed by 22 % of higher secondary levels. Only 11 % of LSPs completed degree-level education. The average length of experience of LSP in VMP operations was three years ranging from two to seven years. Most of them had two years experience. All of the respondents bought VMP by their own cash with planting incentive from the LWR-2010-080: Conservation Agriculture Project. Many LSP owned a number of farm implements namely 2WT (power tiller), power thresher, shallow tube well (STW), sprayer, and hand weeder that were mostly used for renting out to others for earning cash income. All of sampled VMP service providers owned 2WT and sprayer, 72 % owned power thresher, 50 % owned STW and 22 % owned hand weeder (Table 1). Hence none of the LSP were totally reliant on the VMP for earning income by custom hiring. Income from the other custom hiring services is not reported here.

Table 1. Socioeconomic profile of VMP local service providers in the study areas

Items	No. of respondent	Mean
1. Farmers' age (year)	18	34.0
2. Level of education (%)		
a. Primary level (Class I-V)	10	55.6
b. Secondary level (VI-X)	2	11.1
c. Higher secondary level (HSC)	4	22.2
d. Degree & above	2	11.1
3. Experience with VMP service (%)		
a. 7 years (2010)	2	11.1
b. 5 years (2012)	1	5.6
c. 3 years (2014)	4	22.2
d. 2 years (2015)	11	61.1
4. Type of farm machinery owned (%)		
a. Versatile Multi-crop Planter (VMP)	18	100
b. 2-wheel tractor or power tiller	18	100
c. Power thresher	13	72.2
d. Shallow tube well	9	50.0
e. Sprayer	18	100
f. Hand weeder	4	22.2

Source: Field survey, 2016

Uses Pattern of VMP and 2WT at Farm Level

The LSPs in the study areas used VMP for crop cultivation in their own land and provided custom hiring services to other farmers to plant a range of crops including wheat, maize, pulses (lentil & mungbean), and mustard. The seedlings of *Boro* paddy were also transplanted in strip-based non-puddled condition. The strips were made by VMP however, transplanting of rice seedlings was done manually. On the other hand, the LSPs also used 2WT for tillage in their own land and rented out it to other farmers for tillage operation. On an average, each LSP accomplished 21.7 ha of crop establishment (e.g., land preparation, seeding in line, fertilizing near to seeded line and land leveling) by VMP, and a 2WT completed 36.4 ha for only tillage operation in 2015-16. The highest area of land was planted through VMP in Rajshahi district followed by Thakurgaon district. Again, the highest tillage was done by 2WT in Rajshahi district followed by Rajbari and Mymensingh district. The LSPs of Rajbari and Mymensingh districts used VMPs for their own crop cultivation purposes and did not provide any tillage service to other farmers. Similarly, two LSPs of Thakurgaon district did not provide any VMP service to other farmers during 2015-2016 (Table 2).

Table 2. District-wise area planted by VMP and 2WT during 2015-2016

District	No. of VMP/2WT studied	Land area planted by VMP (ha)		Land area tilled by 2WT (ha)	
		Total area	Average area	Total area	Average area
Rajshahi	11	305.8	27.8	469.6	42.7
Thakurgaon	4	77.8	19.5	78.5	19.6
Rajbari	2	5.5	2.8	78.2	39.1
Mymensingh	1	1.9	1.9	29.4	29.4
All area	18	391.0	21.7	655.7	36.4

Source: Field survey, 2016

The sampled LSPs provided VMP services for 4 months in a year (Fig-1). The period from October to November was the peak season of VMP service since most of the *Rabi* crops are sown within these periods. By contrast, the periods from April to September and January to February was treated as lean periods for VMP service. In the June-July period, 2WT was used for puddling the lands for *T. Aman* seedling transplantation. 2WTs were also widely used in December for initial tillage of the land for *Boro* rice seedling transplantation (Fig-1).

Fig 1. Seasonality of VMP and 2WT use in the study areas
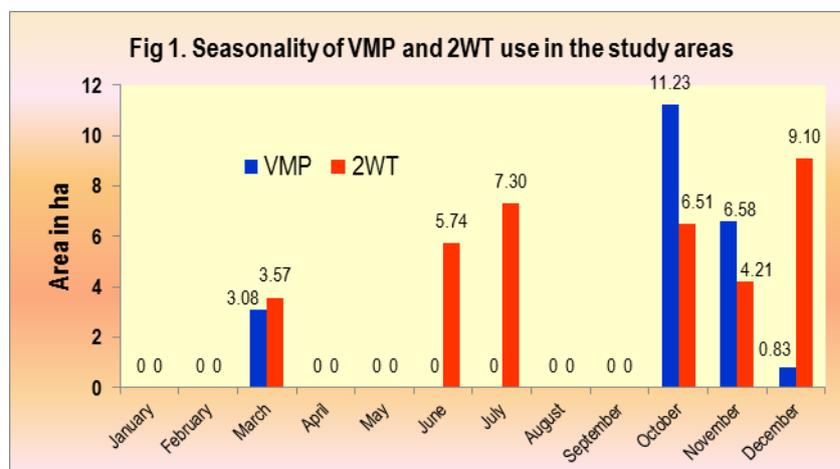
Benefit-Cost Analysis of VMP Operations

The total costs of VMP operations with and without planting incentive for VMP were Tk.1,41,855 and Tk. 1,48,155, respectively. In present situation (with planting incentive), the share of VC and FC were 84.5 % and 15.5 %, respectively. Among cost items, the highest cost was for fuel and oil (70.2 %) followed by driver's wage (11.3 %) and interest on investment to purchase the machinery (8.7 %).

LSPs pointed out that the renting out of VMP services to other farmers was a profitable business in the study areas. The average area under land preparation and seed sowing by VMP per year ranged from 1.9 ha to 57.8 ha with an average of 21.7 ha. Again, the average area of full tillage with 2WT was estimated at 36.4 ha per year. The custom hiring charge of VMP in the last year ranged from Tk. 2,964 to Tk. 3,705 per ha with an average of Tk. 3,273 per ha. By comparison, the average per ha custom hiring charge for 2WT was Tk. 5,852 for full tillage (average 2.72 tillage passes). The custom hiring charges varied in the study areas due to the extent of demand for VMP and 2WT services. Based on this information, the annual gross return received from VMP and 2WT services were calculated. The average gross return received by the LSP was Tk. 2,84,289 per year. The annual net returns over total cost were Tk. 1,44,434 and 1,36,134 with planting incentive and without planting incentive on VMP, respectively. The average benefit cost ratios (BCRs) were 2.00 and 1.92, respectively implying that VMP operations on custom hiring basis at farm level was profitable (Table 3).

The current rate of adoption of VMP at farm level was still low since VMP is in its initial stage of promotion and commercialization. However, it is hoped that the current net return and rate of return will be higher in near future when the rate of adoption will be high.

**Table 3. Benefit-Cost analysis of VMP operations at farm level**

Particular	With planting incentive on VMP	Without planting incentive on VMP
A. Gross income (Tk/year)	**2,84,289**	**2,84,289**
Average area under tillage (ha/LSP)	58.2	58.2
Average rental charge of 2WT&VMP (Tk/ha)	4,888.81	4888.81
B. Variable cost (Tk/year)	**1,19,816 (84.5)**	**1,19,816 (80.9)**
Fuel and oil	99,616 (70.2)	99,616 (67.2)
Wage for driver	15,968 (11.3)	15,968 (10.8)
Repair and maintenance	1,521 (1.1)	1,521 (1.0)
Spare parts	2,711 (1.9)	2,711 (1.8)
C. Fixed cost (Tk/year)	**22,039 (15.5)**	**28,339 (19.1)**
Interest on machineries (2WT+VMP)	12,373 (8.7)	15,973 (10.8)
Depreciation on power tiller (2WT)	6,580 (4.6)	6,580 (4.4)
Depreciation on VMP	2,700 (1.9)	5,400 (3.6)
Depreciation on machinery shed	386 (0.3)	386 (0.3)
D. Total cost (Tk/year)	**1,41,855 (100)**	**1,48,155 (100)**
E. Gross margin (Tk/year)	1,64,473	1,64,473
F. Net income (Tk/year)	1,42,434	1,36,134
G. Rate of return (BCR)		
Over variable cost	2.37	2.37
Over total cost	2.00	1.92
H. Payback period of VMP (year)	**0.72**	**0.98**

Note: Figures in the parentheses are percent of total

Source: Appendix table 1

Break-even Analysis of VMP Operations

With the planting incentive, the annual fixed cost (FC), variable cost (VC) and gross revenue (GR) were estimated at Tk. 22,039, Tk. 2,060 and Tk. 4,889 per hectare respectively. Therefore, the break-even use of VMP is 7.79 ha of planting per year. Break-even point is the point where a LSP can operate the VMP with no loss or profit. Therefore, the LSP in the study area have to cultivate more than 7.79 ha of land per year to make it profitable (Fig 2). In fact their average planting area was 2.8 times higher.

Fig 2. Break-even analysis for VMP operations
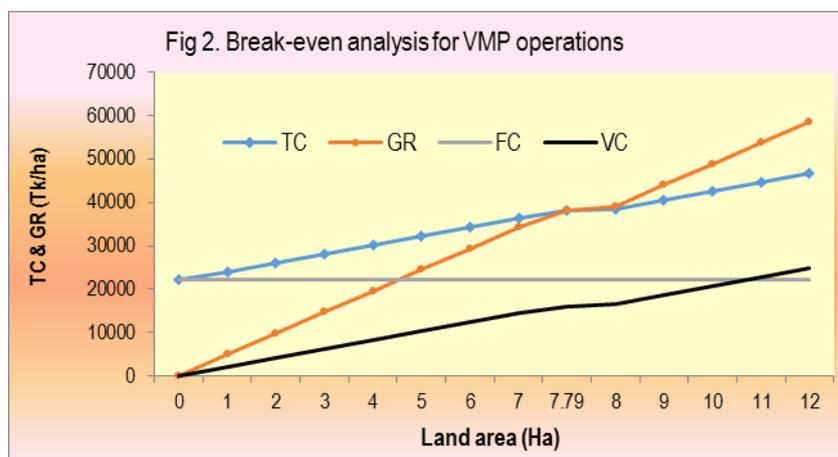
Impacts of VMP on Local Service Providers' Livelihood

Impact on land holdings: Table 4 shows that the land holding of the LSPs increased slightly along with different land categories after having VMP. Irrespective of land categories, the average holding size was increased by 8.3 % mainly due to increase in mortgaged land. However, this change in land holding was insignificant.

Table 4. Status of land holdings before and after ownership of VMP

(Fig .in ha)

Land category	After having VMP	Before having VMP	Mean difference	P(T<=t) value
1. Own land	1.89	1.88	0.01 (0.5)	0.99
2. Rented in	0.06	0.06	0.00	1.00
3. Rented out	0.06	0.11	-0.05	0.67
4. Mortgaged in	0.54	0.40	0.14 (35.0)	0.64
5. Homestead	0.04	0.04	0.00	1.00
6. Orchard	0.23	0.23	0.00	1.00
7. Pond	0.16	0.14	0.02 (14.3)	0.85
*Farm size	**2.86**	**2.64**	**0.22 (8.3)**	

Note: Figures in the parentheses indicate percent increase over pre-ownership period.

*Farm size = (Own land+Rented in+Mortgaged in +Homestead+Orchard+Pond) – (Rented out+Mortgaged out)

Source: Field survey, 2016

Impact on livestock resources: Due to the increased income of the LSPs that was earned from renting out the VMP for service, most livestock and poultry resources were increased to some extent during post-ownership period.

Remarkable increase was found in the quantity and value of bullocks. The value of overall livestock & poultry was increased by 11.2 % (Table 5).

Table 5. Status of livestock resources before and after ownership of VMP

Livestock and poultry	After having VMP		Before having VMP		Mean difference	
	Quantity (no.)	Value (Tk)	Quantity (No.)	Value (Tk)	Quantity (No.)	Value (Tk)
1. Bull/Ox	2.3	103500	1.7	72833	0.7 (40.0)	30667 (42.1)
2. Cow	2.4	155056	2.3	151250	0.2 (8.6)	3806 (2.5)
3. Calves	2.3	56667	2.6	57400	-0.3	-733
4. Goat	3.0	16700	3.0	16556	0.0	144 (0.9)
5. Duck (adult)	3.6	988	3.5	931	0.1 (3.6)	56 (6)
6. Chicken (adult)	11.7	2654	13.0	2850	-1.3	-196
All types	25.5	335563	26.0	301820	-0.6	33743 (11.2)

Figures in the parentheses indicate percent increase over pre-ownership period.

Source: Field survey, 2016

Table 6. Annual income of local service providers before and after ownership of VMP

Income Source	After having VMP (Tk)	Before having VMP (Tk)	Mean difference	P(T<=t) value
1. Crop production	405856 (65.0)	341308 (73.7)	64549	
Rice	139900	136872	3028	0.96
Wheat	122870	94698	28172	0.80
Jute	14422	14172	250	0.98
Maize	52856	50300	2556	0.95
Pulses	41350	15157	26193	0.32
Oilseed	11931	10108	1822	0.75
Vegetables	16806	13917	2889	0.73
Crop residue	5722	6083	-361	0.89
2. Livestock	45556 (7.3)	33667 (7.3)	11889	0.41
3. Poultry	294 (0.0)	59 (0.0)	235	0.35
4. Farm machinery	63806 (10.2)	8889 (1.9)	54917***	0.005
5. Fishery	42118 (6.7)	42118 (9.1)	0	1.00
6. Service	23800 (3.8)	23244 (5.0)	556	0.97
7. Business	43222 (6.9)	13778 (3.0)	29444	0.34
Total income	**6,24,651 (100)**	**4,63,062 (100)**	**1,61,589**	

Note: *** indicate significant at 1% level; Figures within parentheses are the percentages of total income.

Source: Field survey, 2016

Impact on household income: The principal components of household income of the LSPs were crop farming, livestock, farm machinery, fisheries, business, and services. The annual household income of the LSP was increased by 34.9 % during post-ownership period of VMP. In the post-ownership period of VMP, the percent increase in income was only significant in case of farm machineries (618 %). The LSP earned 10.2 % of total income from custom hiring of VMP services (Table 6).

Impact on household assets: Due to increased income, the housing assets of all LSPs of VMP increased to some extent. They have made good improvements in their dwelling and kitchens during the post-ownership period. Table 7 revealed that the number and value of Pacca building were increased by 133 % and 43 %, respectively, during post-ownership period. Improvement was also found in the value of Pacca kitchen. Some of the LSPs of VMP have constructed more valuable storehouses due to increase of crop production.

Table 7. Type of houses owned by LPSs before and after ownership of VMP

	After having VMP		Before having VMP		Mean difference	
	Quantity	Value (Tk)	Quantity	Value (Tk)	Quantity	Value (Tk)
1. Dwelling house	**2.22**	**382972**	**1.94**	**280417**	**0.28 (14.3)**	**102556 (36.6)**
Pacca[1]	0.39	222556	0.17	155556	0.22	67000
Semi-pacca[2]	1.06	139444	0.94	102778	0.11	36667
Katcha-pacca[3]	0.78	20972	0.83	22083	-0.06	-1111
2. Kitchen	**1.06**	**30417**	**1.00**	**24694**	**0.06 (5.6)**	**5722 (23.2)**
Pacca	0.17	23056	0.17	17500	0.00	5556
Semi-pacca	0.11	1667	0.11	1667	0.00	0
Katcha-pacca	0.78	5694	0.72	5528	0.06	167
3. Other houses	**1.89**	**81444**	**1.67**	**74639**	**0.22 (13.3)**	**6806 (9.1)**
Cowshed	0.83	31444	0.78	26389	0.06	5056
Poultry shed	0.56	1056	0.50	472	0.06	583
Store house	0.50	48944	0.39	47778	0.11	1167

Note: [1] House with concrete roof and brick wall.

[2] House with corrugated iron (CI) sheet roof and brick wall.

[3] House with CI sheet roof and thrashed bamboo/jute stick/straw wall.

Figures in the parentheses indicate percent increase over pre-ownership period.

Source: Field survey, 2016

Providing planting services by VMP had a major impact in increasing the household assets in the study areas. Table 8 shows the comparative scenarios of the household asset positions of VMP LSPs. The quantity and quality (in terms of value) of different types of furniture and modern amenities of the LSP were

increased after having VMP. Among different types of furniture, the highest increase was recorded in the number of dining tables and showcases (42.9 %). However, the quantity and value of overall furniture were increased by 23 % and 19 %, respectively. Again, the highest increase was reported in the number of mobile phones (44.7 %) and motor cycles (25 %) among modern amenities. However, the number and value to modern amenities were increased by 25.2 % and 45.5 %, respectively.

Impact on food intake: Due to increase in income from renting out VMP service to others, the frequency and quality of food intake were significantly increased in the study areas. One of the highest improvements was reported in the case of weekly intake of fish. Meat intake also increased remarkably (Table 9).

Table 8. Household assets of LSPs before and after ownership of VMP

Household assets	After having VMP		Before having VMP		Mean difference		% increase over pre-ownership	
	Quantity (No.)	Value (Tk)	Quantity (No.)	Value (Tk)	Quantity (No.)	Value (Tk)	Quantity	Value
1. Furniture	**20.22**	**93783**	**16.44**	**78808**	**3.78**	**14975**	**23.0**	**19.0**
Cot (*Khat*)	2.50	32417	2.06	25722	0.44	6694	21.6	26.0
Chowki[1]	1.50	4750	1.67	3861	-0.17	889	-10.0	23.0
Almirah	1.39	18739	1.17	16878	0.22	1861	19.0	11.0
Dining table	1.11	7611	0.78	6500	0.33	1111	42.9	17.1
Table	2.17	5822	1.78	5172	0.39	650	21.9	12.6
Chair	6.44	4878	4.56	3258	1.89	1619	41.5	49.7
Bench	0.33	156	0.28	144	0.06	11	20.0	7.7
Dress-stand	1.89	2356	1.67	2133	0.22	222	13.3	10.4
Trunk/big box	1.78	7972	1.72	7694	0.06	278	3.2	3.6
Showcase	1.11	9083	0.78	7444	0.33	1639	42.9	22.0
2. M. amenities	**9.65**	**109742**	**7.72**	**75431**	**1.94**	**34311**	**25.2**	**45.5**
Cell phone	3.06	23122	2.11	18139	0.94	4983	44.7	27.5
Motor cycle	0.56	65556	0.44	39556	0.11	26000	25.0	65.7
Bi-cycle	0.83	3361	0.67	2500	0.17	861	25.0	34.4
Television	1.17	13833	1.17	11833	0.00	2000	0.0	16.9
Wrist watch	0.67	1061	0.61	1061	0.06	0	9.1	0.0
Wall clock	1.89	1978	1.44	1811	0.44	167	30.8	9.2
Flash light	1.50	831	1.28	531	0.22	300	17.4	56.5

[1]a four legged wooden bedstead

Source: Field survey, 2016

Impact on LSP's livelihood status: Among different livelihood status indicators, a remarkable change was found in the societal membership of the LSPs during

post-ownership period. Table 10 revealed that the membership of LSP with local level social organizations increased by 255 % in the study areas. The use of toilet tissues were also increased to some extent. It was also reported that better economic standing enabled them to buy more costly new clothes for several social and religious events.

Table 9. Food intake pattern of LSPs after ownership of VMP

Food intake pattern	Frequency of food intake		Mean difference	P(T<=t) value
	After having VMP	Before having VMP		
1. Food intake (times/day)	3.0	3.0	0.0	1.00
2. Fish intake (time/week)	3.7	2.3	1.4***	0.007
3. Meat intake (time/month)	5.6	4.1	1.5*	0.11
4. Egg intake (time/week)	2.5	2.2	0.3	0.60
5. Milk intake (time/week)	5.2	5.1	0.1	1.00
6. Vegetable intake (kg/week)	5.6	5.1	0.5	0.40

Note: *** and * indicate significant at 1% and 10% level, respectively.

Source: Field survey, 2016

Table 10. Livelihood status of LSPs before and after ownership of VMP

Livelihood criteria	% of respondent's opinion		% increase over pre-ownership
	After having VMP	Before having VMP	
1. Use tube well water	100	100	0.0
2. Use sanitary toilet	100	100	0.0
3. Use toilet tissue	100	89	12.4
4. Adopt contraceptive method	100	100	0.0
5. Sending children to school	100	100	0.0
6. Consultation with MBBS doctor	100	100	0.0
7. Offer gifts in social events	100	100	0.0
8. Societal membership	78	22	255

Source: Field survey, 2016

Overall Impacts of VMP on LSP's Livelihood

The LPSs of the study areas were asked to mention the benefits received from custom hiring of VMP service. In this regard, 78 % of LSPs opined that they gained popularity and honour from farmers. Due to receiving extra income from custom hiring services through VMP, about 67 % of LSPs referred to their financial improvement and 28 % commented on the improvement of food intake standard of their families. Twenty eight percent of LSPs were able to get

additional crop lands through mortgage and 22% could build new houses through VMP incomes. It was also possible for some LSPs to start livestock farming and continue children's education due to this custom hiring business (Table 11).

Table 11. Overall impacts of VMP on LSP's livelihood

Type of impact	Frequency	% of respondent
1. Get popularity in the locality	14	78
2. Improve financial status of the family	12	67
3. Improve food intake standard	5	28
4. Get crop lands under mortgage	5	28
5. Build new house	4	22
6. Start livestock farming	3	17
7. Continuation of children's education	3	17
8. Others*	3	17

* Other impacts include repayment of loan, buy motor cycle, mitigate cost of crop cultivation, etc

Source: Field survey, 2016

Challenges encountered by LSPs

Although renting out of VMP service was profitable, the LSP encountered some challenges which were related to VMP generation 9. The VMP machine generally performs well in the sandy-loam and alluvial soils, but not in the wet soil condition. However, the VMP was developed for dry land crop cultivation. By contrast, 2WT are used to wet soil tillage and puddling soil for rice and while 61% of LSPs mentioned this as a problem, they may not have realistic expectations. Seating arrangement on machine is important in reducing drudgery of the LSP. Walking behind the VMP was not preferred by LSP. Fifty six percent LSP noted that there was no seating arrangement on the machine during ploughing. Since version 14, the VMP is fitted with a seating arrangement for road transportation and operating in longer fields. Since the VMP can only plant 4 rows per pass, it requires longer time to complete planting and seeding operations. The 12 hp engine of 2WTs is unable to pull more than 4 furrows/lines in a single pass. Presently, the population of the VMP is low, so many farmers does not know about the benefit of VMP use: that was another crucial problem for LSP in the study areas. Some LSPs (17 %) reported that trained and skilled drivers are scarce, especially in the peak season (*Rabi* season). Seventeen percent of LSPs claimed that the weight of VMP machine is high (Table 12). Later versions of the VMP have reduced weight.

Table 12. Challenges encountered by local service providers of VMP

Type of problem	Frequency	% of respondent
1. Unable to use this machine in the wetland	11	61
2. No seating arrangement on the machine during ploughing	10	56
3. Relatively slow operation to complete planting	9	50
4. Farmers' lack of knowledge on the benefit of VMP	8	44
5. Shortage of trained and skilled driver	3	17
6. Heavy weight of VMP machine	3	17
7. Others*	5	28

* Other problems include lack of spear parts, irregularity in seed/fertilizer dropping, unable to use all fertilizers together, lack of power tiller, higher cost compared to PTOS, etc

Source: Field survey, 2016

Suggestions Provided by LSPs

Respondent LSPs provided some suggestions for wider adoption of VMP in the study areas. Seventy eight percent of LSPs suggested that VMP machine should be popularized among farmers and power tiller owners through conducting field days and demonstrating the performance of VMP. A good number of LSPs opined that the price of VPM is still too high. Therefore, they suggested reducing its price or giving more subsidies on its price so that farmers and LSPs can buy it easily. Some LSPs suggested redesigning the machine with seating arrangement (this has been accomplished in more recent versions of the VMP) and make it applicable for wet condition. The advantages of Conservation Agriculture remain unknown to most of the farmers. In this sense 28 % of LSPs requested the concerned the Department of Agricultural Extension to provide training on CA for farmers. Wider adoption of the VMP mostly depends on skilled and experienced drivers. Seventeen percent of LSPs suggested that hands-on training on VMP operations should be arranged for power tiller drivers on a continuous basis. Some LSPs also suggested providing bank loan for purchasing VMP machine with easy terms and condition (Table 13).

Table 13. Local service provider's suggestions for wider adoption of VMP

Suggestion	Frequency	% of respondent
1. Arrange field days/demonstrate VMP performance	14	78
2. Reduce the price of VMP/Continue subsidy on VMP	11	61
3. Redesign the machine with seating arrangement	8	44
4. Redesign the machine for operating in wetland	6	33
5. Arrange CA awareness training for farmers	5	28
6. Arrange skill training for LSP	3	17
7. Provide bank loan with easy terms and condition	3	17

Source: Field survey, 2016

Conclusions and Recommendations

The study assessed the annual use pattern and profitability of VMP operations and its impacts on LSP's livelihood. The VMP can be used for 12 months in a year, but the highest use occurs in the *rabi* season. The custom hiring of VMP services is a profitable business in the study areas. The payback period and annual break-even size of land were less than a year (0.72 year) and 7.79 ha, respectively. This custom hiring business has made some improvements in the livelihoods of the LSPs of VMP. The incomes from custom hiring business are mostly spent on land mortgage, livestock purchase, construction of houses, purchase of furniture & modern amenities, and purchase of dietary fish & meat that indicate higher standard of living of LSP and their families. Besides, most LSPs gain honour from farmers and popularity in the locality. Although renting out VMP service was profitable, it is constrained by some minor problems, no seating arrangement on the machine during ploughing, slow planting operation time, heavy weight, and lack of trained drivers.

Financial support and technical assistance regarding VMP should be made available by the government or by NGO for LSP and local manufacturers, and redesign (if possible) of the machine for the higher adoption of VMP in the study areas. In addition, necessary steps should be taken for raising farmers' awareness toward CA farming and developing skilled drivers of VMP for wider adoption of this machine.

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Appendix 1. Profitability of VMP operation at farm level

Particular	Only VMP income (subsidy on VMP)	Only VMP income (No subsidy on VMP)	Only tillage income with PT
A. Gross return (Tk/year)	**71,092**	**71,092**	**213197**
Area under tillage (ha/LSP)	21.721	21.721	36.43
Rental charge of VMP/PT (Tk/ha)	3272.95	3272.95	5852
B. Variable cost (Tk/year)	**28517**	**28517**	**91299**
Fuel and oil	17,884	17,884	81732
Wage for driver	7,922	7,922	8046
Repair and maintenance	1,017	1,017	504
Spare parts	1,694	1,694	1017
C. Fixed cost (Tk/year)	**17652**	**23952**	**11352**
Depreciation on power tiller (PT)	2,193	2,193	2193
Depreciation on VMP/plough	2,700	5,400	0
Depreciation on machinery shed	386	386	386
Interest on investment (VMP+PT)	12,373	15,973	8773
D. Total cost (Tk/year)	**46169**	**52469**	**102651**
E. Gross margin (Tk/year)	42575	42575	121898
F. Net return (Tk/year)	24923	18623	110546
G. Rate of return (BCR)			
Over variable cost	2.49	2.49	2.34
Over total cost	1.54	1.35	2.08
H. Payback period (PT+VMP) (year)	**4.14**	**7.15**	**0.66**

Note: Price of VMP = Tk. 60,000; Subsidized price of VMP = Tk. 30,000; Average price of PT = Tk. 73,111 (including ploughing machine); Diesel cost for intensive tillage (Tk/ha) = Tk.2243.54; Diesel cost for minimum tillage (Tk/ha) =Tk. 823.33; Interest rate = Tk.12/year; Life of VMP &PT = 10 years; Salvage value of PT & VMP = 10% of their purchase prices; Depreciation = 12 months for VMP & PT

Conversion rate = 1US$ = 81 BDT;

Figures in the parentheses are percentages of the total cost.

Source: Field survey, 2016.

INTEGRATED MANAGEMENT OF BACTERIAL WILT AND ROOT KNOT NEMATODE OF BRINJAL

M. I. Faruk[1], M. M. Islam[2], F. Khatun[3]
M. A. Hossain[4] and T. K. Dey[5]

Abstract

The field experiment was conducted at Regional Agricultural Research Station (RARS), Hathazari, Chattagram to find out the efficacy of integration of poultry refuse (PR) with stable bleaching powder (SBP) or $CaNO_3$ and Furadan 5G for the management of bacterial wilt (*Ralstonia solanacearum*) and root-knot nematode disease *(Meloidogyne incognita)* of brinjal. Soil was treated with PR @ 3 t/ha 3 weeks before transplanting, stable bleaching powder @ 20 kg/ha during final land preparation and Furadan 5G @ 20 kg/ha on the day of seedlings transplanting while $CaNO_3$ was used as soil drenching 10 days after seedling transplanting. Results showed that integration of poultry refuse with Furadan 5G and stable bleaching powder or $CaNO_3$ reduced root-knot and bacterial wilt diseases and increased plant growth as well as yield of brinjal. The most effective treatment combination was PR + stable bleaching powder + Furadan 5G with early sowing, followed by PR + $CaNO_3$ + Furadan 5G with early sowing for the management of bacterial wilt and root knot nematode diseases and increasing plant growth and yield of brinjal. The technology, poultry refuse+ stable bleaching powder + Furadan 5G was validated at Bangladesh Agricultural Research Institute (BARI), Gazipur, Regional Agricultural Research Station of Jessore, Hathazari and Jamalpur and also at Agricultural Research Station and farmers field at Burirhat, Rangpur and OFRD farm at Alamnagar, Rangpur. The validation trials showed that integration of poultry refuse + stable bleaching powder+ Furadan 5G in early sowing was an effective management package of bacterial wilt and root knot nematode diseases of brinjal which also offered 21.81 to 25.98% higher yield over the conventional practices.

Keywords: Poultry refuse, Stable bleaching powder, Furadan, $CaNO_3$, *Meloidogyne incognita*, *Ralstonia solanacearum* and Brinjal.

Introduction

Availability of quality food and nutrition are the major challenges to achieve healthy and prosperous Bangladesh like other developing countries of the world. Vegetable play a vital role in everyday diet in general and it is an important source of vitamins, minerals and plant proteins in human diets throughout the

[1&2]Senior Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, [3]Chief Scientific Officer and Head, Plant Pathology Division, BARI, Gazipur, [4]Chief Scientific Officer and Head, Soil Science Division, BARI, Gazipur, [5]Ex-Director, Pulse Research Center, BARI, Gazipur, Bangladesh.

world. Among the vegetables, brinjal (eggplant) (*Solanum melongena* L.) is the second most important vegetable crop next to potato in Bangladesh in respect of acreage and production (BBS, 2005). It is cultivated commercially throughout the tropical and subtropical region of the world under field and greenhouse conditions. It is also popular in other countries like Balkan area, France, Indonesia, Italy, Japan, Mediterranean, Turkey and United states (Bose and Som, 1986). But the productivity of brinjal in Bangladesh is low (17.5 t/ha) as compared to Japan (32 t/ha), Italy (28.2 t/ha) and Turkey (30.2 t//ha) FAOSTAT (2012). It is estimated that 10% of crops are lost due to plant diseases worldwide each year which can lead to considerable financial losses for the farmers of underdeveloped countries (Strange and Scott 2005). This crop suffers from the various diseases; about 13 different diseases so far recorded in Bangladesh (Das *et al*., 2000; Khan *et al*., 1998; Rashid, 2000). Among the diseases wilt of eggplant especially Bacterial wilt caused by *Ralstonia solnacearum* is damaging for solanaceous crops worldwide (Ali, 1993; Hayward, 2005). In Bangladesh brinjal and tomato are seriously affected by bacterial wilt (Rahman and Haque, 1986; Rahman *et al*., 2010). Another devastating disease for brinjal root-knot disease caused by *Meloidogyne* spp. is highly damaging and yield reducing factor throughout the country (Mian, 1986). A number of approaches for controlling bacterial wilt and root-knot nematodes including development of resistant variety have been taken, but the success is very few. For management of bacterial wilt disease e.g. intercropping, crop rotation and soil amendment against the pathogen has been reported (Sood *et al*., 1998; Yadessa *et al.*, 2010; Djeugap *et al.*, 2014). In case of root-knot nematodes application of nematicide, (Hossain *et al.,* 1989), organic soil amendments (Faruk *et al.,* 2001*;* Bari *et al.,* 2004; Hassan *et. al.*, 2010, Zakaria *et al*. 2013), cultural management, physical methods like crop rotation and biological measures like *Trichoderma* spp*, Pacecilomyces lilacinus, Pasturia penetrans, Verticillium chlamydosporium* and *Pseudomonas aeruginosa* (Viaene and Abawi, 2000; Moraes *et al*., 2006; Santana *et al*., 2012; Zakaria *et al*., 2013; Lamovšek *et al*., 2013) are being used. Presently, researchers have been diverted their attention to develop integrated approaches against the pest because often any single approach is insufficient to manage bacterial wilt and root knot nematode diseases of brinjal efficiently. Under the above circumstances, the study was undertaken to find out efficacy of poultry refuses, stable bleaching powder, $CaNO_3$ and Furadan 5G to manage bacterial wilt and root-knot nematode of brinjal and to increase plant growth and yield of brinjal.

Materials and Methods

Field experiment: The experiment was conducted at the Regional Agricultural Research Station, Hathazari, Chattagram during 2011-12 to find out the effective management practices against bacterial wilt and root-knot nematode disease of brinjal. The unit plot size was 3 m ×3.75 m keeping 1m distance from plot to plot. The experiment was laid out in a randomized complete block design

with three replications. Thirty days old brinjal seedlings var. BARI Begun-4 was cultivated. Ten treatments *viz.*, T_1 = Poultry refuse @ 3 t/h + Stable bleaching powder @ 20 kg/h + early sowing (Middle of October), T_2 = Poultry refuse@ 3 t/h + $CaNO_3$ @ 1% solution + early sowing, T_3 = Poultry refuse@ 3 t/h + Stable bleaching powder@ 20 kg/h + Furadan 5G@ 20 kg/h + early sowing, T_4 = Poultry refuse@ 3 t/h + $CaNO_3$@ 1% solution + Furadan5G@ 20 kg/h + early sowing, T_5 = Control-1 (as comparison for early sowing) T_6= Poultry refuse @ 3 t/h + Stable bleaching powder @ 20 kg/h + late sowing (1st week of December), T_7 = Poultry refuse@ 3 t/h + $CaNO_3$ @ 1% solution + late sowing, T_8 = Poultry refuse@ 3 t/h + Stable bleaching powder@ 20 kg/h + Furadan 5G@ 20 kg/h + late sowing, T_9 = Poultry refuse@ 3 t/h + $CaNO_3$@ 1% solution + Furadan5G@ 20 kg/h + late sowing and T_{10} = Control-2 (as comparison for late sowing). Partially decomposed poultry refuse @ 3 t/h was added to the soil three weeks before seedling transplanting for proper decomposition. Furadan 5G was added before seedling transplanting, Stable bleaching powder was used during final land preparation and $CaNO_3$ was used as soil drenching after 10 days ages of the plant. Recommended fertilizers (Urea 300 kg/ha, TSP 250 kg/ha, MP 200 kg/ha and Zypsum 100 kg/ha) were added during final land preparation as well as split at three times of different crop growth stages, weeding and irrigation were done as per recommendation of the crop.

Validation of developed technology

The validation trials using BARI Begun-8 for controlling bacterial wilt and root knot nematode diseases were conducted in the field of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur and also in the Regional Agricultural Research Station (RARS), Hathazari and Jamalpur during 2012-13 cropping season. The developed technology that is poultry refuse @ 3 t/ha + Stable bleaching powder @ 20 kg/h + Furadan 5G 25 kg/ha for integrated management of root knot nematode and bacterial wilt of brinjal was applied in the locations of Gazipur, Jamalpur and Hathazari where control treatment was also used for comparison. The unit plot size was approximately 1500 square meter and spacing was 60 cm x 60 cm. Similar trials with BARI Begun-4 and BARI Begun-10 were also conducted at the Regional Agricultural Research Station (RARS), Jessore and Burirhat, Rangpur, farmers' field at Burirhat, Rangpur and OFRD farm at Alamnagar, Rangpur during 2012-13 cropping season where no control treatment was used. Poultry refuse was applied @ 5t/ha before 21 days of seedling transplanting. After application poultry refuse was properly mixed with soil and water was added frequently for complete decomposition of poultry refuse in the soil. Stable bleaching powder @ 20 kg/h was applied at the final stage of land preparation and Furadan 5G was mixed at the time of seedling transplanting. Brinjal seedlings were transplanted at the age of 35-40 days. Intercultural operations were done as per standard recommendation.

Data collection and analysis: Data on different parameters *viz.* shoot height, shoot weight, root length, root weight and yield of brinjal were taken. During the growing period bacterial wilt incidence was recorded from seedling to maturity stage of the crop. Root knot disease severity was recorded as a gall index following 0-10 scale (Zeck, 1971) after final harvesting of brinjal. The percent data were converted into arcsine transformation values before statistical analysis. Data were analyzed statistically by using the MSTAT-C program. The treatment effects were compared by applying the least significant different (LSD) test at P=0.05 level.

Results and Discussion

Shoot growth: Average shoot length of brinjal was significantly increased in case of various treatments and it was comparatively low in control-1 and control-2 (22.33 and 20.00 cm plant^{-1}, respectively) while integrated soil amendments with poultry refuse (PR), stable bleaching powder (SBP), CaNO$_3$ and Furadan 5G showed increased shoot length both in early sowing (ES) and late sowing (LS) condition ranging from 27.52-42.61 cmplant^{-1} and 23.00-37.25 cm plant^{-1}, respectively (Table 1). The highest shoot length (42.61 cm plant^{-1}) was obtained with integration of PR + SBP + Furadan 5G +ES followed by the treatments PR + SBP + Furadan 5G +LS (38.53 cm plant^{-1}), PR + CaNO$_3$ +Furadan 5G+ ES(37.69 cm plant^{-1}). The treatments PR+ SBP +ES, PR + CaNO$_3$ +ES and PR + CaNO$_3$+ Furadan 5G +LS produced comparatively short shoot length. The least effective treatments to increase shoot length was PR+ CaNO$_3$+LS and PR + SBP+ LS (23.00 cm plant^{-1}).

The shoot weight of brinjal was 140.70 and 130.0 g plant^{-1} in control-1 and control-2, respectively. Integration of PR, SBP, CaNO$_3$ and Furadan5G increased shoot weight by 142.50-167.00 g plant^{-1} at early sowing and 141.7-164.6 g plant^{-1} at late sowing conditions. The highest shoot weight was achieved by the treatment of PR + SBP + Furadan 5G +ES followed by PR + SBP +Furadan 5G+LS, PR + SBP + ES, PR + CaNO$_3$+ Furadan 5G +ES and PR + CaNO$_3$+ LS. The least effective treatment to increase shoot weight was PR + CaNO$_3$+ Furadan 5G +LS followed by PR + CaNO$_3$ +ES and PR + CaNO$_3$+ LS which were statistically similar to control treatments (Table 1).

Root growth: Soil amended with PR, SBP and CaNO$_3$ and application of Furadan 5G showed positive effects on root growth of brinjal as compared to untreated control. Minimum root length (8.60 cm plant^{-1}) was recorded in control-2 (Table 1). The highest root length (28.64 cm plant^{-1}) was recorded in PR + SBP + Furadan 5G +ES treatment which was followed by PR + CaNO$_3$+ Furadan 5G +ES, PR + SBP + Furadan 5G +LS and PR + SBP +ES. The rest of the treatments was least effective to increase root length (Table 1). Root weight was 5.18 g plant^{-1} and 7.67 g plant^{-1} in control-2 and control-1, respectively. The highest root weight (22.58 g plant^{-1}) was recorded in integration of PR + SBP +

Furadan 5G +ES, which was followed by PR + CaNO$_3$+ Furadan 5G +ES, PR + SBP + Furadan 5G +LS and PR + SBP + ES. The rest of the treatment was found least effective to increase root weight.

Table 1. Effect of integrated management approaches on vegetative growth of brinjal

Treatment	Shoot Length (cm)	Shoot Weight (gm)	Root Length (cm)	Root Weight (gm)
PR+SBP+ES	37.25 b	164.2 ab	21.04 b	19.43 b
PR+CaNO$_3$+ES	27.52 c	142.5 e	13.47 c	11.11 c
PR+SBP+Furadan+ES	42.61 a	167.0 a	28.64 a	22.58 a
PR+ CaNO$_3$ +Furadan+ES	37.69 b	161.0 bc	23.64 b	21.85 ab
Control-1	22.33 d	140.7 e	12.43 c	7.67 de
PR+SBP+LS	23.00 d	152.0 d	14.33 c	8.17 d
PR+CaNO$_3$+LS	23.20 d	156.0 cd	13.00 c	9.67 cd
PR+SBP+Furadan+LS	38.53 b	164.6 ab	21.58 b	20.17 ab
PR+ CaNO$_3$+Furadan+ LS	27.20 c	141.7 e	14.73 c	12.27 c
Control-2	20.00 d	130.0 f	8.60 d	5.18 e

Values within the same column with a common letter do not differ significantly (P=0.05) by LSD test.

PR= Poultry refuse, SBP= Stable bleaching powder, ES=Early sowing (Middle of October), LS= Late sowing (1st week of December)

Severity of bacterial wilt and root knot nematode: The severity of root knot disease and bacterial wilt of brinjal was significantly reduced compared to control due to integration of PR, SBP, CaNO$_3$ and Furadan 5G for soil treatment (Table 2). Higher bacterial wilt (22.67% and 20.80%) incidence was recorded from control-2 and control-1 plots, respectively. Integration of different treatments reduced bacterial wilt incidence by 38.46-61.54% at early sowing and 32.95-64.71% at late sowing conditions as compared to control. Regarding the root knot nematode disease, average gall index value of 4.34 and 5.12 was recorded in the control-2 and control-1 plots, respectively. The gall index value under integration of different treatments with PR, SBP, CaNO$_3$ and Furadan 5G range from 1.63-2.52 at early sowing and 1.83-2.86 at late sowing conditions. Maximum reduction of gall index value (68.16%) was obtained with PR +SBP + Furadan 5G+ES compared to control followed by PR + SBP + Furadan 5G +LS (56.25%), PR + CaNO$_3$+ Furadan 5G +ES (51.95%), PR +SBP + ES, PR + CaNO$_3$+ +ES (50.78%) and PR + CaNO$_3$+ Furadan 5G +LS (50.69%).

Crop yield: Integration of organic soil amendments with PR, SBP, CaNO$_3$ and Furadan 5G gave significant increase in fruit number/plant, fruits weight/plant and fruit yield per hectare (Table 3). Fruit number per plant was 9.00 and 11.00 under control-2 and control-1, respectively. Fruits yield was increased by 24.00 to 28.33 per plant at early sowing and 20.00 to 24.67 per plant at late sowing in different treatments. Fruits weight per plant 0.64 g/plant and 0.84 g/plant under

control-2 and control-1 treatments, respectively. The highest fruits weight 1.73 kg plant[-1] was achieve by PR +SBP + Furadan 5G+ES followed by and PR + CaNO₃+ Furadan 5G +ES, PR +SBP + Furadan 5G+LS and PR +SBP + ES treatments.

Table 2. Effect of integrated management approaches on bacterial wilt and root knot diseases of tomato

Treatments	Bacterial wilt incidence (%)	Reduction of Bacterial wilt incidence over control (%)	Gall index (0-10 scale)	Reduction of gall index value over control (%)
PR+SBP+ES	11.20 de	46.15	2.46 d	51.95
PR+CaNO₃+ES	12.80 cd	38.46	2.52 d	50.78
PR+SBP+Furadan+ES	8.00 f	61.54	1.63 g	68.16
PR+ CaNO₃ +Furadan+ES	9.60 ef	53.85	2.24 e	56.25
Control-1	20.80 a	-	5.12 a	-
PR+SBP+LS	14.40 bc	36.48	2.18 e	49.77
PR+CaNO₃+LS	15.20 b	32.95	2.14 e	34.10
PR+SBP+Furadan+LS	8.00 f	64.71	1.83 f	57.83
PR+ CaNO₃ +Furadan+ES	12.27 d	45.88	2.86 c	50.69
Control-2	22.67 a	-	4.34 b	-

Values within the same column with a common letter do not differ significantly (P=0.05) by LSD test.

PR= Poultry refuse, SBP= Stable bleaching powder, ES=Early sowing, LS= Late sowing

Table 3. Effect of integrated management approaches on the yield of brinjal

Treatment	No. of fruits/ plant	Weight of fruits/plant (Kg)	Yield (t/ha)	Yield increased over control (%)
PR+SBP+ES	24.00 bc	1.54 abc	22.51 b	43.27
PR+CaNO₃+ES	25.67 ab	1.38 cd	21.59 bc	40.85
PR+SBP+Furadan+ES	28.33 a	1.73 a	24.73 a	48.36
PR+ CaNO₃ +Furadan+ES	26.00 ab	1.64 ab	24.56 a	48.00
Control-1	11.00 e	0.84 f	12.77 d	-
PR+SBP+LS	20.00 d	1.15 e	20.56 c	42.36
PR+CaNO₃+LS	21.67 cd	1.32 de	22.23 b	46.69
PR+SBP+Furadan+LS	23.33 bcd	1.59 ab	22.32 b	46.91
PR+ CaNO₃ +Furadan+ES	24.67 abc	1.48 bcd	22.37 b	47.03
Control-2	9.00 e	0.64 g	11.85 d	-

Values within the same column with a common letter do not differ significantly (P=0.05) by LSD test.

PR= Poultry refuse, SBP= Stable bleaching powder, ES=Early sowing, LS= Late sowing.

The lower yields 11.85 and 12.77 t/ha were found in control-2 and control-1 treatments, respectively. Application of different treatments increased the fruit yield upto 21.59-24.73 t ha[1] at early sowing and 20.56-22.37 t ha^{-1} at late sowing conditions. Integration of PR +SBP + Furadan 5G+ ES gave significantly higher (48.36%) yield compared to control followed by PR + CaNO$_3$+ Furadan 5G +ES (48.00%), PR + CaNO$_3$+ Furadan 5G +LS (47.03%), PR +SBP + Furadan 5G+LS (46.91%) and PR + CaNO$_3$ +LS (46.69%), respectively.

Table 4. Validation of integrated management of bacterial wilt and root knot nematode of brinjal at Gazipur, Hathazari and Jamalpur

| Location | Variety | Treatment | Disease incidence | | Yield (t/ha) | Yield higher over control (%) |
			Bacterial wilt (%)	Root knot (0-10 scale)		
BARI, Gazipur	BARI Begun-8	Treated	0.88	1.50	44.50	25.62
		Control	7.50	2.75	33.10	-
RARS, Hathazari	BARI Begun-8	Treated	4.50	1.40	43.50	25.98
		Control	24.00	3.75	32.20	-
RARS, Jamalpur	BARI Begun-8	Treated	1.5	1.50	43.10	21.81
		Control	11.0	3.50	33.70	-

Validation of the developed technology: Higher bacterial disease incidence ranging from 7.50-24.00% was observed in the control plot while it ranged from 0.88-4.50% in the treated plots of Gazipur, Jamalpur and Hathazari locations (Table 4). Root knot nematode disease severity recorded as the gall index value was also lower range dfrom 1.40-1.50 in the treated plot and higher 2.75 to 3.75 in the control plots. Integrated management package gave higher yield 43.10 to 44.50 t ha[1] of brinjal where the yield was lower 32.20 to 33.70 t ha[1] in the control plots. At RARS Hathazari, Chattagram yield was 25.98% higher in the treated plot compared control plot. While at Gazipur and Jamalpur the yield was 25.62% and 21.81%, respectively higher in the treated plot compared to control plot. At RARS, Jashore, bacterial wilt incidence was 22.06% and the gall index value was 3.00 producing the yield of 36.50 t ha[1] (Table-5). In Rangpur region, no bacterial wilt disease was observed at RARS, Burirhat farm and farmers field at Gangachara, Rangpur but at OFRD, Alamnagar, Rangpur farm only 5% bacterial wilt incidence was recorded (Table 5). The root knot disease severity was lower (1.8) at RARS, Burirhat, Rangpur but OFRD farm of Rangpur and farmers field at Gangachara, Rangpur the gall index value was higher with 2.25 and 3.25, respectively. The crop yield was higher in the OFRD, Alamnagar, Rangpur farm at 45.60 t ha[1] where it was 44.46 and 37.13 t ha[1] at the RARS, Burirhat and farmers field at Gangachara, Rangpur, respectively.

**Table 5. Validation of integrated management of bacterial wilt and root knot
nematode of brinjal at Rangpur and Jeshore regions**

Location	Variety	Disease incidence		Yield (t/ha)
		Bacterial wilt (%)	Root knot (0 – 10 scale)	
ARS, Burirhat, Rangpur	BARI Begun-4	0	1.8	44.46
Anil Chandra Mohanto, Gangachara, Rangpur	BARI Begun-10	0	3.25	37.13
OFRD, BARI, Alamnagar, Rangpur	BARI Begun-10	5	2.25	45.60
RARS, Jeshore	BARI Begun-8	22.06	3.00	36.50

Bacterial wilt, caused by *R. solanacearum* and root knot nematode caused by
Meloidogyne spp. are the major constraints for vegetables production all over the
world (Hayward, 2005; Sakhuja and Jain, 2001). These diseases are particularly
important for cultivation of Solanaceous crops in tropical and subtropical
climates where high temperatures and abundant rainfall during the growing
season promote the disease development and dissemination. Organic
amendments like poultry manure, waste materials, organic compost etc. have
been recognized for soil borne diseases management and improvement of soil
health (Hasan *et al*., 2010; Farahat *et al*., 2010). Results of the present study
showed that integration of poultry refuse, stable bleaching powder and Furadan
5G drastically reduced bacterial wilt and root-knot disease incidence and
increased plant growth parameters as well as yield of brinjal compared to control.
These results were supported by Orisajo *et al*. (2007) and Djeugap *et al.* (2014)
who reported that poultry manure had a suppressive effect on root-knot
nematodes and bacterial wilt incidence. Biswas and Singh (2008) and Kishun
(1981) reported that bleaching powder as effective in decreasing bacterial wilt.
Chen *et al*. (2000) reported that use of organic amendments in the form of
manure and compost could effectively decrease parasitic nematode populations
and disease intensity on plants. The nematicidal effects of organic amendments
had been attributed to several factors including increase in facultative parasites
due to their richness in organic matter and release of toxic substances during
decomposition (Oka, 2010; Sikora *et al.,* 2007; Oka *et al.,* 2007). Farahat *et al*.
(2010) also reported that efficacy of poultry manure against plant-parasitic
nematodes might either be due to stimulation of specific micro-organisms that
were capable of parasitizing eggs and juveniles or production of substances from
decomposition of the manure which were toxic to the nematodes. Similar studies
were also done by Hassan *et al*. (2010) and Orisajo *et al*. (2007) who showed that
amending the soil with organic waste materials such as poultry refuse, rice husk
and saw dust suppressed the populations of *Meloidogyne* spp. both in the soil and
roots of tomato with simultaneous increase in the growth and yield. Oka *et al.*

(2007) and Orisajo *et al.* (2008) also reported that application of soil organic amendments was not only beneficial to nematode management but also to plant growth and productivity. Therefore, it might be concluded that integration of poultry refuse with Furadan 5G and stable bleaching powder was the best treatment combination for reducing root knot nematode and bacterial wilt diseases and increasing plant growth as well as yield of brinjal.

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MORPHO-ANATOMICAL APPRAISAL OF SOME PULSE CROPS UNDER SALINITY STRESS

M. A. H. Khan[1], M. A. Baset Mia[2], J. U. Ahmed[3]

M. A. Karim[4] and M. M. H. Saikat[5]

Abstract

The experiment was conducted at the field and laboratory of the Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during the period from December 2016 to March 2017 to find out the morpho-anatomical appraisal of some pulse crops of Fabaceae family under salinity stress. Seven pulse crops viz., Lentil var. BARI Masur-7, Mungbean var. BARI Mung- 6, Blackgram var. BARI Maskalai-1, Chickpea var. BARI Chola-9, Field pea var. BARI Motor-1, Grasspea var. BARI Khesari-3 and Cowpea var. BARI Felon-2; two levels of salinity 0 and 8 dSm^{-1} were imposed as experimental treatments. It was laid out in a completely randomized design with three replications. The NaCl was directly mixed to the dry soil. The soil (6 kg pot^{-1}) of each treatment was placed in plastic pots with drainage holes in the bottom. The results of the experiment revealed that, salt stress caused decrease in morphological attributes and also changed anatomical features. There was a significant variation in relative values (%) of plant height, root length (%) root dry matter (%) and shoot dry matter (%) of seven selected pulse crop varieties due to the salinity stress. The highest percentage of relative plant height (92), relative root length (98), relative root dry weight (89) and relative shoot dry weight (72.8) were observed in cowpea followed by grass pea and the lowest percentage of relative plant height (51), relative root length (56), relative root dry weight (54) and relative shoot dry weight (48) were observed in lentil. The stem anatomical features were found similar changes in xylem and phloem area. Among the pulse crop varieties, cowpea and grass pea were performed better whereas lentil and black gram were found more susceptible species than the others according to their morphological and anatomical attributes.

Keywords: Salinity, pulse crops, morphology, anatomy

Introduction

Among the food crops grown in Bangladesh pulses occupy only 0.37 million hectares of land that is 4.48% of the total cropped area (BBS, 2017). But these

[1]Ph.D. Fellow, Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur-1706 & Scientific Officer, Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Cumilla, [2&3]Professor, Department of Crop Botany, BSMRAU, Salna, Gazipur-1706, [4]Professor, Department of Agronomy, BSMRAU, Salna, Gazipur-1706, [5]Professor, Department of Genetics and plant breeding, BSMRAU, Salna, Gazipur-1706.

crops have been cultivating since prehistoric times as a source of dietary protein and as an important component of legume crop diversification in the predominantly cereal based cropping pattern. These crops also can improve the soil nutrient status through symbiotic N_2 fixation. Total production of pulse during 2015-16 in Bangladesh was around 0.412 million metric tons from 0.372 million ha of land at the rate of 0.96 metric ton per hectare (BBS, 2017). Among various abiotic stresses, soil salinity is the major factor that hampers the plant growth and development and yield. Salinity can limit growth and yield of plant in three ways through osmotic potential, creating ion toxicity, causing dis-arrangement and imbalance of ion uptake resulting disorders of enzyme activities and membrane vis-a-vis metabolic activities. Salt tolerant plants are capable of minimizing detrimental effects of salt stress by producing a series of anatomical, morphological and physiological adaptations such as an extensive root system and salt screening glands on the leaf surface (Marcum *et al.*, 1998). Although physiological adaptations are crucial in identifying selection criteria against salt stress both halophytes and non-halophytes exhibit remarkable structural modifications when exposed to high salinities, particularly in leaf anatomy (Dickson, 2000). Such structural changes in response to salinity stress are not very much clear, but they certainly play a crucial role in combination with physiological attributes in tolerating the stress. It appears that little information is available regarding the effect of salinity on the morphology and anatomy of pulse crop. The present study, was therefore, carried out to investigate specific anatomical and morphological modifications of some pulse crops of Fabaceae family which might have played a significant role in finding out an avenue of breeding technique for developing salt tolerant crops. As such the experiment was undertaken to observe the morphology and micro morphology (anatomy) of some pulse crops under salt stress situation.

Materials and Methods

The experiment was conducted at the Vinyl house and laboratory of the Department of the Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur from December 2016 to March 2017. Seven cultivated pulse crop varieties of Fabaceae family, developed by the Bangladesh Agricultural Research Institute (BARI), namely, viz., Lentil var. BARI Masur-7, Mungbean var. BARI Mung- 6, Blackgram var. BARI Maskalai-1, Chickpea var. BARI Chola-9, Field pea var. BARI Motor-1, Grasspea var. BARI Khesari -3 and Cowpea var. BARI Felon-2; were used in the experiment. The experiment was conducted in plastic pot using 6 kg of soils. There were three replications of each treatment namely: control and 8 dS m^{-1}. The method suggested by Rowell (1994) was used to calculate the quantity of NaCl required for the preparation of each saline soil treatment. The NaCl was directly mixed to

the dry soil. The soil (6 kg pot^{-1}) of each treatment was placed in plastic pots with drainage holes in the bottom. The pots were arranged on wooden benches of the vinyl house. Another treatment (Control) was maintained by using fresh or tap water on the pot.

Seeds of pulse crops variety were sown on 15 December 2016. After sowing of seeds light irrigation was given to ensure uniform germination of seeds. Recommended fertilizers namely N, P, K, S were used at the rate of 20, 20, 24 and 10 kg ha^{-1} as urea, triple superphosphate (TSP), muriate of potash (MoP) and gypsum respectively. For the anatomical studies, the leaf samples were collected from control and salinity treatment of pulse crop varieties at 50 days after sowing. The cross sections 5 cm slices from the stem were chosen and softened in a mixture of glycerine. Then the sections were photographed with a digital photo-camera attached to light microscope at 10-40 X magnifications and image4.0 software.

For the calculation of area of xylem and phloem, the following formula was used (Hameed et $al.$, 2013):

$$\text{Area} = \frac{\text{Maximum length X Maximum width}}{4} \times \pi \text{ Where, } \pi = \text{Constant (22/7)}$$

This formula was derived from the area of circle, which is πr^2, which was π x d (diameter)/2 x d/2.

After 50 days of germination, plants were harvested for collection of data. The mean root dry weight was calculated for each treatment. After separation of roots the samples of stem, branch and leaf were dried in an electrical oven to a constant weight at 70° C. Thereafter, the mean shoot dry weight was calculated for each treatment.

Relative data were calculated by using the following formula (Shaheenuzzamn, 2014).

$$\text{Relative value} \frac{\text{Value of saline treated genotype}}{\text{Value of control genotype}} \times 100$$

The collected data were analyzed statistically followed Completely Randomized Design by Statistix10 computer package programme (Gomez and Gomez 1984).

Results and Discussion

There was a significant variation in relative values of plant height (%), root length, shoot length, root dry matter and shoot dry matter, stem xylem area of the seven selected pulse crop species due to induced of salt stress.

Relative Plant Height (%)

Significant variation was observed in relative plant height (%) of seven selected pulse species as affected by salt stress (Figure1). However, the highest relative plant height (92%) was observed in cowpea followed by grasspea (84%) and the lowest (51%) in lentil.
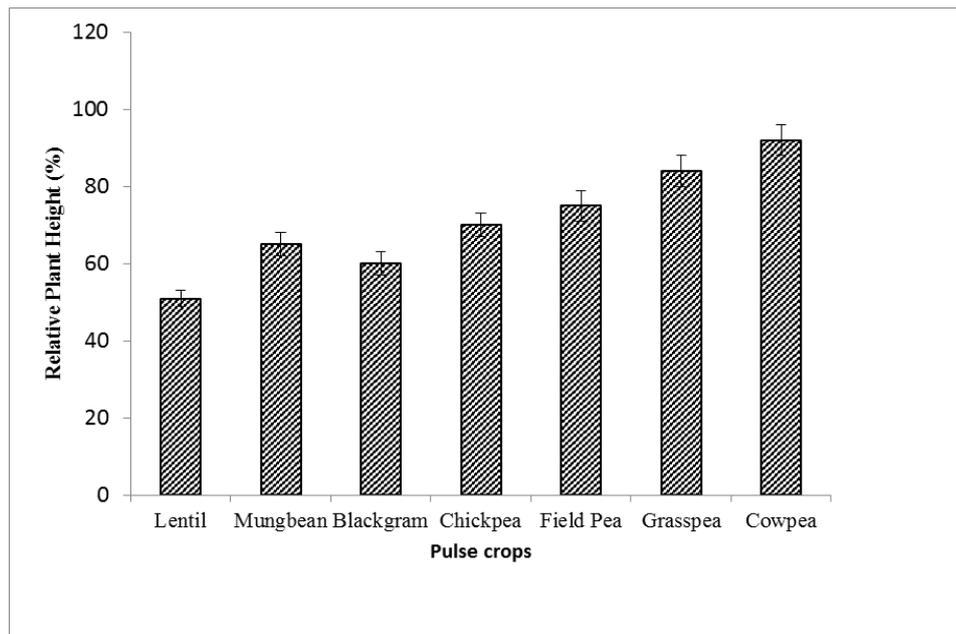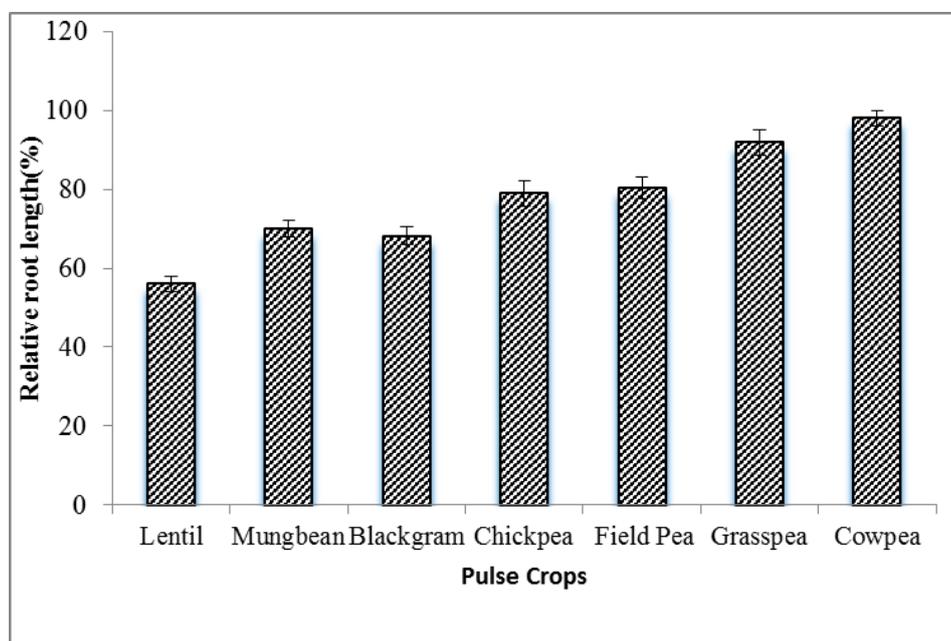

Fig. 1. Relative plant height (%) of seven selected pulse crops under salt stress at 8 dS m^{-1} used as commercial NaCl; vertical bar represents standard error (SE).

Salinity reduces plant growth through osmotic and toxic effects and high sodium uptake ratio values cause sodicity, which increases soil resistance, reduces root growth, and reduces water movement through the root with a decrease in hydraulic conductivity also found that lentil appeared to be very salt sensitive (Katerji, *et al.* (2001).

Relative Root Length (%)

Due to salt stress the relative root length (%) of seven selective pulses crop varieties differed significantly (Figure3). The highest value (98%) was found in cowpea which was statistically similar to grass pea (91.8) and the lowest relative value (56%) was found in lentil.
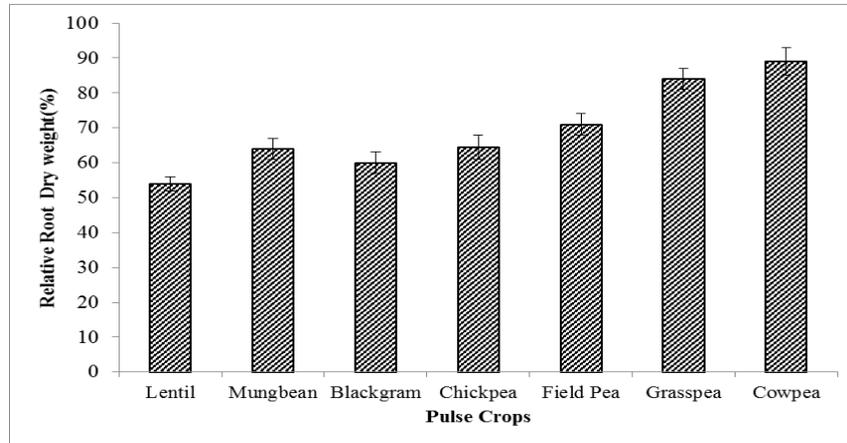
Fig. 3. Relative root length (%) of seven selected pulse crops under salt stress at 8 dSm-1 used as commercial NaCl; vertical bar represents SE.

The result elucidated that the increment of NaCl concentration caused the reduction of seedling root length of lentil. The result is in full agreement with those found by Duzdemiro *et al.* (2009) for pea. Root morphology is not only a very important factor for nutrient absorption by roots, but also it is very important for water uptake by roots from saline soils (Schleiff, 2008)**.** Ouji *et al.* (2017) investigated that, 50 mMol of salinity has a more pronounced effect on root length with respect to shoot length, as roots are directly exposed to salt solution. The reduction in root and shoot development might be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings.

Relative Root Dry Weight (%)

There was a significant variation in relative root dry weight (%) of the seven selected pulse crop varieties due to induced salinity stress (Figure 5).
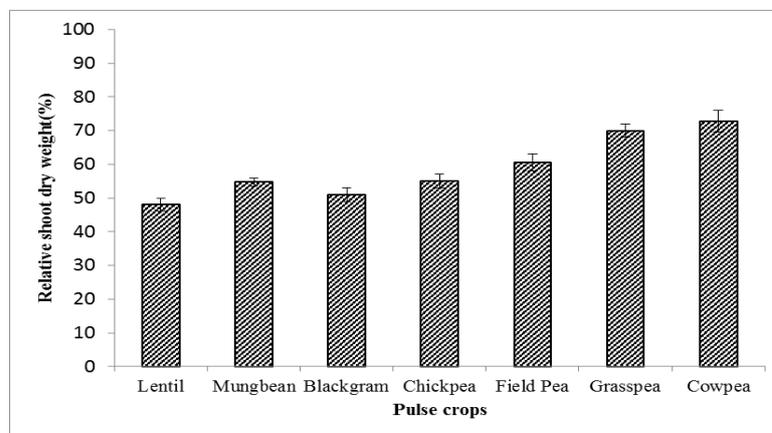
Fig 5. Relative root dry weight (%) of seven selected pulse crops under salt stress at 8 dS m-1 used as commercial NaCl; vertical bar represents SE.

The highest relative root dry weight (89%) was observed in cowpea that was statistically similar to grass pea and the lowest (54%) in lentil. This result is in agreement with Kaya *et al.*, 2005. Taffouo *et al.* (2004) also found that the increase in salt concentration significantly reduced the production of dry weight at 50mM of NaCl in *Glycine max* and *Phaseolus vulgaris.*

Relative Shoot Dry Weight (%)

Relative shoot dry weight (%) was also reduced due to salinity stress of seven selected pulse crop varieties (Figure 6). However, the highest shoot dry weight was observed in cowpea and the lowest in lentil.
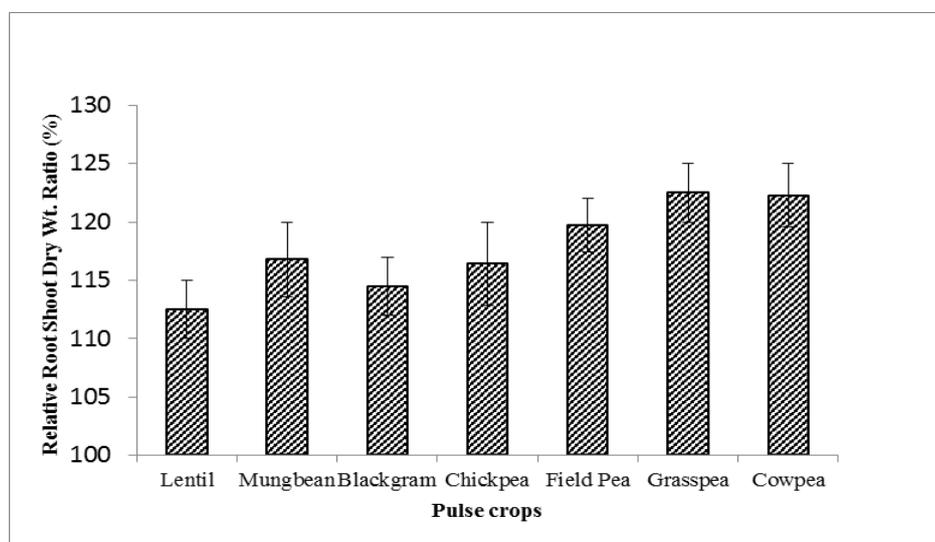

Fig. 6. Relative shoot dry weight (%) of seven selected pulse crops under salt stress at 8 dSm-1 used as commercial NaCl; vertical bar represents SE.

Salt stress reduced the growth of other grain legumes, including mungbean (Kabir *et al*., 2004), lentil (Bandeoglu *et al*., 2004). These growth reductions are often attributed to reductions in tissue water potential, indicating less water availability to cells which results in stomatal closure, reduced photosynthesis and inhibited growth (Garg and Manchanda, 2009).

Relative Root and Shoot Dry Weight Ratio (%)

Due to salinity stress the relative root shoot dry weight ratio (%) of seven selective pulse crop varieties did not differed significantly (Figure 7). However, the highest relative value (122.3%) was found in cowpea and the lowest relative value (112.5%) was observed in lentil.

Fig. 7. Relative root and shoot dry weight ratio (%) of seven selected pulse crops under salt stress at 8 dSm-1 used as commercial NaCl; vertical bar represents SE.

Salinity may directly or indirectly inhibit cell division, cell enlargement, which results in reduction of shoot length, number of leaves, dry matter accumulation, leaf size, mobilization of food material from source to sink and increased root shoot ratio (Mass and Poss, 1989).

Relative Stem Xylem Area (%)

Salinity showed a subtractive effect on shoot anatomy of pulse crops. It is indicated that, with the increase in salinity level, there was a significant decrease in stem xylem and phloem area. Relative stem xylem area (%) was reduced due to salinity stress of seven selected pulse crops (Figure 8).
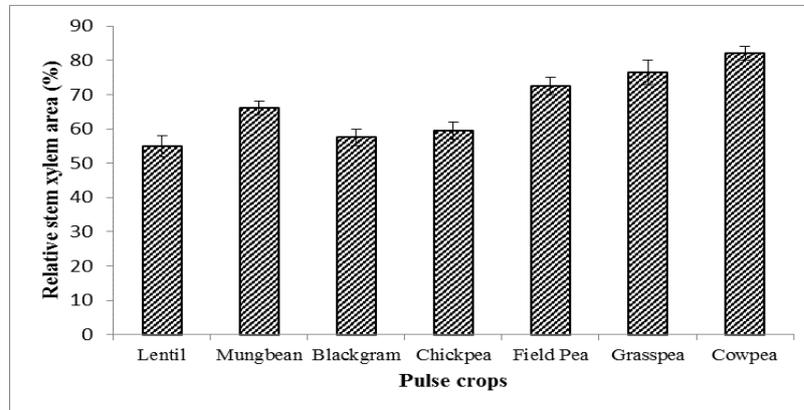
Fig. 8. Relative stem xylem area (%) of seven pulse crops under salt stress at 8 dS m-1 used as commercial NaCl ; vertical bar represents SE.

However, the highest relative xylem area (82%) was observed in cowpea followed by (76.5%) grass pea and the lowest (55%) in lentil. Salinity affects plant growth through osmotic effects and ion toxicity.

Relative Stem Phloem Area (%)

Salinity also reduced the phloem area of different pulse crops compare to control condition (Figure 9). But lower reduction percentage was observed in cowpea and grass pea compare to other pulses due to salinity stress condition. However, the relative stem phloem area (%) was observed in cowpea followed by grasspea and the lowest was found in lentil followed by black gram. Younis *et al.* (2014) reported that xylem and phloem area of stem showed a decreasing trend under salt stress condition which indicates that with the increase in salinity level there was noticeably decrease in the xylem and phloem cell growth.
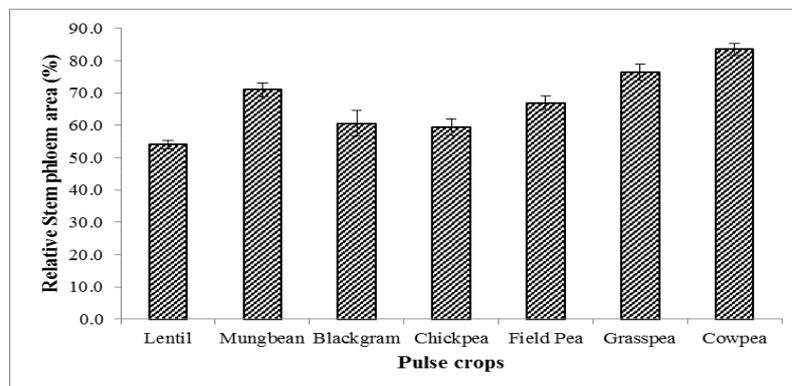

Fig. 9. Relative stem phloem area (%) of seven pulse crops under salt stress at 8 dS m-1 used as commercial NaCl; vertical bar represents SE.

Xylem area and phloem area was maximum under normal soil (control) condition. This indicates that salinity induces structural changes in xylem and phloem of stems. The increasing salinity reduces the stems vascular area while cell thickness increased with salinity level compared to control treatment. Salinity caused the gradual changes in plant internal structure which reduces the xylem and the phloem area (Hameed *et al*., 2010).
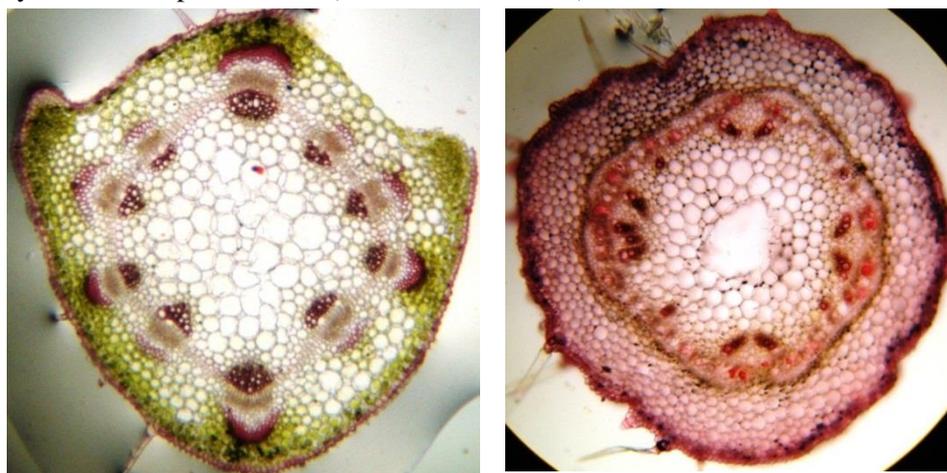
A. BARI Maskalai 1 (Stem) Salt: 8dS m^{-1} B. BARI Maskalai 1 (stem) Control

Plate 1: Transverse section of stem of black gram showing the effect of salinity seen under digital compound microscope (Model Primo star Zeiss) at 40X.
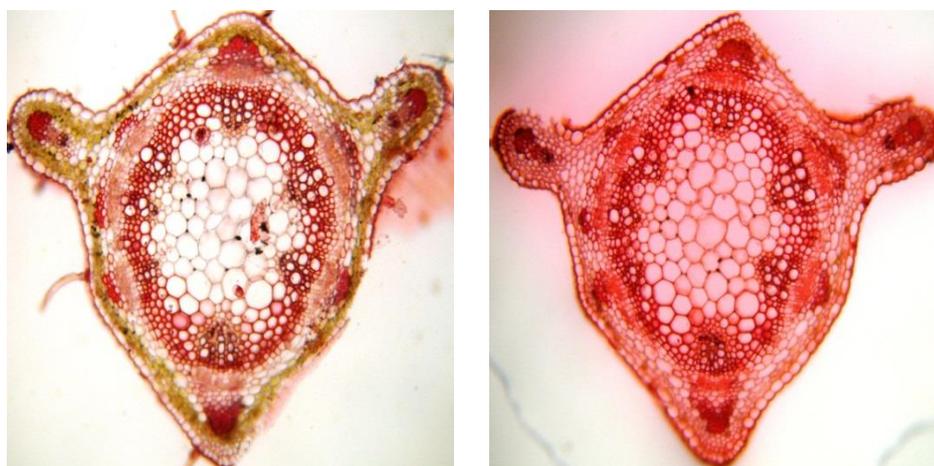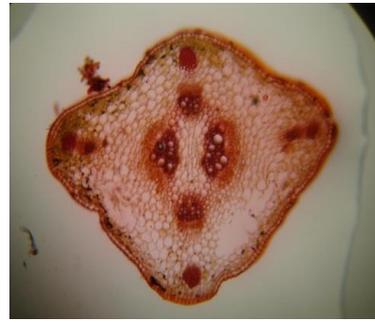

A. BARI Masur 7 (Stem) Salt: 8dS m^{-1} BARI Masur 7 (Stem) Control

Plate 2. Transverse section of stem of lentil showing the effect of salinity seen under digital compound microscope (Model Primo star Zeiss) at 40X.

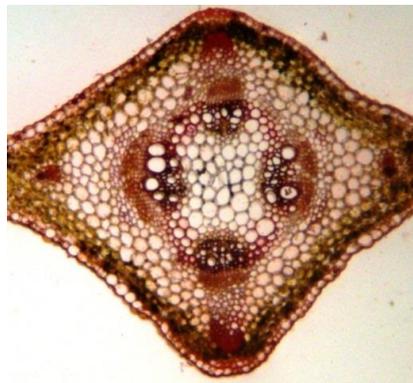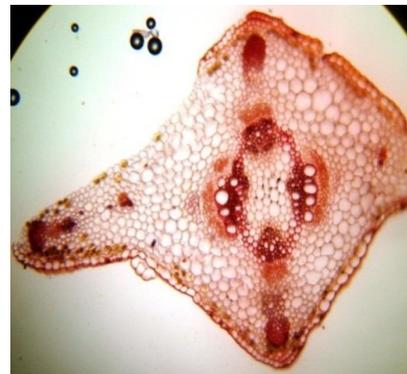A. BARI Chola 9 (Stem) Salt: 8dS m^{-1} BARI Chola 9 (Stem)-Control

Plate 3. Transverse section of stem of chickpea showing the effect of salinity seen under digital compound microscope (Model Primo star Zeiss) at 40X.
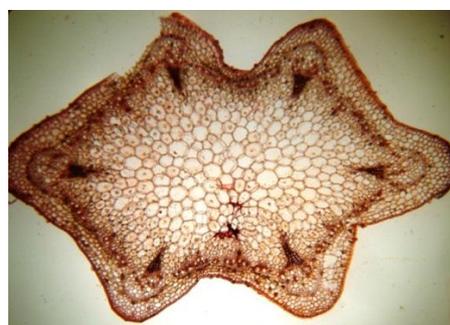

A. BARI Motor 1 (Stem) Salt: 8dS m^{-1} BARI Motor 1 (Stem) Control

Plate 4. Transverse section of stem of field pea showing the effect of salinity seen under digital compound microscope (Model Primo star Zeiss) at 40X.
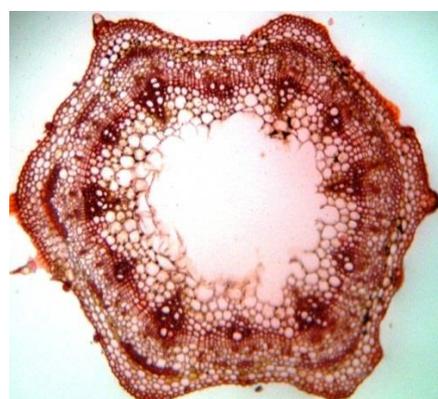

A. BARI Khesari 3 (Stem) Salt: 8dS m^{-1} B. BARI Khesari 3 (Stem)-control

Plate 5. Transverse section of stem of grass pea showing the effect of salinity seen under digital compound microscope (Model Primo star Zeiss) at 40X.

A. BARI Felon 2 (Stem) Salt: 8dS m^{-1} B. BARI Felon 2 (Stem) Control

Plate 6. Transverse section of stem of cowpea showing the effect of salinity seen under digital compound microscope (Model Primo star Zeiss) at 40X.

A. BARI Mung 6 (Stem) Salt: 8dS m^{-1} B. BARI Mung 6 (Stem) Control

Plate 7. Transverse section of stem of mungbean showing the effect of salinity seen under digital compound microscope (Model Primo star Zeiss) at 40X.

Conclusion

In morphological study, it was found that, the plant height, root length were shorter in size and shape under salinity stress condition than normal condition. Root dry weight and shoot dry weight were also reduced due to salinity stress than normal condition. The anatomical features were found some changes in stem xylem and phloem area. Some xylem and phloem size were reduced which might be the cause of lack of nutrients and water supply for physiological drought. Among the pulse crops cowpea (BARI Felon-2) and grass pea (BARI Khesari-3) were given better performance than the others whereas lentil (BARI Masur-7) and black gram (BARI Maskalai-1) were found more susceptible pulse crops according to their morphological and anatomical characteristics.

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EFFECT OF ETHEPHON ON RIPENING AND POSTHARVEST QUALITY OF MANGO

A. A. Sabuz[1], M. G. F. Chowdhury[2], M. M. Molla[3]
M. H. H. Khan[4] and M. Miaruddin[5]

Abstract

The experiment was conducted at the laboratory of Postharvest Technology Division, BARI to evaluate the effect of postharvest application of 6 concentrations (0, 250, 500, 750, 1000 & 10000 ppm) of ethephon on ripening and postharvest qualityof mango (cv. Langra) fruits harvested at mature green stage on 3rd week of June in 2011 and 2012. The treated fruits were assessed for physiological changes such as ripening %, weight loss (%), biochemical aspects such as TSS (^{0}Brix), titratable acidity (%), reducing sugar (%), total sugar (%), ascorbic acid content (mg/100g), total carotenoids (µg/100g), carbon di oxide production (ml/g fruit) and residual level of the applied ethephon during storage period. The observations were recorded at 2 days interval during 6 days storage at ambient condition (23±2°C with 80±5% RH). Complete yellow color (full ripe) was developed on the fruits treated with 500-1000 ppm ethephon at 4 days of storage while yellowish green and greenish yellow color was developed on 250 ppm treated and control fruits, respectively, and 10000 ppm ethephon treated fruits overripened at this period. At 6 days of storage, 250 ppm ethephon treated fruits got ripen and 500-1000 ppm ethephon treated fruits overripened whereas 10000 ppm treated fruits got rotten and control one was still unripe. Irrespective of ethephon treatments, weight loss of fruits, TSS, reducing sugar, total sugar, carbon di oxide production and total carotenoid showed increasing trends upto 6 days whereas titratable acidity, ascorbic acid and residue level of ethephon showed decreasing trends in both years. At 4 days of storage, 750-1000 ppm ethephon dipped fruits induced uniform attractive yellow color while untreated control fruits remained yellowish greenish (unripe) even after 6 days of storage. At 6 days of storage TSS, reducing sugar, toatal sugar, ascorbic acid and total carotenoid content were found maximum in 750-1000 ppm treated fruits compared to 250-500 ppm treated fruits. The residue level of ethephon in mango fruits treated with ethephon concentrations (250-1000 ppm) at 6 days of storage was found below 2 ppm (0.1 1ppm-0.54 ppm), which is safe for human consumption. Therefore, mangoes ripened by using ethephon @ 750-1000 ppm can be consumed safely without any health risk.

Keywords: Mango, Ethephon, Ripening, Residue, postharvest quality.

Introduction

Mango (*Mangiferaindica* L.) belonging to the family *Anacardiaceae* is one of the most important commercially grown fruits in Bangladesh. It is known as 'aam' considered as the king of fruits due to its excellent flavor, taste, nutritive value, processing qualities and its delicacy for the table (Doke *et. al.,* 2018). But, it is highly perishable in nature. Bangladesh occupies 8th position among mango growing countries and produces about 2.74 % of world's total mango production. In Bangladesh, the total production of mango fruits is about 12.88 lakh metric tons from 0.42 lachectors of land in 2016-2017 (BBS, 2018). It is major fruit crop cultivated in tropical and subtropical zones of the world. In Bangladesh it grows well in north-western and south-western region. But recently it is cultivated in all parts of the country. It is an abundant source of carotene, precursor of "vitamin A" after ripening.

Ripening is a process in fruits that causes them to become more palatable. In general, a fruit becomes sweeter, less green and softer as it ripens. The process of fruit ripening is chiefly regulated by a gaseous plant hormone called ethylene. The chemical commonly used to ripen fruits commercially is ethephon (2-chloroethyl phosphonic acid) which penetrates into fruit and decomposes to ethylene(Alexander and Grierson, 2002). Aqueous solution of ethephon is stable below pH 3.5. Above pH 3.5, the hydrolysis of ethephon begins with the release of free ethylene along with chloride and phosphate ions (Sukhjit, 2017).

In natural condition, mango fruits harvested at mature but unripe condition ripen slowly leading to high weight loss, desiccation and ripening is also uneven. Transporting and distributing mango fruits from farmer's field to consumer's basket can take several days. During this time ripen fruits become overripe and inedible. A part of naturally ripened fruits can also be damaged during transportation causing great loss to farmer. For this reason farmers harvest their fruits at mature green stage and apply ethephon to ripen artificially minimizing postharvest loss. Early and uniform ripening and color development can be achieved by dipping of mango fruits (physiologically mature but unripe) in diluted ethephon solution which is recommended for a number of climacteric fruits including mango (Bhandari*et. al.,* 2017; Doke *et. al.,* 2018; Gurjar *et al.,* 2017; Sukhjit, 2017), banana (Mahajan *et. al.,* 2010), tomato (Moniruzzaman *et. al.,* 2015), guava (Mahajan *et. al.,* 2008), and pear (Dhillion and Mahajan, 2011) and their recommended dosage of ethephon is 500-1000 ppm. When the the mature fruits dipped in aqueous solution of ethephon, ethephon enters into the fruit cells, releases ethylene and hastens the ripening process(Zhu *et al.*, 2005).

Farmers use ethylene releasing chemicalsm namely Tomtom, Harvest, Promot, Ripen, Prolong, Ethrel, Goldplus, etc. in high doses (10000 ppm or more) on

mangoes to quicken the ripening process and to increase the shelf life (Anon., 2014). Hakim *et. al.* (2012) opined that ripening chemicals are considered hazardous to human health and they have to be used within safe recommended level. Recently there have been a mixed opinions on the toxicity of ethephon among the consumers all over the country. Ethephon has been registered with EPA (US Environmental Protection Agency) since 1973 as a plant growth regulator used to promote fruit ripening and flower induction. It is a chemical which is irritant to the skin or the eyes but it is not skin sensitizer and carcinogen as classified by IARC(International Agency for Research on Cancer) as group D (not carcinogenic to human) and FAO pointed out maximum allowable daily intake for ethephon at 0.05 ppm (mg/kg) body weight/day (Bui, 2017). Recommended maximum residue level of ethephon is 2 ppm (mg/kg) of treated fruit (Anon., 2001). Optimum dose for ripening of mango fruits has yet not been developed in our country. The present study was therefore, conducted to determine the optimum concentrations of ethephon for ripening of mango without affecting its nutritional and postharvest qualityduring storage at ambient temperature.

Materials and Methods

Site: The present investigation was carried out at the Laboratory of the Postharvest Technology Division in Bangladesh Agricultural Research Institute during 3rd week of June of the year 2011-12 maintaining temperature 23 ± 2^0 C and RH $85\pm5\%$. Fruits were carried from the farmer's field to the laboratory in plastic crates covering themselves in newspaper.

Plant material: The mango (cv.Langra) which were physiologically mature and have attained the full size, light green with tinge of yellow at apical end were collected from farmers field nearby Volahat Upazilla, Chapai-nawabganj to use for the study. Fruits were harvested on 18th June, 2011 and 20th June, 2012. The fruits were selected on the basis of uniformity, maturity and size (200-250 gm). The experiment was laid out in Completely Randomized Design (CRD) with 6 treatments with3 replications for each treatments.Selected fruits were divided into two parts. One for investigating chemical parameters at 2 days interval upto 6 days and other one was kept in plastic box at ambient condition to examine physical parameters at same interval upto 6 days of storage.

Treatment setting: The experiment consisted 6 level of ethephon concentrations (T_1=control, T_2= 250 ppm, T_3=500ppm, T_4=750 ppm, T_5=1000 ppm and T_6= 10000 ppm).T_6(10000 ppm) is being used by the farmers as common ripening practice. Prior to use, fruits were washed with clean water, dipped for 2 minutes in 250 ppm "propiconazole" and dried with air flow before setting the experiment. Then the fruits were dipped for 5 minutes in the following

concentrations of ethephon solution as stated above.The temperature was set at 23 ± 2^0 C and RH $85\pm5\%$.

After that, the fruits were kept at ambient temperature for 10 minutes in an attempt to reduce possible chemical injury and being dried up. The control fruits were dipped for 5 minutes in tap water without using the ethephon solution. The number of fruits treated under each treatment was 12. The source of ethephon was Ripen-15 as it was available at the market.

Parameters studied: The parameters studied were percentage of ripening, physiological weight loss (%), titratable acidity (%), total soluble solid(TSS), reducing sugar (%), total sugar (%), ascorbic acid content (mg/100g), total carotenoid (µg/100 g) and carbon di oxide production (ml/g fruit). Each data were recorded at 2 days interval upto 6 days.

Percentage of ripening: In order to determine the ripening percentage, mango fruits were daily observed for their color development and when skin color turned to full yellow, they were considered as ripe. Ripening percentage was calculated following the formula;

$$\text{Ripening (\%)} = \frac{\text{Number of ripe fruits}}{\text{Total number of fruits}} \times 100$$

Weight loss: Weight loss was calculated by following formula;

$$\text{Weight loss of fruits (\%)} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Ascorbic acid content: For ascorbic acid measurement, 10g pulp was homogenized in 50 mL of 3% cold metaphosphoric acid (HPO_3) using a blender for 2 min and filtered through Whatman filter paper No. 2. The clear supernatant was collected for assaying ascorbic acid by 2, 6-dichlorophenolindophenol titration following the method of Ranganna (1986). Ten milliliters of aliquot were titrated with 0.1% 2, 6-dichlorophenolindophenol solution until the filtrate changed to pink color persisted for at least 15 seconds and the titration volume of 2, 6-dichlorophenolindophenol was recorded. Prior to titration 2, 6-dichlorophenolindophenol solution was calibrated by ascorbic acid standard solution. Ascorbic acid content was calculated according to the titration volume of 2, 6-dichlorophenolindophenol and results were expressed as mg/100g fresh weight.

$$\text{Ascorbic acid content (mg/100g)} \frac{\text{T x D x V1 x 100}}{\text{V2 x W}}$$

Where T= Titre, D = Dye factor, V1= Volume made up, V2= Volume of extract taken for estimation, W= Weight of sample taken for estimation.

Titratable acidity of mango pulp: It was determined following the method described by Ranganna (1986).

The titratable acidity (TA) was analyzed using the titration method. Pulp sample (10 g) were homogenised using a kitchen blender with 40 ml of distilled water. The mixture was then filtered through cotton wool. The filtrate (5 ml) with one to two drops of phenolphthalein (0.1%) as indicator was titrated using0.1 N NaOH to an endpoint pink (pH 8.1). The results were expressed as the percentage citric acid per 100 g fresh weight.

$$\text{Total titratable acidity (\%)} \frac{T \times N \times E \times V1 \times 100}{V2 \times W}$$

Where T= Titre, N = Normality of NaOH, V1= Volume made up, E= Equivalent weight of malic acid, V2= Volume of extract taken for estimation, W= Weight of sample taken for estimation.

TSS content of mango pulp: Total soluble solid (TSS) in the extracted juice of fruits was measured by a Digital Hand Refractometer (ATAGO (Brix = 0 to 32) by placing a drop of pulp solution on its prism and direct reading was recorded and the results were expressed as ^{0}Brix.

Total sugar & reducing sugar (%) measurement: Total sugar and reducing sugar content of fruit was estimated by the following procedures described by Lane and Eynon (1923).

Standardization of Fehling's solution

50 milliliter of both Fehling's solution A and Fehling's solution B were mixed together in a beaker. Ten milliliter of mixed solution was pipetted into a 250 ml conical flask and 25 ml distilled water was added to it. Standard sugar solution was taken in a burette. The conical flask containing mixed solution was heated on a hot plate. When the solution began to boil, 3 drops of methylene blue indicator solution were added to it without removing the flask from the hot plate. Mixed solution was titrated by solution. The end point was indicated by de-colorization of the indicator.

Fehling's solution was calculated by using the following formula;

Fehling's Factor (g of invert sugar) = (Titre x 2.5)/1000

Preparation of sample

50 ml of fruit juice was mixed with 100 ml of distilled water and 5 ml of neutral lead acetate solution and then kept for 10 minute and the mixture was homogenized. Then the blended material was transferred to a 250 ml volumetric

flask. The volume was made up to the mark with distilled water. Then solution was filtered.

Titration of reducing sugar

10milliliter of mixed Fehling's solution was taken in a 250 ml conical flask and made 250 ml with distilled water. Purifier juice solution (filtrate) was taken in a burette. Conical flask confining mixed Fehling's solution was heated on a hot plate. 3-5 drops of methylene blue indicator were added to the flask when boiling started and titrated against solution taken in burette. The end point was indicated by de-colorization of indicator. Percent reducing sugar was calculated according to the following formula;

$$\% \text{ reducing sugar} = \frac{F \times D \times 100}{T \times W \times 1000}$$

Where,F= Fehling's factor,D= Dilution, T= Titer andW= Weight of sample.

Non-reducing sugar was estimated by using the following formula;

% Non-reducing sugar = % Total invert sugar - % Reducing sugar

> Estimation of total sugar

    % Total Sugar = % Reducing sugar + % Non- reducing sugar

Measurement of total carotenoids:

Total carotenoid was measured by taking 5 grams of the sample, grounded with acetone and anhydrous sodium sulphate in a pestle and mortar (Ranganna, 1986). Total carotenoids (µg/100g) was measured by spectrophotometer (T-80, PG Instrument Ltd. UK) at 451ηm (Alasalvar*et al.*, 2005).

Carbon-di-oxide measurement:

The level of CO_2 volume ml/g fruit weight was measured by Archimedes' principle and expressed as ml/g of fruits.

*Residue level of ethephon:*The residue level in treated mango was measured by Gas chromatography flame ionized detector in Toxicology laboratory, Entomology Division, BARI, Gazipur and SGS Laboratory, Dhaka and expressed as ppm (mg/kg). The method is stated by Rahman *et. al.* (2012).

*Statistical analysis***:** Data analysis were performed by one-way ANOVA using software SPSS 20.0 (IBM INC: New York). Mean comparison was done by Tuky w test at 5% level of probability. All data were expressed in triplicate as means ± standard deviation.

Results and Discussions

Since there was no significant difference between two year's analytical data, pooled analysis was done and presented.

Effect on ripening: The investigation revealed that ethephon application enhanced the onset of ripening in mango and the response varied according to the concentrations ((Tables 1&2). 100% ripening was found when the fruits were treated with ethephon @10000ppm after 4 days of ambient storage. Almost all the fruits were fully ripened at 6 days of storage except control treatment. The ethephon (2-chloroethyl phosphonic acid) penetrates into fruit and decomposes to ethylene. Ethylene regulates the expression of several genes involved in fruits ripening so as to modulate the activity of various enzymes involved in the process of ripening (Beitz *et al.* 1977). These enzymes act to soften the skin of the fruit and also convert complex polysaccharides into simple sugars. It was explained by Holl (1977) in other way that the ethylene probably brings about the climacteric, since in many fruits the rise in respiration is directly preceded by an elevation in the ethylene concentration. This respiratory climacteric can be induced by ethylene treatment without a simultaneous change in tissue permeability. It has also been reported that ethylene alters the proportion of individual transfer RNA species.In support of the present study, the color development in mango fruits was remarkably affected by post-harvest application of ethephon. Out of all the concentrations, 1000 ppm ethephon gave the most attractive and deep colored fruits. However, the specific mode of action of ethephon in accelerating color development is not clearly understood. Nour and Goukh (2010) observed that peel color score progressively increased during ripening of guava fruits. They observed that fruits treated with ethephon (250-1000 ppm) reached the full yellow stage 3, 4 and 6 days earlier than untreated fruits, respectively. They also reported ethephon treated fruits had reached the soft stage 2-6 days earlier than the control treatment.Color development was better due to rapid degradation of chlorophyll and higher synthesis of carotenoid pigmentation and alteration in pigment due to different applied ethephon treatment. These findings are more or less similar to the findings of Gurjar *et. al* (2017) and Sukhjit (2017) in mango.

Effect on weight loss: Table2 indicates that the physiological loss in weight was significantly increased with the increase of applied ethephon concentrations. This might be due to rapid respiration and transpiration. The maximum weight loss 3.73% was observed at T_6(10000 ppm) where as it was only 1.74% in control treatment (T_1) at 6 days of ambient storage. Similar type of decrease in fruit weight during storage was also observed by Sharma and Singh (1981) in dates when dipped in 250-500 ppm ethephon for 5 minutes and by Gurjar*et. al* (2017) in 'Amrapali' when dipped into 250-1250 ppm ethephon.

Effect on titratable acidity: It was observed from Table 2, that acidity of the mango fruit was not clearly understood by post-harvest application of ethephon and the response varied within the concentrations. Minimum titratable acid (0.704%) was found in mango fruits treated with 1000 ppm ethephon at 6 days of ambient storage. Similar finding was also noted in guava (Singh *et al*.,1979) and in date (Sharma and Singh, 1981). Riberau-Gayaon (1968) suggested that transformation of organic acids into sugars was one of the reasons for deceasing organic acids during fruit ripening. Therefore, another possibility seemed that ethephon might enhance the conversion of organic acids to sugars since present findings revealed that sugar content was increased and acidity was decreased following ethephon application.

Effect on total soluble solid (TSS): The maximum TSS (20.7^0Brix) was observed in 750 ppm closely followed by 1000 ppm (20.50^0Brix) at 6 days of storage which was significantly different compared to control fruits (12.8%) after 6 days of storage (Table 3). Similarly, increased total soluble solids due to post harvest application of ethephon was also reported by Singh *et al.* (1979) in guava, Sharma and Singh (1981) in date, Meitei *et al.* (1983) in peaches. The initial increased rate of TSS might be due to rapid loss of water from the fruits and the conversion of starch into sugar at a faster rate (Fernandez *et al.*, 2006).

Effect on reducing sugar and total sugar: It revealed that the total sugars and reducing sugar increased with increasing ethephon concentration (Table 3). The maximum total sugar (10.20%) and reducing sugar (7.16%) was observed in treatment T_5 (1000 ppm) treated fruits at 6 days of ambient storage. The extent of sugar content increased up to 6 days of storage. Probably ethephon enhanced the rate of accumulation of reducing sugar in mango fruits. Similarly, high percentage of reducing sugar with ethephon application in dates was observed by Sharma and Singh (1981). The finding is corroborated with the result of Kumar and Singh (1993) who observed that higher percentage of sugar in ethephon (750 ppm and 500 ppm) treated mango fruits over control treatment.

Effect on ascorbic acid: The ascorbic acid decreased significantly up to 6 days after storage for all the treatment (Table 4). At 6 days of storage maximum ascorbic acid content was recorded in 1000 ppm treated fruits (29.12 mg/100 g) followed by 750 ppm treated fruits (28.12 mg/100 g). The fruits during storage, in general showed a declining trend in ascorbic acid content significantly irrespective of the treatments applied. A reduction in ascorbic acid content with the subsequent prolongation of storage might be due to rapid oxidation phenomenon of organic acid in later stage of storage (Orzolek and Argell, 1974).

Effect on total carotenoid: A significant increase in total carotenoids was observed up to 6 days after storage in all the treatment (Table 4). The maximum total carotenoid was observed in 1000 ppm treated mango fruits (13.24 µg/100gm) at 6 days of ambient storage. Ethylene might increase the carotenoid content through its synthesis. This fact was established by Young and Jahn (1972) while working in citrus.

Effect on Carbon-di-oxide (CO_2) production: It was obvious from the experiment that CO_2 level of the air-tight mango sample gradually increased with the increased ethephon application rate as well as with the storage period (Table 4). Maximum CO_2 was found in 1000 ppm treated fruits at 6 days of storage which was 8.60 ml/g fruits and the lowest was observed in control treatment (5.17 ml/g). It might be resulted from the increase in respiration rate of the fruit sample.

Residue analysis: The residue level was observed in all treated mangoes at 2, 4 and 6 days of storage (Table 5). Initially the maximum residue (ethephon) was observed in treatment T_5 (1.86 ppm) followed by treatment T_5 (1.80 ppm) and the minimum was found in treatment T_2 (0.41 ppm) after 2 days of storage. It was observed that residue level decreased below themaximum residue level (MRL) of ethephon (2ppm). The residue level in treated fruits decreased with the increasing storage period and found the value was only 0.50-0.54 ppm at 6 days of storage in case of 750 and 1000 ppm ethephon treated fruits. The reason behind this is that it is very volatile compound and it completely agrees with Beitz *et al.,* (1977).

Table 1. Effect of ethephon on color development during storage ofmango (cv. Langra)

Treatments (ppm)	Storage Days			
	0 DAS	2 DAS	4 DAS	6 DAS
T_1 = Control	Green	Green ,slight yellow	Green > yellow	Yellow >green
T_2 = 250 ppm	Green	More green than yellow	Yellow > green	Ripe
T_3 = 500 ppm	Green	More yellow than green	Full ripe	Over-ripe
T_4 = 750 ppm	Green	More yellow than green	Full ripe	Over-ripe
T_5 = 1000 ppm	Green	Yellowish	Full ripe	Over-ripe
T_6 = 10000 ppm	Green	Yellow	Over-ripe	Rotting

DAS=Days after storage

Table 2. Effect of ethephon on ripening (%), weight loss (%), titratable acidity (%) of mango during storage *(pooled of year 2011 and 2012)*

Treatment (ppm)	Ripening (%)			Weight loss (%)			Titrable acid (%)		
	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS
T$_1$ = Control	0.00 ± 0.00 e	18.00± 1.00 e	27.54± 0.20 d	1.13± 0.10 c	1.34± 0.10 e	1.74± 0.10 d	0.786± 0.005 b	0.760± 0.01 d	0.745± 0.01 b
T$_2$ = 250 ppm	26.00± 1.00 d	58.75± 0.20 c	64.37± 1.00 c	1.35± 0.05 c	1.74± 0.10 d	2.12± 0.10 c	0.816± 0.01 a	0.792± 0.002 b	0.780± 0.01 a
T$_3$ = 500 ppm	25.00± 1.00 d	64.00± 1.00 b	95.00 ± 1.00 b	1.75± 0.10 b	2.35± 0.10 c	2.84± 0.10 b	0.816± 0.01 a	0.774± 0.002 c	0.729± 0.004bc
T$_4$ = 750 ppm	28.30± 0.50 c	50.00± 1.00 d	100.00± 0.00	1.87± 0.10 b	2.87± 0.10 b	3.43± 0.10 a	0.826± 0.01 a	0.768 ± 0.001 c	0.723 ± 0.004 cd
T$_5$ = 1000 ppm	38.18± 0.20 b	65.55± 0.10 b	100.00± 0.00 a	2.64± 0.10 a	3.13± 0.10ab	3.64± 0.10 a	0.776± 0.01 b	0.712± 0.002 e	0.704± 0.004 d
T$_6$ = 10000 ppm	42.34± 0.30 a	100.00± 0.00 a	100.00± 0.00 a	2.75± 0.10 a	3.23± 0.10 a	3.73± 0.20 a	0.828± 0.007 a	0.710± 0.004 a	-

*DAS=Days after storage

**All values are means of triplicate determinations ± SD. Means within columns with different letters are significantly different at 5% level of probability by Tukey w test

Table 3. Effect of ethephon on TSS (°B), reducing sugar (%), total sugar (%) of mango during storage(*pooled of year 2011 and 2012*)

Treatment (ppm)	Total Soluble Solid (°Brix)			Reducing sugar (%)			Total sugar (%)		
	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS
T₁ = Control	10.6± 0.10ab	12.0± 1.00 d	12.8± 0.50 c	3.24± 0.03 c	4.01± 0.05 e	4.25± 0.10 f	5.53± 0.05 b	6.61± 0.03 e	6.82± 0.05 c
T₂ = 250 ppm	10.7± 0.10 a	16.8± 0.50 c	18.3± 0.10 b	3.90± 0.10ab	5.01± 0.05 d	5.48± 0.05 d	6.55± 0.10 a	8.64± 0.10 c	9.61± 0.03 b
T₃ = 500 ppm	10.5± 0.10ab	16.3± 0.50 c	20.4± 0.10 a	3.77± 0.10 b	5.26± 0.02 c	5.06± 0.02 e	6.51± 0.03 a	8.25± 0.04 b	9.68± 0.03 b
T₄ = 750 ppm	10.6± 0.10ab	17.4± 0.20bc	20.7± 0.10 a	3.82± 0.04ab	5.63± 0.10 b	5.81± 0.01 c	6.43± 0.04 a	8.69± 0.06 c	9.62± 0.04 b
T₅ = 1000 ppm	10.5± 0.10ab	21.3± 0.20 a	20.5± 0.10 a	3.95± 0.075ab	5.93± 0.05 a	6.16± 0.02 b	6.45± 0.03 a	9.49± 0.04 a	10.2± 0.04 a
T₆ = 10000 ppm	10.4± 0.10 b	19± 1.00 b	20.9± 0.10 a	4.00± 0.10 a	5.63± 0.05 b	7.88± 0.01 a	6.53± 0.04 a	9.32± 0.01 b	-

DAS=Days after storage

All values are means of triplicate determinations ± SD. Means within columns with different letters are significantly different at 5% level of probability by Tukey w test.

Table 4. Effect of ethephon on ascorbic acid (mg/100g), total carotenoids (μg/100g) and CO₂ production (ml/gm fruit) of mango during storage (*pooled of year 2011 and 2012*)

Treatment(ppm)	Ascorbic acid (mg/100g)			Total carotenoids (μg/100g)			CO₂ production (ml/g fruit)		
	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS
T₁= Control	36.38± 0.05 a	29.12± 0.04 c	27.15± 0.10 d	4.67± 0.10 b	7.71± 0.03 b	7.90± 0.10 e	3.91± 0.02 e	4.58± 0.04 e	5.17± 0.05 d
T₂ = 250 ppm	35.77± 0.05 c	29.83± 0.05 b	26.40± 0.10 e	5.00± 0.10 a	7.82± 0.03 b	8.80± 0.10 d	5.48± 0.04 c	6.36± 0.02 d	6.87± 0.04 c
T₃= 500 ppm	36.23± 0.05bc	29.45± 0.10bc	27.95± 0.10 c	5.25± 0.10 a	7.26± 0.02 c	9.07± 0.10 c	5.25± 0.10 d	6.26± 0.01 d	7.47± 0.02 b
T₄ = 750 ppm	36.33± 0.10a	29.74± 0.10 b	28.19± 0.10 b	4.67± 0.10b	7.74± 0.10 b	13.10± 0.04 b	5.97± 0.05 b	6.90± 0.10 c	7.59± 0.02 b
T₅ = 1000 ppm	35.47± 0.10 d	30.50± 0.50 a	29.12± 0.10 a	5.12± 0.10 a	8.34± 0.10 a	13.24± 0.02 a	5.99± 0.05 b	7.13± 0.10 b	8.60± 0.10 a
T₆ = 10000 ppm	36.03± 0.10 b	27.66± 0.04 d	25.19± 0.05 f	5.15± 0.10 a	8.25± 0.10 a	13.34± 0.02 a	6.53± 0.01 a	9.28± 0.05 a	-

DAS=Days after storage

All values are means of triplicate determinations ± SD. Means within columns with different letters are significantly different at 5% level of probability by Tukey w test

Table 5. Estimated residue level (ppm) of ethephon in treated mango *(pooled of year 2011 and 2012)*

Treatments	Days after storage		
	2 DAS	4 DAS	6 DAS
T_1 = Control	0.00 ± 0.00 f	0.00 ± 0.00 e	0.00 ± 0.00 d
T_2 = 250 ppm	0.41 ± 0.03 e	0.26 ± 0.02 d	0.11 ± 0.02 c
T_3 = 500 ppm	0.64 ± 0.02 d	0.51 ± 0.03 c	0.49 ± 0.02 b
T_4 = 750 ppm	0.85 ± 0.02 c	0.53 ± 0.02 c	0.50 ± 0.01 ab
T_5 = 1000 ppm	1.80±0.01 b	1.28 ± 0.02 b	0.54 ± 0.02 a
T_6 = 10000 ppm	1.86 ± 0.01 a	1.40±0.02 a	-

DAS=Days after storage

All values are means of triplicate determinations ± SD. Means within columns with different letters are significantly different at 5% level of probability by Tukey w test.

Conclusion

It can be concluded from the present investigation that the use of ethephon had a significant impact on the ripening and postharvest quality of mango fruits. Among the treatments ethephon application @ 1000 ppm was best for retaining the various physical and chemical parameters followed by ethephon application @ 750 ppm till the 6 days of storage. Therefore, at matured green stage, ethephon can be applied@750-1000 ppm for uniform ripening of mango at ambient condition (23±2°C) with 85±5% relative humidity. The estimated residue level in 750-1000 ppmethephon treated mango fruits at 4 and 6 days of storage remains lower than maximum residue limit (MRL) of ethephon (2ppm).

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EVALUATION OF SALT TOLERANT MUNGBEAN (*Vigna radiata* L.) GENOTYPES ON GROWTH THROUGH BIO-MOLECULAR APPROACHES

M. M. Rohman[1], I. Ahmed[2], M. R. Molla[3]

M. A. Hossain[4] and M. Amiruzzaman[5]

Abstract

This study was conducted to obtain saline tolerant mungbean genotypes through evaluating growth, biochemical and molecular parameters, and possible salt tolerant mechanisms were studied in different salt sensitive genotypes. Thirteen prescreened mungbean genotypes were grown on 0, 40, 80 and 120 mM NaCl induced salinity and evaluated by germination percentage, shoot and root length, superoxide ($O_2{}^{\bullet-}$) generation rate, concentration of H_2O_2, lipid peroxidation (as malondialdehyde, MDA), methylglyoxal (MG), K^+/Na^+ and proline content in leaves. Based on these parameters, genotypes BD-10588, BD-6894 and IR-01 were selected as tolerant genotypes. For studying oxidative stress tolerance mechanism, BD-10588 and IR-01 were used as tolerant and BD-6887 and BD-10741 as susceptible genotypes, and comparative ROS ($O_2{}^{\bullet-}$ and H_2O_2), and MDA as well as LOX activity between the two groups were determined. Analysis of activities of ROS metabolizing antioxidant enzymes strongly suggested that superoxide dismutase in tolerant genotypes provided first line protection from salt induced $O_2{}^{\bullet-}$. Higher catalase (CAT) and ascorbate peroxidase (APX) played major role in H_2O_2 metabolism in tolerant genotypes. Both specific and in-gel activities of glutathione peroxidase (GPX) strongly proved the H_2O_2 metabolism for reducing oxidative damage in both tolerant and susceptible genotypes. However, higher peroxidase activity was important for mitigating salt stress in susceptible mungbean genotypes. Therefore, SOD, APX and GPX are very important for protecting salt mediated oxidative damage in mungbean genotype.

Keywords: Salinity, bio-molecular approach, mungbean, oxidative stress

Introduction

The productivity of mungbean has remained low due to susceptibility to various biotic (Mungbean yellow mosaic virus, powdery mildew and Cercospora leaf spot) and abiotic (salinity, drought, temperature, and water-logging) stresses at different growth stages. Salinity stress is a serious problem in arid and semi-arid tropics and in the Indo-Gangetic plains in irrigated areas and also planting areas of mungbean where it is especially grown after Aman rice crop in the coastal

[1&5]Molecular Breeding Lab, Plant Breeding Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, [2&3]Molecular Biology Lab, Plant Genetic Resources Centre, BARI, Gazipur, [4]Soil Science Division, BARI, Gazipur, Bangladesh.

regions. Mungbean is a short duration moderate saline tolerant crop where 50 mM NaCl can cause yield losses $\geq 70\%$ (Saha *et al*., 2010; Gosh *et al*., 2015). However, salt tolerant mungbean variety is still limited. Though significant knowledge has been achieved for saline tolerance mechanism, it is not fully understood. Therefore, efficient selection criteria for salt tolerant genotypes of crops including mungbean are essential for obtaining salt tolerant genotypes.

In Bangladesh, salinity is the most important constraints for crop production in coastal zone throughout the year. The coastline of Bangladesh is 710 km long with pH ranges 6.0-8.4 which is composed of the interface of various ecological and economic systems, including mangroves, tidal flat (Ahmad, 2019; Haque, 2006). Salinity problem has been increasing in Bangladesh, and over the last 35 years, salinity has increased around 26 percent in the coastal region of Bangladesh (Mahmuduzzaman *et al*., 2014). Therefore, for sustainable crop production in coastal region, saline tolerant crop species and crop varieties are urgent issues.

Salinity stress is the most devastating stress, and increasing soil salinity in coastal regions has focused attention on the possibility of crop damage in fields located in sea regions worldwide. The problem due to salinity will affect the production of agricultural crops in the upcoming years, particularly in arid and semiarid regions (IPCC, 2014). This situation is a great threat to ensure food security for densely populated countries like Bangladesh where more than one million hectare (ha) of land remain fallow in coastal area, because crops that have a higher yield but have lower adaptability to salinity will need to be replaced by crops that have higher adaptive potential but are likely to have a lower yield. As a result, there is an urgent need to develop highly adaptive crops that also have a higher yield under salinity. Salinity affects plant growth and development in two ways. First, it imposes osmotic stress by reducing the soil water potential, leading to limited water uptake. Second, it causes excessive uptake of ions, particularly Na^+ and Cl^-, that ultimately interfere with various metabolic processes. Plant responses to the osmotic and ionic components of salt stress are complicated and involve many gene networks and metabolic processes (Hasegawa *et al*., 2000; Munns and Tester, 2008). Such responses depend mainly on the inherent salt tolerance of the plant, the severity of salt stress (the concentration of salt in the soil solution), and the duration of the plant roots' exposure to the salt. Salinity tolerance is a complex trait, and plant breeders' efforts to produce crops with higher yields have largely been unsuccessful because of mutagenic adaptive responses to these traits. Moreover, a significant number of physiological activities are involved in the tolerance process.

Salinity causes negative consequences for gas exchange, resulting in low CO_2 assimilation for photosynthesis and consequently a significant reduction in electron transportation. As a result, reactive oxygen species (ROS) are generated, such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2),

perhydroxy radicals ($HO_2^{\bullet}$), and alkoxy radicals (RO**.**) (Gill and Tuteja, 2010; Moller *et al*., 2007). Normally, generation of ROS is balanced with scavenging by various antioxidants (Foyer and Noctor, 2005). This balance between the generation and scavenging of ROS is broken down by various biotic and abiotic stresses, including salinity drought. At higher concentrations, ROS are highly reactive and cause damage to proteins, DNA, lipids, and carbohydrates, resulting in cell death (Rohman *et al*., 2019). As a result, accumulation of ROS under environmental stresses is the foremost reason for reduced productivity of crops (Mittler, 2002; Apel and Hirt, 2004; Mahajan and Tuteja, 2005). On the other hand, methylglyoxal (MG) is a potentially cytotoxic compound, which can react with and modify other molecules, including DNA and proteins (Yadav *et al*., 2005a). Therefore, both ROS and MG must be removed or detoxified or kept below toxic level for cellular survival. Therefore, ROS and MG can be important criteria for selecting saline tolerant genotypes of crops (Yadav *et al*., 2005a, b; Singla-Pareek *et al*., 2008; Azooz *et al*., 2009). ROS cause oxidation of polyunsaturated fatty acid (PUFA) to produce important stress marker melondialdehyde (MDA) for cell wall leakage. Moreover, imbalance between K^+ and Na^+ ions under salinity can be an important indicator. Therefore, these parameters were used as selection criteria for obtaining tolerant mungbean genotypes.

Overproduction of ROS under abiotic stress including salinity causes oxidative damage in plant cell (Gill and Tugeta, 2010; Rohman et al., 2019). Plants possess ROS metabolism system through enzymatic and nonenzymatic antioxidants for cellular protection from oxidative damage. Among the enzymatic antioxidant, superoxide dismutase (SOD), peroxidase (POD) catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidases (GPX) are major ROS scavengers (Apel and Hirt, 2004; Gill and Tujeta, 2010). SOD provides first line protection from $O_2^{\bullet-}$ and produce H_2O_2 which is further metabolized by POD, CAT, APX and GPX (Gill and Tujeta, 2010). Considering all, we designed the study to obtain salt tolerant genotypes as well as to study oxidative stress tolerance in mungbean seedlings. In this study, we screened some preselected mung bean genotypes at seedling stage by evaluating growth parameters and content of ROS, MDA, MG and K^+/Na^+ as selecting criteria to obtain tolerant mungbean genotypes. Later, the possible oxidative damage in susceptible genotypes were studied.

Materials and Methods

Evaluation of mungbean genotypes through germination and growth parameters

For screening of mungbean accessions, the laboratory experiment was conducted in the Molecular Biology lab, PGRC, BARI, Gazipur. In this study 13 germplasm preselected from 91 were used. The laboratory experiment was made to screen

best performing accessions against salinity tolerance of mungbean at germination and seedling growth stage under room temperature using procedures followed by Taffouo *et al*. (2009). In order to investigate the response of mungbean accessions to different concentrations of NaCl solution (0, 50, 80 and 120 mM NaCl) were used. Glass petridishes (10 cm diameter) were thoroughly washed and sterilized in hot air oven. After sterilization, petridishes were lined with Whatman No.1 filter paper. Ten ml of distilled water as a control and salt solutions [50, 80 and 120 mM NaCl (Laboratory grade)] were added in to separate petridishes. Ten uniform seeds of each mungbean accession were placed in each Petri dish (five petridishes per genotypes per treatment) at uniform distances. Each petridish was filled with 10 ml of the respective treatment solution of NaCl on alternate days. The petridishes were put in a hood to avoid the loss of moisture through evaporation. Germination was started after three days of sowing, and a seed was considered to have germinated when the lengths of the emerging plumule and radicle were about 0.5 cm. Seeds were checked for germination every other day and the germination count was continued for 10 days. After 10 days, growth parameters like germination percentage, seedling shoot and root length were measured.

Determination of proline

Proline was measured according to Bates *et al.* (1973) based on proline's reaction with ninhydrin.

Evaluation of mungbean genotypes through biochemical parameters

Different biochemical parameters as selection criteria were measured at Molecular Breeding Lab, Plant Breeding Division, BARI. Thirteen accessions screened from germination test were grown in petridishes and one-week old seedlings were subjected to NaCl induced salinity of 0, 40 and 80 mM for one week and data were taken on different parameters from leaves. For comparative oxidative stress, two relative tolerant genotypes (BD-10588 and IR-01) and two susceptible genotypes (BD-6887 and BD-10741) were grown again and after germination, salinity of 0 and 80 mM were imposed for one week. After one week, data were taken from leaves.

Measurement of the $O_2^{\cdot-}$ generation rate

Superoxide radical was determined in 3rd leaf according to the method described in Rohman *et al*. (2016a). Leaves (0.3 g) were homogenized in 3 ml of 65 mmol phosphate buffer (pH 7.8) on an ice bath and were then centrifuged at 4°C and $5,000 \times g$ for 10 min. The supernatants (0.75 ml) were mixed with 0.675 ml of 65 mM K-phosphate buffer (pH 7.8) and 0.07 ml of 10 mM hydroxylamine chlorhydrate and were placed at 25°C. After 20 min, 0.375 ml of 17 mM

sulfanilamide and 0.375 ml of 7 mM α-naphthylamine were added, and the mixture was placed at 25°C for another 20 min before it was mixed with 2.25 ml of ether. The absorbance was measured at 530 nm and the $O_2^{•-}$ concentration was calculated from a standard curve of $NaNO_2$.

Measurement of H_2O_2

H_2O_2 was assayed according to the method described by Yu *et al*. (2003). Third leaf tissue (0.5 g) was homogenized in 3 ml of 50 mM K-P buffer (pH 6.5) at 4°C. The homogenate was centrifuged at $11,500 \times g$ for 15 min. The supernatant (3 ml) was mixed with 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), and the mixture was then centrifuged at $11,500 \times g$ for 15 min at room temperature. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content ($\in = 0.28$ μM^{-1} cm^{-1}) and expressed as micromoles per gram FW.

Measurement of MDA

The level of lipid peroxidation was measured by estimating melondialdehyde (MDA), a decomposition product of the peroxidized polyunsaturated fatty acid component of the membrane lipid, using thiobarbituric acid (TBA) as the reactive material following the method of Heath and Packer (1968). Briefly, leaf tissue (0.5 g) was homogenized in 3 ml 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at $11,500 \times g$ for 10 min. The supernatant (1 ml) was mixed with 4 ml of TBA reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95°C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged at $11,500 \times g$ for 15 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nanomole of MDA per gram FW.

Measurement of K^+/Na^+: The sap was extracted from leaves and was put on compact Na^+ ion meter (Horiba-731, Japan) and compact K^+ ion meter (Horiba-722, Japan) to estimate the Na^+ and K^+ ions in leaves. The K^+/Na^+ ratio was measured from the estimated values.

Extraction of soluble protein for activity assay

Fresh leaf (0.5g) was homogenized in 1 ml of 50 mM ice-cold K-P buffer (pH 7.0) by mortar and pestle containing 100 mM KCl, 1 mM ascorbate, 5 mM β-mercaptoethanol and 10 % (w/v) glycerol. The homogenates were centrifuged at $11,500 \times g$ for 10 min and the supernatants were used for determination of enzyme activities. All procedures were performed below 4 °C.

Determination of protein

The protein concentration in the leaf extracts was determined according to the method of Bradford (1976) using BSA as a protein standard.

Assay activities of enzymes

Lipoxygenase (LOX, EC: 1.13.11.12): LOX activity was measured following Doderer *et al.* (1992). For the measurement of activity, the substrate solution was prepared by adding 35µl linoleic acid to 5 ml distilled water containing 50 µl Tween-20. LOX activity was determined spectrophotometrically by adding 10 µl of sample to 590 µl substrate solution. The increase in absorbance at 234 nm was measured for 1 min at 25°C. The activity was expressed as µM hydroperoxide formed min^{-1} mg^{-1} protein using a molar extinction coefficient of 25,000 M^{-1} cm^{-1}.

SOD (EC: 1.15.1.1): To determine SOD activity of whole cell homogenate, the reaction was prepared on ice in 50 mM potassium phosphate buffer (p^H 7.8, with 1.34 mM diethylenetriaminepentaacetic acid) and an indirect competitive inhibition assay was used for the measurement (Spitz, and Oberley, 1989). This assay is based on the competition between SOD and an indicator molecule nitroblue tetrazolium (NBT), for superoxide production from xanthine and xanthine oxidase. One unit of activity was defined as the amount of protein required to inhibit NBT reduction by 50%.

POD (EC: 1.11.1.7): POD activity was estimated according to Hemeda and Klein (1990). The reaction mixture contained 25 mM K-P buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and the protein solution). Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation for 1 min using extinction coefficient of 26.6 mM^{-1} cm^{-1}.

APX (EC: 1.11.1.11): APX activity was assayed following the method of Nakano and Asada (1981). The reaction solution contained 50 mM K-P buffer (pH 7.0), 0.50 mM ascorbic acid (ASA), 0.10 mM H_2O_2, 0.1 mM EDTA, and enzyme extract in a final volume of 0.7 ml. The reaction was started by the addition of H_2O_2, and the activity was measured by observing the decrease in absorbance at 290 nm for 1 min using an extinction coefficient of 2.8 mM^{-1} cm^{-1}.

GPX (EC: 1.11.1.9): GPX activity was measured as described by Elia *et al.* (2003) using H_2O_2 as a substrate. The reaction mixture consisted of 100 mM sodium-phosphate buffer (pH 7.5), 1 mM EDTA, 1 mM NaN_3, 0.12 mM NADPH, 2 mM GSH, 1 unit glutathione reductase (GR), 0.6 mM H_2O_2, and 20 µl of sample solution. The reaction was stated by the addition of H_2O_2. The oxidation of NADPH was recorded at 340 nm for 1 min, and the activity was calculated using the extinction coefficient of 6.62 mM^{-1} cm^{-1}.

CAT (EC: 1.11.1.6): CAT activity was measured according to the method of Csiszár *et al*. (2007) by monitoring the decrease of absorbance at 240 nm for 1 min caused by the decomposition (or degradation) of H_2O_2. The reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2, and enzyme solution in a final volume of 0.7 ml. The reaction was initiated with the addition of enzyme extract, and the activity was calculated using the extinction coefficient of 39.4 M^{-1} cm^{-1}.

SDS-PAGE and Native In-gel activity staining

Changes in proteins having isoenzymic activity of the ROS scavenging enzymes were studied using PAGE under non-reduced, non-denatured conditions at 4^0C according to Laemmli (1970). Native PAGE analysis was performed for SOD 10% PAGE for other enzymes 8% were done. SOD gel activity was assayed following Beauchamp and Fridovich (1971). After completion of electrophoresis, the gel was incubated in a solution containing 2.45 mmol NBT for 20 min, followed by incubation in 50 mmol potassium phosphate buffer (pH 7.8) containing 28µmol riboflavin and 28 mmol TEMED under dark condition. SOD expression was observed after light exposure for 10 to 20 min at room temperature.

APX isozymes were separated by native PAGE (gels containing 10% glycerol) for 4.5 h at 4°C at a constant current of 35 mA per gel. Ascorbate (2 mM) was included in the carrier buffer. The gels were pre-run for 30 min to allow ascorbate present in the carrier buffer to enter the gel prior to the application of samples, according to the method of Rao *et al*. (1996). Following electrophoretic separation, gels were equilibrated with 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM ascorbate for 30 min. The gels were incubated in the above buffer containing 4 mM ascorbate and 2 mM H_2O_2, for 20 min. After a brief wash in the buffer for 1 min, the gels were submerged in a solution of 50 mM potassium phosphate buffer (pH 7.8) containing 28 mM TEMED and 2.45 mM nitroblue tetrazolium, and subjected to gentle agitation. The reaction was continued for 10 to 15 min and stopped by a brief wash in water. The gel was exposed to light to visible the APX bands. In-gel activity of GPX were visualized using 2mM o-Dianisidine. The gel washed for 15 min in 2.5% Triton-X-100 following wash in distilled water for 15 minutes. The gel was immersed in 10 mM K-P buffer (pH 7.2) containing 2mM o-Dianisidine for 1 hour following immerse in 10 mM H_2O_2 to appear band.

Statistical Analysis:

All data obtained was analyzed by STATISTIX 10 program following complete randomized design (CRD) with at least three replications. The mean differences among the treatments were compared by least significant (LSD) tests. P value of ≤0.05 was considered to be significant.

Results

Effect of salinity on germination percentage of mungbean genotypes

The effects of different concentrations of NaCl solution on germination of mungbean seeds are shown in Table 1. The results showed germination percentage varied among the accessions, and different salinity levels. Increase in NaCl concentration reduced germination percentage. At 120 mM of NaCl only BD-10588, BD-6894, BD-10733, IR-01 showed few germinations. At 80 mM salinity, BD-6894, BD-10588, BD-10733 and IR-01 showed 80% germination; whereas, BD-6887, BD-10589 and BD-10741 had 50% germination.

Table 1. Germination percentage and germination rate of selected mungbean genotypes against different salinity levels. Different letters within a column are significant at P≤0.05. Mean separation was not performed for at 120 mM salinity

Genotypes	Germination percentage			
	Control	40 mM	80 mM	120 mM
BD-6887	80c	70c	50d	0
BD-6894	100a	100a	80a	5
BD-10588	98a	90ab	80a	10
BD-10589	100a	100a	50d	0
BD-10590	100a	100a	70b	0
BD-10733	90b	90ab	80a	2
BD-10740	90b	80bc	52cd	0
BD-10741	100a	80bc	50d	0
BD-10744	100a	100a	70b	0
IR-01	100a	100a	80a	6
IR-03	100a	100a	70b	0
IR-04	100a	100a	60c	0
IR-05	100a	100a	60c	0

Effect of salinity on seedling shoot and root length of mungbean genotypes

All accessions responded differently to salt stress for shoot and root length. Increment of salinity level reduced seedling shoot and root length in all accessions (Table 2). At 80 mM salinity, IR-01 showed the highest shoot length (6.25 cm) followed by BD- 10588 (6.16 cm) and BD-6894 (6.07 cm) while BD-10740 had the lowest shoot length (3.56 cm). In case of root length, under 80 mM salinity, BD-6894 had the highest root length (2.03 cm) followed by BD-

10588 (1.96 cm) and IR-01 (1.51 cm) while BD-10744 had the lowest root length (0.46 cm).

Table 2. Shoot and root length of selected mungbean genotypes against different salinity levels. Different letters within a column are significant at P≤0.05.

Genotypes	Root length (cm)			Shoot length (cm)		
	Control	40 mM	80 mM	Control	40 mM	80 mM
BD-6887	13.97de	9.86de	4.81cd	4.16c	1.83d-f	1.27bc
BD-6894	17.83ab	12.43bc	6.07ab	6.17a	2.33bc	2.03a
BD-10588	15.62b-d	13.05b	6.17ab	2.76e	2.08b-d	1.96a
BD-10589	16.76a-c	15.57a	5.20bc	3.63d	3.16a	0.83de
BD-10590	14.60c-e	12.28bc	5.83ab	2.76e	2.06b-d	1.49b
BD-10733	17.37a-c	13.67b	4.16de	2.96e	2.5b	0.66ef
BD-10740	15.76b-d	7.12g	3.56e	3.40d	1.23g	1.02cd
BD-10741	13.83de	7.83fg	4.17de	2.76e	2.12b-d	1.04cd
BD-10744	17.56ab	9.26d-f	4.83cd	4.6b	1.46e-g	0.46f
IR-01	18.96a	15.22a	6.25a	2.16f	1.93cd	1.51b
IR-03	12.56e	11.13b-d	4.47cde	2.93e	2.50b	1.36b
IR-04	12.36e	8.68e-g	4.67cd	1.66g	1.43fg	0.77d-f
IR-05	13.06de	10.63c-e	4.25c-e	4.42bc	1.90c-e	1.20bc
Mean	15.40	11.29	4.95	3.41	2.04	1.20
CV (%)	5.41	4.04	3.54	3.52	4.91	3.05

The mean seedling root length was 3.41 cm in control, 2.19 cm in 40 mM NaCl and 1.09 cm was in 80 mM NaCl. In this study, with the increment of salt concentration, seedling shoot and root length gradually decreased in all accessions of mungbean, but the effect of salinity varied among genotypes. The present study is in good agreement with Shitole and Dhumal (2012) who reported that shoot and root length in mungbean seedlings reduced with increasing NaCl concentration.

Biochemical changes in leaves of munbean genotypes

Salinity stress causes morphological, physiological, biochemical and molecular changes in cell which causes functional loss of cell organelles and finally cell death (Gill and Tujeta, 2010). Salinity stress increased the $O_2^{\bullet -}$ generation rate in leaves of all the mungbean genotypes (Fig. 1). At 80 mM salinity, BD10741 produced the highest $O_2^{\bullet -}$ followed by BD10590 and IR-04 and being statistically similar to each other. Importantly, BD-10588 produced the lowest $O_2^{\bullet -}$ followed by IR-01 and BD-6894.
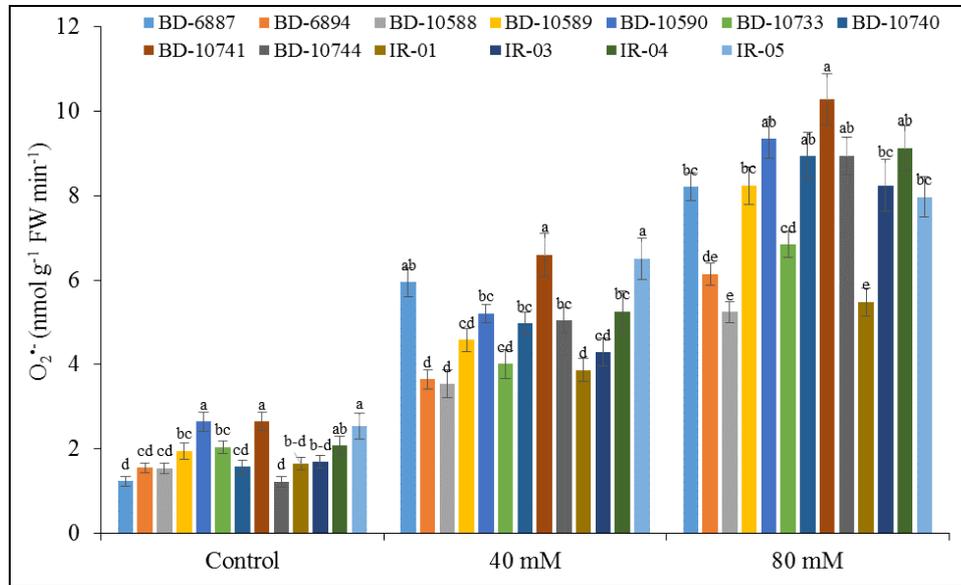
Fig.1. Comparative $O_2^{\bullet-}$ content in leaves of different among mungbean genotypes under salinity. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents Mean ± SE.

Fig. 2. Comparative H_2O_2 content in leaves of different among mungbean genotypes under salinity. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents Mean ± SE.
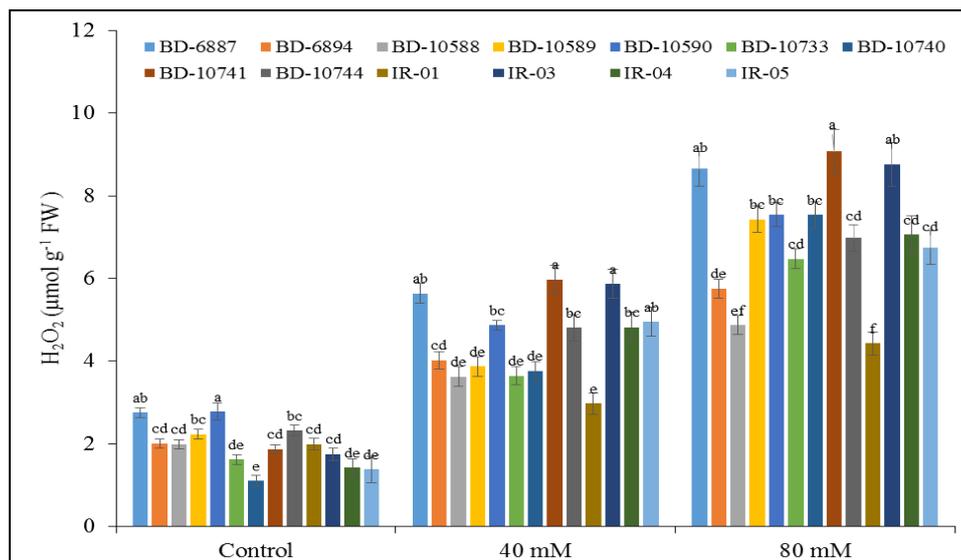
Like $O_2^{\bullet-}$ generation, H_2O_2 was also enormously increased with increasing NaCl concentration (Fig. 2). Here, also significant variation was observed among

different genotypes. At 80 mM salinity, BD10741 produced the highest H_2O_2 followed by BD-6887 and IR-03. Importantly, IR-01 produced the lowest H_2O_2 followed by BD-10588 and BD-6894.

Malondialdehyde is a lipid peroxidation product of PUFA of cell wall. In this study, MDA content increased significantly with increasing salinity, and the content varied among different genotypes (Fig. 3). Higher content of MDA in BD-10741, BD-6887 and IR-03 at 80 mM salinity indicated their susceptibility to salinity. On the other hand, lower MDA in BD-10588, BD-6894 and IR-01 suggested their better cell membrane integrity.

Methyl glyoxal (MG) level significantly increased in leaves of all mungbean genotypes under salinity stress (Fig. 4). At 80 mM salinity, IR-03 produced the highest MG followed by BD-10741, IR-05 and BD-6887. Importantly, BD-10588, BD-6894, BD-10589 and IR01 produced lower MG.
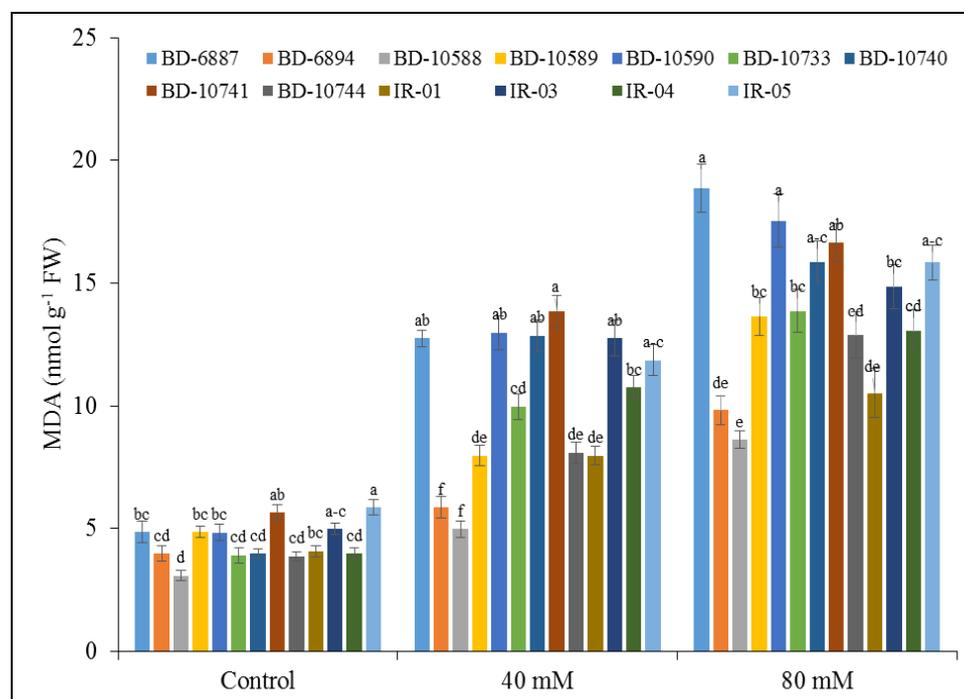

Fig. 3. Comparative MDA content in leaves of different among mungbean genotypes under salinity. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents Mean ± SE.
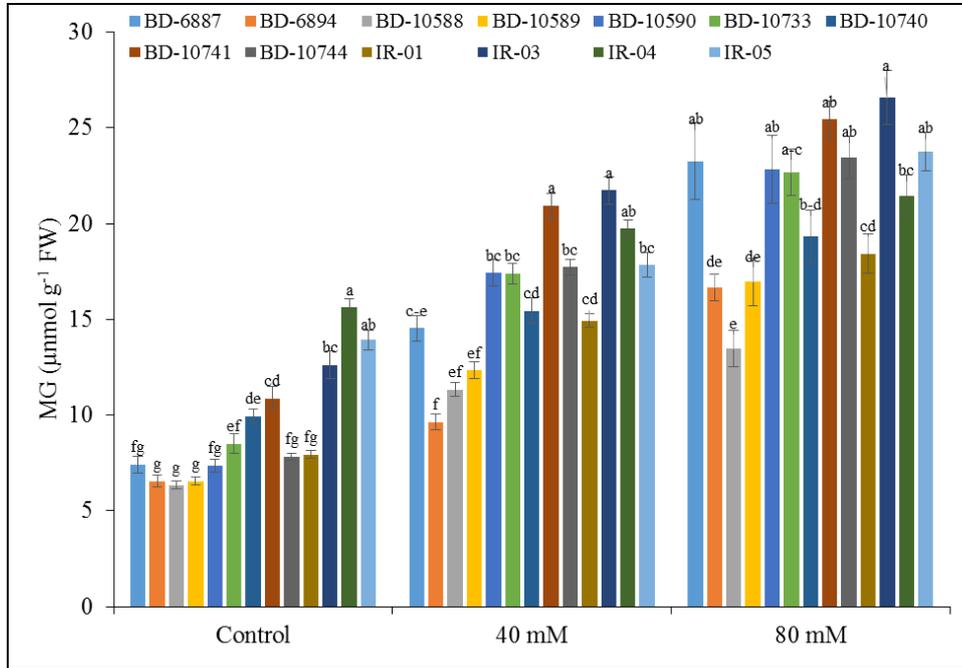
Fig. 4. Comparative MG content in leaves of different among mungbean genotypes under salinity. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents Mean ± SE.
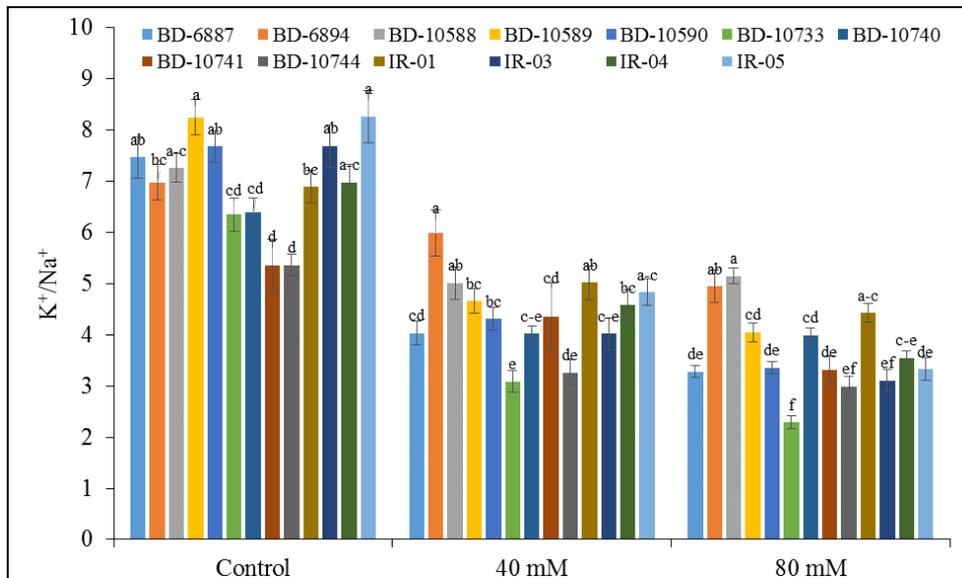

Fig. 5. Comparative K^+/Na^+ in leaves of different among mungbean genotypes under salinity. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents Mean ± SE.

Comparative K⁺/Na⁺ in leaves and roots of mungbean genotypes

Salinity stress increases Na^+ uptake which severely reduces K^+ content in both leaves and roots of plants. However, reduction of K^+ uptake varies among genotypes. In this experiment, generally the genotypes which had lower ROS and MG showed higher K^+/Na^+ than the other genotypes (Fig. 5) depicting their higher tolerance to salinity.
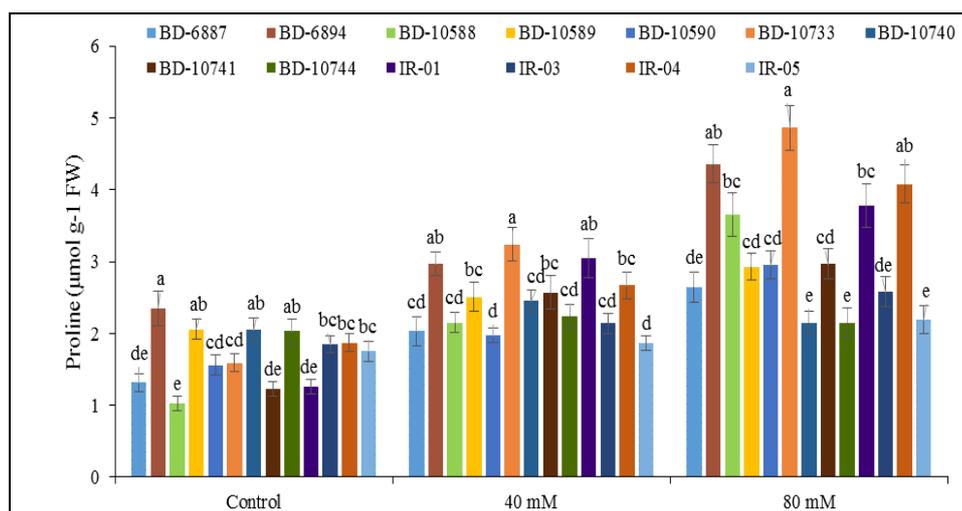

Fig. 6. Comparative proline content in leaves of different among mungbean genotypes under salinity. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents Mean ± SE.

Comparative proline content in leaves of mungbean genotypes

Proline, a free amino acid with adaptive role, accumulated differently in leaves of mungbean genotypes (Fig. 6). Among the genotypes, BD-10733 showed the highest concentration (4.86 µmol g⁻¹ FW) followed by BD-6894 (4.36 µmol g⁻¹ FW) while BD-10740 and BD-10744 had the least concentration (2.15 µmol g⁻¹ FW) at 80 mM salinity. The genotypes BD-10588 and IR-01 which had lower ROS, MDA and MG showed moderate concentration of proline (3.65 and 3.78 4.86 µmol g⁻¹ FW, respectively). Contrarily, BD-6887 and BD-10741 showing higher ROS, MDA and MG contained comparatively lower proline content.

Selection of genotypes based on physiological and biochemical parameters for studying ROS regulation mechanism

Based on germination, root-shoot length, ROS, MDA, MG, K^+/Na^+ and proline content, BD-10588, BD-6894 and IR-01 seemed to be capable to tolerance salinity. Contrary, BD-6887 and BD-10741 were not germinated under 120 mM salinity. To study the ROS regulation mechanism, genotypes BD-10588 and IR-01 were selected as relative tolerant genotypes and BD-6887 and BD-10741 as

susceptible genotypes. It should be mentioned that phenotypically differently saline sensitive genotypes are required for such type of research.
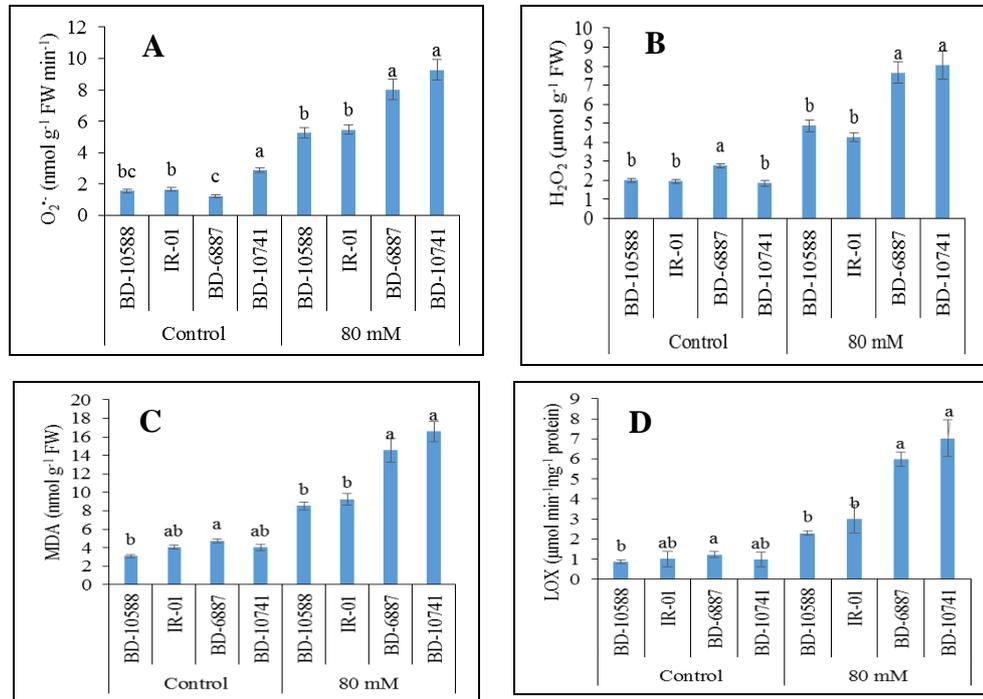

Fig. 7. Comparative $O_2^{\cdot-}$ (A), H_2O_2 (B), MDA (C) contents and LOX activity (D) in leaves of selected mungbean genotypes. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents mean ± SE.

Comparative ROS, MDA content and LOX activity in leaves of selected mungbean genotypes

The content of ROS, MDA and LOX activity in tolerance and susceptible genotypes were found to be increased at 80 mM salinity as compared to the control treatment (Fig. 7). Importantly, $O_2^{\cdot-}$ generation rate, concentrations of H_2O_2, MDA as well as LOX activity in tolerant genotypes were significantly lower than those in susceptible genotypes under saline condition.

Regulation of ROS metabolizing antioxidant enzymes in leaves of selected mungbean genotypes

Superoxide dismutase (SOD) activity:

Activity of SOD varied significantly among tolerant and susceptible genotypes under stress although in control condition significant variation was not observed (Fig. 8). As compare to control treatment, SOD activity at 80 mM was increased

by 36% and 29% in BD-10588 and IR-01, respectively. On the other hand, the activity was increased by 27% and 16% in Bd-6887 and BD-10741, respectively (Fig. 8).

Fig. 8. Activity of SOD in leaves of selected mungbean genotypes. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents mean ± SE.
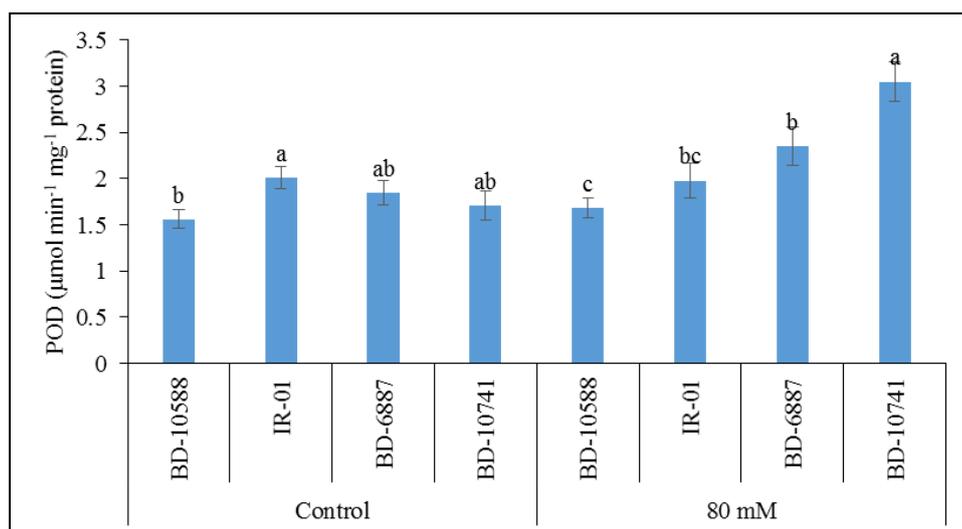
Fig. 9. Activity of POD in leaves of selected mungbean genotypes. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents mean ± SE.

Peroxidase (POD) activity:

The peroxidase (guaiacol) activity varied significantly among the genotypes under both control and salinity (Fig. 9). The POD activity in tolerant genotypes

remained almost similar at 80 mM salinity as compare to control. On the other hand, the activity increased in susceptible genotypes under salinity by 27% and 78% in BD-6887 and BD-10741, respectively, as compare to control treatment.

Catalase (CAT) activity:

The catalase activity among the genotypes were almost similar in control condition (Fig. 10). At 80 mM salinity, significant variation was not observed in tolerant genotypes. However, the activity in susceptible genotypes decreased by 35% and 34% in BD-6887 and BD-10741 as compared to respective control. Importantly, the activity was significantly lower than the tolerant genotypes.
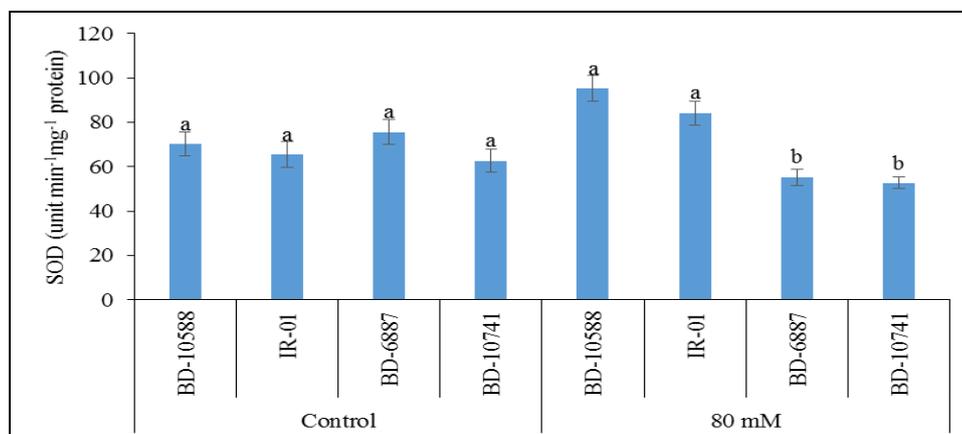

Fig. 10. Activity of CAT in leaves of selected mungbean genotypes. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents mean ± SE.

Ascorbate peroxidase (APX) activity:

The APX activity varied among different genotypes under both control and stress condition (Fig. 11A). Remarkably, tolerant genotypes, BD-10588 and IR-01 maintained higher activity under salinity as compare to susceptible ones. The in-gel activity also showed the similar result (Fig. 11B).

Glutathione peroxidase (GPX) activity:

As compared to control, GPX activity increased under salinity stress in all the genotypes (Fig. 12A). As compare to respective control, 86%, 21%, 55% 80% higher activity under salinity were observed in BD-10588, IR-01, BD-6887 and BD-10588, respectively. In in-gel analysis of GPX activity, two isozymes were found (Fig. 12B). Here increased activity was also observed in these genotypes.

Fig. 11. Specific activity (A) and in-gel activity (B) of APX in leaves of selected mungbean genotypes. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents mean ± SE.
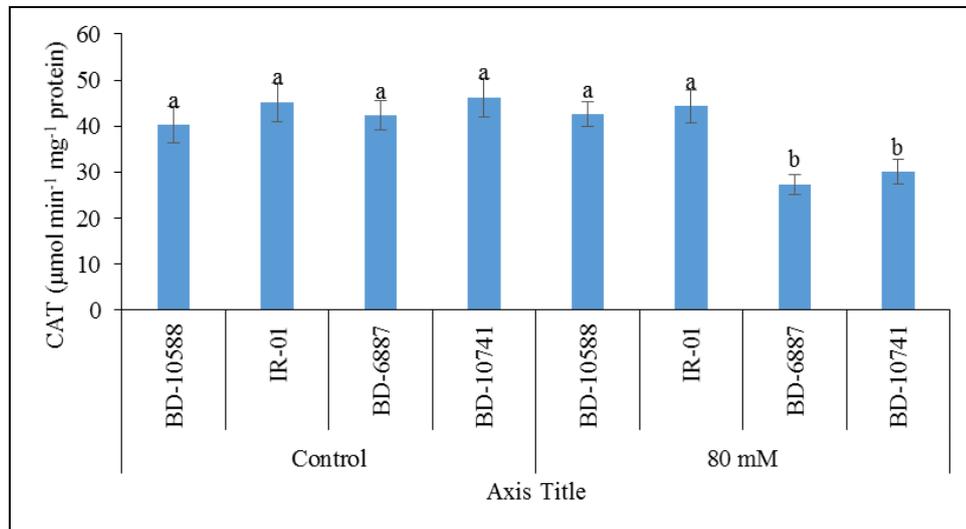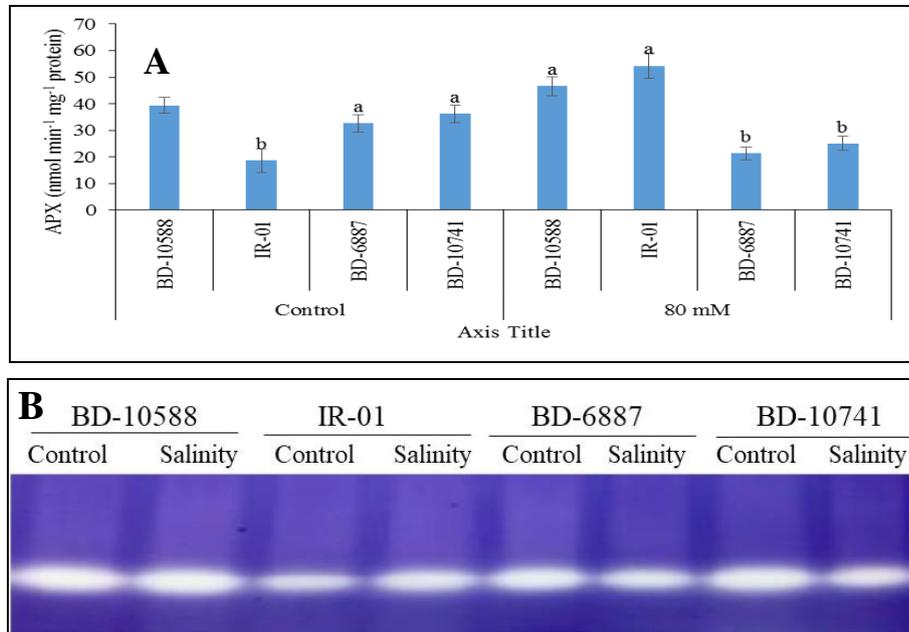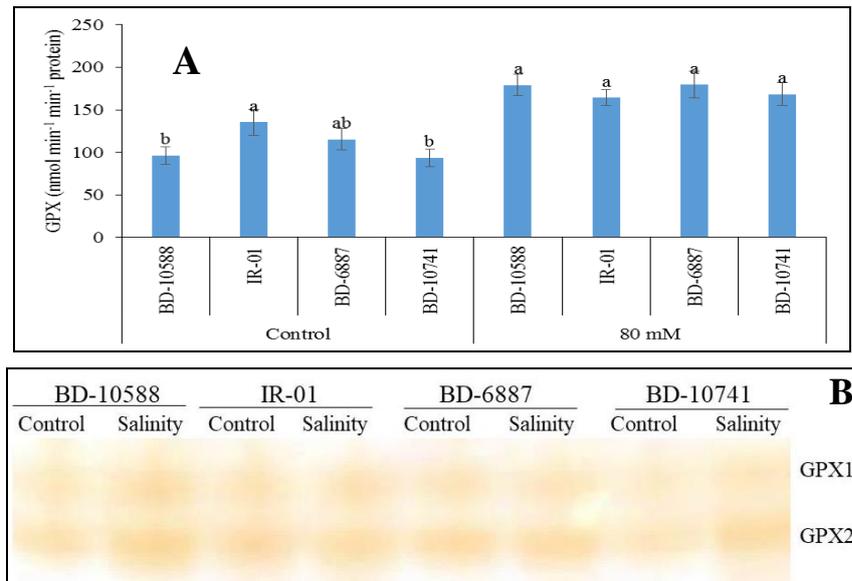

Fig. 12. Specific activity (A) and in-gel activity (B) of GPX in leaves of selected mungbean genotypes. Different letters above the bars within a treatment are significant at P≤0.05. Vertical bar represents mean ± SE.

Discussion

Selection of salt tolerant genotypes by growth and biochemical parameters

As salinity level increased, germination percentage, root and shoot length decreased (Table 1 and 2). Genotypes with higher germination percentage along with higher root and shoot length suggested their saline tolerance ability. At higher salinity (120 mM), only BD-6894, BD-10588, BD-10733 and IR-01 showed few germinations. Hence, for growth and biochemical parameters, 0, 40 and 80 mM concentrations were considered. The length of root and shoot also decreased with increasing salinity level (Table 2). Genotypes like BD-10588, BD-6894 and IR-01 had higher shoot length at 80 mM concentration. On the other hand, roots of BD-10744, IR-04 and BD-10733 were reduced remarkably at 80 mM salinity. Naseer *et al*. (2001) reported that the reduction in shoot length occured due to excessive accumulation of salt in the cell wall, which modifies the metabolic activities and limits the cell wall elasticity. The possible reason for the reduced shoot development could also be due to the toxic effects of the NaCl as well as the unbalanced nutrient uptake by the seedlings. These results can further be explained by biochemical parameters like ROS, MDA, MG, proline contents and LOX activity. Under abiotic stress like, salinity causes over production of ROS which interact with cellular organelles like protein, DNA, lipids and pigments to oxidation and functional loss, finally to cause cell death (Rohman *et al*., 2019). In this study, Genotypes like BD-10588, BD-6894 and IR-01 produced lower ROS like $O_2^{\cdot-}$ and H_2O_2 (Fig. 1 and 2). On the other hand, other genotypes exhibited higher $O_2^{\cdot-}$ and H_2O_2. It can be critical factor to cell wall damage, because these genotypes had higher MDA with higher LOX activity (Fig. 7A, B). It is established that in presence of LOX, higher ROS oxidize the PUFA of cell wall to MDA causing electroleakage (Doderer *et al*., 1992; Gill and Tujeta, 2010). Therefore, higher shoot and root growth of BD-10588, BD-6894 and IR-01 were associated with lower ROS, MDA and LOX activity. Moreover, lower MG content can least damage of root and shoot (Saxena *et al*., 2011). Higher MG with poor growth in mungbean also reported under drought stress (Nahar *et al*., 2015). The lower ration of K^+/Na^+ in these three genotypes also suggested lower Na^+ mediated injury along with higher K^+. Since the remaining genotypes had comparatively higher ROS, MDA, MG and K^+/Na^+, cell death and ionic toxicity would be higher. Thus, these genotypes were sensitive to salinity stress. Among the sensitive genotypes, BD-6887 and BD-10741 were most sensitive due to containing higher ROS, MDA, MG as well as K^+/Na^+ contents with very poor germination and shoot and root length. Previously, susceptible genotypes with higher ROS, MDA, MG as well as K^+/Na^+ were reported in maize (Rohman *et al*., 2016a, b; 2018), and rice (Hasanuzzaman *et al*., 2014). Generally, plants accumulate compatible solutes like proline under abiotic stress including salinity stress. It maintains cellular water status and membrane stability, inhibits protein oxidation and scavenges free radicals through antioxidative action under osmotic stress (Ashraf and Foolad, 2007). Therefore,

genotypes with higher proline content are considered as tolerant to saline and other abiotic stresses (Gilll and Tujeta, 2010). In this study, considerable proline accumulation under salinity increased in BD-10588 and IR-01, although BD-10733 and BD-6894 showed the highest accumulation (Fig. 6). Conversely, BD-6887 and BD-10741 with higher ROS, MDA, LOX and MG showed comparatively lower proline accumulation under salinity. Therefore, salt tolerance could be partly associated with proline content in mungbean. Similar results were also found in maize (Moussa and Abdel-Aziz, 2008; Rohman *et al*., 2016a).

Comparative oxidative stress tolerance mechanism in mungbean genotypes

Based on germination percentage, length of shoot and root, contents of ROS, MDA, MG and K^+/Na^+, BD-10588 and IR-01 were considered as most tolerant and BD-6887 and Bd-10741 as most susceptible genotypes to study oxidative stress tolerance mechanism. ROS generation is a common phenomenon in crop under abiotic stress including salinity (Bartoli *et al*., 1999; Noctor *et al*., 2012). In this study, leaves of BD-10588 and IR-01 had higher $O_2^{\cdot-}$ and H_2O_2 concentrations under 80 mM salinity stress suggesting more oxidative damage in cell. It might be due to limited capacity of scavenging of $O_2^{\cdot-}$ and H_2O_2. This capacity is explained by the activities of different antioxidant enzymes. SOD is considered as the first line defense against ROS, being responsible for the dismutation of $O_2^{\cdot-}$ to H_2O_2 (Apel and Hirt, 2004; Gill and Tujeta, 2010). On the other hand, enzymes like CAT, POD, GPX and APX catalyze the conversion of H_2O_2 to water and O_2 (Gratao *et al*., 2005). The balance between ROS production and activities of antioxidative enzymes determines whether oxidative signaling and/or damage will occur (Moller *et al*., 2007). Tolerance of a plant to salinity depends on the capability of scavenging ROS and reducing their damaging effects as well as Na^+ and Cl^- uptake (Farooq *et al*., 2015). Tolerance level also depends upon intensity and duration of stress as well as plant species and its developmental stage (Chaves *et al*., 2003; Jung, 2004). Like ROS, MDA is regarded as a marker for evaluation of lipid peroxidation or damage to plasmalemma and organelle membranes that increases with increasing ROS accumulation under environmental stresses (Garg and Manchanda, 2009). In this study, $O_2^{\cdot-}$ generation rate and H_2O_2 and MDA contents were significantly lower in tolerant genotypes (Fig. 7A, B, C). Previously, Rohman *et al*. (2019) also reported comparatively higher ROS and MDA levels in a number of saline susceptible maize. However, such types of results were not reported in mungbean previously.

LOX enzyme catalyzes the peroxidation of polyunsaturated fatty acids to their corresponding hydroperoxides (Doderer *et al*., 1992). The increased LOX activity was assumed as a reason for increased lipid peroxidation of polyunsaturated fatty acids as reported in many plants (Demiral and Türkan, 2004; Azooz *et al*., 2009; Sánchez-Rodríguez *et al*., 2012; Rohman *et al*., 2016a,

b). However, LOX activity was significantly higher in susceptible genotypes under saline stress. LOX activity was also correlated with increased MDA content in susceptible genotypes (Fig. 7C, D). Previously, Hossain *et al*. (2010) and Nahar *et al*. (2015) reported higher MDA with higher LOX activity. In this report, we found that susceptible genotypes were associated with higher LOX and MDA.

The SOD activity was higher in both tolerant genotypes, BD-10588 and IR-01 while susceptible genotypes BD6887 and Bd-10741 had comparatively lower activity under salinity stress (Fig. 8). Previously, we reported higher SOD activity in tolerant maize genotype (Rohman *et al*., 2016a). Higher SOD activity under salinity in tolerant genotypes may convert cytotoxic $O_2^{\cdot-}$ to H_2O_2, and thus, lower $O_2^{\cdot-}$ mediated cytotoxicity. Previously, Nahar et al. (2015) reported increased SOD activity in mungbean under salinity stress. In that study, they did not use any susceptible genotypes. Therefore, in susceptible mungbean genotypes, SOD activity can decrease.

POD catalyze the decomposition of H_2O_2 to water, thus alleviating the damaging effects (Jin *et al*., 2011). In this study, POD activity increased only in susceptible genotypes (Fig. 9). Therefore, increment of POD activity can be important strategy of salt sensitive mungbean genotypes. Besides, CAT, GPX and APX are associated with oxidative tolerance by scavenging of H_2O_2 (Gill and Tujeta, 2010; Miller *et al*., 2010). However, CAT, as compared to APX, GPX and POD, shows low affinity to H_2O_2 with a high processing rate (Scandalios, 2005). This is essential because, unlike other H_2O_2 scavenging enzymes (APX, GPX and POD), CAT is not substrate dependent with low affinity to of peroxide (Scandalios, 2005). In this study, CAT activity remained almost similar in saline condition, but it was reduced in susceptible genotypes. (Fig. 10). Therefore, higher CAT activity can reduce H_2O_2 mediated oxidative damage in tolerant genotypes.

APX, the most important antioxidant enzyme in plants which reduces H_2O_2 mediated oxidative damage by using ascorbic acid as electron donor (Gill and Tujeta, 2010; Rohman *et al*., 2019). The spectrometric and in-gel activity results strongly proved that APX activity increased in tolerant mungbean genotypes (Fig. 11A, B). The decreased activity in susceptible genotypes may spell them cellular damage. Comparatively lower APX activity was also reported in susceptible genotypes of maize and rice (Hasanuzzaman *et al*., 2014; Rohaman *et al*., 2016a).

In glutathione (GSH) dependent metabolism, H_2O_2 is removed by GPX where GSH is converted to oxidized glutathione (GSSG). In this study, the increased GPX activities in both types of mungbean genotypes suggested a role of this enzyme in H_2O_2 metabolism under salinity (Fig. 12A). This result is supported by Nahar *et al*. (2015). Additionally, the GPX isozymes provided strong proof of increased GPX activity in mungbean genotypes under saline stress (Fig. 12B).

Conclusion

Considering growth, biochemical and molecular parameters, genotypes, BD-10588, BD-6894 and IR-01 were selected as tolerant genotypes. Higher SOD, CAT and APX activities played major role in $O_2{}^{\cdot -}$ and H_2O_2 metabolism as well as lower MDA production in tolerant genotypes. On the other hand, GPX was equally important for both tolerant and susceptible genotypes while POD played better role in susceptible genotypes. Through in-gel activity analysis, we provide strong evidence of salt mediated oxidative stress mitigation through in-gel activity of enzymes. As GPX had equal role in both tolerant and susceptible genotypes, both GPX1 and GPX2 can further be investigated through biotechnological and molecular approaches to obtain detailed roles in mungbean.

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POPULATION FLUCTUATION OF JASSID, AND SHOOT AND FRUIT BORER OF OKRA

A. Mohammad[1], S. N. Alam[2], M. R. U. Miah[3]
M. R. Amin[4] and R. S. Smriti[5]

Abstract

A field study was conducted at the research field of Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during April to July, 2016 to measure the population fluctuation of jassid (*Amrasca biguttula biguttula*), and shoot and fruit borer of okra *(Earias vittella)*. Results revealed that jassid was always found in okra leaf during the whole study period. The highest jassid population was found during June and population reduced at the last week of July. Jassid population had significant positive relationship with temperature and significant negative relationship with relative humidity and rainfall. The weather parameters contributed 63.1% abundance of jassid population. But temperature had the most important effect which contributed 31.4% population fluctuation of jassid. Okra shoot and fruit borer population was the lowest during May and the highest at the end of June. There were non-significant relationship between okra shoot and fruit borer population and weather parameters. Multiple regression equation exerted 15.2% effect of weather parameters on okra shoot and fruit borer population abundance.

Keywords: *Amrasca biguttula biguttula*, *Earias vitella*, Temperature, Relative humidity, Rainfall.

Introduction

Okra (*Abelmoschus esculentus* L.) is a favourite vegetable in Bangladesh and is widely grown in tropical and subtropical regions (Obeng and Sackey, 2003; Oyelade *et al.*, 2003). In Bangladesh, okra is produced mainly in the kharif season but it can be grown throughout the year. In the year 2014-2015, the total production of okra was 51.8 thousand tons from 11.4 thousand hectares of land with an average yield of 4.56 tha^{-1} (BBS, 2015). The yield is very low compared to other countries.

Okra production in Bangladesh is affected by many factors, among them insect pest attack is major one. Nineteen insect species and four mite species have been reported on okra (Anon., 2000). These insect pest complex damages the crop during their different growth stages, right from germination to harvest and

[1]Scientific officer, Entomology section, Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur, [2]Chief scientific officer, Entomology Division, BARI, Gazipur, [3&4]Department of Entomology, Bangabandhu Sheikh MujiburRahman Agricultural University (BSMRAU), Gazipur, [5]Department of Soil Science, BSMRAU, Gazipur, Bangladesh.

resulting lower yields (Gulati, 2004). The yield losses due to insect pests have been reported up to 69% (Mani *et al.*, 2005).Among the insect pests, jassid (*Amrasca biguttula biguttula* Ishida) and okra shoot and fruit borer (OSFB) (*Earias vittella*) are the most serious pests and major limiting factors in okracultivation (Rahman, 1983). Jassid infestation begins at very early stages of crop growth and continues up to harvest depending upon agro-climatic conditions (Faleiro and Rai, 1985). The nymphs and adults suck the sap from under side of leaves and cause phytotoxic symptomsknown as hopper burn which results in complete desiccation of plants. The insect has been reported in okra throughout the year (Senapati and Khan, 1978). Okra shoot and fruit borer larvae cause damage both in vegetative and reproductive phase of the crop. When the crop is young, larvae bore into the tender shoots and feed on the internal tissue. In reproductive stage, larvae bore into the flower buds and fruits. For this reason, infested flower buds drops off and fruits become deformed resulting low market value (Dahiya *et al.*, 2008).

Knowledge on the population fluctuation of jassid and shoot and fruit borer throughout the growing season of okra is very much important prior to develop their sound IPM program. Therefore, this study was undertaken to measure the population dynamics of jassid, and shoot and fruit borer of okra in relation to meteorological factors.

Materials and Methods

The field study was laid out in a Randomized Complete Block Design (RCBD) with three replications in the field of the Entomology Division, Bangladesh Agricultural Research Institute. The unit plot size was 9 m × 4 m. The inter block distance was 2 m and inter plot distance was 1m. There were three blocks andeach block was composed of three experimental unit plots along with an untreated control plot. Five pheromone traps of okra shoot and fruit borer were set in the experimental at as equal distance (108 m^2).

Number of jassid was counted and recorded every 3 days intervals during early morning from 5 leaves of randomly selected 5 plans plot^{-1}. Mean jassid numberleaf^{-1}was calculated by using the collected data from all plots. Population of adult okra shoot and fruit borer were counted from pheromone trap catches and recorded at 3 days interval. The mean number okra shoot and fruit borertrap^{-1} was determined from total trap catches. The weather data of temperature, relative humidity and rainfall during the experimental period were collected from the meteorological observatory section of Bangladesh Rice Research Institute (BRRI), Gazipur.

The relationship of weather parameters on the population abundance of jassid and OSFB were determined by simple regression. The combined effects of temperature, relative humidity and rainfall on the population abundance of

insects were measured by using a Multiple Linear Regression Equation. All the analyses were performed using IBM SPSS statistics 21.

Results and Discussion

Population fluctuation of jassidis shown in Figure 1. Jassid population was slightly fluctuated throughout the season and took the highest peak (4.98 leaf^{-1}) during first week of June (7th week after sowing).The second highest (4.96 leaf^{-1}) population was observed at 1st July and after that population declined. Jassid population was the lowest (2.97 leaf^{-1}) during the last week of July (14th week after sowing) when crop was at late harvesting stage and rainfall increased. Continuous rainfall hindered the population build-up of jassid as reported by Lal *et al.,* 1990. Inee *et al.* (2000) observed that meteorological parameters played an important role on the population build-up of jassid and they found that maximum jassid population reached to the peak at the last week of May.

Present study showed significant positive relationship of jassid population with temperature (y =0.230x – 2.437, R^2= 0.314, F$_{1,20}$= 9.1, P<0.01) but significant negative correlation with relative humidity (y =-0.042x + 7.652, R^2 =0.42, F$_{1,20}$=14.47, P<0.01)and rainfall (y = -0.028x + 4.550, R^2 =0.40, F$_{1,20}$= 13.28, P<0.01) (Fig. 2).All the regression coefficients were significant at 1% level. That means jassid population was increased with the increasing of temperature and decreased with increasing relative humidity and rainfall. Similar results were reported by Mahmood *et al.* (2002). During the study, the temperature, relative humidity and rainfall ranged from 26.7^{o}c to 31.6^{o}c, 66.5% to 95.5% and 0 mm to 38.8 mm, respectively.
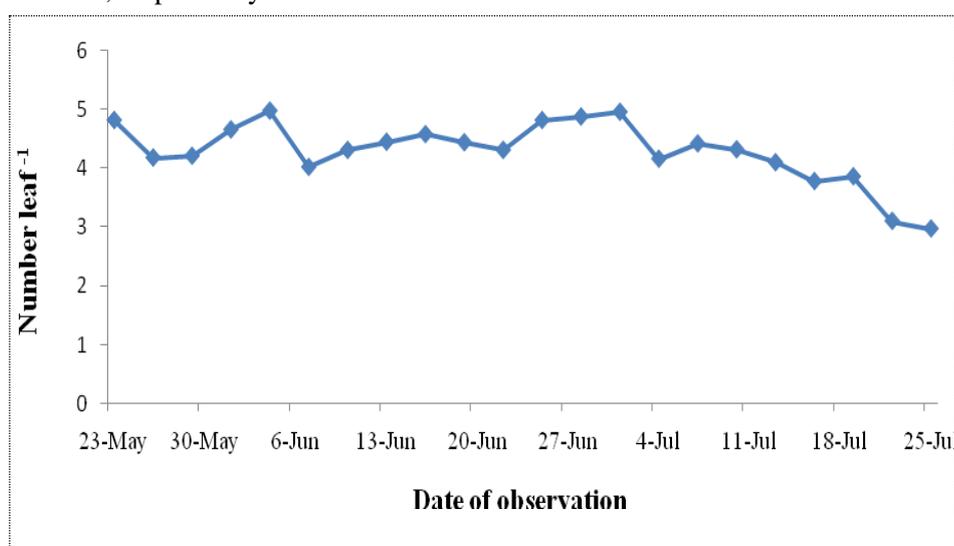
Fig.1. Population fluctuation of jassid on okra during 23 May to 25 July, 2016.
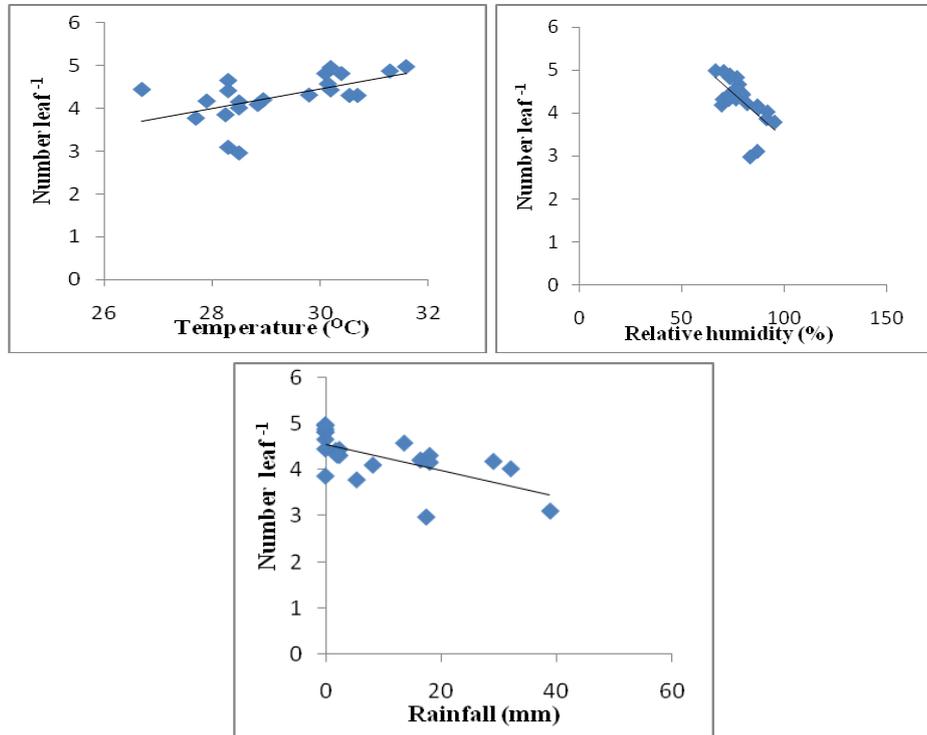
Fig. 2. Relationship of jassid population with temperature, relative humidity andrainfall.

Table 1. Multiple regression models along with coefficients of determination (R^2) and the impact of weather parameters on the seasonal abundance of Jassid during April to July 2016

Regression equation	R^2	100 R^2	Role of Individual factor (%)	F statistic	
$Y = - 2.437 + 0.230X_1$	0.314	31.4	31.4	$F_{1,20}= 9.1$	P < 0.01
$Y = 4.015 + 0.097 X_1 - 0.032X_2$	0.451	45.1	13.7	$F_{2,19}= 7.8$	P < 0.01
$Y = 4.829 + 0.060X_1 - 0.027X_2 - 0.020X_3$	0.631	63.1	18	$F_{3,18}= 10.3$	P < 0.01

Y, jassid population leaf^{-1}, X_1, temperature (^{0}C), X_2, relative humidity (%), X_3, rainfall (mm)

Table 1 showed the effect of temperature, relative humidity and Rainfallwas31.4%, 13.7%and 18% respectively, on jassid population abundance. The contribution of temperature with combination of relative humidity revealed 45.1% abundance. The multiple linear regression analysis showed that the

weather parameters together contributed 63.1% abundance of jassid population. All the equations were insignificant at 1% level.
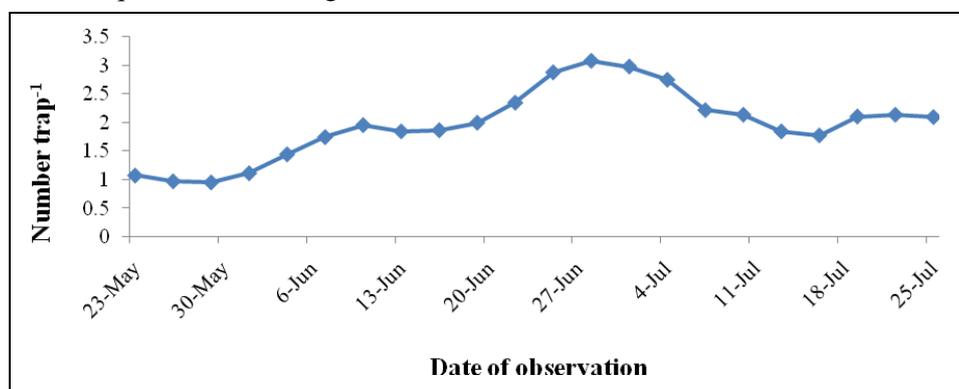
Fig. 3. Population fluctuation of okra shoot and fruit borer during 23 May to 25 July, 2016.
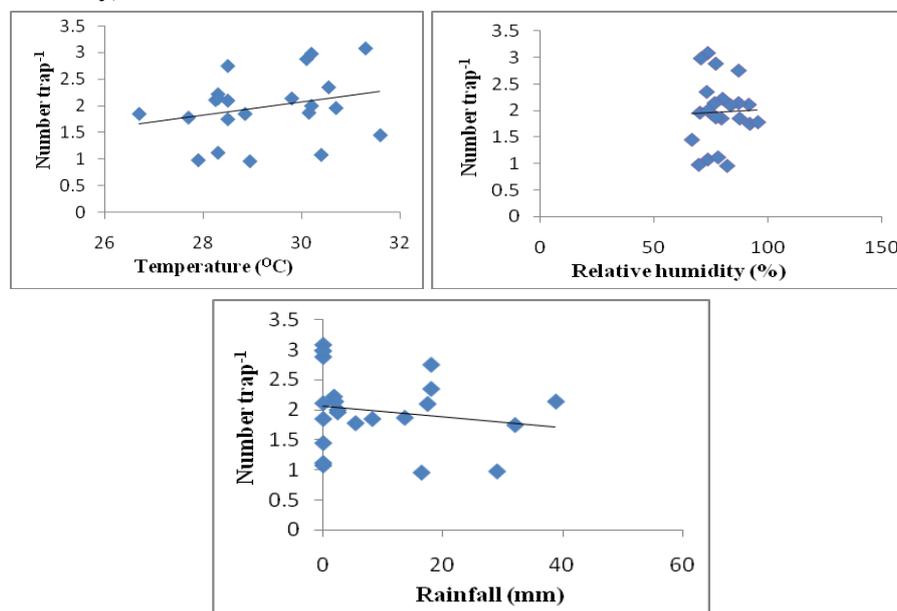
Fig. 4. Relationship ofokra shoot and fruit borer population with temperature, relative humidity and rainfall.

Population fluctuation of okra shoot and fruit borer is shown in Figure 3. Okra shoot and fruit borer population was low during early crop stage and found the lowest (0.96trap[-1]) during the last week of May (6th week after sowing). After that population was gradually increased with single fall and reached to the peak (3.08 trap[-1]) during the last week of June (10th week after sowing). After June population decreased up to third week of July and again slightly increased during

last week of the same month. Almost similarly, Dutta and Saha (1990) observed the highest population of okra shoot and fruit borer in the month of May and June.

Present study revealed that okra shoot and fruit borer population was positively correlated with mean temperature (y = 0.124x – 1.649, R^2 = 0.068, $F_{1,20}$= 1.4, P = 0.24) and relative humidity (y = 0.002x + 1.821, R^2 =0.0006, $F_{1,20}$ = 0.013, P = 0.91) but negatively correlated with rainfall (y = - 0.009x +2.053, R^2 =0.028, $F_{1,20}$ = 0.577, P =0.46)(Fig. 4).All the regression coefficients were non-significant. Dutta and Saha (1990) reported increased incidence of *E.vittella* with the increasing temperature and relative humidity.

Table 2.Multiple regression models along with coefficients of determination (R^2) and the impact of weather parameters on the seasonal abundance of OSFB during April to July 2016

Regression equation	R^2	100 R^2	Role of individual factor (%)	F statistic	
Y = - 1.649 + 0.124X_1	0.068	6.8	6.8	$F_{1,20}$= 1.4	P = 0.24
Y = - 6.898 + 0.232 X_1 + 0.026X_2	0.135	13.5	6.7	$F_{2,19}$= 1.5	P = 0.25
Y = - 6.610 + 0.219X_1 + 0.028X_2 - 0.07X_3	0.152	15.2	1.7	$F_{3,18}$= 1.1	P = 0.38

Y, OSFB population leaf^{-1}, X_1, temperature (^{0}C), X_2, relative humidity (%), X_3, rainfall (mm)

The multiple linear regression analysis showed that temperature individually contributed 6.8% population abundance of OSFB and its effect was insignificant. The temperature with combination of relative humidity contributed 13.5% abundance, which was statistically non-significant. The individual effects of relative humidity demonstrated 6.7% abundance. The individual effect of rainfall was 1.7%. The weather parameters together contributed 15.2% abundance of OSFB and equations were insignificant (Table 2).

Present study showed population fluctuation of the jassid and OSFB in summer season. The highest jassid, and okra shoot and fruit borer population was found during June. The weather parameters showed significant effect on jassid population abundance but insignificant effect on okra shoot and fruit borer population abundance.

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GENOTYPE AND ENVIRONMENT INTERACTION OF SWEETPOTATO VARIETIES

S. Sultana[1], H. C. Mohanta[2], Z. Alam[3]
S. Naznin[4] and S. Begum[5]

Abstract

The article presents results of additive main effect and multiplicative interaction (AMMI) and genotype (G) main effect and genotype by environment (GE) interaction (G × GE) biplot analysis of a multi environmental trial (MET) data of 15 sweetpotato varieties released from Bangladesh Agricultural Research Institute conducted during 2015–2018. The objective of this study was to determine the effects of genotype, environment and their interaction on tuber yield and to identify stable sweetpotato genotypes over the years. The experimental layout was a randomized complete block design with three replications at Gazipur location. Combined analysis of variance (ANOVA) indicated that the main effects due to genotypes, environments and genotype by environment interaction were highly significant. The contribution of genotypes, environments and genotype by environment interaction to the total variation in tuber yield was about 60.16, 10.72 and 12.82%, respectively. The first two principal components obtained by singular value decomposition of the centred data of yield accounted for 100% of the total variability caused by G × GE. Out of these variations, PC1 and PC2 accounted for 71.5% and 28.5% of variability, respectively. The study results identified BARI Mistialu- 5, BARI Mistialu- 14 and BARI Mistialu- 15 as the closest to the "ideal" genotype in terms of yield potential and stability. Varieties 'BARI Mistialu- 8, BARI Mistialu- 11 and BARI Mistialu- 12' were also selected as superior genotypes. BARI Mistialu- 3 and BARI Mistialu- 13 was comparatively low yielder but was stable over the environment. Among them BARI Mistialu-12, BARI Mistialu-14 and BARI Mistialu-15 are rich in nutrient content while BARI Mistialu-8 and BARI Mistialu-11 are the best with dry matter content and organoleptic taste. Environments representing in 1st and 3rd year with comparatively short vectors had a low discriminating power and environment in 2nd year was characterized by a high discriminating power.

Introduction

Sweetpotato (*Ipomoea batatas* L.) is used in a variety of ways for food, feed and processed products, with the principal uses varying by region. The literature on nutritional value of cooked and fried sweetpotatoes as well as processing

[1&4]Scientific Officer (Plant Breeding), Tuber Crops Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, [2]Principle Scientific Officer, Tuber Crops Research Centre, BARI, Gazipur-1701, [3]Scientific Officer, Tuber Crops Research Centre, BARI, Gazipur-1701, [5]Scientific Officer, Plant Breeding Division, BARI, Gazipur-1701, Bangladesh.

sweetpotato into food products such as bread, ready-to-eat breakfast, french fries, syrup, starch and beverages was comprehensively reviewed by Woolfe (1992), Bovel-Benjamin (2007) and Padmaja (2009). In developing countries, the crop is mainly grown for homestead food and feed use and to sell to local markets for fresh consumption. Use of both vines and roots for pig feeding is important in China, Vietnam and Papua New Guinea (Peters, 2004). Padmaja (2009) provides details on use of the crop for cattle, poultry and fish feed.

Sweetpotato was domesticated in tropical America about 6000 BC and reached Polynesia, Hawaii and New Zealand naturally or by early seafarers in pre-Columbian times. The Spanish introduced the crop to the Philippines in the 16th century, from whence it spread to other islands and the Asian main land. By 1594, the crop was recorded in south China, where it was promoted to mitigate drought during the Qing Dynasty (ruling from 1644 to 1912). Portuguese seafarers introduced the crop into western Mediterranean Europe, Africa, India and parts of South-east Asia (O'Brien, 1972; Yen, 1976; Jia, 2013). According to the Food and Agriculture Organization (FAO) of the United Nations, sweetpotato is currently cultivated in 117 countries in all tropical and subtropical regions of the world, with 112 million metric ton (MT) of production in 2017. Asia is the world's largest sweetpotato producing region, with about 70.5% of annual production (FAOSTAT, 2017).

Trends in area cultivated from 2000 to 2016 (Fig. 1), notably show declines in Bangladesh (from 41.28 to 24.89 thousand ha). The total production decreased accordingly from 3,83,000 to 2,59,372 MT (FAOSTAT 2017). But storage root yield trends for the same period show slight increases (Fig. 1). Thus, there is significant potential to increase national yields through the use of improved cultural practices and varieties. For developed countries, about 50% of yield progress across crops is usually attributed to breeding progress (Wricke and Weber, 1986).
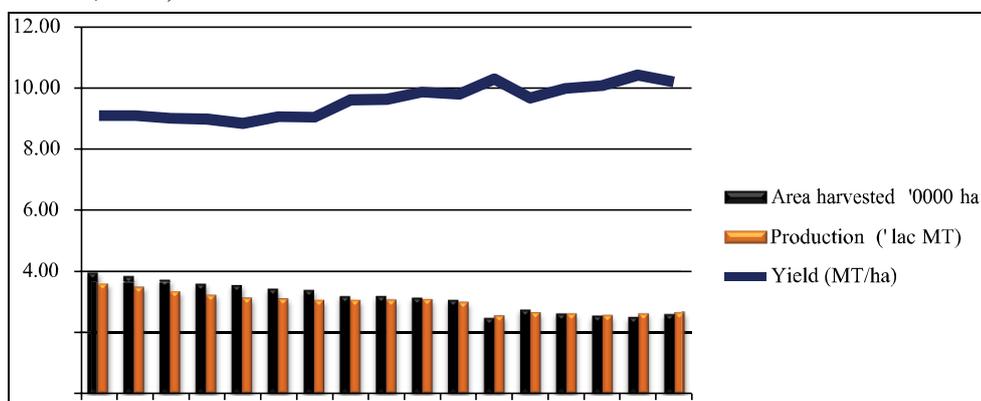

Fig. 1.Sweetpotato production in Bangladesh

In Bangladesh, in spite of having different technologies the crops like sweetpotato are being pushed to marginal areas. Major reason is farmers are not properly acknowledged with high yielding variety as well as their nutritional status. Awareness of the high nutritional value of sweetpotato is increasing consumer demand among health conscious consumers in many other countries (USDA, 2015). Orange-fleshed sweetpotato (OFSP) can be used effectively to combat vitamin A deficiency (VAD) among vulnerable populations (Low *et al*., 2007; Hotz *et al*., 2011). Thus, sweetpotato can play an important role in the context of food security in Bangladesh. Tuber Crops Research Centre (TCRC) already has developed sixteen (16) sweetpotato varieties and some other promising lines are in the process of recommendation and release. However there is no target based recommendation available for sweetpotato production. Considering this, we went for a fine tuning of our varietal data and tried to make it more target oriented.

With this view, the objective of this study was assessment of variations in yield performance of sweetpotato varieties across years based on the AMMI and GGE biplot and identification of the most valuable genotypes. The basic cause of difference in the performance of genotypes over environments is the occurrence of genotype by environment interaction (Gedif, Yigzaw, 2014). The best tool for estimating G and GE effects is multi environment trials (METs). METs are an optimal method to select better genotypes for any specific environment and to identify genotypes that consistently realize their genetic potential in a wide range of environments.

Data from METs are usually quite large, and it is difficult to deduce general patterns of such data without graphical representation. Yan et al. (2000) developed the GGE biplot technique for graphical analysis of multi environment trial data. GGE biplot analysis considers that only the G and GE effects are relevant and that they need to be considered simultaneously when evaluating genotypes. The GGE biplot has therefore been used in crop variety trials to effectively identify the best performing genotype across environments, to identify the best genotypes for mega-environment delineation, whereby specific genotypes can be recommended for specific mega-environments, and to evaluate the yield and stability of genotypes (Yan, Kang, 2003; Yan, Tinker, 2006). An additive main effect and multiplicative interaction (AMMI) model is also commonly used to analyse GE interaction during yield trials.

Materials and methods

The experiment was conducted at Tuber Crops Research Centre (TCRC) in Gazipur during 2015-18. Fifteen (15) varieties of BARI released sweetpotato were included in the study. Vines were planted on 18th, 15th and 22nd of November in three consecutive years during 2015-2017 in the field having plot

size 3.0 x 3.0 m. The crop was fertilized with 250-280 kg/ha urea, 140-170 kg/ha TSP, 230-260 kg/ha MOP, 60-80 kg/ha gypsum, 10-12 kg/ha zinc sulphate, 90-120 kg/ha magnesium sulphate, 6-8 kg/ha boric acid and 10,000 kg/ha cowdung. All cowdung, TSP, gypsum, zinc sulphate, boric acid and half urea & MP should be applied during final land preparation. Rest of the urea and MP should be applied after 35-40 DAP in furrow system. Harvesting time were 17th , 20th and 25th March of 2016, 2017 and 2018 accordingly.

Both qualitative and quantitative data were collected in the study. The qualitative data included predominant skin colour, predominant flesh colour, sweetness, fibreness and texure etc. Sweetness were evaluated by organoleptic taste, where $\leq$ 3= slightly sweet, 4-6= moderately sweet, 6$\leq$ =highly sweet. In case of fibreness, $\leq$ 3= high fibre content, 4-6= moderate fibre content, 6$\leq$ = low fibre content and texture marked as $\leq$ 3= highly moist, 4-6= moderately dry, 6$\leq$ =highly dry.

The quantitative data included root dry matter content, expressed as percentage of root dry weight (g) to fresh root weight (g). Samples (100–200 g) were taken from roots of representative plants in a plot and the roots were cut into smaller pieces and oven dried at 70 °C for 72 h. Fresh root yield, expressed as harvested fresh roots weight of 9 m^2 converted to ton per hectare. Data were processed and analyzed using Cropstat 7.2 program and PB Tools 1.4.

The β-carotene content was estimated according to the method of the Association of Official Analytical Chemists (AOAC,1980) in Post Harvest Technology Division of BARI . In to a conical flask containing 50ml of 95% ethanol,10g of the macerated sample was placed and maintained at 70-80ºC in a water bath for 20 minutes with periodic shaking. The supernatant was decanted, allowed to cool and its volume was recorded as initial volume. The ethanol concentration of the mixture was brought to 85% by adding 15ml of distilled water and it was further cooled in a container of ice water for about 5minutes. The mixture was transferred in to a separating funnel and 25ml of petroleum ether (pet-ether) was added and the cooled ethanol was poured over it. The funnel was swirled gently to obtain a homogenous mixture and it was later allowed to stand until two separate layers were obtained. The bottom layer was run off into a beaker while the top layer was collected in to a 250ml conical flask. The bottom layer was transferred in to the funnel and re-extracted with 10ml pet-ether for 5-6 times until the extract became fairly yellow. The entire pet-ether was collected in to 250ml conical flask and transferred in to separating funnel for re-extraction with 50ml of 80% ethanol. The final extract was measured and poured in to sample bottles for further analysis. The absorbance of the extracts was measured using a spectrophotometer (model 22UV/VIS) at a wavelength of 451nm. Samples of each extract were placed in cuvettes and readings were taken when the figure in

the display window became steady. The operation was repeated 5-6 times for each sample and average readings were recorded. The concentration of β-carotene was calculated using Bear-Lamberts Law, which states that the absorbance (A) is proportional to the concentration(C) of the pigment, as represented by the equation:

A ∞L   Where: C= concentration of carotene
(if concentration(C) is constant).   A= absorbance
A=ECL; C=A/EL   E=extinction coefficient
                                                        L= thickness of cuvettes (path length)
                                                        =1cm E of β-carotene =1.25x104μg/l

Table 1. Meteorological conditions during sweetpotato growing season in three consecutive year in Gazipur.

Month	2015-16		2016-17		2017-18	
	Air Temperature (°C)	Precipitation mm	Air Temperature (°C)	Precipitation mm	Air Temperature (°C)	Precipitation mm
November	23.79	0	25.38	127	26	0
December	18.83	0	16.27	0	23	3
January	18.85	10	19.59	0	18	0
February	21.31	9	23.5	0	22	2
March	25.07	53	24.79	190	26	2

Results and Discussion

Table 2 shows the phenotypic characteristics of sweetpotato varieties used in this study. All the plants are semi-erect to semi spreading type. The skin color of the tuber varied from cream color to pink color while their flesh color varied from light yellow to deep orange. The shape of tuber also varied from oval to long irregular form.

The yield capacity of the varieties varied both across environmental conditions, suggesting influence of the environmental factor (Table 3). The analysis of variance showed that the effects of genotype (G), environment (E) and their interaction (G × E) on grain yield were statistically significant (Table 4). The *ANOVA* for tuber yield revealed that genotype, environment and GE interaction effects accounted for 60.16%, 10.72% and 12.82 % of the total sum of squares, respectively. The E portion in multi environment trials (METs) is known to be the smallest among all sources of variation, which is regarded as irrelevant for genotype evaluation (Yan and Kang, 2003). This is the reason that E is removed from the phenotypic data observed, which helps to concentrate on G and GE effects, which are relevant for genotype evaluation (Yan and Kang, 2003; Fan *et al.*, 2007).

Table 2. Description of 15 varieties of sweetpotato tested

Variety	Source	Year	Skin color	Shape	Skin smooth-ness	Flesh color	BCC	DM %
				Tuber characteristics				
BARI Mistialu-1	Philippines	1985	cream	Elliptic	MS	Pale yellow	0.99	29.82
BARI Mistialu-2	Taiwan	1985	red	Elliptic	MS	Dark orange	13.59	21.67
BARI Mistialu-3	local collection	1988	white	Long Elliptic	MS	Cream	0.03	28.56
BARI Mistialu-4	Hybridiz^n	1994	Pale orange	Obovate	MS	Dark orange	7.23	27.97
BARI Mistialu-5	Hybridiz^n	1994	Red	Oblong	S	Pale orange	4.41	22.79
BARI Mistialu-6	CIP	1998	Pale orange	Oblong	MS	Pale orange	1.04	24.15
BARI Mistialu-7	CIP	1998	white	Oblong	MS	Pale yellow	0.69	28.99
BARI Mistialu-8	CIP	2008	pink	Long irregular	MS	Pale yellow	1.76	35.82
BARI Mistialu-9	CIP	2008	red	Long irregular	MS	Int .orange	4.41	20.04
BARI Mistialu-10	Hybridiz^n	2013	white	Oblong	MS	Dark cream	0.12	27.14
BARI Mistialu-11	Hybridiz^n	2013	pink	Long elliptic	MS	Cream	0.04	35.70
BARI Mistialu-12	CIP	2013	cream	Long oblong	MS	Pale orange	5.46	21.93
BARI Mistialu-13	CIP	2013	yellow	Long oblong	MS	Int. orange	6.12	29.35
BARI Mistialu-14	CIP	2018	pink	Long irregular	MS	Dark orange	14.67	25.06
BARI Mistialu-15	CIP	2018	pink	Long irregular	MS	Dark orange	12.35	21.94

BCC= β-carotene contentmg $100g^{-1}$, MS=Medium smooth, S=Smooth, DM%= Dry matter %

Table 3. Tuber yield capacity of BARI sweetpotato varieties in the multi environment trial, t ha^{-1}

Variety code	Variety	2015-16	2016-17	2017-18	Mean
1	BARI Mistialu-1	11.63	11.85	18.31	13.93
2	BARI Mistialu-2	17.86	16.00	13.62	15.82
3	BARI Mistialu-3	12.95	6.000	9.294	9.41
4	BARI Mistialu-4	15.23	14.85	11.12	13.73
5	BARI Mistialu-5	21.83	15.56	15.46	17.61
6	BARI Mistialu-6	15.61	12.44	9.821	12.62
7	BARI Mistialu-7	14.14	10.26	11.31	11.90
8	BARI Mistialu-8	26.60	21.70	15.95	21.41
9	BARI Mistialu-9	15.15	15.67	12.12	14.31
10	BARI Mistialu-10	25.21	11.59	17.50	18.10
11	BARI Mistialu-11	33.87	13.96	27.10	24.97
12	BARI Mistialu-12	38.93	29.30	31.14	33.12
13	BARI Mistialu-13	10.08	10.41	8.065	9.517
14	BARI Mistialu-14	23.76	13.59	16.34	17.89
15	BARI Mistialu-15	22.06	14.45	17.65	18.05

Table 4. Analysis of variance (*ANOVA*) for effects of genotype, environment and their interaction on sweetpotato tuber yield during 2015–2018.

Source	Degree of freedom	Sum of squares	Mean square	% SST
Genotype (G)	14	1600.20	114.300***	60.16
Environment (E)	2	285.114	142.557***	10.72
G × E	28	341.072	12.1811*	12.82
Error	116	433.3	3.7353	
Total		2659.69		

% SST – percentage relative to the sum of squares total; *** – significant at the 0.1% level of probability

* – significant at the 5% level of probability

The GGE biplot was constructed using the first two principal components (PC1 and PC2) derived from subjecting the data to singular-value decomposition. The GGE biplot graphically displays G plus GE of the MET data in a way that facilitates visual variety evaluation and mega-environment identification. Only two PC (PC1 and PC2) are retained in the model because such a model tends to be the best model for extracting patterns (Yan, 2002; Yan *et al*., 2007; Yan, 2014). Via this model, PC1 and PC2 can be readily displayed in a two dimensional biplot so that the interaction between each genotype and each environment can be visualized (Fig. 3).

The first two principal components obtained by singular value decomposition of the centred data of tuber yield accounted for 100% of the total variability caused by G + GE. Out of these variations, PC1 and PC2 accounted for 71.5% and 28.5 % of variability, respectively (Fig 3& 4).
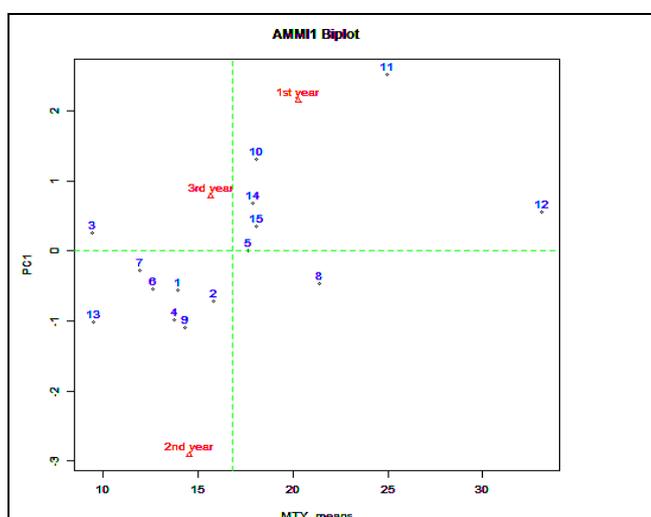
Fig. 2. AMMI biplot of the first interaction (IPCA1) score plotted against mean tuber yield (t/ha))

In AMMI biplot model, some genotype in one environment exposed higher yield than in other environment. Individual genotype performance can be measured based on their position relative to the X and Y axis. The suitable genotype are those which have high yield with stable performance over the year. Year 1 and year 2 were highly varied for tuber yield. Year 1 represented the most favorable environment and the environment of 2nd year was most unfavorable for tuber yield (Fig 2). According to Fig. 2, genotype no. 3, 5, 7 and 15 have differences for tuber yield only for main (additive) effects. They had low interaction and thus they were stable. These varieties can be recommended for all environments. While the genotype no. 1, 2, 6, 8 and 14 have main and interaction effects. The other genotypes had high interaction with the environments hence they were specific to certain environment.

The IPCA1 scores were schemed against the IPCA2 scores for supplementary assessment of adaptation for tuber yield (Fig. 3). According to Fig. 3 the genotypes 3, 5,7 and 15 were stable over years for tuber yield as located within the circle. The performances of other genotypes were unstable due to their dispersed position.

Fig. 3. AMMI Biplot 2 interaction (IPCA1 and IPCA2) of 15 sweetpotato varieties in three consecutive years for tuber yield

In contrast, the genotype main effect plus genotype-environmental interaction (GGE) biplot model provides better graphical illustration and identify the performance of cultivars under multiple stress environment, ideal cultivars and

mega environment. In other words, the GGE biplot analysis was used for estimation of discriminating power and representativeness of an 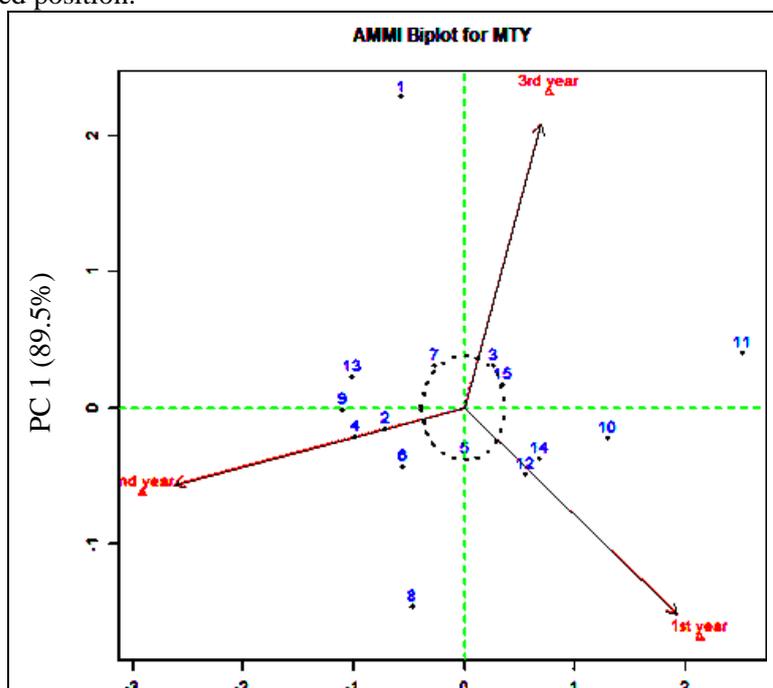environment as a test one for assessing genotypes (Fig.4). The graph "which-won-where" enables to identify potential mega- environments (Yan *et al*., 2000 and Yan and Hunt, 2001). GGE biplot allows visualizing environment vectors lengths, which are proportional to standard deviations of genotype yields in a corresponding environment. If the marker of a test environment is close to the biplot centre, i.e. has a short vector, all genotypes in it are similar, and this environment is not informative about their differentiation. Thus, environments representing in 1st and 3rd year with comparatively short vectors had a low discriminating power, and environment in 2nd year was characterized by a high discriminating power (Fig. 4).

The genotypes that are utmost from the origin are connected with a straight line forming a polygon. The lines starting from the origin divide the polygon into several sectors. The genotype at the vertex of the polygon performs the best in the environment falling within the sectors (Yan 2002). The locations within one sector are the ones where the certain genotype had the best yield and can be considered as mega- environments only for that genotype. Which-won-where biplots for tuber yield were presented in Fig. 4.

Genotype no. 8 and 12 were high tuber yielder in 2016-17 but genotype no.11 was more stable in mega environments (Fig. 4). Genotype no. 5, 14 and 15 are more stable to different environment.
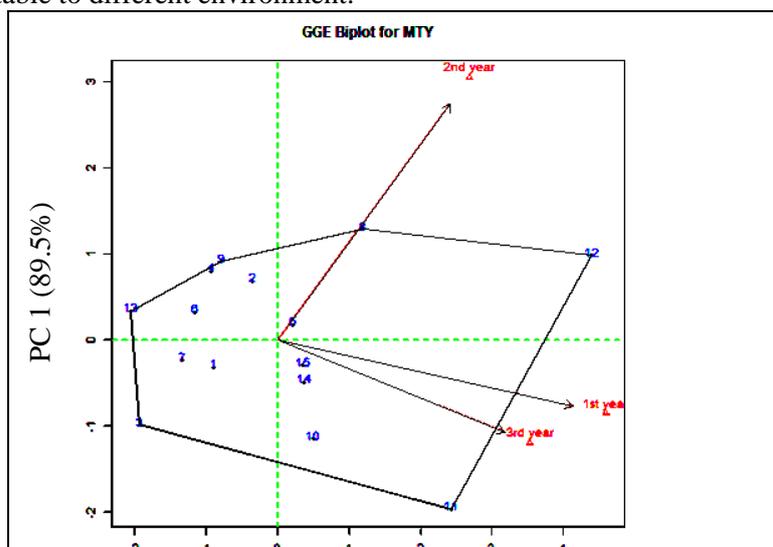

Fig. 4. GGE biplot showing "which won where" for tuber yield of 15 sweetpotato varieties in three consecutive years .

However, many breeders rank yield and quality equally, because clones that do not meet consumer quality preferences are simply not permanently adopted. With respect to the 'dessert type' in Asia and the Pacific, yield was ranked number five after: (i) eating qualities; (ii) nutritional value; (iii) appearance and uniformity; and (iv) early maturity (Lin *et al*., 1983). However, dry matter, starch content and sugars do not exclusively control taste and flavor. Hence, storage roots must be assessed by eating for quality breeding. Sensory attributes of sweetpotato hybrid clones are shown in Table 5. Statistical analysis was not done on these parameters. From mean value, BARI Mistialu-11 (17), BARI Mistialu-8 (15) gave the best mouth feel and got the superiority in terms of overall acceptability. BARI Mistialu-14 (13) also has good taste even contrasted with high beta-carotene (14.67 mg $100g^{-1}$). Yellow and orange colour in sweetpotato storage roots is determined by carotenoids. Fortunately, the proportion of β-carotene as dominant provitamin A is greater than 80% among the total carotenoid content in OFSP (Woolfe, 1992). For this reason, flesh colour alone can be used to predict beta-carotene content of storage roots. In the ways of this aspect, most of the varieties have extra bonus of nutrition.

Table 5. Mean sensory evaluation of sweetpotato varieties

Variety	Boiled Color	Fiberness (a)	Sweetness (b)	Texture (c)	Overall Acceptability Score (a+b+c)
BARI Mistialu-1	Yellow	5	4	3	12
BARI Mistialu-2	Orange	4	2	2	8
BARI Mistialu-3	Cream	4	4	5	13
BARI Mistialu-4	Pale Orange	3	4	3	10
BARI Mistialu-5	Pale Orange	4	3	3	10
BARI Mistialu-6	Light yellow	2	4	3	9
BARI Mistialu-7	Yellow	3	3	5	11
BARI Mistialu-8	Yellow	2	7	6	15
BARI Mistialu-9	Light orange	2	3	3	8
BARI Mistialu-10	Yellow	4	2	3	9
BARI Mistialu-11	Light yellow	4	7	6	17
BARI Mistialu-12	Orange	4	3	3	10
BARI Mistialu-13	Light orange	3	3	3	9
BARI Mistialu-14	Deep orange	3	5	5	13
BARI Mistialu-15	Deep orange	3	4	4	11

Fibreness : ≤ 3= High fibre content, 4-6= moderate fibre content, 6≤ = Low fibre content
Sweetness : ≤ 3= slightly sweet, 4-6= moderately sweet, 6≤ =highly sweet, Texure: ≤ 3= Highly moist, 4-6= moderately dry, 6≤ =highly dry

Conclusion

In the current study, BARI Mistialu- 8, BARI Mistialu- 11 and BARI Mistialu- 12 were selected as superior genotypes. BARI Mistialu- 5, BARI Mistialu- 14 and BARI Mistialu- 15 were found stable for marketable tuber yield. The study also revealed that sweetpotato yield was highly influenced by the differences among genotypic effects, followed by genotype by environment interaction (G × E) variable and cultivation environments. This study also clearly demonstrated that the GGE biplot model was effective for the determination of the magnitude and pattern of G × E effect and visualizing the yield potential and stability of sweetpotato genotypes as well as discriminating ability and representativeness of the test environments. Considering the seasonal variations, such stable genotypes were regarded to be climate smart and could be used as parents in a breeding programme. However, there is need to increase the number of sites to better reveal the difference among genotypes.

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EGG DEPOSITION AND WEIGHT LOSS OF SEEDS BY PULSE BEETLE, *Callosobruchus chinensis* L. ON DIFFERENT GENOTYPES OF PULSES

S. Ahmed[1], A. Haque[2], H. Mahmud[3]
and K. M. Khalequzzaman[4]

Abstract

Lentil *Lens culinaris* Medik., mungbean *Vigna radiata* L. R. Wilczek, chickpea *Cicer arietinum* L. and blackgram *Vigna mungo* L. Hepper of different genotypes were tested against pulse beetle, *Callosobruchus chinensis* L. in the laboratory of the Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh to evaluate the number of eggs deposition and percent weight loss of seeds. Maximum number of eggs (73.1) was oviposited on chickpea and minimum (19.5) was found on blackgram. The highest (19.9%) weight loss in seed was noted in chickpea and the lowest (7.6%) was recorded in blackgram. On the basis of weight loss of seed, blackgram and mungbean exhibited as tolerant genotype, lentil with moderately susceptible and chickpea as susceptible. Genotypes ML-22 of lentil, MC-21 of mungbean, Hyprosola of chickpea and MAK–1-79 of blackgram were found tolerant as compared to other tested genotypes considering the reduced number of egg deposition and lowest loss of seed weight. Evidently, the percent weight loss was marked as positive correlation with number of eggs deposition in all the genotypes and the regression line resulted in increasing the number of eggs laid with the increase in percent weight loss.

Keywords: *Cicer arietinum* L., *Callosobruchus chinensis* L., Egg deposition, Seed, Susceptible, *Vigna mungo* L. Hepper.

Introduction

Pulses are valuable crops and different varieties are grown in most parts of the world (Alemayehu and Getu, 2015). Pulses play a vital role in the diet of common people of Asian countries including Bangladesh. Pulses are excellent sources of proteins (20 - 40%), carbohydrates (50 - 60%) and are fairly good sources of thiamin, niacin, calcium and iron (Bhalla *et al*., 2008).

Bangladesh has fertile land in most of the area all over the country which provides productive cultivation of pulses as well as satisfies the demand for native people. Pulses are damaged by a number of insect pests both in field and

[1]Deputy Secretary, Ministry of Textile and Jute, Secretariat, Dhaka, [2]Professor, Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh-2202, [3]Chief Instructor, Agriculture Training Institute, Ishwardi, Pabna, [4]Principal Scientific Officer, Spices Research Centre, Bangladesh Agricultural Research Institute (BARI), Shibganj, Bogura, Bangladesh.

storage. Amongst many insect pests viz., legume pod borer *Maruca vitrata* (Geyer), gram pod borer, *Helicoverpa armigera* (Hubner), aphids *Aphis craccivora* Koch, whitefly *Bemisia tabaci* Genn., tobacco caterpillar *Spodoptera* spp., leaf hopper *Empoasca* spp. and thrips *Megaleurothrips distalis* Karny and *Caliothrips indicus* Bag. caused extensive damage to grain legumes under field conditions while bruchids *Callobruchus* spp. damaged the grain in storage (Reddy, 2009). Pulse beetle, *Callosobruchus chinensis* L. (Bruchidae: Coleoptera) is also of significant importance as a major insect pest of stored chickpea (Rajasri and Rao, 2012). Higher egg laying of *C. maculatus* on some pulses (cowpea, pigeonpea, greengram, and blackgram) might be due to their larger seed size and smooth surface in comparison to other pulses (chickpea and pea) and maximum oviposition on smooth surfaced seeds while wrinkled surface was least preferred for oviposition (Satya Vir, 1980). Seed volume also showed direct relationship with oviposition preference by *C. maculatus* on various pulses (Sharma *et al.*, 2016). Increase weevilisation was noticed with increase protein content in gram (*Cicer arietinum*) (Saxena and Saxena, 2011). Moreover, bruchids have the unusual habit of laying eggs on unsuitable surfaces under conditions of host deprivation as observed by Wang, 2004. Phenol and protein contents of seeds also resulted significant contribution in influencing the egg deposition behavior (Chakraborty and Mondal, 2016). Higher phenol content of different pulses also extended the developmental period of pulse beetle (*C. chinensis*) and showed inhibitory effect in the developmental process of pulse beetle (Patel, 2002).

One of the major constraints in production of pulses is the insect pests which inflict severe losses both in the field and storage (Bhalla *et al.*, 2008). Among these, pulse beetle, *Callosobruchus maculatus* (Fabricius) [Coleoptera: Bruchidae] is the major pest that causes serious damage and is cosmopolitan (Bhalla *et al.*, 2008). However *C. chinensis* is not only considered to be the most destructive insect but also causing severe damage to the extent of 93.33% in different pulse crops ((Parsai *et al.*, 1989). The insect spends its entire immature life in individual legumes seeds, where they cause weight loss of seed, decrease germination potential and diminish the market price as well as nutritional value. The eggs of *C. chinensis* are laid on pulse seeds, and the larvae and pupae complete their development inside the grain. Seeds damaged by bruchids were not fit for consumption (Deshpande *et al.*, 2011; Bae *et al.*, 2014) or planting and also reduced their aesthetic value (Singh, 2011; Sarwar, 2015). *C. chinensis* L. is also known to be prolific and rapid in breeding and can quickly cause a serious quantitative reduction as well as diminish nutritive value of stored grains (Alemayehu and Getu, 2015).

Bangladesh Institute of Nuclear Agriculture (BINA) has Chosen some genotypes of chickpea, lentil, mungbean and black gram in order to develop new variety. It is very important to study the resistance of these genotypes against the pulse beetle. The present study was undertaken to assess the egg deposition and the

weight loss of seeds by *Callosobruchus chinensis* L. on different genotypes of pulse grain.

Materials and Method

The study was conducted in the laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh, Bangladesh during June - September, 1999. Effect of different pulse genotypes was tested to assess the egg deposition and weight loss on pulse seeds by *C. chinensis*. The present study was carried out at room temperature from 28 to 33 °C with relative humidity of 68 to 75%.

Collection of Test Materials

Four species of pulses, lentil *Lens culinaris* Medik., munghean *Vigna radiata* L., chickpea *Cicer arietinum* L. and blackgram *Vigna mungo* L. with five genotypes of each pulse were selected for this study. The seeds of all the pulse genotypes were collected from the Department of Genetics, Bangladesh Institute of Nuclear Agriculture, Mymensingh.

Stock culture of pulse beetle

Pulse beetles were collected from the Department of Entomology, Bangladesh Agricultural University, Mymensingh. They were kept in a large glass jar (12 cm × 18 cm) with 100 g seeds of gram to oviposit. The adult pulse beetles were shifted after oviposition and seeds along with eggs were left for emerging as adult in the jar. The newly emerged adults were again allowed to oviposit on new seeds and the procedure was continued to maintain a steady stock culture.

Oviposition test

Ninety grams of seed from each genotype of pulses were separated into three equal parts (each 30 g) and kept in three Petri dishes (8.5 cm × 1.5 cm). Each dish was considered as a replicate and total number of Petri dish was 60. Four pairs of newly emerged adult pulse beetles were released in each Petri dish and were allowed to lay eggs. Randomly, five gram seeds of each replicate were used. The insects were separated and the number of eggs laid on seeds was counted with the help of magnifying glass after five days. Then the Petri dishes along with infested seeds were covered with lid and kept on the table for weight loss assessment.

Weight loss assessment

Initial weight of healthy grain was taken before exposing to pulse beetle. After completion of the counting of damaged seeds of each replication, the weight of all seeds exposed to attack by the pulse beetle were taken from each genotype for

assessing the weight loss of pulse seeds due to infestation. The percentage of weight loss was calculated using following formula:

$$\text{Percent weight loss} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Data analysis

Data were analysed by using analysis of variance and the mean values were compared by Duncan's Multiple Range Test (DMRT). Before statistical analysis the percent values were transformed by angular transformation. Correlation and regression coefficients were also estimated between number of eggs laid and percent weight loss of different genotypes of pulse grain.

Results

Effect of different pulses on egg deposition and weight loss of seed

The numbers of eggs deposited by the females of *C. chinensis* on different pulses are presented in Table 1. Significant difference was found among different pulses with number of eggs (P<0.01) deposition. Maximum number of eggs (73.1) was laid on chickpea, while the minimum (19.5) was found on blackgram. The number of eggs laid on chickpea (73.1) was more than three times compared to that of blackgram (19.5). In case of weight loss of seed, percent seed weight loss differed significantly among the pulses (P<0.01). Significantly the highest seed weight loss (19.9%) was recorded in chickpea, while the lowest (7.6%) was observed in blackgram and this was statistically identical to that of mungbean (8.9%). On the basis of seed weight loss, blackgram and mungbean were tolerant genotype, but lentil was moderately susceptible while chickpea was susceptible.

Table 1. Number of eggs laid and percent weight loss by *C. chinensis* on seeds of different pulses

Pulses	Number of eggs (Mean ± SE)	Mean weight loss (%)
Lentil	56.0 ± 1.7 b	13.4 b
Mungbean	59.5 ± 2.4 b	8.9 c
Chickpea	73.1 ± 0.9 a	19.9 a
Blackgram	19.5 ± 0.07 c	7.6 c

* Means in a column followed by the same letter are not significantly different

Effect of different genotypes of lentil on egg deposition and wt. loss of grain

The number of eggs laid on different genotypes of lentil was presented in Table 2. The number of eggs laid differed significantly among the lentil genotypes (P<0.01). The highest (96.33) number of eggs were laid on BARI Masur-4, while

the lowest on ML-22 (44.33) which was statistically similar to that of ML-432 (46.33), ML-9 (47.66) and ML-478 (45.33). The percentage of seed weight loss varied significantly among the genotypes (P<0.05). The maximum (17.2%) seed weight loss was found in BARI Masur-4 whereas the minimum in ML-22 (11.4%) was recorded and it was statistically identical to that of ML-432 (12.2%), ML-9 (12.6%) and ML- 478(13.4%). Consequently all the genotypes categorized as tolerant except BARI Masur-4.

Table 2. Number of eggs laid and percent weight loss by *C. chinensis* on seeds of different genotypes of lentil

Genotypes	Number of eggs (Mean ± SE)	Mean weight loss (%)
ML- 432	46.33 ± 2.18 b	12.2 b
BARI Masur-4	96.33 ± 8.74 a	17.2 a
ML-22	44.33 ± 1.76 b	11.4 b
ML-9	47.66 ± 2.33 b	12.6 b
ML-478	45.33 ± 1.45 b	13.4 b

* Means in a column followed by the same letter are not significantly different

Relationship between number of eggs laid and percent weight loss by *C. chinensis* on seeds of different lentil genotypes were presented in Fig. 1. The regression equation of y = 0.0955x + 8.01 and the straight line in the figure indicated that linear relationship between number of eggs laid and percent weight loss were observed to be strongly positive correlation (r = 0.9527**).
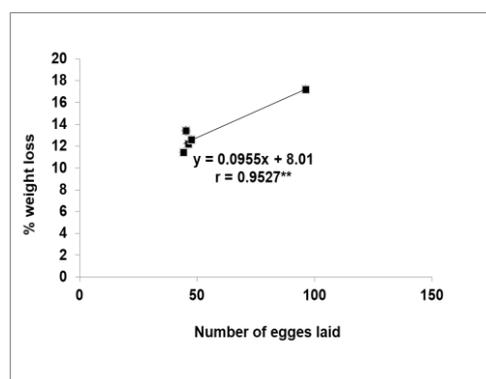
Fig. 1. Relationship between no. of eggs laid and % weight loss by *C. chinensis* on seeds of lentil genotypes
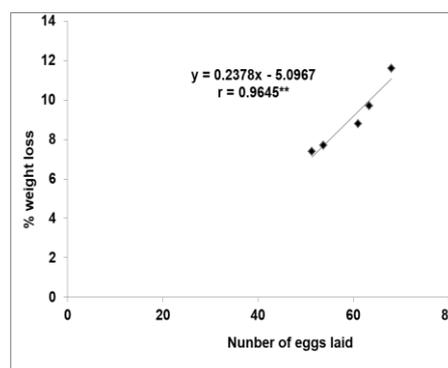
Fig. 2. Relationship between no. of eggs laid and % weight loss by *C. chinensis* on seeds of mungbean genotypes

The regression line signified that increase of number of eggs laid with increase the percent weight loss of lentil genotypes.

Effect of different mungbean genotypes on egg deposition and wt. loss of grain

Significant differences among different genotypes of mungbean (P<0.05) with number of eggs laid by the beetles were shown in Table 3. The maximum

number (68.0) of eggs was recorded on MC-26 which was statistically similar to that of Binamoog-5 (63.3) and MC-36 (61.0). The minimum number of eggs (51.3) was deposited in MC-21, which was statistically identical to Binamoog-2 (53.7). The difference among the genotypes in respect to seed weight loss was statistically significant (P<0.01). The highest loss (11.6%) in weight was found with MC-26. The lowest loss in weight (7.4%) was observed in MC-21 and this was statistically similar to that of Binamoog-2 (7.7%). On the basis of this result, MC-21 and Binamoog-5 may be categorized as tolerant, on the other hand Binamoog-2 and MC-36 were moderately susceptible and MC-26 was susceptible.

Table 3. Number of eggs laid and percent weight loss by *C. chinensis* on seeds of different genotypes of mungbean

Genotypes	Number of eggs (Mean ± SE)	Mean weight loss (%)
MC-26	68.0 ± 2.1 a	11.6 a
Binamoog -2	53.7± 1.8 bc	7.7 cd
MC - 36	61.0 ± 5.0 ab	8.8 bc
Binamoog-5	63.3 ± 2.3 a	9.7 b
MC-2I	51.3 ± 1.5 c	7.4 d

*Means in a column followed by the same letter (s) are not significantly different

Correlation and regression coefficients were estimated between number of eggs laid and percent weight loss by *C. chinensis* on seeds of mungbean genotypes presented in Fig. 2. The regression and correlation between number of eggs laid and percent weight loss were obtained with the equation of $y = 0.2378x - 5.0967$ and correlation coefficient was $r = 0.9645^{**}$. The relationship showed that the percent weight loss was strongly correlated (positive) with number of eggs laid in mungbean genotypes, while the regression line exhibited the increase of number of eggs laid, increased the percent weight loss.

Effect of different genotypes of chickpea on egg deposition and wt. loss of grain.

C. chinensis laid eggs on all tested genotypes of chickpea and their number differed significantly as shown in Table 4. Significantly the lowest number of eggs (55.7) on Hyprosola was recorded. The highest (84.3) number of eggs was laid on Barisola-3 which was statistically similar to that of P-34 (74.3), L-84 (76.7) and Binasola-5 (74.7). Significant difference among different genotypes of chickpea was found in respect of percent weight loss caused by the beetles (p < 0.05). Significantly the maximum weight loss (23.6%) was observed in Binasola-5 and categorized as susceptible, while the lowest (16.6%) was considered as tolerant in Hyprosola and as moderately susceptible with P-34 (19.9%), Barisola-3 (19.7%) and L-84 (20.0%) genotypes (Table 4).

Table 4. Number of eggs deposition and percent weight loss by C. *chinensis* on seeds of different genotypes of chickpea

Genotypes	Number of eggs (Mean ± SE)	Mean weight loss (%)
P - 34	74.3 ± 4.91 a	19.9 ab
Hyprosola	55.7 ± 2.6 b	16.6 b
Barisola - 3	84.3 ±3.28 a	19.7 ab
L - 84	76.7± 2.33 a	20.0 ab
Binasola-5	74.7 ± 5.78 a	23.6 a

*Means in a column followed by the same letter are not significantly different

Number of eggs laid and percent weight loss of *C. chinensis* on seeds of chickpea genotypes were presented in Fig. 3. The regression equation $y = 0.138x + 9.8639$ and the straight line in the Figure resulted positive correlation ($r = 0.587^*$) between number of eggs laid and percent weight loss through linear relationship. The regression line suggested that the increase in number of eggs laid was found to be increase the percent weight loss in chickpea genotypes.

Effect of different blackgram genotypes on egg deposition and wt. loss of grains

Egg depositions of *C. chinensis* on all the seeds of five blackgram genotypes were observed. Laying of eggs did not show any statistical differences among the genotypes. Maximum (22.0) number of eggs on M-25-54 was marked, while the lowest was recorded in MAK-1-79. Significantly the highest (9.0%) weight loss in MAK-1 was considered as susceptible. The lowest weight loss (6.1%) in MAK-1-79 was categorized as tolerant and this was statistically identical to that of M-25-54 (7.4%) and M–25-58 (6.4%) and was considered as moderately susceptible (Table 5).

Table 5. Number of eggs deposition and percent weight loss of grain by *C. chinensis* on different genotypes of blackgram

Genotypes	Number of eggs (Mean ± SE)	Mean weight loss (%)
Binamash-1	19.0 ± 1.2	8.9 a
MAK-1-79	17.3 ± 1.2	6.1 b
M-25-54	22.0 ± 2.1	7.4 ab
M–25-58	18.13 ± 1.2	6.4 b
MAK-1	20.7 ± 0.9	9.0 a

*Means in a column followed by the same letter are not significantly different

Correlation and regression coefficients were evaluated between number of eggs laid and percent weight loss on seeds of blackgram genotypes (Fig. 4). Number of eggs laid and percent weight loss of grain was obtained in $y = 0.3732x +$

0.3109 and correlation coefficient was noted as r = 0.5256*. The relationship showed that the percent weight loss was positive correlation with number of eggs laid on blackgram genotypes. The regression line indicated the number of eggs laid as increased the percent weight loss increased.
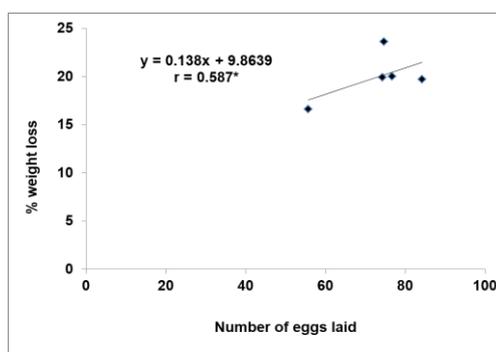
Fig. 3. Relationship between no. of eggs laid and % weight loss by *C. chinensis* on seeds of chickpea genotypes
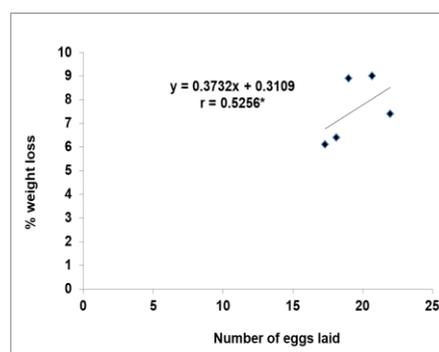
Fig. 4. Relationship between no. of eggs laid and % weight loss by *C. chinensis* on seeds of blackgram genotypes

Discussion

Maximum eggs were deposited on chickpea and minimum eggs were laid on blackgram among the tested pulses. Cope and Fox (2003) reported that variation in seed size was important during oviposition periods. The seeds of chickpea were larger in size having larger surface area which favoured larger egg deposition. Chakraborty and Mondal (2016) also reported that pulse beetle laid maximum number of eggs in larger surface area of seed, and penetration and initial development was resulted with loss of grain weight. The results were also supported with the observation of Singh (1976) who reported that *C. chinensis* preferred oviposition on chickpea followed by mungbean, lentil, blackgram and bean in order of decreasing suitability. Bhaduria and Jakhmola (2006) reported that the ovipositional preference and survival of the pulse beetles on blackgram were less preferred. This was in conformity with the observation of Teotia and Singh (1968) who reported that wrinkled, depressed or rough seed coat of the host seed was relatively less preferred for oviposition and were unsuitable for development of insects. Kamble *et al.* (2016) reported that medium size seed with thin seed coat characteristics were found to be least preferred for oviposition as compared to bold seeded varieties, while the seeds of lentil and mungbean were smaller in size than blackgram but the egg deposition was higher on lentil and mungbean seeds.

The highest (19.9%) weight loss in chickpea seed was recorded and this pulse was most susceptible, while the lowest (7.6%) was observed in blackgram. The number of larvae of *C. chinensis* feed inside the seeds responsible for higher

damage and weight loss. Bharathi *et al.* (2016) reported that *C. chinensis* was evaluated on eight different host-grains viz., greengram *Vigna radiate* L., blackgram *Vigna mungo* L., bengalgram *Cicer arietinum* L., redgram *Cajanus cajan* L., cowpea *Vigna sinensis* L., soybean *Glycine max* L., pea *Pisum sativum* L. and pillipesara *Phaseolus trilobus* L. and bengalgram exhibited significantly maximum percentage of weight loss of grains (58.55%). These findings were also in accordance with the observation of Radha and Susheela, 2014 and Osman *et al.*, 2015 who reported that the grain legumes affected by seed weight loss due to infestation. Demnati and Allache (2014) reported that chickpea seed weight loss was observed due to chickpea beetle infestation. These findings were supported by Aslam (2004). Similar findings were reported by Hossain *et al.* (2014) and found the highest seed infestation (64.34%) and (4.17%) weight loss in chickpea seeds. Besides this, chickpea was found more suitable and blackgram was less suitable for feeding by the pulse beetle larvae. Mungbean seeds were the medium sized with weight loss of 8.9%, while lentil seeds were the smallest sized with 13.4% weight loss. However, it was evident from the literature that higher seed weight and thick seed coat prolonged developmental period of beetle (Chakraborty *et al.,* 2004).

The pulse beetle laid eggs on the seed coat and the larvae fed inside the seed. The rate of oviposition varied significantly among the genotypes of respective pulse species. Minimum number of eggs was deposited on the genotype ML-22 of lentil, MC-21 of mungbean, Hyprosola of chickpea and MAK.-1-79 of blackgram. Lower moisture content of seeds, seed weight and seed coat thickness showed negative correlation with oviposition, adult emergence percentage and total developmental period, while phenol, OD phenol and protein content exhibited positive correlation with oviposition and developmental period as reported by Chakraborty and Mondal (2016).

Evidently, the percent weight loss was observed as positive correlation with number of eggs laid of all the genotypes and the regression line indicated the number of eggs laid was increased with increase in percent weight loss. Weight loss in seed was varied significantly among the pulse genotypes. Minimum seed weight loss was noted in ML-22 of lentil, MC-2I of mungbean, Hyprosola of chickpea and MAK-1-79 of blackgram among the pulses. The present findings in seed weight loss by pulse beetle were noticed that the twenty tested genotypes of four pulses were not found free from seed weight loss due to pulse beetle infestation. This variation of eggs deposition on greengram genotypes might be attributed to some physical (seed size, seed coat texture) and biochemical parameters. These results are concomitant with some previous works, where bruchid laid maximum number of eggs on susceptible genotypes than the resistant one (Shivanna *et al.*, 2011; Badii *et al.*, 2013). Sarwar (2012) also studied that resistant lentil harboured significantly lower number of eggs, inhibited adult progeny development and decreased grain weight loss which indicating resistance.

Conclusion

Pulse beetle exhibited higher susceptibility on chickpea and showed the lowest susceptibility in blackgram measured by egg deposition and percent seed weight loss. Small seed size with thin seed coat and smooth seed surface of seed characteristics were found to be least preferred for oviposition as compared to bold seeded pulses. Physical and various chemical parameters may also showed significant contributions in influencing the egg deposition and seed weight loss.

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EFFECT OF INTEGRATED NUTRIENT MANAGEMENT ON THE YIELD, YIELD ATTRIBUTES AND PROTEIN CONTENT OF LENTIL

S. Aktar[1], M. A. Quddus[2], M. A. Hossain[3]
S. Parvin[4] and M. N. Sultana[5]

Abstract

A field experiment was conducted at the Pulse Research Sub-station of Bangladesh Agricultural Research Institute (BARI), Gazipur during *rabi* season of 2015-16 and 2016-17 to evaluate the effectiveness of organic and inorganic sources of nutrients in terms of growth and yield maximization of lentil. The experiment was laid out in a randomized complete block design (RCBD) having six treatments with three replications. The treatments were T_1= Recommended dose ($N_{20}P_{15}K_{30}S_{10}Zn_3B_{1.5}$ kg ha^{-1}), T_2= IPNS (Inorganic) +2.5 t ha^{-1} cowdung, T_3= IPNS (Inorganic) +5 t ha^{-1} cowdung, T_4= IPNS (Inorganic) +1.5 t ha^{-1} poultry manure, T_5= IPNS (Inorganic)+3 t ha^{-1} poultry manure and T_6= Control. The results reveal that the integrated nutrient management had significant effects on the plant height, number of branches per plant, number of pods per plant, number of seeds per pod, 1000-seed weight, and seed yield of lentil. The maximum seed yield (1216 kg ha^{-1}) as well as protein content (26.1%) were recorded with T_4 treatment. The results advocate that satisfying the recommended dose through application of poultry manure @ 1.5 t ha^{-1} with IPNS inorganic fertlizer could be suggested for achieving yield miximization of lentil in chhiata soil series of Gazipur.

Keywords: Integrated nutrient management, yield attribute, quality, lentil yield

Introduction

Lentil (*Lens culinaris* Medik) is an important food legume with various uses as food because of its protein-rich grains. Lentil is the second most important pulse crop of Bangladesh in terms of area (2.704 lakh hectares) and production (3.555 lakh metric ton) (BBS, 2016) and it ranks the highest in terms of consumer preference as total pulse consumption (Krishi Diary, 2018). Lentil is also important in crop diversification in the cropping systems of Bangladesh. Lentil productivity is below potential due to low input usage and limited usage of modern agronomic practices. Declining soil fertility is major constraints to crop production. Organic manures facilitate improve most of the physical, biological and chemical characteristics of soil, thus improving soil fertility (Vishnoi *et al.*,

[1]Scientific Officer, Pulse Research Sub-station, Bangladesh Agricultural Research Institute (BARI), Gazipur, [2]Senior Scientific Officer, Soil and Water Management Section, HRC, BARI, Gazipur, [3]Chief Scientific Officer, Soil Science Division, BARI, Gazipur, [4]Senior Scientific Officer, TCRC, BARI, Gazipur, [5]Ex-Agricultural Development Officer, IRRI-Bangladesh.

2013; Zeidan, 2007). Nutrients play an important role for increasing the yield of lentil through their effect on the plant itself and on the nitrogen fixing by symbiotic process (Sahu *et al*., 2017). However, balanced fertilization including manures can increase the yield of lentil. Conjunctive use of manure along with chemical fertilizers reduces organic carbon depletions and the gap between potential yield and actual yield is bridged to a large extent (Tolanur and Badanur, 2003). Cowdung and poultry manure are the two common sources of organic manure. Between them, poultry manure is a rich source of nutrients as it contains 3.03 percent of nitrogen, 2.63 percent of phosphorus and 1.4 percent potassium (Vishnoi *et al.*, 2013). Hence, attention is needed to increase organic matter content in soil through balanced fertilization accompanied with organic manure. Therefore, the experiment was undertaken to evaluate the proper organic source and to determine the optimum dose of organic and inorganic sources of nutrients for achieving higher yield of lentil.

Materials and Methods

The field experiment was conducted at research field of Pulse Research sub-station, Bangladesh Agricultural Research Institute (BARI), Gazipur during *rabi* seasons of 2015-16 (1st year) and 2016-17 (2nd year). The land of Gazipur is medium high with fine-textured (clay loam) grey terrace soils. It belongs to Chhiata soil series under the agroecological zone - Madhupur Tract (AEZ-28). The experimental area received rainfall from 1.40 to 118 mm during October to March. The mean minimum and maximum air temperatures during October to March of the experiment were 21.4 & 28.6°C in the 1st year and 20.3 & 29.0°C, respectively in the 2nd year. The average minimum and maximum humidity (%) were 51 and 88 during October to March. The initial soil (0-15 cm) sample and all manure samples were analyzed as outlined by Page *et al*. (1982). Cowdung and poultry manure that used in the experiment were analysed by the standard methods. The results of chemical analysis are shown in Tables 1 and 2.

The land was prepared by a tractor operated chisel plough and then rotavator was used for breaking the clod and finaly the land was leveled by the leveler. The experiment was planned with six treatments such as T_1= Recommended dose ($N_{20}P_{15}K_{30}S_{10}Zn_3B_{1.5}$ kg ha^{-1}) as per FRG, (2012), T_2= IPNS (Inorganic) +2.5 t ha^{-1} cowdung, T_3= IPNS (Inorganic) +5 t ha^{-1} cowdung, T_4= IPNS (Inorganic) +1.5 t ha^{-1} poultry manure, T_5= IPNS (Inorganic) +3 t ha^{-1} poultry manure and T_6= Control (no addition of fertilizer or manure). The treatments of the experiment were laid out in randomized complete block design (RCBD) with three replications. The unit plot size was 12 m^2 (4 m × 3 m). Nutrients N, P, K, S, Zn and B were applied as urea, TSP, MoP, gypsum, zinc sulphate (heptahydrate) and boric acid; respectively during final land preparation.The test crop variety was BARI Masur-7. Seeds were sown in the 3rd week of November in both years at a rate of 35 kg ha^{-1}, sowing was done continuously in rows at a depth of 2-3 cm maintaining row to row spacing of 30 cm. The seeds were treated using the fungicide Provex 200 (at

Table 1. Fertility status of initial soil sample of the experimental field at Gazipur

	pH	OM (%)	Total N (%)	Ca	K	P	S	Zn	B
				meq. 100 g^{-1}			mg kg^{-1}		
	6.1	1.35	0.066	6.2	0.12	17	20	0.80	0.14
Critical level	-	-	0.12	2.0	0.12	7	10	0.60	0.20
Interpretation*	acidic	low	very low	high	low	optimum	medium	low	low

*FRG, (2012)

Table 2. Nutrient status of cowdung and poultry manure of the experiment

Type of manure	Moisture (%)	pH	OM (%)	Total N (%)	K	P	S	Zn	B
					meq. 100 g^{-1}		%		
Cowdung	12.54	6.9	20.1	1.09	0.58	0.62	0.36	0.012	0.12
Poultry manure	12.36	8.1	21.9	1.42	0.84	0.85	0.49	0.015	0.16

2.5 g kg^{-1} seeds) before sowing for controlling of root rot disease. Hand weeding as well as thining of seedlings was done at 25 days after sowing (DAS). Again, hand weeding was done at around 50 DAS.Two sprays were done with fungicide of Rovral starting from 55 DAS to control *Stemphylium* blight disease and two times insecticide (Karate @ 2 ml L^{-1} of water) was sprayed at 10 days interval starting from 60 DAS to overcome insect infestation. The crop was harvested at maturity.

Data on the seed yield (kg ha^{-1}) at around 10% moisture basis were recorded from the whole plot technique. For straw yield (kg ha^{-1}), mature plants were collected from two 1m^2 quadrates in each plot at harvest time. Harvest index (%) was determined as a ratio of economic yield to biological yield (Zerihun *et al.*, 2013). The data of yield attributes included plant height, number of branches plant^{-1} and number of pods plant^{-1} were recorded from ten plants selected randomly from each unit plot. Ten pods were detached randomly from ten plants and the number of seeds per pod was counted and averaged. Thousand seed weight (g) was determined by counting of 500 seeds randomly from each plot and weighing through electronic balance and converting it into 1000-seed weight. Five plants from each plot were chosen randomly at seedling, vegetative, flowering and pod formation stages for recording nodulation per plant. Plants were smoothly uprooted and the soil from roots was removed carefully using tap water. Nodules were separated and counted from each plant and averaged. Separated nodules were sliced into two pieces to observe the inside color for determining of nodule activity. The light-pink or red coloured nodules were considered as active.

Seed samples were digested with di-acid mixture (HNO$_3$-HClO$_4$) (5: 1) as described by Piper (1966) for determination of N concentration (Micro-Kjeldahl method). Protein contein in lentil seed was calculated by multiplying %N by a factor 5.30 (FAO, 2018).

All the data of growth, yield attributing characters, number of nodules per plant and yield of lentil were statistically analysed by ANOVA procedure. Then, multiple comparisons were done by LSD at 5% level (Statistix 10., 1985).

Results and Discussion

Growth attributes of lentil

Growth attributes like plant height and number branches per plant were influenced significantly due to imposing different integrated nutrient treatments (Table 3). In the experiment, the tallest plant (31.2 cm) was found in T$_4$ treatment which was statistically similar with T$_1$, T$_2$ and T$_3$ treatments. The most dwarf plant (27.5 cm) was observed in T$_6$ treatment (control).The plant height increased progressively due to application of cowdung and poultry manure with inorganic fertilizers. Similar observation was noted by Singh *et al.* (2011).

Table 3. Effects of integrated nutrient management on the growth and yield attributes of lentil

Treatment	Plant height (cm)	No. of branches plant^{-1}	No. of pods plant^{-1}	No. of seeds pod^{-1}	1000-seed weight(g)
T_1	30.7	2.82	52.8	1.90	20.3
T_2	29.6	2.84	55.4	1.93	21.0
T_3	28.7	2.80	51.8	1.83	20.6
T_4	31.2	2.86	55.9	1.93	22.0
T_5	28.0	2.73	53.6	1.80	20.5
T_6	27.5	2.46	50.8	1.73	19.6
LSD $_{0.05}$	2.7	0.2	3.9	0.05	0.9
CV (%)	5.01	3.29	4.03	1.51	2.28

Note: T_1= Recommended dose (FRG, 2012), T_2= IPNS (Inorganic) +2.5 t ha^{-1} cowdung, T_3= IPNS (Inorganic) +5 t ha^{-1} cowdung, T_4= IPNS (Inorganic) +1.5 t ha^{-1} poultry manure, T_5= IPNS (Inorganic) +3 t ha^{-1} poultry manure and T_6= Control.

Table 4. Effect of integrated nutrient management on the yields and harvest index of lentil

Treatment	Seed yield (kg ha^{-1})			Straw yield (kg ha^{-1})			HI (%)		
	1st yr.	2nd yr.	Mean	1st yr.	2nd yr.	Mean	1st yr.	2nd yr.	Mean
T_1	1105	949	1027	1700	1584	1642	39.4	37.3	38.4
T_2	1274	1095	1185	1886	1788	1837	40.3	37.8	39.1
T_3	1078	973	1026	1657	1600	1629	39.4	37.8	38.6
T_4	1312	1120	1216	1956	1817	1887	40.3	38.1	39.2
T_5	1065	967	1016	1703	1594	1649	38.5	37.8	38.2
T_6	820	735	778	1497	1302	1400	35.3	36.2	35.7
LSD $_{0.05}$	182	228	-	345	298	-	1.21	ns	-
CV (%)	9.06	12.9	-	10.9	10.1	-	1.71	3.63	-

Note: T_1= Recommended dose (FRG, 2012), T_2= IPNS (Inorganic) +2.5 t ha^{-1} cowdung, T_3= IPNS (Inorganic) +5 t ha^{-1} cowdung, T_4= IPNS (Inorganic) +1.5 t ha^{-1} poultry manure, T_5= IPNS (Inorganic) +3 t ha^{-1} poultry manure and T_6= Control.

The maximum number of branches per plant (2.86) was recorded from T_4 treatment which was statistically similar to T_1, T_2, T_3 and T_5 treatments and the minimum number (2.46) being noted in T_6 treatment was significantly inferior to others treatments (Table 3). Application of cowdung or poultry manure along with chemical fertilizers enhanced the branches per plant of lentil. Krishnan (2016) reported the maximum numbers of branches per plant in green gram (11.6 and 12.9) due to application of NPK fertilizers (20:40:20 kg ha^{-1}) with vermicompost and farmyard manure. However, integrated nutrient management is one of the important issues for sustainable crop production.

Yield attributes of lentil

Significant variation in the number of pods per plant of lentil was observed between the treatments (Table 3). The highest number of pod per plant (55.9) produced in treatment T_4 was statistically identical to T_2 (55.4), T_5 (53.6) and T_1 (52.8). The lowest number of pod per plant was recorded in T_6 (50.8). Mohammed et al. (2016) observed the possitive effect of poultry litter on the number of pods per plant. This particular parameter is a significant component that directly imparts the effects on potential yield.

The number of seeds per pod also differed significantly among the treatments and the highest was in T_4 (1.93) followed by T_2 and T_1 while the lowest in T_6 (1.73). Vishnoi et al. (2013) found that the number of seed per pod was significantly influenced by organic and inorganic sources of phosphorus in lentil. The 1000-seed weight ranged from 19.6 to 22 g and differed significantly among the treatments, the highest weight (22.0 g) was recorded in T_4 and the lowest value (19.6 g) being noted in T_6 treatment (Table 3). Nandini Devi et al. (2013) noted that seed index of soybean was maximum (12.86 g) with the integrated application of 75% RDF coupled with vermicompost at a rate of 1 t ha^{-1}. The above yield attributes was improved due to an adequate supply of nitrogen, phosphorus, potassium and sulphur to the crop at the early stages as well as steady supply of nutrient at later stages for application of manure.

Yield of lentil

Seed and straw yields were increased due to application of cowdung or poultry manure along with inorganic fertilizers over the control treatment (Table 4). The highest seed yield of lentil was recorded in T_4 (1312 kg ha^{-1} in the 1st year and 1120 kg ha^{-1} in the 2nd year) which was statistically similar to T_2 in the 1st year and similar to T_2, T_3, T_5 and T_1 in the 2nd year. The lowest seed yield of lentil (820 kg ha^{-1} in the 1st year and 735 kg ha^{-1} in the 2nd year) were noted in T_6 treatment. The mean seed yield ranged from 778 to 1216 kg ha^{-1} across the treatments (Table 4). Singh et al. (2018) reported that grain and straw yield were significantly ($P < 0.05$) enhanced in balanced (with

FYM) fertilization treatments compared with the unfertilized control. Organic manure (poultry manure) with mineral fertilizer might had long residual effect which enhances the growth and yield of the crop. However, it is reported in this study that poultry manure contained high percentage of nitrogen, phosphorus, potassium and other nutrients which ultimately improved the crop productivity. Ewulo (2005) corroborated the similar result that poultry manure contained higher amount of nitrogen and phosphorus for the healthy growth of plants. Significantly the highest straw yield was produced in T_4 (1956 kg ha^{-1} in the 1st year and 1817 kg ha^{-1} in the 2nd year) which was statistically alike with T_5, T_3, T_2 and T_1 treatments and the lowest in T_6 (1497 kg ha^{-1} in the 1st year and 1302 kg ha^{-1} in the 2nd year). The mean straw yield of lentil (average of two years) varied from 1400 to 1887 kg ha^{-1} across the treatments (Table 4). The harvest index (HI) varied between 35.3-40.3% in the 1st year and 36.2-38.1% in the 2nd year across the the treatments. However, the highest harvest index (40.3% in the 1st year and 38.1% in the 2nd year) was recorded in T_4 treatment and the lowest in T_6 treatment in both years. Krishnan (2016) noted that maximum harvest index of green gram (29.5%) was recorded under NPK (20:40:20 kg per hectare) with farmyard manure.

Table 5. Effect of integrated nutrient management on the number of nodules per plant in different dates (pooled data of 2- years)

Treatment	No. of nodules plant^{-1} after 30 DAS	No. of nodules plant^{-1} after 45 DAS	No. of nodules plant^{-1} after 60 DAS	No. of nodules plant^{-1} after 75 DAS
T_1	8.2	18.4	24.5	21.1
T_2	10.5	21.3	30.8	23.5
T_3	9.0	22.0	32.0	28.9
T_4	12.7	26.6	35.1	30.0
T_5	10.0	23.2	32.6	28.7
T_6	8.0	16.9	23.8	22.8
LSD (0.05)	2.7	3.5	2.7	3.5
CV (%)	15.0	8.93	4.97	7.48

Note: T_1= Recommended dose (FRG, 2012), T_2= IPNS (Inorganic) +2.5 t ha^{-1} cowdung, T_3= IPNS (Inorganic) +5 t ha^{-1} cowdung, T_4= IPNS (Inorganic) +1.5 t ha^{-1} poultry manure, T_5= IPNS (Inorganic) +3 t ha^{-1} poultry manure and T_6= Control.

Table 6. Effect of integrated nutrient management on protein content in seed of lentil (pooled data of 2-years)

Treatment	N content	Protein content	Protein yield
	(%)		kg ha^{-1}
T$_1$= Recommended dose (FRG, 2012)	4.56	24.2	249
T$_2$= IPNS (Inorganic)+2.5 t ha^{-1} cowdung	4.86	25.6	304
T$_3$= IPNS (Inorganic)+5 t ha^{-1} cowdung	4.78	25.3	260
T$_4$= IPNS (Inorganic)+1.5 t ha^{-1} poultry manure	4.92	26.1	317
T$_5$= IPNS (Inorganic)+ 3.0 t ha^{-1} poultry manure	4.77	25.3	257
T$_6$=Control	4.53	24.0	186
LSD (0.05)	0.24	1.31	40.3
CV (%)	2.79	2.87	8.45

Nodulation of Lentil

The number of nodules per plant was influenced significantly by the different treatments. Per plant nodules in each treatment progressively increased from 30 days after sowing (DAS) to 60 DAS, and then decreased in irrespective of treatments (Table 5). Per plant nodules ranged across the treatments from 4.0-12.7 at 30 DAS; 16.9-26.6 at 45 DAS; 23.8-35.1 at 60 DAS and 22.8-30.0 at 75 DAS. The highest number of nodules (12.7, 26.6, 35.1 and 30.0, respectively) was always recorded in T_4 treatment and the lowest number of nodules noted in T_6 (control) treatment (Table 5). Nandini Devi *et al.* (2013) reported that the maximum nodules per plant of soybean (43.00) were found in the integration of 75% RDF with organic vermicompost at a rate of 1 t ha^{-1}. It reveals that the minimum numbers of nodules per plant were formed at the earlier stage (30 DAS) of the crop which gradually increased with time and reached the maximum value at the mid-flowering stage (60 DAS) and then declined to the reduced number of nodules after completion of flowering of the crop. This finding was supported by Kevin Zaychuk (2006). Albiach *et al.* (2000) noted that organic fertilizers are not only the source of organic matter and nutrient, but also boost microbial population and improve physical, biological and chemical properties of the soil. Thus, with long-term retention of organic matter in agricultural systems, survival and growth condition of rhizobia may improve with time and allow nodulation without the addition of lime.

Protein content of lentil

The N content as well as protein content of lentil seed were significantly influenced by integrated nutrient management. In the experiment, the highest protein content (26.1%) was achieved in T_4 treatment that was statistically identical with T_2, T_3 and T_5 treatments and lowest protein content (24.0%) was recorded in control (T_6) treatment (Table 6). Among various treatments, the treatment T_4 {IPNS (Inorganic)+1.5 t ha^{-1} poultry manure} produced significantly higher protein yield (317 kg ha^{-1}) in lentil seed (Table 6). Nandini Devi *et al.* (2013) reported that integration of 75% RDF with vermicompost at the rate of 1.0 t ha^{-1} produced significantly higher protein content of soybean seed.

Conclusion

From the two years study, the lentil performed better in T_4 {IPNS (Inorganic) +1.5 t ha^{-1} poultry manure} treatment regarding to plant height, branches per plant, pod number, seed weight, nodule formation and protein yield. The highest seed yield was produced from the treatment of IPNS (Inorganic) + 1.5 t ha^{-1} poultry manure (T_4). The results suggest that satisfying the recommended fertilizer dose through application of poultry manure @ 1.5 t ha^{-1} with IPNS

inorganic fertlizer could be suggested for achieving the maximum yield potential of lentil at chiata series soil of Gazipur.

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PERFORMANCE OF LENTIL-MUNGBEAN-T.AUS RICE-T.AMAN RICE AGAINST EXISTING CROPPING PATTERN LENTIL-JUTE-T.AMAN RICE IN FARIDPUR REGION

S. Ahmed[1], A. F. M. R. Quddus[2], M. Mohiuddin[3]
M. R. Islam[4] and M. A. Hossain[5]

Abstract

A trial was conducted in the farmers' field at Farming Systems Research and Development (FSRD) site, Hatgobindapur, Faridpur during 2014-15 and 2015-16 to develop an alternate cropping pattern over existing cropping pattern for increasing cropping intensity, total productivity as well as farmers' income. The alternate cropping pattern Lentil (var. BARI Masur-7)- Mungbean (var. BARI Mung- 6)- T. Aus rice (var. BRRI dhan48)- T.Aman rice (var. BRRI dhan62) was tested against the existing cropping pattern Lentil (var. Local)-Jute (var. JRO 524)- T.Aman rice (var. BRRI dhan33). All the four crops in the AP were grown successfully within stipulated time frame following recommended practices. Crop duration and turn around time in alternate cropping pattern were 340 days and 25 days while that in existing cropping pattern were 313 days and 52 days, respectively. Cropping intensity increased from 300% (in existing pattern) to 400% in the alternate cropping pattern. Two years results showed that, average yield of T.Aus rice, T.Aman rice, Lentil and Mungbean in the alternate cropping pattern were 3.75, 4.17, 1.31 and 0.80 t ha^{-1}, respectively. The rice equivalent yield and production efficiency was increased by 18 and 35%, respectively over existing pattern. The gross margin of the whole alternate cropping pattern was Tk.106304 ha^{-1} whereas Tk. 75480 ha^{-1} from existing cropping pattern. The alternate pattern provided 41% higher gross margin over existing cropping pattern. The MBCR was 4.86 over existing cropping pattern.

Keywords: Four crops based pattern, cropping intensity, production efficiency, rice equivalent yield, MBCR

Introduction

Agriculture in Bangladesh is the largest economic sector that provides more than 16% of the country's GDP and employs 45% of labour forces (BBS 2016). Bangladesh is one of the most densely populated countries of the world with population growth rate of 1.34% (BBS, 2017). Food requirement is estimated to be doubled in the next 25 years. With increase in population agricultural land is decreasing at an alarming rate for construction of housing, roads and other

[1]Senior Scientific Officer, [2]Scientific Officer, [3]Principal Scientific Officer, On-Farm Research Division (OFRD), BARI, Faridpur; [4]Chief Scientific Officer and [5]Senior Scientific Officer, On-Farm Research Division, BARI, Gazipur.

infrastructures. Thus major challenge is to produce more and more food to feed the ever increasing populations from limiting land area. In this situation, increasing cropping intensity is indispensable and as such more crop(s) should be accommodated in the existing two or three crops based cropping patterns. Sustain crop production in Bangladesh through improvement of cropping pattern in the rice based cropping system is regarded important to address national issues such as, food security, poverty alleviation, land degradation and pollution control (Aziz and Rahman, 2011). The main challenge of the new millennium is increase yield per area unit by at least 50% through manipulating the limited land resource. In this regard, the challenges for the agronomist are to understand crop production problems and process to develop the best ways of production technologies for the management of problems and sustain production. In case of production agronomy, targeting high yield with highest cropping intensity and productivity are the most logical way to raise the total production. In order to produce more food within a limited area, two most important options to be adopted are i) to increase the cropping intensity producing three or more crops in the same piece of land round the year and ii) to increase the production efficiency of the individual crop. Lentil-Jute-T.Aman rice covers 5% of total cultivable land in Faridpur. Jute is one of the major cash crops in Faridpur region which covers about 77 thousand hectares of land in 2011-12 but it was 71 thousand hectares in 2012-13 (DAE, 2014). Unavailability of retting water on time, poor quality seed, pest attack and abrupt fluctuation in market price are main reasons behind decline in jute area. It this context mungbean could be introduced after harvesting of lentil in February to replace jute. Then T.Aus rice (var. BRRI dhan 48) could easily be transplanted in the 2nd week of May after 1st picking of Mungbean. Inclusion of pulse crop like, mungbean in the cropping pattern would maintain a good health of soil (Sharma and Prasad, 1999). T.Aman rice (var. BRRIdhan 62) could be transplanted in the 3rd week of August and thus harvested within last week of October. Hence, Lentil-Mungbean-T.Aus rice-T.Aman rice could be developed as an alternate cropping to increase cropping intensity as well as maintain soil fertility. The study was therefore carried out to find out the performance of the alternative cropping pattern with a view to increase cropping intensity and total productivity as well as farmers' income.

Materials and Methods

The experiment was carried out in farmers' field at FSRD site, Hatgobindapur, Faridpur during two consecutive years (2014-15 and 2015-16) to develop an alternate cropping pattern to increase cropping intensity and productivity against the existing cropping Lentil-Jute-T.Aman rice. The experimental field belongs to the agro-ecological zone of Low Ganges River Floodplain Soil (AEZ#12). The initial soil of the experimental plots was clay loam in texture with average organic matter content 1.26% and pH 7.6 (slightly alkaline). The total nitrogen content was 0.07% (very low) and K content was 0.39 meq/100 g soil (high).

Available P and S were 33.5µg/g soil (high) and 15.2 µg/g soil (medium), respectively (Table 8). The average maximum temperature of two experimental years was 34.62^0C (recorded in the month of April) and that of minimum was 12.48^0 C (recorded in the month of January); while the average maximum total rainfall was 518.30 mm (in July) and minimum was 2.85 mm (in January) during the study period. No rainfall occurred during the period of November (Appendix Table 3). The alternate cropping pattern Lentil (var. BARI Masur-7)- Mungbean (var. BARI Mung-6)- T.Aus rice (var. BRRI dhan48)- T.Aman rice (var. BRRI dhan62) was tested against the existing cropping pattern Lentil (var. Local)- Jute (var. JRO 524)- T.Aman rice (var. BRRI dhan33). The trial was conducted in five selected farmers' field, considering each farmer as a replication (dispersed). The four crops of alternate pattern were grown in lands near to lands having existing cropping pattern following the time frame. The stipulated time frame for alternate and existing cropping pattern is shown in Table 1.

Table 1. Time frame of the alternate and existing cropping pattern

Alternate Pattern	Lentil (var. BARI Masur-7)	Mungbean (var. BARI Mung-6)	T.Aus rice (var. BRRI dhan48)	T.Aman rice (var. BRRI dhan62)
Total duration (340 days)	30 Oct to 20 Feb (113 days)	25 Feb to 30 Apr (65 days)	10 May to 05 Aug (86 days) Seed sowing: 16 April	10 Aug to 25 Oct (76 days) Seed sowing: 01 July
Turn around time (day)	05	05	10	05

Existing Pattern	Lentil (var. Local)	Jute (var. JRO 524)	T.Aman rice (var. BRRI dhan33)
Total duration (313 days)	25 Oct to 16 Feb (115 days)	25 Mar to 20 July (117 days)	01 Aug to 20 Oct (81 days)
Turn around time (day)	05	36	11

The crop management practices followed in the alternate cropping pattern of two years (2014-15 and 2015-16) are stated below:

Lentil: Lentil var. BARI Masur-7 was used in the cropping pattern of Lentil-Mungbean-T. Aus rice and T.Aman rice where fertilizers were used at the rate of 24-32-22-9-2-1 kg ha^{-1} N-P-K-S-Zn-B in the field. All the fertilizers were applied at final land preparation. The seeds were sown on 31 October to 6 November in 2014 and 5-6 November in 2015. One hand weeding was done at 25 to 29 days after sowing (DAS) for uprooting of weeds mainly *Shiyal Kata* (*Argemone*

Mexicana). Pre-sowing irrigation was done for bringing optimum *joe* condition in each year. The fungicide, Rovral @ 2 g lit^{-1} water at 82 to 96 DAS was sprayed to control *Stemphylium* blight. The crop was harvested at 114 to 118 DAS (26 -28 Feb) in 2015 and 115-116 DAS (March 1-2) in 2016.

Mungbean: Mungbean var. BARI Mung-6 was used in the alternate cropping pattern of Lentil-Mungbean-T. Aus rice and T.Aman rice where fertilizers were used at the rate of 24-32-22-9-2-0 kg ha^{-1} N-P-K-S-Zn-B. All the fertilizers were applied at final land preparation. The seeds were sown on 5 to 6 March in 2015 and 6-7 March in 2016. One hand weeding at 20 to 25 DAS was done. Pre sowing irrigation was applied for bringing optimum *joe* condition. Only one picking of BARI Mung- 6 was done on 8-9 May 2015 (64 DAS) but 18-19 May in 2016 (71 DAS). After harvest mungbean plants were incorporated with the soil.

T.Aus rice: T. Aus rice var. BRRI dhan48 was used in the alternate cropping pattern of Lentil-Mungbean-T. Aus rice and T.Aman rice with fertilizer dose of 80-20-20-8-1-0 kg ha^{-1} N-P-K-S-Zn-B. All of phosphorus, potassium, sulphur and zinc was applied as broadcast and incorporated during final land preparation. Nitrogen was applied in two equal splits as top dress at 10-15 and 30-35 DAT (days after transplanting). Twenty two days old seedlings were transplanted on 16-17 May, 2015 (21-22 days left) due to delay sowing of seeds in seedbed and 25 days old seedlings were transplanted for second cycle in 2016. One to two hand weeding were done in both the cycle. Irrigation was done 12-15 times in the month of May (148.90 mm rainfall) due to insufficient rainfall in 2015 but only three irrigations were applied in June 2015 due to higher amount of rainfall (345.3 mm). Maximum temperature (^{o}C), no. of rainfall days and amount of rainfall recorded during T.Aus rice cultivation (May to June 2015) under Lentil-Mungbean-T.Aus rice-T.Aman rice are stated below in Table 2. However, weather data of October 2014 to September 2015 and October 2015 to September 2016 are presented in Appendix Table-1 and Appendix Table-2, respectively.

Table 2. Weather data during May to June 2015 for T.Aus rice cultivation

Parameter	May 2015	June 2015
Maximum temperature (^{o}C)	36.8	36.7
Total rainfall (mm)	148.9	345.3
Number of Rainfall days	14	19
Rainfall range (mm)	0.2-78.4	0.2-103.4

The integrated pest management (Perching, light trap) approach was given priority than using of chemical pesticide. BRRI dhan48 was harvested on 5 to 13 August in 2015 (109 to 116 DAT) and 7-8 August for second cycle in 2016.

Table 3. Crop management practices of existing and alternate cropping pattern at Faridpur during 2014-15

Parameters	Alternate Cropping Pattern				Existing Cropping Pattern		
	Lentil	Mungbean	T.Aus rice	T.Aman rice	Lentil	Jute	T.Aman rice
Crop	Lentil	Mungbean	T.Aus rice	T.Aman rice	Lentil	Jute	T.Aman rice
Variety	BARI Masur-7	BARI Mung-6	BRRI dhan48	BRRI dhan62	Local	JRO 524	BRRI dhan33
Spacing	Broadcast	Broadcast	20 cm × 15 cm	20 × 15 cm	Broadcast	Broadcast	20 × 15 cm
Fertilizer dose (NPKSZnB kg/ha)	24-32-20-18-2-1	24-32-22-9-2-0	85-20-20-8-1-0	80-15-35-8-1-0	20-20-25-0-3.5-2	85-20-50-0-0-0	80-25-40-5-2.6-1.2
Date of planting/ sowing	Nov. 5-6	Mar. 5-6	May 16-18	Aug. 13-14	Oct. 25	Mar. 25	Aug. 1
Irrigation (no.)	1	1	12	2	1-2	1-2	1-2
Weeding (no.)	1	1	1	1	0-1	2-3	1
Date of harvesting	Feb.26-28	May 5	Aug. 5-13	Oct.26	Feb. 16	July 18	Oct. 20
Field duration (days)	114-115	61	81-87	75	115	116	81
Turnaround time (days)	-	5-6	11-13	8	04	36	13

Table 4. Crop management practices of existing and alternate cropping pattern at Faridpur during 2015-16

Parameters	Alternate Cropping Pattern				Existing Cropping Pattern		
Crop	Lentil	Mungbean	T.Aus rice	T.Aman rice	Lentil	Jute	T.Aman rice
Variety	BARI Masur-7	BARI Mung-6	BRRI dhan48	BRRI dhan62	Local	JRO 524	BRRI dhan33
Spacing	Broadcast	Broadcast	20 cm × 15 cm	20 × 15 cm	Broadcast	Broadcast	20 × 15 cm
Fertilizer dose (NPKSZnB kg/ha)	24-32-20-18-2-1	24-32-22-9-2-0	85-20-20-8-1-0	80-15-35-8-1-0	20-20-25-0-3.5-2	95-20-50-0-0-0	80-25-40-5-2.6-1.2
Date of planting/ sowing	Nov. 5-6	Mar. 6-7	May 16-17	Aug. 13-14	Oct. 26	Mar. 25	July 24-26
Irrigation (no.)	1	1	3	2	1-2	1-2	2
Weeding (no.)	1	-	1	1	0-1	2-3	1
Date of harvesting	Mar.1-2	May 8-9	Aug.7-8	Oct. 26	Feb. 18	July 20	Oct. 19
Field duration (days)	117	64	84	75	116	118	84-86
Turn around time (days)	9	4	7	5	5	34	3-5

T.Aman rice: T.Aman rice var. BRRI dhan62 was used for T.Aman rice - the fourth crop of the cropping pattern Lentil-Mungbean-T. Aus rice and T.Aman rice. About 25-26 days old seedlings were transplanted in both 2015 and 2016 due to delay in harvesting of T.Aus rice. Fertilizers were used at the rate of 80-15-35-8-1-0 kg ha^{-1} N-P-K-S-Zn-B in the field. All of phosphorus, potassium, sulphur and zinc was applied as broadcast and incorporated during final land preparation. Nitrogen was applied in three equal splits. The first one-third was applied immediately after seedling establishment stage (7-10 DAT), second top dress at rapid tillering stage (28-30 DAT) and third one was applied at panicle initiation stage (43-46 DAT). One weeding was done by spraying weedicide (Changer) at 7-8 DAT. Irrigation was done twice in both the years. The integrated pest management (Perching, light trap, etc.) approach was used along with insecticide (Regent, Virtako, etc.) to control the pest. BRRI dhan62 was harvested on 26-27 October, 2015, (100 to 101 seed to seed) for both years irrespective of farmers.

Thus total field duration of the alternate pattern was 331 and 340 days in 2015 and 2016, respectively. On the other hand, field duration of whole existing pattern was 312 and 318 days in 2015 and 2016, respectively. The crop management practices of two years (2014-15 to 2015-16) of the existing cropping pattern and alternate cropping pattern are presented in Table 3 and Table 4. Data regarding existing cropping pattern (Lentil-Jute-T.Aman rice) were recorded from fields adjacent to the experimental plots.

Data on yield parameters and yield of each of the crops were collected following standard procedures. Agronomic performance like, field duration, production efficiency and rice equivalent yield of cropping patterns were calculated. Production efficiency value in terms of kg/ha/day was calculated by total main product in a cropping pattern divided by total duration of crops in that pattern (Tomar and Tiwari, 1990).

$$\text{Production Efficiency (kg/ha/day)} = \frac{\sum Y_i}{\sum d_i}$$

Where, Y_i= Yield (kg) of ith crop and d_i= Duration (day) of ith crop of the pattern

i= 1, 2, 3, 4

Rice Equivalent Yield (REY): For comparison between crop sequences, the yield of every crop was converted into rice equivalent on the basis of prevailing market price of individual crop (Verma and Modgal, 1983). Rice equivalent yield (REY) was computed as yield of individual crop multiplied by market price of that crop divided by market price of rice.

$$\text{Rice equivalent yield (t/ha/yr)} = \frac{\text{Yield of individual crop} \times \text{market price of that crop}}{\text{Market price of rice}}$$

For the economic analysis, gross return, gross margin and marginal benefit cost ratio was calculated on the basis of prevailing market price of the produces. Economic analysis involved collection of data on prices and quantities of inputs used and output produced. The inputs used included seed, fertilizer, labour, herbicide and insecticides. The MBCR of the existing cropping pattern and any replacement for it can be computed as the marginal value product ((MVP) over the marginal value cost (MVC). The Marginal of prevalent pattern (F) and any potential replacement (E) for it was computed as (CIMMYT, 1988).

$$\textbf{Marginal Benefit Cost Ratio (MBCR)} = \frac{\text{Gross return (E) - Gross return (F)}}{\text{TVC (E) - TVC (F)}} = \frac{\text{MVP}}{\text{MVC}}$$

Where, TVC= Total variable cost; MVP = Marginal value of product; MVC= Marginal value cost

The nutrient uptake by different crops in both alternate and existing pattern was calculated according to Fertilizer Recommendation Guide (FRG, 2012).

Results and Discussion

Yield performance: Year-wise (2014-15 and 2015-16) information regarding yield and cropping duration in the alternate cropping pattern (AP) and existing pattern (EP) are presented in Table 5 & Table 6. All of parameters showed the highest value in AP compared to that of EP. During 2014-15, the rice equivalent yield (17.90 t/ha/yr) and production efficiency (29.79 kg/ha/day) was higher in AP than EP (Table 5). Similar results were also found in 2015-16, alternate cropping pattern provided higher rice equivalent yield (16.47 t/ha/yr) and production efficiency (28.42 kg/ha/day) than existing cropping pattern (Table 6). Average yield of lentil, mungbean, T.Aus rice and T.Aman rice in the AP were 1.31, 0.80, 3.75 and 4.17 t/ha; while the yield of lentil, jute and T.Aman rice in the EP were 1.00, 2.48 and 3.40 t/ha, respectively (Table 7). On an average, AP gave higher rice equivalent yield (REY) of 17.19 t/ha/yr against EP (14.59 t/ha/yr). Higher rice equivalent yield was obtained from AP due to introduction of two new crops mungbean and T.Aus rice instead of jute. Thus REY obtained from the AP was 18% higher than that of EP. The EP (Lentil-Jute-T.Aman rice) required on an average 313 days field duration to complete the cycle while AP (Lentil-Mungbean-T.Aus rice-T.Aman rice) required 340 days (excluding seedling age of rice) to complete one cycle. The AP showed 35% production efficiency over EP (average production efficiency of alternate and existing pattern was 28.90 and 21.50 kg/ha/day, respectively, as shown in Table-7). Results indicated that mungbean (BARI Mung-6) and lentil (BARI Masur-7) could easily be fitted in the existing cropping pattern keeping 25 days turnaround time in a year.

Table 5. Performance of alternate cropping pattern and existing cropping pattern at Faridpur during 2014-15

Parameters	Alternate pattern				Existing pattern		
Crop	Lentil	Mung	T.Aus	T.Aman	Lentil	Jute	T.Aman
Variety	BARI Masur-7	BARI Mung-6	BRRI dhan48	BRRI dhan62	Local	JRO 524	BRRI dhan33
Grain (t ha^{-1})	1.50	0.710	4.03	3.92	1.21	2.45	3.74
BY-product (t ha^{-1})	0.93	--	4.19	4.12	0.75	3.81	4.21
Rice equivalent yield (t ha^{-1})	6.44	2.06	4.70	4.70	4.65	6.7	4.47
Gross return (Tk ha^{-1})	99825	31950	72940	73020	80747	97180	64755
Total variable cost (Tk ha^{-1})	33757	24692	70010	54380	28524	84266	58820
Gross margin (Tk ha^{-1})	66068	7258	2930	18640	52223	12914	5935
Total gross return (Tk ha^{-1}) (whole pattern)	277735.00				242682.00		
Total expenditure (Tk ha^{-1}) (whole pattern)	182839.00				171610.00		
Total gross margin (Tk ha^{-1}) (whole pattern)	94896.00				71072.00		
Total rice equivalent yield (t/ha/yr)	17.90				15.82		
Production efficiency (kg/ha/day)	29.79				22.81		
MBCR (whole pattern)				4.29			

Price of output (Tk kg^{-1}): Lentil 65.00, Mungbean-45.00, Rice-15.50, Rice straw-2.50

Price of input (Tk kg^{-1}): Urea- 16.00, TSP-22.00, MP-17.00, Gypsum-8.00, Boron-150.00, Zn-150.00

Insecticide: Regent- 175.0, Weedicide: Changer- 85.00 (Each 100 g packet)

Labor cost (Tk labor $^{-1}$): 300.00

Table 6. Performance of alternate cropping pattern and existing cropping pattern in Faridpur during 2015-16

Observation	Alternate pattern				Existing pattern		
Crop	Lentil	Mung	T.Aus	T.Aman	Lentil	Jute	T.aman
Variety	BARI Masur-7	BARI Mung-6	BRRI dhan48	BRRI dhan62	Local	JRO 524	BRRI dhan33
Grain (t ha^{-1})	1.12	0.89	3.46	4.42	0.79	2.51	3.01
By-product (t ha^{-1})	0.70	--	4.01	3.81	0.55	3.80	3.23
Gross return (Tk ha^{-1})	108150	40275	81230	79830	76425	116820	69890
Total variable cost (Tk ha^{-1})	36292	34672	67280	53530	36218	88880	58150
Gross margin (Tk ha^{-1})	71858	5603	13950	26300	40207	8940	11740
Rice equivalent yield (t ha^{-1})	5.41	2.01	4.06	4.99	4.01	5.84	3.50
Total gross return (Tk ha^{-1}) (whole pattern)		309485.00				263135.00	
Total expenditure (Tk ha^{-1}) (whole pattern)		191774.00				183248.00	
Total gross margin (Tk ha^{-1}) (whole pattern)		117711.00				79887.00	
Rice equivalent yield (t/ha/yr)		16.47				13.35	
Production efficiency (kg/ha/day)		28.42				19.66	
MBCR (whole pattern)			5.43				

Price of output (Tk kg^{-1}): Lentil 95.00, Lentil by-product: 2.50, Jute fibre: 42.00, Jute stick: 3.00, Mungbean-45.00, Both Rice-20.00, Rice straw-3.00

Price of input (Tk kg^{-1}): Urea- 16.00, TSP-22.00, MP-17.00, Gypsum-8.00, Boron-240.00, Zn-180.00

Insecticide: Regent- 175.0, Weedicide: Changer- 85.00 (Each 100 g packet)

Labor cost (Tk labor $^{-1}$): 350.00

Table 7. Average performance of alternate cropping pattern and existing cropping pattern at Faridpur (2014-2016)

Observation	Alternate pattern				Existing pattern		
Crop	Lentil	Mung	T.Aus	T.Aman rice	Lentil	Jute	T.Aman rice
Variety	BARI Masur-7	BARI Mung-6	BRRI dhan48	BRRI dhan62	Local	JRO 524	BRRI dhan33
Grain (t ha⁻¹)	1.31	0.8	3.75	4.17	1.00	2.48	3.40
Rice equivalent yield (t/ha/yr)			17.19			14.59	
Total gross return (Tk ha⁻¹) (whole pattern)			293610.00			252909.00	
Total expenditure (Tk ha⁻¹) (whole pattern)			187307.00			177429.00	
Total gross margin (Tk ha⁻¹) (whole pattern)			106604.00			75480.00	
Production efficiency (kg/ha/day)			28.90			21.50	
MBCR (whole pattern)			4.86				

Table 8. Initial and post-harvest nutrient status of soils (soil depth 0-15cm) under Lentil-Mungbean-T.Aus rice-T.Aman rice cropping pattern (SRDI, Faridpur)

Item	Texture	Land type	pH	OM (%)	Total N (%)	Available P	K (meq/100g soil)	S (µg/g soil)
Initial soil status								
Average	Clay Loam	MHL (Irrigated)	7.6	1.26	0.07	33.5	0.39	15.2
Interpretation			Slightly alkaline	Low	VL	High	High	Medium
Post-harvest soil status								
Average	Clay Loam	MHL (Irrigated)	7.7	1.28	0.06	33.9	0.44	19.2
Interpretation			Slightly alkaline	Low	V. Low	High	High	Medium

Table 9. N uptake (kg ha⁻¹) by grains and straw of crops and the system as influenced by different cropping pattern (Average of 2014-15 and 2015-16)

CP	Lentil		Mungbean/Jute		T. Aus rice		T. Aman rice		System		
	Grain	Stover	Grain/Fibre	Stover	Grain	Straw	Grain	Straw	Grain/Fibre	Straw/stover	Total
AP	45.06	9.72	25.20	16.00	50.63	27.88	56.29	26.93	177.18	80.53	257.71
EP	34.40	7.80	81.01	0.00	0.00	0.00	45.89	25.30	161.31	33.10	194.41

Alternate Pattern (AP)= Lentil-Mungbean-T.Aus rice-T.Aman rice; Existing Pattern (EP) =Lentil-Jute-T.Aman rice

Table 10. P uptake (kg ha⁻¹) by grain and straw of crops and the system as influenced by different cropping patterns (Average of 2014-15 and 2015-16)

CP	Lentil		Mungbean/Jute		T. Aus rice		T. Aman rice		System		
	Grain	Stover	Grain/Fibre	Stover	Grain	Straw	Grain	Straw	Grain/Fibre	Straw/stover	Total
AP	3.93	1.87	4.40	5.50	11.25	5.33	22.93	5.15	42.51	17.84	60.35
EP	3.00	1.50	16.53	0.00	0.00	0.00	18.69	4.83	38.23	6.33	44.56

Alternate Pattern (AP)= Lentil-Mungbean-T.Aus rice-T.Aman rice;   Existing Pattern (EP) =Lentil-Jute-T.Aman rice

Table 11: K uptake (kg ha⁻¹) by grain and straw of crops and the system as influenced by different cropping pattern (Average of 2014-15 and 2015-16)

CP	Lentil		Mungbean/Jute		T. Aus rice		T. Aman rice		System		
	Grain	Stover	Grain/Fibre	Stover	Grain	Straw	Grain	Straw	Grain/Fibre	Straw/stover	Total
AP	14.41	8.83	12.24	21.50	11.63	69.71	12.93	67.32	51.20	167.36	218.56
EP	11.00	7.09	165.33	0.00	0.00	0.00	10.54	63.24	186.88	70.33	257.20

Alternate Pattern (AP)= Lentil-Mungbean-T.Aus-T.Aman rice; Existing Pattern (EP) =Lentil-Jute-T.Aman rice

Cost and return: The details of economic analysis of two years (2014-15 to 2015-16) are presented in Table 5 and Table 6. Economics of system productivity of the two cropping patterns showed that the gross return and gross margin varied between the cropping patterns. From average of two years, higher gross return (Tk. 293610/ha) and gross margin (Tk.106304/ha) were obtained from AP (Lentil-Mungbean-T.Aus rice-T.Aman rice) and lower gross return (Tk. 252909/ha) and gross margin (Tk. 75480/ha) was found in EP (Lentil-Jute-T.Aman rice). Thus, the AP provided 42% higher gross margin than EP due to harvesting additional yields from two crops mungbean and T.Aus rice instead of growing jute only. Higher (Tk.187307/ha) total variable cost was recorded from AP due to inclusion of T.Aus rice and mungbean which was 5.57% higher than the total variable cost in EP (Tk.177429/ha). The average MBCR was 4.86 which indicating that alternate pattern could produce more economic returns over the farmers' existing pattern (Table 7).

Soil analysis: Initial and post-harvest soil sample analytical data were shown in Table 8. The sol analysis showed that, except N all other nutrient contents increased slightly which might be due to inclusion of two pulse crops (lentil and mungbean) in the alternate cropping pattern (AP). There was also possibility of increasing N content in the soil as two legume crops were grown in the alternate pattern, but it did not happen which might be due to leaching, runoff and volatilization loss of N through cultivation of subsequent T.Aus rice and T.Aman rice shortly after harvest of mungbean and keeping standing water in both T.Aus and field.

Apparent nutrient uptake and balance

N uptake in grain was 177.18 kg ha^{-1} for whole AP whereas 161.31 kg ha^{-1} for EP (Table 9). The higher N uptake in grain (56.29 kg ha^{-1}) was found from T.Aman rice and lower from mungbean (25.20 kg ha^{-1}) in AP (Table 9). In EP, due to lower yield of T.Aman rice, N uptake was 23% lower than that of AP. The total N uptake was 257.71kg ha^{-1} and 194.41 kg ha^{-1} in AP and EP, respectively. In both the system, negative N balance was found. But, deficit was higher in EP than AP might be due to use of lower fertilizer dose (185 kg ha^{-1}) and addition of no residues to soil (Fig. 1). In case of P uptake, total uptake in AP and EP was 60.35 kg ha^{-1} and 44.56 kg ha^{-1}, respectively (Table 10). Nutrient balance was in surplus for both the system (Fig. 1). In case of K uptake, the highest amount of K uptake (165.33 kg ha^{-1}) was found in jute in EP which was higher than all the crops in both the systems. Total K uptake was 18% higher in EP compared to AP might be due to higher fertilizer dose (Table 11). The balance was consistently negative being higher (-142.20 kg ha^{-1}) in EP and lower (- 81.00 kg ha^{-1}) in AP (Fig. 1).
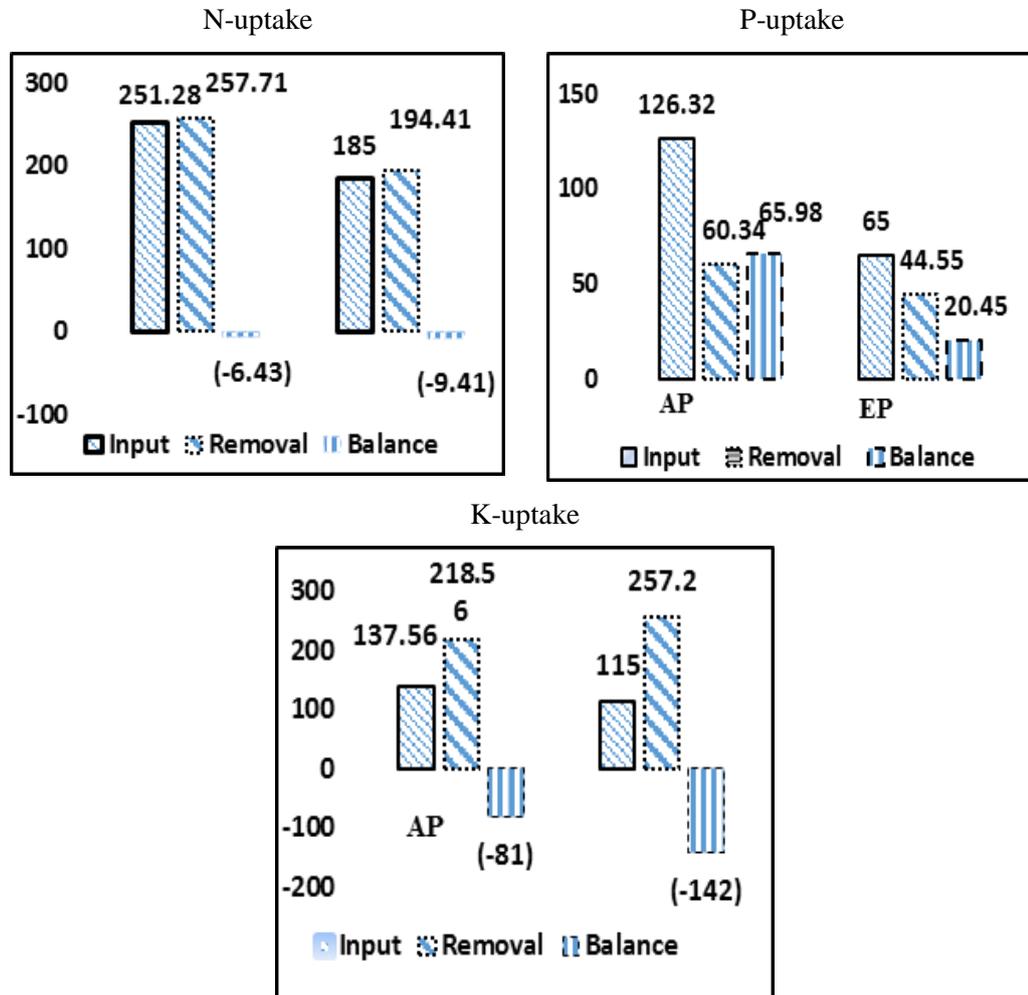
Fig 1. Inputs, outputs and balance of N, P and K (kg ha^{-1}) in response to alternate and existing cropping patterns in 2014-15 and 2015-16.

Conclusion

From the results, alternate cropping pattern (Lentil-Mungbean-T.Aus rice-T.Aman rice) was found agronomically viable in terms of REY and production efficiency. Besides, MBCR from the alternate cropping pattern showed the pattern economically viable. On an average, 340 days required to complete the one cycle of the alternate cropping pattern with turn around time of 25 days only. So, more efforts should be given in labour management, timely supply of inputs and land preparation. In this alternate cropping pattern, two pulse crops (Mungbean and lentil) could be fitted in the rice based pattern to increase the pulse production as well as enhance soil fertility.

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Appendix Table 1. Weather data at Faridpur from October 2014 to September 2015

Month	Avr. Max (°C)	Avr. Min (°C)	Avr. RH (%)	Total rainfall (mm)
October 2014	32.56	23.58	81.03	83.2
November 2014	30.42	18.08	78.13	000.0
December 2014	24.72	13.90	85.19	000.0
January 2015	24.59	13.10	81.93	5.7
February 2015	28.50	15.43	76.75	21.2
March 2015	32.75	18.73	65.32	2.8
April 2015	33.12	22.83	76.27	160
May 2015	36.8	21.4	79	148.9
June 2015	36.7	23.0	84.86	345.3
July 2015	32.07	26.03	87.77	539.3
August 2015	32.68	26.73	86.90	312.2
September 2015	33.46	26.22	85.9	213.37

Appendix Table 2. Average weather data at Faridpur from October 2015 to September 2016

Month	Avr. Max (°C)	Avr. Min (°C)	Avr. RH (%)	Total rainfall (mm)
October 2015	32.92	23.89	81	79.4
November 2015	30.62	19.06	80.03	000.0
December 2015	25.93	15.15	81.51	006.0
January 2016	25.16	11.86	80.51	000.0
February 2016	30.06	17.78	75.24	15.8
March 2016	34.07	21.05	68.68	024.4
April 2016	36.11	26.23	74.50	57.2
May 2016	33.22	24.71	79.80	222
June 2016	34.08	26.22	82.73	282.2
July 2016	32.28	26.35	88.13	497.3
August 2016	33.13	26.67	83.84	303.10
September 2016	33.31	26.36	86.03	124.7

COMBINATION OF BIOLOGICAL CONTROL AGENTS AND GARLIC (*ALLIUM SATIVUM*) EXTRACT IN REDUCING DAMPING-OFF DISEASE OF TOMATO

V. O. Dania[1] and J. A. Omidiora[2]

Abstract

Damping-off *(Pythium aphanidermatum)* is a soil-borne disease which accounts for seedling mortality and significant yield losses in tomato production. Laboratory and screenhouse experiments were conducted in 2017, with a repeated field trial in 2018 to evaluate the efficacy of combining three biological control agents (BCAs), *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* with *Allium sativum* extract for the integrated management of the disease in tomato crop. Treatments were laid out in a completely randomized design and randomized complete block design in the screenhouse and field experiments, respectively with eighteen treatments and three replications. The BCAs and extract were formulated and applied using seed treatment and soil sprinkling methods. Treatment combinations of BCAs with *A. sativum* were more effective in the reduction of mycelial growth of the pathogen with inhibitory values that ranged between 77.6-91.2% than single inoculation. Seed treatment before planting was more effective than soil sprinkle method, reducing pre-emergence and damping-off incidence to between 6.8-18.3% and 9.7-26.3% under screenhouse and field conditions, respectively than the sprinkling method. Soil sprinkle with *T. harzianum* in combination with *A. sativum* extract had the highest cumulative tomato fruit yield of 902 kg/ha^{-1} under field conditions. This study showed that combined application of BCAs and *A. sativum* extract reduced damping-off disease and thereby improved the fruit yield of tomato.

Keywords: *Bacillus subtilis*, damping-off, integrated management, Seed treatment, Soil sprinkle, Tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is the most commonly grown and consumed vegetable worldwide (Kurze *et al*., 2018), because of its rich source of vitamin A and it plays an important role in maintaining the human health being a high source of lycopene which acts as an antioxidants and free radical scavenger.

Nigeria is ranked second largest producer of tomato in Africa, with an output of 1.81 million metric tonnes of tomato annually under rainfed production (Ibitoye *et al*., 2015). It is a food and cash crop which contributes significantly to the economy of the local population (Kator *et al*., 2015).

[1]Department of Crop Protection and Environmental Biology, University of Ibadan, PMB 001, Ibadan, Nigeria, [2]International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Nigeria.

The crop is propagated mainly through seeds by farmers, especially in Nigeria, that rely on their own saved seeds from previous season for planting. These helpless farmers often suffer from seed and seedling losses resulting in significant yield reduction arising from cultivation of unhealthy seeds. Among the main constraints to tomato production is the damage caused by soil-borne pathogens, mainly bacteria and fungi, which result in severe losses in production (Barone and Frusciante, 2007). Tomato plants are susceptible to many soil-borne fungal genera such as *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora*, causing serious diseases such as root rot, wilt and damping-off (Srinon *et al.,* 2006).

Pre and post emergence damping-off disease of tomato is caused by *Pythium aphanidermatum*, a soil-borne pathogen which results in seed rot or death of young seedlings (Ashwathi *et al.,* 2017). The pathogen can survive in the soil through the production of perennating structures such as oospores or sporangia (Agrios, 2005).

Currently, damping-off disease *(Pythium aphanidermatum)* of tomato is being managed in Nigeria through the use of synthetic fungicides such as Benlate. Although this approach has produced some desired results, the indiscriminate use has contributed to adverse environmental effects including reduction in beneficial soil-borne microbes and evolvement of new virulent races of pathogens (Thakur and Tripathi, 2015). The use of biocontrol agents (BCAs) in combination with other organic materials is a suitable alternative to chemical applications which ensures sustainable disease management within the concept of an integrated approach. The biocontrol agents are highly effective, inexpensive with excellent shelf life and suitable method of delivery (Someshwar *et al.,* 2013). The antimicrobial activities of garlic, a common Nigerian spice, has been reported (Obagwu and Korsten, 2003). Botanicals are cheap, biodegradable and eco-friendly. However neither of these strategies could achieve a complete control.

Therefore, this study was designed to evaluate the effect of different formulation and combination of control options for the integrated management of damping-off disease of tomato under screenhouse and field conditions

Materials and Methods

Experimental site

The *in vitro* experiment was conducted in the Plant Pathology laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. The *in-vivo* experiments were carried out in the screen house during rainy seasons in 2017 with a repeated field trial in 2018 to validate the screenhouse results. Ibadan is located on latitude 7°33´N 3°56´E with an elevation of 213 m above sea level and annual rainfall ranging between 1250 mm and 1500 mm in the Derived savanna agroecological zone of Nigeria.

Isolation and preparation of fungal inoculum

Pythium aphanidermatum was isolated from roots of tomato seedlings that showed damping-off symptoms such as water-soaked and shriveled stem base leading to rotting of the collar immediately above soil level. Soil debris was washed off the roots, surface-sterilized in 10 % sodium hypochlorite for one minute and rinsed in three changes of sterile distilled water. Infected tissues were then cut into small sizes measuring 2mm× 2mm washed in sterile distilled water for 1 minute and dried on sterile filter paper. Tissue samples were plated on water agar medium in 9 cm Petridishes and incubated at 28±2°C for 10 days under alternating 12 h photoperiod of light and darkness. The fungus was then transferred to 1.7 % corn meal agar (CMA) medium amended with 100μl pimaricin (Davet and Rouxel, 2000). Pathogen cultures were maintained on corn meal agar slants at 8°C. A 3-mm diameter agar plug of mycelia was sub-cultured on a freshly prepared water agar in Petridishes and incubated in the dark at 28-30°C (Zamanizadeh *et al*., 2011). The culture plates were observed for 5 to 10 days for the development of reproductive structures such as sporangia and oospores. Identification of species was carried out using a standard monograph guide for the *Pythium* species (Plaats-Niterink, 1981) and following confirmation at the Mycological Herbarium at the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Substrate for the growth of *P. aphanidermatum* was prepared using sorghum grains as carrier according to modified method of Khiyami *et al*. (2014). Grain weights of 50 g sorghum were poured into a 500-ml Erlenmeyer flasks, each containing 100 ml of water. Contents of bottles were autoclaved at 121°C and 1.05 kg/cm^2 pressure for 30 min. Excess water in the sterilized sorghum grains was drained off using sterile cheese cloth. Fungal inoculum was taken from the edge of a 7-day old culture on Potato Dextrose Agar and introduced into bottles and allowed to colonize sorghum grains for 14 days. At full mycelial ramification, the substrate in each bottle was emptied on sterile filter paper, air-dried in a Laminar flow hood for two days and then ground to fine powder using a rotary blender and sieved. Sandy loam soil was sterilized at using an Sussman Electrical Sterilizer Model SG 12-04-220, USA at 71°C for 1h prior to inoculum application at 10 g/kg of sterilized soil (Adandonon *et al*., 2006).

Isolation and formulation of biological control agents

Trichoderma species were isolated from tomato rhizosphere from four different locations using serial dilution method. Ten grammes of soil adhering to roots of each sample were dissolved in 100 ml of sterile distilled water, shaken for 20 minutes on a rotary shaker, and diluted serially in sterile distilled water. One ml from each of 10^{-4} to 10^{-6} dilutions was dispensed on acidified potato dextrose agar with ten replicates and incubated at 28±2°C for 3 days. Selected cultures

with typical green *Trichoderma* colonies were purified and identified using standard taxonomical keys for *Trichoderma* (Rifai, 1969, Bissett, 1991). Mass production of *Trichoderma* species was carried out using modified Richards medium (Harman *et al.*, 1991) which contained 10 g KNO_3, 5 g KH_2PO_4, 1.3 g anhydrous $MgSO_4$, 8 g sucrose, 20 mg $FeCl_3$ and 100 ml of tomato juice in 1 litre of sterile distilled water. An aliquot of 100 ml of the medium was dispensed in 250- ml conical flask and inoculated with a 5-mm agar plug from a 7-day-old culture on acidified potato agar medium and incubated at 28±2°C. After 21 days incubation period, the mycelia were harvested and air-dried in plastic plates under sterile conditions at room temperature (28-30°C) for two days. They were thereafter ground to a fine powder in a blender and stored at 4°C in polyethylene bags until it was required for the study. Colony forming units in formulation of *Trichoderma* spp. mixtures was adjusted to 3 x 10^7 cfu/g (Nashwal *et al.,* 2008). A powder formulation of *Bacillus subtilis* containing 10^8 colony forming units per gram (cfu/g^{-1}) used in this experiment was collected from the Microbiology unit of the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Inhibitory effect of biological control agents and *Allium sativum* extract on radial mycelial growth of *Pythium aphanidermatum in vitro*

Fresh healthy bulbs of *A. sativum* were washed with sterile distilled water (SDW) and ground to a fine pulp using a rotary blender MJ-BL 40G1 JA. Four samples of 50 g, 100 g, 200 g and 250 g were weighed separately. The weights were ground to a pulp of each sample weight and dissolved in 1litre of SDW to produce final extract concentrations of 5%, 10%, 20% and 25% w/v, respectively. The extract suspensions were agitated intermittently for 10 minutes and left in the Laminar flow hood overnight to enable the active constituents dissolve in the medium. The suspension was then filtered through sterile cheese cloth into Erlenmeyer flask. An aliquot of 1ml was dispensed into 15 ml acidified PDA in 9 cm diameter Petridishes and swirled gently to allow proper mixing of extract and medium. After solidification of the PDA, a 3-mm cork borer was used to obtain mycelial disc from the edge of 7-day old colonies of *P. aphanidermatum* and placed at the centre of each Petridish. Biological control agents, *T. viride, T. harzianum* and *B. subtilis* were inoculated at four equidistant points relative to the pathogen at the center of each Petridish. The control consisted of the pathogen grown on unamended PDA. While a synthetic mancozeb fungicide applied at the rate of 0.5 g/l served as positive check. Each concentration consisted of four replicates in a completely randomized design with 19 treatments. Petridishes were incubated at 28±2°C and the radial growth of the colony treatment was measured at 5 days of incubation and expressed as a percentage of the control.

Screenhouse and field evaluation of effect of single and combined application of *Allium sativum* extract and biological control agents on incidence of damping-off disease

Healthy seeds of a susceptible tomato variety UC82B used in this experiment were obtained from a certified agricultural commercial seed center in Ibadan, Nigeria. Treatments were applied using two methods i.e. seed treatment and soil sprinkle (Harman *et al.*, 1991). The seeds were slightly moistened with sterile distilled water and then treated with the powdered formulation of *Trichoderma* species and *B. subtilis* with spore concentration of 3 x 10^7 cfu/g^{-1} and 10^8 cfu/ g^{-1} respectively as previously described, at the rate of 10 g/kg seeds. Sterilized starch was added to the powdered seed mixture at 5 g/kg as adhesive and mixed thoroughly in aseptic plastic bowls before being planted in soil artificially inoculated with the pathogen at 10 g/kg of soil 2 days before planting. In the seed treatment with the extract, a 25% w/v *A. sativum* concentration which proved to be most effective in the *in vitro* trial was selected and used in the screenhouse and field experiments. It involved soaking the tomato seeds in the extract solution for 10 minutes. The seeds were then air-dried at room temperature (28±2°C) for 1 h before planting. Soil inoculation with *P. aphanidermatum* was done at 10 g/kg of sterilized soil as previously described. The soil sprinkle method involved mixing of 0.5 g powdered formulation of BCAs with 5 g of sterilized soil and sprinkling of the mixture in each planting hole. Experimental pots measuring 25 cm in diameter were each filled at 90% capacity with 5 kg sandy loam soil sterilized using a Sussman Electrical Sterilizer Model SG 12-04-220, USA at 71°C for 2 hours. Four seeds were planted per pot which were later thinned to two and the experimental layout was a randomized complete design with three replications. Screen house environmental conditions varied between 74±2% to 90±2% relative humidity with a temperature range of 28±2°C. The experiment consisted of 18 treatment combinations: T_1=*Trichoderma viride* seed treatment, T_2=*Trichoderma viride* soil sprinkle, T_3=*Trichoderma harzianum* seed treatment, T_4=*Trichoderma harzianum* soil sprinkle, T_5=*Bacillus subtilis* seed treatment, T_6=*Bacillus subtilis* soil sprinkle, T_7=*Allium sativum* seed treatment, T_8=*Allium sativum* soil sprinkle, T_9=*Trichoderma viride* seed treatment + 25% *A. sativum,* T_{10}=*Trichoderma viride* soil sprinkle + 25% *A. sativum,* T_{11}=*T. harzianum* seed treatment + 25% *A. sativum,* T_{12}=*T. harzianum* soil sprinkle + 25% *A. sativum,* T_{13}=*Bacillus subtilis* seed treatment + 25% *A. sativum,* T_{14}=*Bacillus subtilis* soil sprinkle + 25% *A. sativum,* T_{15}=Inoculated and untreated, T_{16}=Uninoculated and untreated, T_{17}=Mancozeb seed treatment and T_{18}= Mancozeb soil sprinkle.

The percentage of pre-emergence and post emergence damping-off were recorded weekly and calculated:

$$\text{Pre-emergence damping-off} = \frac{\text{No. of ungerminated seeds}}{\text{Total number of planted seeds}} \times \frac{100}{1}$$

$$\text{Post-emergence damping-off} = \frac{\text{No. of infected seedlings}}{\text{Total number of germinated seedlings}} \times \frac{100}{1}$$

The effect of treatment combinations on growth and yield parameters were determined according to Khiyami *et al*. (2014). Disease incidence was calculated by expressing the number of symptomatic plants as a percentage of the total plants evaluated. Disease control was calculated and expressed as a percentage:

Disease control = [1-DT/DC]$\times$ 100

Where DR = Disease reduction, DT = Disease incidence on treatment

DC = Disease incidence on control.

Disease severity rating was evaluated on a scale of 1-5 using the modified method of Adandonon *et al*. (2006):

1=No symptoms, 2=No wilting of leaves, seedlings fell on the ground after fourth day, 3=Wilting of leaves on 3rd day with plants falling to the ground on fourth day, 4=Wilting of leaves on third day with plants falling on ground same day, 5=Wilting of leaves on second day with seedlings falling to the ground same day or next day. 6=Wilting of leaves on first day with seedlings falling over on second day.

Disease severity values obtained were converted to percentages using the method os Assad *et al*. (2010):

$$\text{Disease severity} = \frac{\text{Sum of disease ratings}}{\text{Total number of ratings}} \times \frac{100}{\text{Maximum disease grade}}$$

The field experiment was conducted in 2018 to validate results obtained in the screenhouse in 2017 at the University Teaching and Research Farm, Ibadan, Nigeria. Therefore, the same treatments that were used in the screenhouse were also evaluated in the field trials. However, the experiment was conducted under natural conditions in a planting site with history of damping -off disease. Each plot was 3x3 m in area with three rows of 3m in length and 50 cm in width. The trial consisted of plots (treatments) in a randomized complete block design with three replications. Four tomato seeds were planted per hole at a spacing of 60 cm $\times$ 50 cm which were later thinned to two. All parameters that were assessed in the screenhouse experiment were also evaluated in the field. Seed-to-plant transmission of damping off disease was evaluated by planting seeds harvested from control plants in the previous screenhouse experiment that were inoculated with *P. aphanidermatum* but untreated. Seeds that were harvested from uninoculated plants served as control. Data collected included percentage pre- and, post-emergence damping-off, and seedling mortality as described above.

Plant growth parameters such as plant height, number of leaves per plant, stem diameter, fresh weight of plant, and yield were determined according to Abd-El-Khair *et al.* (2010).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the Statistical Analysis System Version 9.1, Institute (SAS institute Inc., 2002). ANOVA was made by F variance test and the pair comparisons were done by Duncan's Multiple Range Test (DMRT) at P=0.05.

Results and Discussion

The inoculation of individual biological control agent reduced radial mycelial growth of *P. aphanidermatum* to between 64.8-67.2% (Table 1). Similarly, the four extract concentrations of *A. sativum* evaluated also had inhibitory effect on growth of the test pathogen varying from 54.2-67.3% relative to the control which was neither amended with BCA nor extract with 100% growth and no inhibition. However, the efficacy of the extract increased with concentration, being most effective at 25% w/v. Generally, treatment combinations of BCAs with *A. sativum* proved to be more effective in the reduction of mycelial growth of the pathogen with inhibitory values that ranged between 77.6-91.2%. Their effects were significantly higher than those of single inoculation of individual BCA or extract against the pathogen.

Table 1. Inhibitory effect of *Allium sativum* extract combinations with BCAs on mycelia growth of *P. aphanidermatum* in-vitro

Treatment	Mycelial growth (mm)	Reduction (%)
Pathogen alone	100a	0.0c
Pathogen + *T. viride*	34.8bc	65.2bc
Pathogen + *T. harzianum*	32.8bc	67.2b
Pathogen + *B. subtilis*	39.2b	64.8bc
Pathogen + 5% *A. sativum*	45.8ab	54.2c
Pathogen + 10 % *A. sativum*	41.2b	58.8c
Pathogen + 20 % *A. sativum*	34.8bc	65.2bc
Pathogen + 25 % *A. sativum*	32.7bc	67.3b
Pathogen + *T. viride* + 5% *A. sativum*	22.4cd	77.6ab
Pathogen + *T. viride* + 10% *A. sativum*	13.5d	86.5a
Pathogen + *T. viride* + 20% *A. sativum*	11.7d	88.3a
Pathogen + *T. viride* + 25% *A. sativum*	12.3d	87.7a
Pathogen + *T. harzianum* + 5% *A. sativum*	19.1cd	80.9ab
Pathogen + *T. harzianum*+ 10% *A. sativum*	10.3de	89.7a
Pathogen + *T. harzianum*+ 20% *A. sativum*	13.2d	86.8a
Pathogen + *T. harzianum*+ 25% *A. sativum*	8.8de	91.2a
Pathogen + *B. subtilis* + 5% *A. sativum*	32.2bc	67.8b
Pathogen + *B. subtilis* +10% *A. sativum*	27.1c	72.9b
Pathogen + *B. subtilis* + 20% *A. sativum*	20.4cd	79.6ab
Pathogen + *B. subtilis s* + 25% *A. sativum*	18.1cd	81.9ab
Pathogen + Mancozeb	9.6dde	90.1a

Means with same letters along a column are not significantly different using DMRT test at P=0.05.

However, inoculation of *T. harzianum* in combination with 25% w/v *A. sativum* concentration had significantly higher inhibition of the the test pathogen than other treatments and control. In contrast, inoculation of *B. subtilis* in the dual culture was least effective among the BCAs. All the BCAs significantly ($P<0.05$) inhibited radial mycelial growth of *P. aphanidermatum* when applied either singly or in combination with varying concentrations of *A. sativum* in the *in vitro* trial compared to the control. This result was consistent with the findings of Idowu *et al.* (2016) who reported the ability of *Trichoderma* species to inhibit *Pythium* spp, causing damping-off disease in seedlings of sweet pepper variety. A zone of inhibition was observed between *B. subtilis* and the pathogen with an apparent lysis of the pathogen mycelia, which may have been due to the production of metabolites or antibiotics by the BCA (Demoz and Korsten, 2006); while *Trichoderma* spp. grew on the mycelia of the test pathogen, under microscopic examination suggesting that the mode of control by the BCAs could be hyper parasitism (Howell, 2003; Harman *et al,.* 2004). *Allium sativum* extract also reduced mycelial growth of the test pathogen, but was more effective when applied in combination with the BCAs. Its inhibitory action may be due to its active ingredient 'allicin', which enhances its potency against plant pathogens (Obagwu and Korsten, 2003).

Screenhouse and Field evaluation of single and combined application of *A. sativum* leaf extract and Biological control agents on incidence of damping-off disease

Tomato seeds that were treated with inoculum powder of either the BCA or extract alone had better inhibitory effect on the test pathogen than the control in the screenhouse experiment of 2017 and the repeated field experiment in 2018 (Table 2). Seed treatment before planting was more effective than soil sprinkle method, reducing pre-emergence damping off incidence to between 6.8-18.3% and 9.7-26.3% under screenhouse and field conditions respectively. Treatment of tomato seeds with *T. harzianum* in combination with *A. sativum* extract proved to be most effective in the single application of the BCAs with a pre and post emergence disease reduction. In contrast, the application of *B. subtilis* either as seed treatment or soil sprinkle was least effective among the BCAs in overall disease incidence, severity and control. Although single dosages of the BCAs and *A. sativum* extract significantly ($P<0.05$) reduced damping-off disease, better results were obtained with the treatment combinations. Seeds that were treated with a mixture of BCA and *A. sativum* extract significantly ($P<0.05$) reduced disease incidence relative to single treatments and compared well to mancozeb fungicide.

Table 2. Effect of *Allium sativum* extract combinations with BCAs on pre and post emergence damping- off in the screenhouse and field conditions

Treatment	Pre-emergence				Post emergence					
	Disease Incidence %		Disease control (%)		Disease incidence %		Disease severity (%)		Disease control (%)	
	Screenhouse	Field	Screenhouse	Field	Screenhouse	Field	Screenhouse	Field	Screenhouse	Field
Trichoderma viride seed treatment	9.0b	14.8bc	67.9bc	53.8bc	22.2ab	23.8ab	48.0bc	24.0bc	72.8b	76.1ab
Trichoderma viride Soil sprinkle	7.3bc	1.5bc	55.2bc	65.8b	26.1b	33.4b	42.0bc	32.0bc	71.6b	65.5bc
Trichoderma harzianum seed treatment	12.1b	11.5bc	77.1b	74.4ab	25.7b	31.7cd	42.0bc	54.0b	81.7ab	75.2ab
Trichoderma harzianum soil sprinkle	11.1b	15.0bc	73.6b	58.2bc	26.1b	20.7cd	34.0c	46.0b	80.2ab	73.1b
B. subtilis seed treatment	16.7ab	22.2b	54.8bc	54.1bc	33.8b	49.4ab	74.0ab	74.0ab	63.1bc	62.5bc
B. subtilis soil sprinkle	18.3ab	18.8b	51.1bc	58.1bc	21.5ab	47.1bc	76.0ab	81.0a	54.7c	56.4c
A. sativum seed treatment	15.3ab	22.3b	51.7bc	47.6bc	34.3ab	47.2b	68.0ab	62.0ab	49.6c	50.2c
A. sativum soil sprinkle	15.1ab	26.3ab	53.6bc	50.7bc	28.3ab	31.3bc	62.0b	50.0b	53.1c	51.8c
T. viride seed treatment + 25% *A. sativum*	10.2b	9.7c	82.8ab	72.1ab	28.2cd	17.6c	46.0bc	44.0b	88.8a	80.6ab
T. viride soil sprinkle treatment + 25% *A. sativum*	9.2b	11.8bc	75.7b	78.5a	22.4b	21.5c	32.0c	46.0b	90.1a	81.2ab
T. harzianum seed treatment + 25% *A. sativum*	6.8bc	19.3c	88.3a	80.0a	19.7b	20.4cd	26.0c	28.0bc	93.7a	81.6ab
T. harzianum Soil sprinkle + 25% *A. sativum*	4.3bc	7.7c	64.8bc	71.1ab	21.2b	23.7cd	32.0c	54.0b	80.3ab	77.1ab
B. subtilis seed treatment + 25% *A. sativum*	14.8ab	25.3ab	63.4bc	33.2c	42.8ab	46.1b	62.0b	72.0ab	59.7c	54.2c
B. subtilis Soil sprinkle + 25% *A. sativum*	13.1ab	18.4b	67.7bc	24.8cd	43.3ab	49.2ab	74.0ab	70.0ab	53.4	66.3bc
Inoculated and untreated	72.7a	78.3a	0.0c	0.0d	78.8a	81.7a	84.0a	92.0a	0.0cd	0.0cd
Uninoculated and untreated	0.0c	10.6c	0.0c	0.0d	0.0d	22.1bc	20.0cd	30.0bc	0.0cd	0.0cd
Mancozeb seed treatment	5.1bc	9.2cd	70.4b	72.8ab	17.5b	11.2cd	44.0bc	26.0bc	86.7a	83.2ab
Mancozeb soil sprinkle	4.1bc	8.2c	87.4a	78.3a	9.5b	14.2c	58.0b	28.0bc	88.4.7a	87.2a

Means with same letters along a column are not significantly different using DMRT test at P=0.05.

These results agree with the earlier report of Adanonon *et al.* (2006) that found seed treatment with BCAs to be more effective than using the soil drench method in the control of soil-bone *Sclerotium rolfsii*. Also, *Trichoderma* spp. used as seed treatment was reported to significantly reduce the incidence of damping-off disease than other formulation methods (Idowu *et al.*, 2016). Disease incidence was apparently higher under field conditions than in the screenhouse. The better disease control achieved in the screenhouse may be due to the asepetic condition that was maintained in the screenhouse which included sterilization of soil that was used in planting and the controlled environment. On the contrary, the environmental conditions could not be regulated in the field, where planting was also done without soil sterilization under natural conditions. However, under field conditions, it was observed that some of the uninoculated and untreated plants were symptomatic, although with a comparatively lower disease incidence. Disease incidence and severity were significantly (P<0.05) lower in case of treatment combinations than single treatment dosage and untreated control. The application of the treatment combination as seed treatment was generally more effective than the sprinkle method in reducing both incidence and of disease severity. Seed treatment with *T. harzianum* in combination with *A. sativum* extract had significantly (P<0.05 lower incidence when compared to other treatments and the untreated control.

Trichoderma harzianum when applied in combination with 25% w/v concentration of *A. sativum* extract was most effective in reducing damping-off incidence, severity and control. Several reports had corroborated the efficacy of *T. harzianum* in the control of soil-borne pathogens (Howell 2003; Manjula *et al.,* 2005; Kumari *et al.,* 2015). In contrast, *Bacillus subtilis* was least effective among the BCAs in disease control both in the screenhouse and field. While *Trichoderma* spp. are fortified with several methods of pathogen control such as rapid competition and colonization of substrate, hyperparasitism and production of lytic enzymes, *B. subtilis* rely mainly on the mechanism of antibiosis (Dania *et al.,* 2016).

All treatments and their combinations significantly (P<0.05) influenced plant height and stem diameter both in the screenhouse and field trials (Table 3). However, tomato plants that were planted under field conditions had significantly higher shoot weight, number of leaves and fruit yield than those grown in the screenhouse. These might be due to the inability of the tomato plant roots in the screenhouse to expand fully due to the reduced space in each experimental pot, hence limited access to nutrients which ultimately influenced the overall yield compared to those that were planted in the open and unrestricted field (Muriungi *et al.*, 2014).

Table 3. Effect of *Allium sativum* extract combinations with BCAs on growth and yield parameters of tomato in screenhouse and field conditions

Treatment	Plant height (cm)		Number of leaves		Stem diameter (cm)		Fresh shoot wt (g)		Yield (kg ha⁻¹)	
	Screenhouse	Field	Screenhouse	Field	Screen house	Field	Screenhouse	Field	Screenhouse	Field
T. viride seed treatment	63.2bc	67.2ab	98.2b	106.4bc	0.60a	0.61a	94.4d	111.7d	722.5f	731.1e
T. viride Soil sprinkle	71.1ab	68.2ab	107.3a	101.3cd	0.63a	0.72a	105.2bc	101.2de	692.2g	852.5b
T. harzianum seed treatment	64.3bc	68.6ab	85.1c	117.2ab	0.65a	0.64a	98.4cd	128.4cd	801.7c	790.6d
T. harzianum soil sprinkle	64.8bc	72.8ac	98.0b	114.5b	0.61a	0.58ab	103.5c	153.5b	775.4de	802.1cd
B. subtilis seed treatment	68.4b	64.0bc	96.1b	104.3c	0.38b	0.56ab	112.3ab	182.3a	784.2d	807.5c
B. subtilis soil sprinkle	73.0ab	75.1a	104.2ab	100.2cd	0.49ab	0.54ab	101.5c	151.5bc	774.5de	781.2de
A. sativum seed treatment	63.2bc	60.8b	98.1b	109.4bc	0.72a	0.69a	123.9a	143.9bc	770.6e	806.7c
A. sativum soil sprinkle	66.1b	64.6b	103.2ab	101.5cd	0.55ab	0.61ab	120.8a	180.8a	801.2c	867.4ab
T. viride seed treatment + 25% *A. sativum*	63.7bc	68.9sb	90.8bc	107.4bc	0.52ab	0.54ab	107.1b	142.1bc	831.2b	777.7de
T. viride soil sprinkle treatment + 25% *A. sativum*	77.1a	75.2a	92.6bc	111.2b	0.61a	0.68a	114.3ab	164.1b	820.7bc	851.3b
T. harzianum seed treatment + 25% *A. sativum*	64.5bc	66.8b	103.9ab	118.4ab	0.64a	0.67a	109.7b	177.3ab	886.1a	688.5ef
T. harzianum soil sprinkle + 25% *A. sativum*	68.2b	70.6ab	96.6b	108.8bc	0.60a	0.58ab	101.7c	126.4cd	882.3ab	902.2a
B. subtilis seed treatment + 25% *A. sativum*	63.3bc	67.1ab	94.3bc	117.6ab	0.52ab	0.57ab	108.3b	103.8de	770.3e	707.5ef
B. subtilis Soil sprinkle + 25% *A. sativum*	62.7bc	65.5b	102.4ab	122.2a	0.58ab	0.65a	107.8b	114.2d	788.8d	835.6bc
Inoculated and untreated	66.2b	69.9ab	103.2ab	100.1cd	0.63a	0.78a	115.2ab	132.2c	603.3g	634.1ef
Uninoculated and untreated	66.1b	65.7b	97.0b	113.3b	0.43b	0.56ab	100.7c	114.7d	732.2ef	808.4c
Mancozeb seed treatment	66.3b	65.2b	105.5ab	112.7b	0.57ab	0.55ab	120.9a	150.9b	702.5fg	865.4ab
Mancozeb soil sprinkle	74.3ab	69.3ab	109.5a	117.7ab	0.63ab	0.58ab	122.9a	148.9bc	794.6d	728.4e

Means with same letters along a column are not significantly different using DMRT test at P=0.05.

Table 4. Effect of treatments on percent incidence of seed-to-plant transmission of damping-off disease in seedlings grown from infected tomato seeds

Treatment	Screen house trial in 2017					Field trial in 2018				
	7WAS	14DAS	21DAS	28DAS	Mortality (%)	7WAS	14DAS	21DAS	28DAS	Mortality (%)
Trichoderma viride seed treatment	3.8b	7.0ab	8.9b	13.1ab	7.3b	4.1bc	9.2b	10.7	11.3bc	9.2b
Trichoderma viride soil sprinkle	5.1b	10.1ab	10.2b	12.3ab	7.1b	3.5bc	8.1b	12.1b	12.8bc	7.4bc
Trichoderma harzianum seed treatment	3.2b	4.9b	9.3b	8.8b	6.7b	6.1b	8.4b	7.7bc	10.4c	7.7bc
Trichoderma harzianum soil sprinkle	3.4b	6.3ab	8.4b	9.7b	5.4b	10.8ab	7.8b	9.5b	11.8bc	8.6b
Bacillus subtilis seed treatment	7.5ab	9.5ab	12.5ab	14.2ab	8.2ab	4.8b	10.5ab	11.2b	13.4bc	13.6ab
Bacillus subtilis soil sprinkle	6.1b	12.3ab	14.6ab	15.8ab	10.1ab	7.7b	10.2ab	14.2ab	16.1b	14.3ab
Allium sativum seed treatment	4.1b	9.4ab	10.8b	15.0ab	9.5ab	6.8b	8.4b	10.1b	14.8b	11.3ab
Allium sativum soil sprinkle	6.2b	8.2ab	12.1ab	17.3ab	13.3ab	5.8b	11.2ab	14.4ab	17.4b	11.8ab
T. viride seed treatment + 25% *A. sativum*	2.5b	3.5b	8.1b	9.9b	7.1b	3.7bc	7.3b	10.5b	12.4bc	8.1b
T. viride soil sprinkle +25% *A. sativum*	1.7bc	4.0b	7.7b	13.3ab	6.3b	3.4bc	5.9bc	9.9b	10.7c	7.4bc
T. harzianum seed treatment + 25% *A. sativum*	2.9b	3.8b	6.5b	7.9b	3.7b	5.7b	8.8b	11.8b	14.0b	9.3b
T. harzianum soil sprinkle + 25% *A. sativum*	6.6b	7.8ab	9.2b	10.6b	10.44ab	6.3b	6.0bc	8.7bc	12.6bc	9.5b
B. subtilis seed treatment + 25% *A. sativum*	6.0b	10.4ab	15.3ab	16.9ab	9.2ab	8.8ab	12.6ab	12.0b	20.2ab	10.8b
B. subtilis soil sprinkle + 25% *A. sativum*	7.9ab	11.2ab	18.2ab	18.8ab	11.6ab	8.1ab	13.9ab	16.6ab	17.5b	12.3ab
Infected and untreated	12.7a	26.1a	47.6a	77.4a	36.8a	15.1a	38.6a	53.5a	83.3a	68.7a
Healthy and untreated	0.0b	0.0b	0.0c	0.0bc	0.0bc	3.7bc	4.8bc	5.7bc	10.2c	10.8b
Mancozeb seed treatment	2.3b	3.5b	4.1bc	7.7b	3.0b	4.3bc	5.4bc	5.9bc	5.9cd	8.4b
Mancozeb soil sprinkle	6.2b	4.1b	7.4b	5.9b	4.0b	6.3b	7.0b	8.5 bc	12.2bc	10.1ab

DAS= Days after sowing. Means with same letters along a column are not significantly different using DMRT test at P=0.05.

Soil sprinkle with *T. harzianum* in combination with *A. sativum* extract had the highest cumulative tomato yield of 902 kg/ha^{-1} under field conditions. Fruit yield was significantly higher in plots that were treated with either BCAs or extract alone and their combinations than those of inoculated but untreated that served as control. The inherent ability of *Trichoderma harzianum* to reduce disease incidence of several pathogens by stimulating vegetative growth and enhancing root development and overall yield of the treated plants were also reported (Harman *et al*., 2004; Singh and Singh, 2004).

The application of treatments reduced the incidence of seed-to-plant transmission of damping-off disease among seeds that were inoculated before planting (Table 4). Seed-to-plant transmission was reduced to between 7.9-18.8% and 10.2-20.2% in screenhouse and field trials respectively relative to control. Similarly, the treatment combinations reduced seedling mortality better than individual application at 28 days after inoculation.

Disease incidence was significantly lower among plants that were inoculated and treated with combinations of BCAs and *A. sativum* extract than control plants in both trials. Also, seedling mortality was significantly reduced (P<0.05) among the treatments compared to control. However, seedling mortality was comparatively higher under field conditions than the screenhouse. Healthy seeds that were uninoculated and untreated were asymptomatic in the screenhouse; however, some of the emerging seedlings became symptomatic under field condition.The combination of biological control agents with *Allium sativum* extract significantly reduced the incidence, severity and seedling mortality caused by damping-off disease. Therefore, the treatment combination as shown in this study could be explored to reduce annual seedlings and yield losses due to the damping-off disease among tomato farmers in Nigeria.

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INCIDENCE OF RHIZOME ROT OF GINGER IN SOME SELECTED AREAS OF BANGLADESH AND THE CAUSAL PATHOGENS ASSOCIATED WITH THE DISEASE

M. M. Islam[1], F. Khatun[2], M. I. Faruk[3]
M. M. Rahman[4] and M. A. Hossain[5]

Abstract

Ginger (*Zingiber officinale* L.) belongs to family Zingiberaceae is an important oriental spice crop. Ginger is high value crop which grows well in warm and humid climate and is cultivated from sea level to an altitude of 1500 meters above sea level (Kandinnan, *et al.* 1996). It has special significance for tropical countries where it is produced and consumed in large quantities (Islam, 2017; BARI, 2012, 2013 and 2014). The aromatic rhizomes are used as spice and medicine. Major producers of ginger in the world are India, Jamaica, Sierra Leone, Nigeria, China, Japan, Taiwan and Australia (Rana and Sharma, 1998). Ginger is much more used in Bangladesh as a spice and is cultivated more or less all over the country. In the country produced only 74380 metric tons of ginger from 9120 ha of land and the yield per hectare was 8.15 kg (BBS, 2011). But average yield is low as compared to other ginger growing countries of the world. The production is not enough to fulfill the annual requirement of the country. So every year a good amount of ginger is imported in exchange of foreign currency. Like many countries diseases are the major limiting factors for ginger cultivation in Bangladesh. Among the diseases, rhizome rot is the most devastating one caused by *Pythium aphanidermatum*, *Fusarium oxysporum*, *Sclerotium rolfsii* and *Ralstonia solanacearum* throughout the world (Chauhan and Patel, 1990; Dohroo *et al.* 1987 and Iyer, 1987). The pathogens involved decide the nature of the damage and symptom expression .The major pathogens involved with rhizome rot are viz., species of *Pythium* causing soft rot, *Fusarium* spp. causing yellows or wilt and *Ralstonia solanacearum* causing bacterial wilt (Elliot, 2003). Basal rot caused by *Sclerotium rolfsii* which appears later in the season in some cases. All these pathogens are known to form complexes with nematodes leading to synergistic effect on the severity of the disease. They predispose the crops to secondary pathogens (Sarma, 1994).Loss due to rhizome rot is estimated in many countries and the main pathogens associated with rhizome rot are the fungi such as *Pythium* spp. and *Fusarium* spp, bacteria like *Ralstonia solanacearum* and nematode (Elliot, 2003). It may cause losses to the extent of 50% or more due to soft rot ((Islam, 2017; BARI, 2012, 2013 and 2014; Joshi and Sharma, 1982) and sometimes total failure of the crops in the tropical regions of India (Fageria *et al*, 2006); 70% rhizome

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[1,3&4]Senior Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, [2]Chief Scientific Officer, Plant Pathology Division, BARI, Gazipur, [5]Chief Scientific Officer & Head, Farm Division, BARI, Gazipur, Bangladesh.

production is reduced due to the infection caused by *Pythium* spp. and *Fusarium* spp. in Nepal (Anon., 2012) and 5-30% losses occurred in Fiji and Australia by *Pythium myriotylum* (Stirling *et al.*, 2009). Soil, water and infected planting materials are the main source of perpetuation of these pathogens (Dohroo, 2005). *Pythium* spp. is able to persist in soil over decay by means of encysted zoospores, oospores and sporangia. *Pythium* spp also can survive in air dry muck soil for up to 12 years (Hoppe, 1966). *Pythium* spread via infested rhizomes (Trujillo, 1964) and as oospores surviving in debris in the soil (Dohroo, 2005). Infection started from contaminated planting materials, saprophytically living fungus in the soil or on trash of previous ginger crops. The soft rot pathogen generally have quite a wide host range and can survive on other host plants so this makes it difficult to control in the field. In the past *Pythium aphanidermatum* was known as the sole causal agent of rhizome rot of ginger and was successfully controlled by the technology developed by Plant Pathology Division, BARI. But during the last few years that technology was not working well and it was noticed that some other fungal and bacterial association were involved with such rot. As the crop is cash crop so it is prime need to identify the causal agents of rhizome rot through intensive survey which will help to modify the existing technology for managing the disease in future. The present research work was undertaken to determine the incidence of rhizome rot of ginger and its causal agents.

A survey program was conducted in five districts namely Nilphamari, Rangpur, Bogra, Tangail and Khagrachari with a view to survey the incidence of rhizome rot during 2013-14. A total of 1156 farmer's field representing two upazilla from each district and at least 10 ginger fields from each upazilla were surveyed. The locations of survey were Upazilla of Sadar and Sayedpur of Nilphamari district, Sadar and Gangachara upazilla of Rangpur, Shibgong and Gabtali upazilla of Bogra, Madhupur and Ghatail upazilla of Tangail and Sadar and Dighinala upazilla of Khagrachari. Incidence of rhizome rot was estimated from each field following the formula of Sagar *et al*. (2008).

$$\text{Percent Disease Incidence} = \frac{\text{Number of diseased plants counted}}{\text{Total number of plants counted}} \times 100$$

All samples were brought to laboratory and then tested in different media viz. V_8 juice agar, Penta-chloro nitro-benzene (PCNB), Potato dextrose agar (PDA), nutrient agar and Tetrazolium Chloride (TZC) for isolation of fungus and bacteria.

Individual infected specimen of ginger rhizomes were washed with plain water and cut into small pieces of 1-1.5 cm^2. The pieces were surface disinfected with 5 % chlorox for 1 min. Four pieces of pseudostems and rhizomes from each sample are placed in Petri plates containing V8 juice agar (Tsao and Ocane 1969) for Pythium, PCNB medium for *Fusarium* spp. (Nash and Snyder,

1962) and PDA for other fungi. The inoculated plates were incubated at 26^0C. The V8 juice agar plates were incubated in the dark for 96 hours. The hyphal tips of fungi growing from those tissues samples were transferred to PDA slants for maintenance.

Pieces of infected pseudostems were placed in a small quantity of sterile distilled water for 20 min to allow bacteria to ooze out. A loop full of bacterial suspension was streaked on to TZC agar plates and the plates were incubated at 28^0C (Kelma, 1954). Fluidal individual colony transferred to Casamino Acid-Peptone Glucose Agar (CPG) and incubated for 48 hours; then again transferred to CPG slants and stored in sterile distilled water.

Pathogenicity of the isolates of *Pythium, Fusarium, Sclerotium* and *Ralstonia* was performed in the pothouse following standard protocol (Kelma, 1954). The ginger sprouts were raised by planting 30g bits of seed rhizome in sterilized potting mixture with soil, sand and cowdung in 3:1:1 ratio. Forty five days old plants were used for inoculation and a control treatment without inoculation was maintained. Isolates of *Pythium* was grown on V8 juice agar. *Fusarium* and *Sclerotium* isolates were grown separately on potato dextrose agar. *Ralstonia* was grown on CPG medium. Seven days old cultures of *Pythium, Fusarium* and *Sclerotium* were harvested by washing mycelial mass with oospores/conidia and then suspended in 100 ml of tap water and maintained by a magnetic stirrer. The mycelia/spore suspension was added to the bags around the hypocotyls at the rate of 50 ml per bag. A 24 h old bacterial culture was harvested with a cotton swab and applied at the base of ginger plants by making injury to the roots of ginger plants in pots.

Observations were made every alternate day regarding development of rhizome rot and wilt symptoms of ginger. After the development of the typical symptoms of the disease, the pathogens were re-isolated and compared with original culture to prove the pathogenicity. Fungi under the genus *Pythium aphanidermatum* were identified with the use of keys of Hendrix and Papa (1974), and *Fusarium oxysporum* and *Sclerotium rolfsii* with Booth's key and for bacterium (Jenkins and Kelma, 1976). Incidence of *Ralstonia solanacearum* from pilai (mother seed rhizome) of ginger at different locations viz. Ramgarh, Tangail, Gazipur, Bogra, Khagrachari and Nilphamari during 2013-14 cropping season were also recorded.

The characteristic initial symptom of rhizome rot appearance was yellowing of leaves and in the early stages, a few tillers show symptom of yellowing. The yellowing symptom later spread to all the tillers and leaves become drooping, withering and drying. The infected shoot could be easily pulled out from the soil. The rhizome becomes water-soaked and mushy appearance (Plate 1).

Plate 1. (a) & (b) Plant, rhizome infected by *Pythium*, (c) tissue disintegration

Rhizome rot was noticed in all the locations with a range of 34.50% to 78.0% incidence ((Fig.1). The maximum disease incidence of 78.00% was recorded from Tangail followed by Nilphamari (46.70%), Rangpur (38.00%) Khagrachari (35.50%) and Bogura (34.50%). The information indicated that the disease incidence varied from locations to locations. The present findings are in agreement with Sagar *et al*. (2008). The authors stated that in Shimoga district, Karnataka of India the rhizome rot incidence was maximum 23.70% followed by Kadagu (22.90%), Kannada (20.24%) and Bidar (13.00%) and the considered all the locations as 'hot spot' of rhizome rot disease of ginger.

Three fungal pathogens viz. *Pythium aphanidermatum, Fusarium oxysporum* and *Sclerotium rolfsii*; and a bacterium *Ralstonia solanacearum* were found to be associated with ginger (Plate 2). *Pythium aphanidermatum* found to be most predominant pathogen among the fungi.
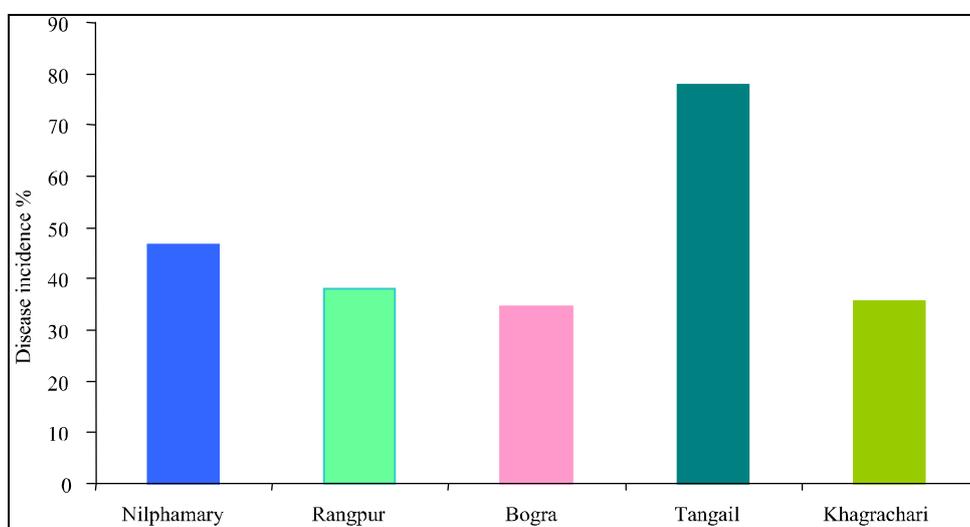
Fig.1: Incidence of rhizome rot disease of ginger in different districts

Next predominant pathogen was *Fusarium oxysporum*; *Sclerotium rolfsii* and *Ralstonia solanacearum*. Results of the present study indicate that several pathogens were involved to cause rhizome rot of zinger. *Pythium aphanidermatum* and *P. myriotylum*; *Fusarium oxysporum* f.sp. *zingiber* and *Ralstonia solanacearum* were also reported from Kerala, India during a survey of rhizome rot of ginger in 1984 and 1985 (Dake and Edison, 1989).

Table 1 Pervalence of pathogens of rhizome rot of ginger collected from five different districts of Bangladesh during 2013-2014.

Locations/Districts	*Pythium aphanidermatum* (%)	*Fusarium oxysporum* (%)	*Sclerotium rolfsii* (%)	*Ralstonia solanacearum* (%)
Nilphamari	41	25	14	12
Rangpur	35	25	9	15
Bogra	44	19	16	11
Tangail	46	26	11	8
Khagrachari	52	15	23	7

It found that *Pythium aphanidermatum* caused maximum rhizome rot incidence in Khagrachari (52%), followed by Tangail (46%), Nilphamari (41%) and Bogura (44%) (Table 1). The pathogen caused the lowest infection in Rangpur (35%). The highest (26%) infection of *Fusarium oxysporum* was observed in Tangail followed by Nilphamari (25%) and Rampur (25%) while the lowest infection was found in Khagrachari and Bogura. The incidence of *Sclerotium rolfsii* varied from 9-23% rhizomes in five districts. The pathogen caused the highest 23% infection in Khagrachari while only 9% rhizome infection was observed in Rangpur. The bacterium *Ralstonia solanacearum* was also found in some collected ginger samples. The highest 15% association of bacteria was found in Rangpur followed by 12% in Nilphamari and 11% in Bogra. The bacterial incidence was the lowest 7% in Khagrachari might be due to non-stagnant water in the field as compared to the plain land during rainy season. Results of the present findings show sharp variation of percentages of organisms in different locations. In a survey report made by Dake and Edison (1989) stated that 19.79% samples yielded *Pythium aphanidermatum* and *P. myriotylum*; 6.6% gave *Fusarium oxysporum* f.sp. *zingiber*i and 26.71% had *Ralstonia solanacearum* during a survey for rhizome rot of ginger in Kerala.

Symptom became apparent after 6 days of inoculation at 28^0C in case of *Ralstonia solanacearum* where as it take 10 days to develop symptom on inoculated plant with *Pythium aphanidermatum* and *Fusarium oxysporum.* The symptoms exhibited wilting by the inoculated fungi and bacteria. Leaf margins with drooping symptoms were noticed five days after inoculation in case of *R. solanacearum*. Yellowing started from the inoculated leaf and progressed upward until all leaves gave a golden yellow appearance. The bacterium was present in

infected pseudostem and rhizome, whereas in plants inoculated with *Fusarium oxysporum*; they became pale yellow followed by yellowing of the tips and then the infection spread along with the leaf margin and pseudostem became brown at collar region. The conspicuous symptoms exhibited by plants inoculated with *Fusarium oxysporum* were yellowing of leaves followed by drooping and drying of leaves and whole plants. The discoloration was also noticed at the hypocotyl portion of pseudostem.
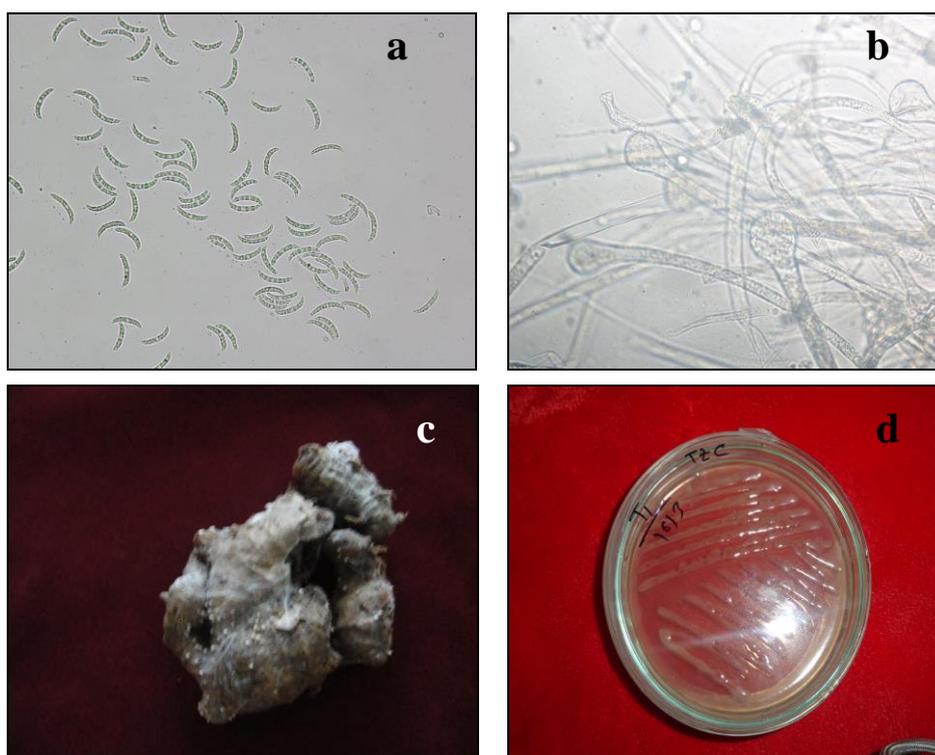

Plate 2. (a) *Fusarium oxysporum* **(b)** *Pythium aphanidermatum* **(c)** *Sclerotium rolfsii* **and (d)** *Ralstonia solanacearum.*

The incidence of *Ralstonia solanacearum,* the wilt causing bacterium from mother seed rhizome of ginger at different locations ranges from 40-67% (Table 2). The highest infection 67% was found in Tangail followed by 55% in Nilphamri and the lowest (40%) incidence recorded in Gazipur preceded by 45% at Bogra during 2013-14 cropping season (Table 2). Disease survey showed that 46.7, 38, 34.5, 78 and 35.5% disease incidence was recorded in Nilphamari, Rangpur, Bogura, Tangail and Khagrachari, respectively. Findings of the present investigation show that causal pathogens of rhizome rot of ginger were *Pythium aphanidermatum, Fusarium oxysporum, Sclerotium rolfsii* and *Ralstonia solanacearum* in Bangladesh. These are involved in developing rhizome rot disease complex.

Table 2. Incidence of *Ralstonia solanacearum* from mother seed rhizome of ginger at different locations during 2013-14 cropping season

Location	Seed rhizome investigated	Seed rhizome infected by *Ralstonia solanacearum*	Incidence (%)
Khagrachari	100	53	53.0
Tangail	100	67	67.0
Gazipur	100	40	40.0
Bogra	100	45	45.0
Rangpur	100	52	52.0
Nilphamari	100	55	55.0

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