Efficacy of Monosodium Glutamate Against Larvae of Culex Quiquefasciatus Say (1823) (Diptera: Culicidae)

D. Akter[1], H. R. Khan[1], M. M. Rahman[2] and M. Begum*[1]

[1]*Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh*
[2]*Bangladesh Atomic Energy Commission*

Abstract

The present experiment was done with 0.00, 0.01, 0.02, 0.03, 0.04 and 0.05 g/ml Monosodium Glutamate (MSG) solution (in 0.35mg of average body weight of larvae) against laboratory reared 4th instar larvae of *Cx. quinquefasciatus*. Mortalities of the larvae were recorded after 48 hours of application. Histo-morphological changes were observed in the untreated and treated larvae. Mean percentage of mortalities were subjected to one way ANOVA and multiple comparisons. Dose response curve were also produced by regression analysis. The highest (93.33%) and the lowest (7.22%) mortalities were recorded at dose 0.05 g/ml and 0.01 g/ml, respectively. Mean mortalities among different doses of MSG were found to be significantly ($p<0.05$) different. Histological study revealed that the tissue disruption in the stomodeal valve and the peritrophic membrane of anterior mid gut at 0.01 g/ml dose of MSG. The 0.3 and 0.5g/ml dose of the MSG caused the tissue destruction of the microvilli and muscular sheath of the rectal pad situated in the hindgut which indicates the major alteration of hind gut has been found at 0.05 g/ml dose of MSG. In untreated of MSG larvae, no such abnormalities were observed.

Keywords: *Culex quiquefasciatus*, monosodium glutamate, food additives, histology

1. Introduction

The various food additives and chemicals are used as preservatives or enhancer of palatability of food. Monosodium glutamate, one of the most common food additive largely consumed since 1909, has been doubted these last twenty years to potential adverse impact on health [19, 12]. As a food additive, it provides a flavoring function through the stimulation of organo sensory receptors and makes the food appetizingwhen added in the proper concentration [17]. MSG elicits a taste described in Japanese as umami, which is translated to ‘‘savory’’ and it is different from four basic taste (sweet, sour, salty and bitter) [22]. It is the naturally occurring amino acid containing 78% of glutamic acid 22% of sodium and water which is most used in India, China, Japan, Thailand,Vietnam and some other tropical countries including Bangladesh where it is generally known as testing salt [4]. MSG is currently found in thousands of diff erent processed foods, including soups, salad dressings, mayonnaise, canned vegetables and frozen dishes [4]. In 1991, the average intake of MSG in United Kingdom was 580 mg/day for general population individual and 4.68 g/day for extreme users [21]. The assessed average daily MSG intake per person in industrialized countries is 0.3–1.0 g, though it depends on the MSG content in foods and an individual’s taste preferences [13]. A typical Chinese restaurant meal contains between 10 and 1500 mg of MSG per 100 g [12]. Asia was responsible for approximately 88 percent of world MSG consumption, with China alone accounting for 55 percent of world consumption and approximately 65 percent of global production [2].

In spite of being flavor enhancer, different studies indicate that MSG is toxic to human and experimental animals [10]. In a review studies on animal and human, “MSG effects on central nervous system, adipose tissue, liver, reproductive organs and other systems have been found” [14]. An adverse reaction to MSG on nervous system, killing brain cells, causing retinal degeneration, endocrine disorder and other pathological symptoms have been reported [15]. Researches indicate potential adverse effects of glutamate may be due to its rapid assimilation and the resulting quick and large increase of glutamic acid in the organism. Histological studies have been showed that monosodium glutamate (MSG) has adverse effect on kidney of mammals (e.g. Adult Wister Rats) [10]. Besides these, monosodium glutamate also induced damage of stomach, testis, liver and kidney of mammals [20]. It has been claimed that MSG has physiological effects in both vertebrates and invertebrates [20]. A report showed that repeated use of MSG is responsible for constriction of Mulpighian tubules of *Lucilia cuprina* [4].That means long time use of MSG as a flavoring agent can cause tissue damage of any internal organ, which ultimate result is early death (4). Repeated application of MSG through ingestion instigated inhibition of ovarian development of *Chrysomya megacephala* [5]. MSG as a food additive increases the consumption of meat food of ants and largely decreases their precision of reaction, response to pheromones, cognition as well as their learning and memorization abilities [8]. Most biological processes are similar for all animals including humans (i.e. genetics, metabolism, nervous cells functioning). Besides vertebrates, invertebrates are used more because of being offered advantages to scientists such as a short life cycle [23]. Insects have similarities with the higher animals, in many cases in structure and physiological functions [23]. Present study on toxic effects of monosodium glutamate has initiated to investigate the efficacy of the stated material on *Cx. Quinquefasciatus* Say,which was used as a biological model.

2. Materials and Method

The experiment was carried out between May, 2016 and July, 2017 in the laboratory of Entomology, Department of Zoology, University of Dhaka in an ambient laboratory

Corresponding author: murshida.begum@du.ac.bd

condition (28 ± 2ºC temperature and 70-80% Relative Humidity).

2.1 Development of mosquito generations in the laboratory

To make sure constant supply of mosquito larvae for the experiment, mosquito rearing started after collecting larvae from their natural breeding places. By separating the larvae in different bowls with cleaned tap water, were provided routinely with baby cereal powder (Nestle Bangladesh), yeast etc. After 6-8 days larvae were moulted into a non-feeding pupal stage. To avoid movement a fixed numbers of pupae were kept in different bowls within adult rearing cages which were made by an iron rod frame (size: 30x30x30 cm). The cages were covered with mesh mosquito net and basal part of that cage made of wood. The pulpal period were lasted for about 2 to 3 days. Adult mosquitoes were provided with 10% glucose solution soaked cotton ball in a petridish as their food for first 2-3 days. Adult females were fed pigeon blood for oocyte development. Large numbers of eggs were observed in a raft by a stereomicroscope (Meiji SKT-3BT) [3]. The egg rafts were collected with the help of a spoon with water carefully and were transferred into several earthen bowls containing tap water to get emergenceof subsequently 1st, 2nd, 3rd and 4th instar larvae. It took one months in summer and one and half months in winter to establish a generations of mosquito for this experiment. The reared 4th instar larvae were used to determine the efficacy of monosodium glutamate against *Cx. quinquafasciatus.*

2.2 Treatment with MSG and mortality tests

Crude Monosodium Glutamate was added with fixed amount of tap water to prepare five doses (0.01, 0.02, 0.03, 0.04 and 0.05 g/ml). A number of 20 actively swimming larvae of 4th instar were taken into conical flask (250 ml) containing 100 ml different doses of MSG solution diluted in tape water. The flasks were stored at room temperature (28±2ºC) and 70-80% of relative humidity and at 12L: 12D (Photoperiod). A control was selected in which only tap water provided with larvae for each dose. The mortality of larvae in each concentration was recorded after 48 hours of exposure and the waning larvae were counted as dead and the mortality values were calculated. All experiments were replicated nine times. The percentage of mortality was calculated by using following formula-

Percentage of mortality

$$=\frac{Number\ of\ died\ in\ a\ test}{Number\ of\ used\ in\ test}\times 100$$

Test mortality records were corrected with the Abbott's formula, whenever there was some control mortality in a test. Abbott's correction formula [1]-

Corrected Mortality

$$=\frac{Mortality\ in\ treatment-Mortality\ in\ control}{100-Control\ mortality}\times 100$$

Histological procedure: Histological slides of MSG treated and untreated 4th instar larvae of *Cx. Quiquefasciatus* were prepared by transverse sectioning the tissues from the head to tail region. Ethanol, Myer's albumin and Xyline were used as fixatives. Serial transverse sections of the tissues were cut at 0.4 µm thickness with the help of a rotary microtome machine (model 08–260–02, ERMA INC, Japan). The tissue sections were stained with eosin and Heidenhein's haematoxylene in the laboratory condition. Histo-pathological observations were performed with the help of a compound microscope (Humascope Classic 2006/95/EC). Photographs were taken by a Canoon powershot S200 camera (16 mega pixel).

2.3 Statistical analyses

The data were reported as arithmetic mean ± Standard deviation (SD). One way of ANOVA and Multiple Range Test was applied on the data to assess the treatment effect [6, 7]. Dose response curve were prepared by regression analysis. All the statistical analyses were done on a computer using statistical software package for SPSS.

3. Results and Discussion

Different doses (0.00, 0.01, 0.02, 0.03, 0.04, and 0.05g/ml) of monosodium glutamate (in 0.35mg of average body weight of larvae) were used to study the efficacy of monosodium glutamate against 4th instar larvae of *Cx. quinquefasciatus* Say. Results of the mortality tests and hist-pathological observations are stated below.

3.1 Mortality of treated Culex quinquefasciatus (Say)

The mortalities of the treated 4th instar larvae of the *Cx. quinquefasciatus* after 48 hours exposure were presented in Table 1. The result of mortality test from these table showed that mortality was increasing along with increasing the doses of monosodium glutamate. Results indicated that the highest percent (93.33%) of mortality was taking place at 0.05 g/ml dose, while the lowest percent (7.22%) of mortality was observed at 0.01 g/ml. No larval mortality of the mosquito was observed in control treatment. The results of the one way ANOVA and multiple range test showed that mean mortalities were significantly different from each other ($p<0.05$) ($F = 431.143$). A dose response curve were produced from the regression analysis also expressed that increasing of the doses of MSG increased the mortality rate of 4th instar larval *Cx. quinquefasciatus* Say and decreased the mean percent of live larvae (Fig. 1).

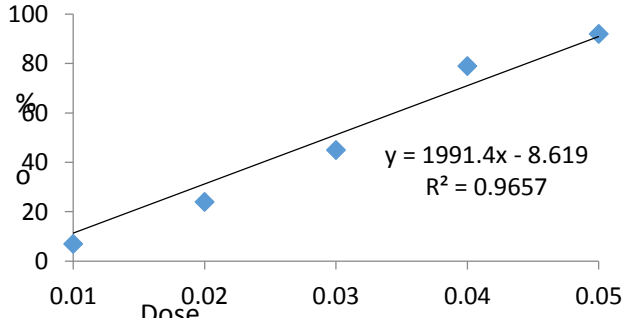

Fig. 1 Dose response curve showed the effectiveness of Monosodium Glutamate against 4th larval instar of C. quinquefasciatus

Table 1: Mortality of 4th instar larvae of *Culex quinquefasciatus* (Say) exposed to 48 hours at different doses of Monosodium Glutamate (N=180)

Dose (g/ml)	Total number of larvae survived	Mean no. of larvae survived (Mean±SD)	Larvae survived (%)	Total number of larvae died	Mean no. of larvae died (Mean±SD)	Mortality (%)
0.01	167	18.56±0.72a	92.78%	13	1.44±0.0.72e	7.22
0.02	138	15.33±1.00b	76.67%	42	4.67±1.00d	23.33
0.03	104	11.56±0.88c	57.78%	76	8.44±0.88c	42.22
0.04	47	5.22±1.39d	26.11%	133	14.78±1.39b	73.89
0.05	12	1.33±1.00e	6.67%	168	18.67±1.00a	93.33
Control	180	20.00±0.00	100%	0	0.00±0.00	0

*Mean larval mortalities after treated with different doses of MSG were compared by Multiple Range Test (Bonferoni, 1935 and 1936).
*Mean mortalities of different doses were significant different from each other ($p<0.05$)*MSG=Monosodium glutamate.

3.2 Histo-pathlogical observations

Permanent slides of transverse section of gut showed tissue damages at 0.05 g/ml and 0.03 g/ml and 0.01g/ml dose of MSG (Figs. 8, 6 and 4, respectively). In control, no such abnormalities were observed (Fig. 2 and 3).

After application of 0.01g/ml MSG, tissue damage was found in the stomodeal valve of anterior mid gut (Fig. 4). The major histological alterations were the structural disorganization hind gut tissue at 0.05 g/ml in which tissue damages found in rectal pads including destruction of

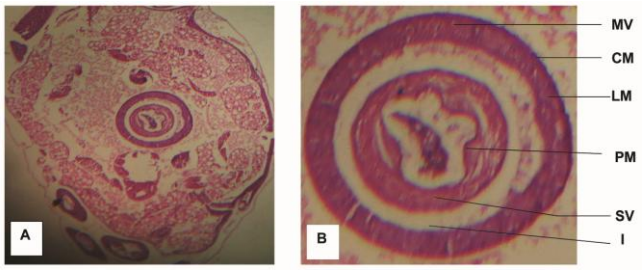

Fig. 2 A. Transverse section through the anterior mid gut area of an untreated 4th instar larvae of Cx. quinquefasciatus. B. Larger view of the transverse section through the anterior mid gut area of an untreated larvae. MV=microvilli, CM=circular muscle, LM=longitudinal muscle, PM= peritrophic membrane, MC= mid gut caeca and I= intima, SV=stomodeal valve

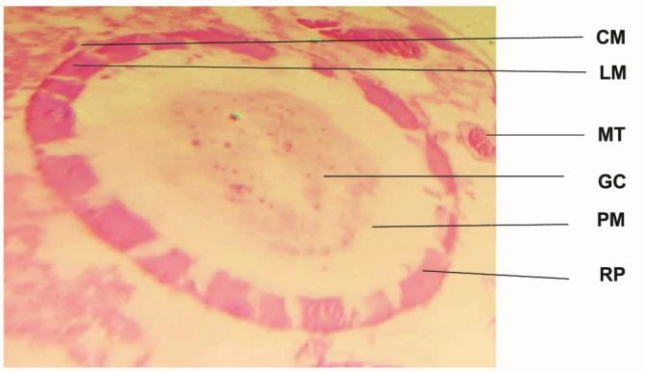

Fig. 3 Transverse section through the hind gut area of an untreated 4th instar larvae of Cx. quinquefasciatus. MV=microvilli, CM=circular muscle, LM =longitudinal muscle, PM= peritrophic membrane and RP= rectal pad, GC=gut content

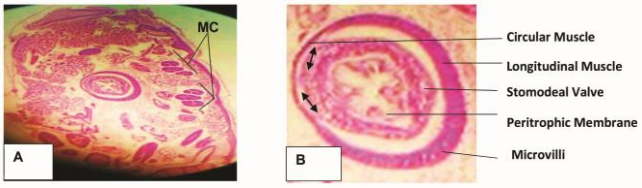

Fig. 4 Transverse section through the anterior mid gut area of a treated (0.01g/ml) 4th instar larvae of Cx. quinquefasciatus. B. Larger view of the transverse section through the anterior mid gut area the treated (0.01 g/ml) larvae.MC=mid gut caeca

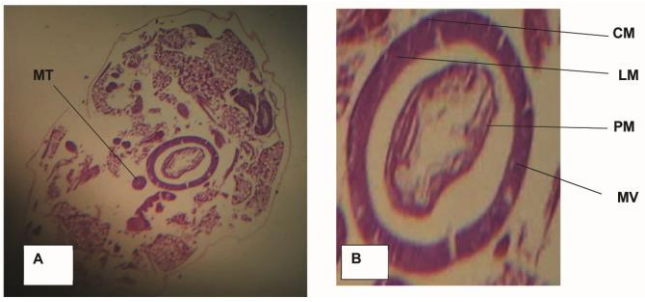

Fig. 5 A. Transverse section through the posterior mid gut area of an ttreated (0.02 g/ml) 4th instar larvae of Cx. quinquefasciatus. B. Larger view of the transverse section through the posterior mid gut area of the treated (0.02 g/ml) larvae. CM=circular muscle, LM =longitudinal muscle, PM= peritrophic membrane

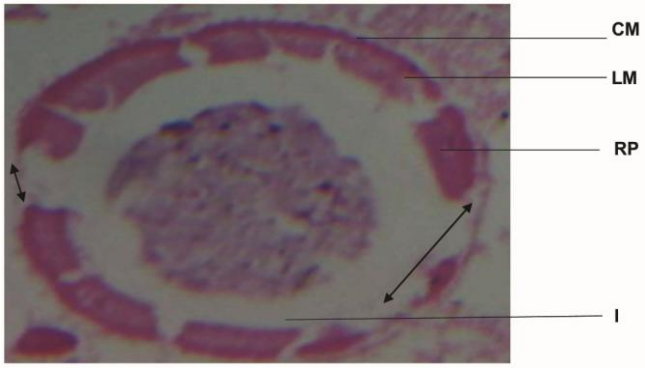

Fig. 6. Transverse section through the hind gut area of a treated (O.03 g/ml) 4th instar larvae of Cx. quinquefasciatus.MV= microvilli, I=intima, CM=circular muscle, LM=longitudinal muscle, and RP=rectal pad.(Arrow indicates the damage area)

peritrophic membrane, the inner longitudinal muscle and outer circular muscle disruption. The microvilli also had been damaged (Fig. 8). Lesions in some tissues occurred at 0.03 g/ml dose in the rectal pad of hind gut and had also longitudinal muscles disruption (Fig. 6). Scrapes in the peritrophic membrane were found in the tissues of treated animals from the doses of 0.02g/ml and 0.04 g/ml also (Fig. 5 and 7). The circular muscle, microvilli, peritrophic membrane, rectal pad were found to in their normal characteristic view in the untreated larval anterior mid gut and hind gut (Fig. 3 and 4).

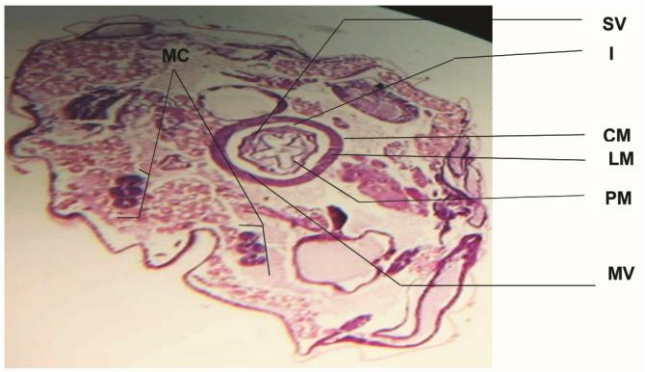

Fig. 7. Transverse section through the anterior mid gut area of a treated (0.04 g/ml) 4th instar larvae of Cx. quiquefasciatus. MV= microvilli, CM=circular muscle, LM=longitudinal muscle, PM= peritrophic membrane, MC= mid gut caeca and I=intima, SV=stomodeal valve

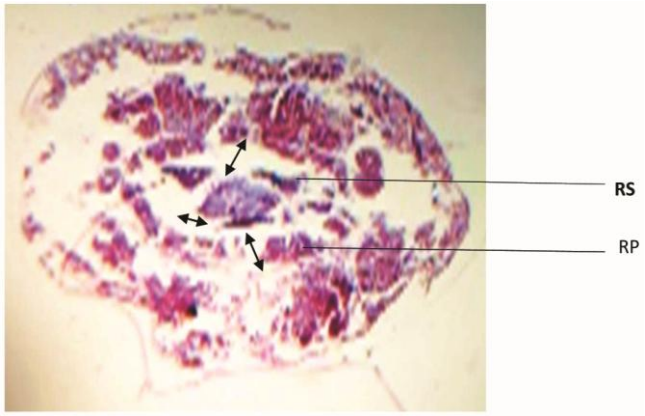

Fig 8.Transverse section through the hind gut area of a treated (0.05 g/ml) 4th instar larvae of Cx. quinquefasciatus. RP= Rectal Pad, RS=Rectal Spine. (Arrows indicate the damage area)

4. Discussion

Results indicated that 0.3 and 0.5g/ml dose of the MSG (in 0.35mg of average body weight of larvae) cause the tissue demolition of the rectal pad situated in the hindgut, microvilli, the peritrophic membrane and muscular sheath (Figs. 6 and 8). Hind gut of the alimentary canal is absorption site of the excretory material of insects [9]. The ionic balance of the body in insect through 'active transport' is maintained by malpighian tubules (MTs) [4]. Most likely the target of MSG is to the Na+/K+ channel of excretory system. The ionic balance of the body was broken down by the MSG and ultimately the MTs were constricted and tissue was also disrupted in the MTs of grey flesh fly *L. cuprina* [4]. However, in insects the MTs originate from the posterior mid gut and hind gut function as excretory organ somehow connected to the rectal pads [9]. Ingestion of any toxic material ionic balance of the body can be broken down [16]. Some of the former worker suggested that MSG somewhat may affect the renal system of vertebrates and invertebrates [18, 16]. Results of the present investigation had a precise connection to the results of the previous examiners. Apart from the renal system other internal organ arrangements of vertebrates were also affected by MSG. For instance, histological changes were observed in the stomach and testes of adult rat [10, 11]. Present researcher also observed the tissue disruption in the stomodeal valve of anterior mid gut at 0.01 g/ml dose of MSG (Fig. 4) which supports the previous results.

Being an Entomologist we have chosen the insects as a research model to observe the efficacy of Monosodium glutamate and assumed that may have some toxic effects on insect [4]. The present investigations have supported the assumption. Insects have similarities with higher animals, in many cases in structure and physiological functions [24]. Long term consumption of MSG exerts serious health hazards on oxidation state, antioxidant enzymes and the neurotransmitter cholinesterase which affects brain tissue structure [25]. So it can be concluded that MSG may effect on human health as well as vigor condition on other animals. Ultimate results may goes on the economic condition of our mother terrain.

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