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# Some Aspects of Biology, Toxicity and Esterase Variability of Melon Fly, *Bactrocera Cucurbitae*

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**Abstract**: Investigations were conducted on some aspects of biology, toxicity and esterase variability of melon fly, *Bactrocera cucurbitae*. Time periods required to develop into adult from egg were recorded 12-23 days in the present rearing condition (28-30 $^{0}$C of temperature and 70-80% of relative humidity). Three types of insecticides (pyrithroid, organophosphate and biopesticides) were tested against the 3$^{rd}$ instar larvae, where malathion was found to be the most toxic followed by cypermethrin and BT crude extracts. $LC_{50}$ for malathion and cypermethrin against the 3$^{rd}$ instar larvae were found to be $6.07\times10^{-7}$ and $8.13\times10^{-6}$ ppm at one hour of exposure respectively. In case of BT crude extracts the $LC_{50}$ for 34L, 47L and R1 strains were 2.19, 1.01 and 2.19 mg/ml at 72 hours of exposure in order. 7.5% polyacrylamide gel electrophoresis was employed to investigate the esterase isozyme variability of this species in terms of sex, age groups, body parts and pesticidal effects. Altogether, five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were identified in this species. All five bands were present in female, whereas only four in male (Est-1 absent). Est-3 was present in all stages of life, Est-4 and Est-5 only in adults, while other two bands showed switch on and off pattern of expression. Relatively, higher esterase bands were observed in the anterior part of the body (head). All the bands were predominantly present in the cypermethrin-killed adults but Est-2 and Est-3 disappeared from the malathion killed adults.

**Key words:** Melon fly, Developmental biology, toxicity, Electrophoresis, Esterase variability

## INTRODUCTION

The melon fruit fly, *Bactrocera cucurbitae* (Diptera: Tephritidae) is a biologically interesting and economically very important group of insects that infests a wide range of plant species causing severe loss to fruits and vegetable crops[1] and is a major threat to cucurbits[2]. The damages result from oviposition in fruit and soft tissues of vegetative parts of hosts, feeding by the larvae and decomposition of plant tissue by invading secondary microorganisms[3]. It attacks flowers, fruits, stems and root tissues that greatly curtailed the production of economically important fruits and vegetables[4]. This fly is a serious pest of fruits and vegetables in all over Bangladesh[5, 6], the control strategy of which mainly depends on vast knowledge about the species. Several numbers of methods are used to reduce direct losses of harvesting locally grown crops including mechanical, cultural, biological control and chemical control[3]. Chemical insecticides are the backbone of insect pest control[7] and most commonly used insecticides for fruit fly control includes malathion, cypermethrin, DDT, diazinon, lindane, chlordane etc. Malathion is an organophosphate parasympathomimetic which binds irreversibly to cholinesterase and widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs[8]. On the other hand, cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes[9]. Oppositely, BT is endotoxins that are extracted from different strains of *Bacillus thureingenesis,* could be used for the management of melon fly infestation. Due to drawback of chemical pesticides, biopesticides may be potential for melon fly control reducing reliance on sprayed insecticides[10].

Isozymes that are the variants of same enzyme and almost identical in function, may differ in other ways viz. amino acid substitutions that change the electric charge of the enzyme. This forms the basis for separating different isozymes by gel electrophoresis to be used as molecular markers. Esterases are hydrolyzing enzyme that may used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture. This enzyme is very important for insecticide breakdown and its isozymes have been amongst the most widely used molecular markers for this purpose. Several studies have been shown that changes in esterase sensitivity to inhibition by organophosphorus and carbamate insecticides can confer high levels of resistance[11, 12]. Aim of the present investigation was to observe the general biology, toxicity effects (malathion, cypermethrin, BT crude extracts) and esterase isozyme variability in terms of sex, age groups, body parts and effects of pesticides in *B. cucurbitae.*

## EXPERIMENTAL

*Bactrocera cucurbitae* was collected from naturally infested fruits and vegetables and reared on sweet gourd, in steel frame cages covered with nylon net in the laboratory (Zoological Garden, Department of Zoology, University of Dhaka) at room temperature (28-32 $^0$C of temperature and 65-80% of relative humidity) and esterases were studied in Genetics and Molecular Biology Lab. of the same University. Adults laid eggs on sweet gourd and the fertilized eggs grew up to 3$^{rd}$ instar larvae on this. The infested gourds were then placed on sawdust in medium sized plastic bowl covered with two folds of nylon nets to pupate successfully. Time required for each developmental stage was recorded and sample from each stage was identified both in naked eye and with the help of stereo microscope. Additional food (10% sugar solution) was supplied in the adult rearing cages with the help of ringed cotton into petri-dishes. After colonizing a sufficient number of adults, 3$^{rd}$ instar larvae were chosen for toxicity test and different doses of cypermethrin, malathion, several strains of BT crude extracts were applied on this. Serial dilution of cypermethrin and malathion was prepared following Islam[13] and BT crude extracts considering the concentration and volume of sugar solution and BT crude extracts. Each dose was wetted with cotton to facilitate the sucking of adults and to prevent unusual death at the time of preliminary trial. Chemical pesticides (malathion and cypermethrin) were tested only against the 3$^{rd}$ instar larvae into petridishes in submerged condition. For larval test against BT crude extracts, fresh ripped pumpkin was heat treated up

to molt and each dose was mixed well with molted pest (10 g). 10 individuals (3rd instar larvae/adults) were subjected to toxicity test at each dose concentration and for explanatory test; each dose was replicated three times at the same environmental condition (28-30 $^0$C of temperature and 70-80% of relative humidity). The dead adults were counted and the percentage mortalities were calculated by the following method:

$$\% \text{ Mortality} = \frac{\text{Total No. of dead}}{\text{Total No. of exposed}} \text{ X } 100$$

Computations of dose response data was performed by Biostat-2009 program based on Finney[14]. Electrophoretic investigation was carried out on 7.5% polyacrilamide gels to see the esterase isozyme variability in terms of Sex, Toxicity, Age group and Body parts followed by Shahjahan et al.[15]. Samples were prepared into separate eppendrof tubes and squashed separately in TBE buffer (proportionate to the sample weight; 0.016 g ~ 40 µl), centrifuged at 12500 rpm for 15 min and aliquots from each sample (15 µl) was loaded on the gel slots for electrophoresis[16]. The electrophoretic bands of esterase isozymes resulting from stained gels with napthyl acetates were assigned to increasing numbers based on decreasing mobility following Richardson[17].

## RESULTS AND DISCUSSION

**General biology:** Different life stages (1st-, 2nd- and 3rd- instar larvae, pupae and adults) of melon fly were observed **(Figure 1)** that required about 12-23 days at present rearing condition. Time required to develop from egg to adult may vary depending on temperature and geographical distribution viz. it takes 13 days at 29° C in Solomon Islands[18]. Each female deposited approximately 65 white elliptical eggs, inserted into fruit that hatched in 2 to 3 days. However, it may vary 1 to 4 days[3]. All three instars of larvae were cylindrical-maggot shape, elongated, legless, narrowed anterior end with mouth hooks, flattened caudal end and white in color, except that were altered by the color of the food within the alimentary canal. The first, second and third instar larvae ranged from 3.2 to 4, 5.2 to 6.9 and 7.5 to 11.8 mm in length respectively. The larval period lasted from 6 to 11 days and each stage was lasting 2 or more days on sweet gourd. The larval period lasts for 3 to 21 days depending on temperature and the host[18, 19, 20]. The pupae were about 5 to 6 mm in length and varies reddish brown to yellowish brown in color (F**igure 1**) that were distinctly ringed by narrow yellow bands around each segment. They took place in the sawdust at 0.7 to 1.2 cm beneath the infected host. In natural condition, larvae pupate in the soil at a depth of 0.5 to 15 cm depending on soil texture and moisture[21, 22, 4]. The pupal stage lasted from 4 to 9 days that may vary depending on host speices viz. 7 days on bitter gourd and 7.2 days on pumpkin[23] or temperature viz. up to 90 days under cool conditions[24]. The adult flies were 6 to 8 mm in length with distinct characteristics of wing pattern, long third antennal segment, reddish yellow thorax and a distinctive black 'T' pattern at the base of the abdomen. Females had a slender pointed ovipositor, which they used to lay eggs under the skin of the host fruit. Oviposition occurred after about 7 days of emergence and adults lived for about 1 month. Survival of the adult flies may depend on host species, temperature and sex of the individuals[23, 24, 25 26]. In general, the life cycle of melon fly lasts from 21 to 179 days[27, 20] spending about 1.73 days for eggs, 4 to 9 days for larvae, and 7 to 11 days for pupae[28].

**Toxicity:** Three types of insecticides were tested against the 3rd instar larvae of *B. cucurbitae*, of which malathion was found to be more toxic followed by cypermethrin and BT crude extracts (Table 1, 2). $LC_{50}$ values of malathion and cypermethrin against the 3rd instar larvae were found to be $6.07\times10^{-7}$, $3.77\times10^{-10}$, $6.08\times10^{-12}$ ppm and $8.13\times10^{-6}$, $7.86\times10^{-9}$, $2.29\times10^{-11}$ ppm at one, two and three hours of exposure respectively. $LC_{90}$ values of malathion and cypermethrin were $7.24\times10^{-2}$, $1.18\times10^{-4}$, $7.68\times10^{-7}$ ppm and 0.198, $1.73\times10^{-3}$, $1.51\times10^{-6}$ at one, two and three hours of exposure respectively **(Table 1 and 2)**. Probit graphs obtained from different dose response data were shown in **Figure 2.**

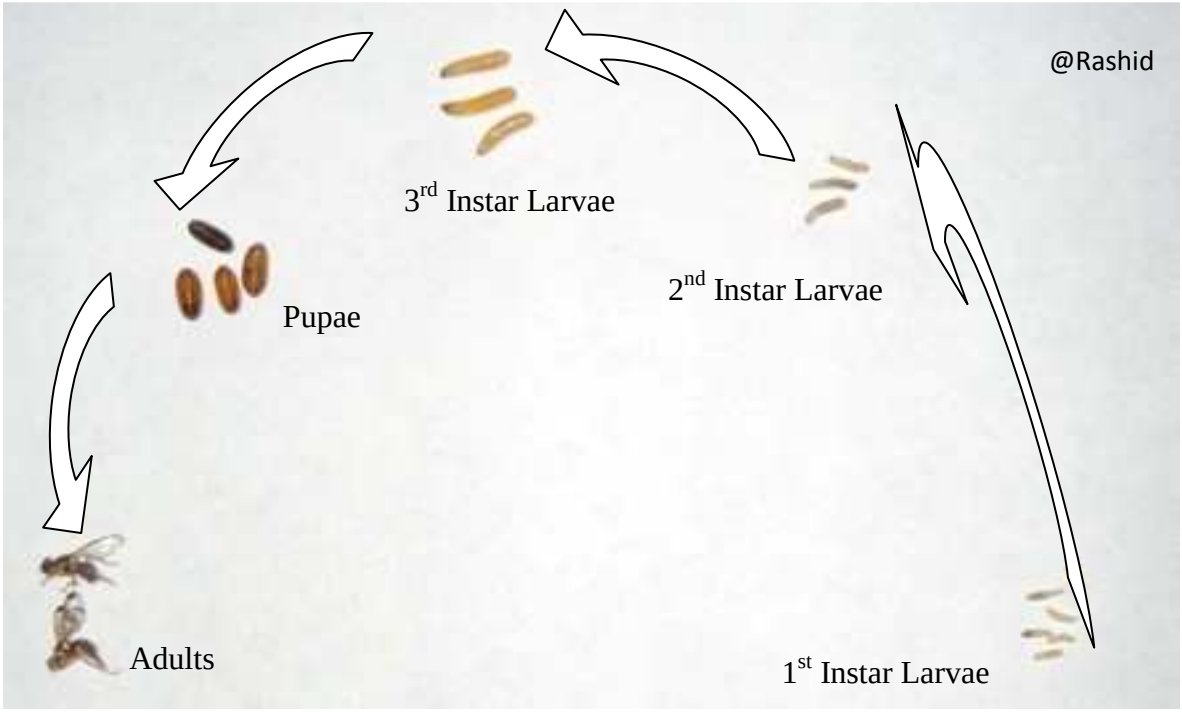


**Figure 1**: Life cycle of Melon fly, *B. cucurbatae* (except eggs).

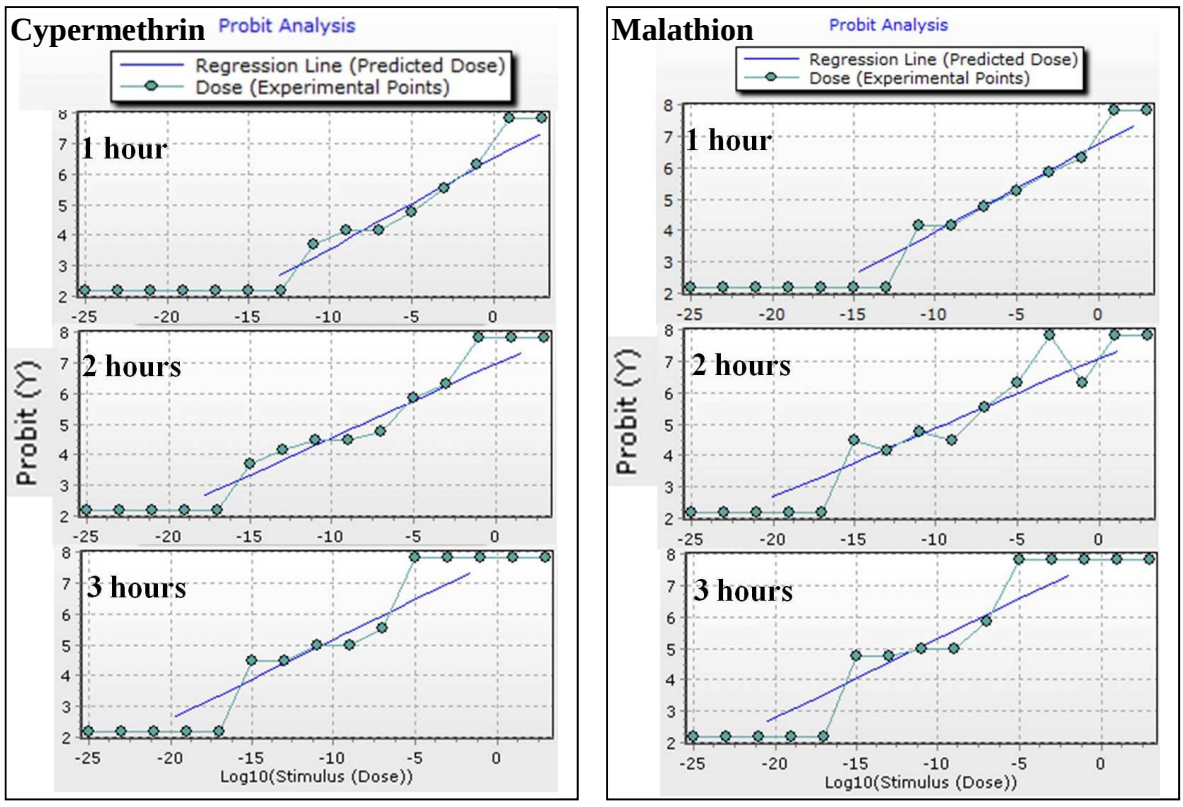


**Figure 2:** Adjusted probit values and predicted regression line of cypermethrin and malathion for the exposed 3rd instar larvae of *B. cucurbitae.*

Four strains of BT crude extracts (1i, 04S, 25L and 15S) were preliminarily applied on adults of melon fly to test the susceptibility level. Among these 1i, 04S and 25L were moderately susceptible with maximum mortality of 25, 5 and 5% respectively. 15S was not susceptible against adults of *B. cucurbitae* (**Table 1 and 2**). Stark et al.[29] found similar feeding toxicity of the natural insecticide spinosad in three economically important fruit fly species; *C. capitata*, *B. cucurbitae* and *B. dorsalis.*

**Table- 1: Mortality rate (average of three replicas) in the 3rd instar larvae of melon fly (B. cucurbitae) at different concentrations of Cypermethrin and Malathion.**

| Doses (ppm) | Cumulative mortality (%) | | | | | | | Statistical analysis at 95% confidence limits | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1h | | 2h | | 3h | | | (ppm) | 1h | 2h | 3h |
| | C | M | C | M | C | M | C | $LC_{50}$ | $8.13\times10^{-6}$ | $7.86\times10^{-9}$ | $2.29\times10^{-11}$ |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | | $LC_{50}$ U | $3.53\times10^{-5}$ | $6.07\times10^{-8}$ | $2.58\times10^{-10}$ |
| $10^{-25}$ | 0 | 0 | 0 | 0 | 0 | 0 | | $LC_{50}$ L | $1.89\times10^{-6}$ | $1.02\times10^{-6}$ | $2.03\times10^{-12}$ |
| $10^{-23}$ | 0 | 0 | 0 | 0 | 0 | 0 | | $LC_{90}$ | 0.198 | $1.73\times10^{-3}$ | $1.51\times10^{-6}$ |
| $10^{-21}$ | 0 | 0 | 0 | 0 | 0 | 0 | M | $LC_{50}$ | $6.07\times10^{-7}$ | $3.77\times10^{-10}$ | $6.08\times10^{-12}$ |
| $10^{-19}$ | 0 | 0 | 0 | 0 | 0 | 0 | | $LC_{50}$ U | $1.51\times10^{-6}$ | $8.77\times10^{-9}$ | $9.54\times10^{-11}$ |
| $10^{-17}$ | 0 | 0 | 0 | 0 | 0 | 0 | | $LC_{50}$ L | $2.44\times10^{-7}$ | $1.64\times10^{-11}$ | $3.87\times10^{-13}$ |
| $10^{-15}$ | 0 | 0 | 10 | 30 | 30 | 40 | | $LC_{90}$ | $7.24\times10^{-2}$ | $1.18\times10^{-4}$ | $7.68\times10^{-7}$ |
| $10^{-13}$ | 0 | 0 | 20 | 20 | 30 | 40 | Number of insect exposed = 10 | | | | |
| $10^{-11}$ | 10 | 20 | 30 | 40 | 50 | 50 | C = Cypermethrin | | | | |
| $10^{-9}$ | 20 | 20 | 30 | 30 | 50 | 50 | M = Malathion | | | | |
| $10^{-7}$ | 20 | 40 | 40 | 70 | 70 | 80 | h = Hours | | | | |
| $10^{-5}$ | 40 | 60 | 80 | 90 | 100 | 100 | | | | | |
| $10^{-3}$ | 70 | 80 | 90 | 100 | 100 | 100 | | | | | |
| $10^{-1}$ | 90 | 90 | 100 | 90 | 100 | 100 | | | | | |
| 10 | 100 | 100 | 100 | 100 | 100 | 100 | | | | | |
| 1000 | 100 | 100 | 100 | 100 | 100 | 100 | | | | | |

Fifteen different strains each with three doses of BT against the 3rd instar larvae were observed where $L_{50}$ and $L_{90}$ values of both 34L and R1 strains were 2.19 mg/ml and 4.82 mg/ml at 48 and 72 hours of exposure respectively, where as 47L shown 1.01 mg/ml and 1.60 mg/ml only at 72 hours **(Table 2).** Strains 28S, R2, 1i, R3, 19S, 30S, 31L and 15S were moderately susceptible with maximum mortality of 5, 5, 10, 15, 40, 5, 25 and 20 % respectively in 1 mg/ml of dose concentration, except 1i where the dose concentration were 0.66 mg/ml. Rest of the strains was not susceptible against these larvae **(Table 1, 2)** that could be explained by the high degree of specificity of each strain of *Bacillus thuringiensis* to host insects[30]. Gujar et al.[31] reported that transgenic cotton that produces insecticidal proteins from *B. thuringiensis* (BT) from a single *cry1A* gene and stacked also with *cry2A* gene has provided satisfactory protection against the damage by the lepidopteron bollworms.

**Table-2: Mortality rate (average of three replicas) in the 3rd instar larvae of melon fly (B. cucurbitae) at different concentrations of different strains of BT crude extract [Number of insect exposed = 10, D1 and D2 represent concentration of doses (mg/ml) in 10 gm of sweet gourd pest and in 10% sugar solution respectively).**

| Strains | $D_1$ | Cumulative mortality (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24h | 48h | 72h | Strains | 24h | 48h | 72h | Strains | 24h | 48h | 72h |
| | Control | 0 | 0 | 0 | Control | 0 | 0 | 0 | Control | 0 | 0 | 0 |
| 34L | 1 | 10 | 10 | 10 | 33S | 0 | 0 | 0 | R3 | 0 | 5 | 15 |
| | 0.8 | 0 | 5 | 5 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| | 0.6 | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| 08S | 1 | 0 | 0 | 0 | 47L | 0 | 0 | 45 | 1i | 5 | 10 | 10 |
| | 0.8 | 0 | 0 | 0 | | 0 | 0 | 30 | | 0 | 0 | 0 |
| | 0.6 | 0 | 0 | 0 | | 5 | 0 | 5 | | 0 | 0 | 0 |
| 28S | 1 | 5 | 5 | 5 | 51S | 0 | 0 | 0 | 24S | 0 | 0 | 0 |
| | 0.8 | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| | 0.6 | 0 | 0 | 0 | | 0 | 10 | 20 | | 0 | 0 | 0 |
| R1 | 1 | 10 | 10 | 10 | 31L | 0 | 15 | 25 | R2 | 5 | 5 | 5 |
| | 0.8 | 0 | 5 | 5 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| | 0.6 | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| 38L | 1 | 0 | 0 | 0 | 30S | 5 | 5 | 5 | 19S | 0 | 20 | 40 |
| | 0.8 | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| | 0.6 | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| | $D_2$ | Preliminary trial on adult flies | | | | | | | | | | |
| 1i | 1 | 5 | 10 | 25 | 04S | 5 | 5 | 5 | Statistical analysis* | | | |
| | 0.6 | 0 | 0 | 0 | | 0 | 0 | 0 | mg/ml | 34L | 47L | R1 |
| | 0.2 | 0 | 10 | 0 | | 0 | 0 | 0 | $LC_{50}$ | 2.19 | 1.01 | 2.19 |
| 25L | 0.7 | 0 | 5 | 5 | 15S | 0 | 0 | 0 | $LC_{50}$ U | 8.01 | 1.13 | 8.01 |
| | 0.6 | 0 | 0 | 0 | | 0 | 0 | 0 | $LC_{50}$ L | 0.6 | 0.94 | 0.6 |
| | 0.5 | 0 | 0 | 0 | | 0 | 0 | 0 | $LC_{90}$ | 4.82 | 1.60 | 4.82 |

*** After 72 hours of exposure and at 95% confidence limits**

**Table- 3: Esterase isozyme variability of B. cucurbatae stained in both α and β naphthyl acetates on 7.5% polyacrylamide gels.**

| Parameter | Sample | Est-1 | Est-2 | Est-3 | Est-4 | Est-5 | T |
|---|---|---|---|---|---|---|---|
| Sex | Female | +++ | +++ | +++ | +++ | +++ | 100 |
| | Male | - | +++ | +++ | + | +++ | 80 |
| Developmental stages | Adults | + | + | ++ | ++ | +++ | 100 |
| | Pupae | - | +++ | ++ | ++ | ++ | 80 |
| | 3rd IL | - | ++ | ++ | - | - | 40 |
| | 2nd IL | ++ | - | + | - | - | 40 |
| | Nc | - | - | - | - | - | 00 |
| Effects of pesticides | Pc | ++ | ++ | ++ | + | + | 100 |
| | Ck | +++ | ++ | +++ | +++ | +++ | 100 |
| | Mk | + | - | - | + | ++ | 60 |
| Body parts | Head | ++ | - | + | - | +++ | 60 |
| | Thorax | - | - | + | - | ++ | 40 |
| | Abdomen | - | - | + | - | ++ | 40 |
| C1 | | 58 | 58 | 92 | 58 | 75 | 70 |

**(-, +, ++ and +++ denote absent, faint, medium and deep stained bands; T1 represents the frequency (%) of esterase bands (out of five bands) present in a certain sample of above mentioned species; C1 personates the frequency (%) of each esterase band)**

Result indicated that the 3rd instar larvae were much susceptible to malathion and least to the strains of BT. Adults were not susceptible enough to the strains of BT crude extract in the dose concentrations of present study. Magana et al.[32] found that field populations of *Ceratitis capitata* showed lower susceptibility to malathion (6- to 201-fold) compared with laboratory populations. After certain periods of exposure, some sort of inactivity was observed as the mortality rates went down but cumulative mortality yet increasing that could be due to lose of toxicological potency of each strain[33]. The use of pathogens and their metabolic products for the control of insects have been found to be advantageous, such as host specificity, absence of resistance problem, non pollutant to environment and non toxic for humans[34].

## ESTERASE VARIABILITY

**Life cycle:** Altogether, five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were found in different life stages of *B. cucurbitae* **(Figure 3).**

Variation in the expression of these isozymes may vary species to species[35], even strain level[36]. As for example, three, four and eight esterase bands were found in the developmental stages of *B. papayae* and *B. columbolae*[37], *B. dorsalis* and *B. tau*[38] and *Aedes aegypti*[39] in order.

All the bands were found in adults, where as pupae and larvae showed four (Est-2, Est-3, Est-4 and Est-5) and two (Est-2 and Est-3 in 3rd instar, Est-1 and Est-3 in 2nd instar larvae) bands respectively. Stage specific expression of these isozymes was also observed in *Cimex hemipterus*[40], *Pediculus humanus*

*capitis*[41], Chironomids[42], *B. dorsalis* and *B. tau*[38], *Aedes aegypti*[39] and many more, where they found a variety of responses. Switch on or off of the particular allele was frequent in the above mentioned species that could be due to regulatory mechanisms acting in agreement with the requirements of a variable number of processes in which esterases were involved[43]. Among the five esterase bands, only Est-3 was present in all developmental stages of the studied species. Similar results were also found in the life cycle of *B. papayae* and *B. columbolae*[37], *Cimex hemipterus*[40], *B. dorsalis* and *B. tau*[38] and in *Aedes aegypti*[39]. Expression pattern of this enzyme indicated that with subsequent developmental stages the allelic expression increased that could be helpful to the organism to become more resistant against poisonous agent[44].

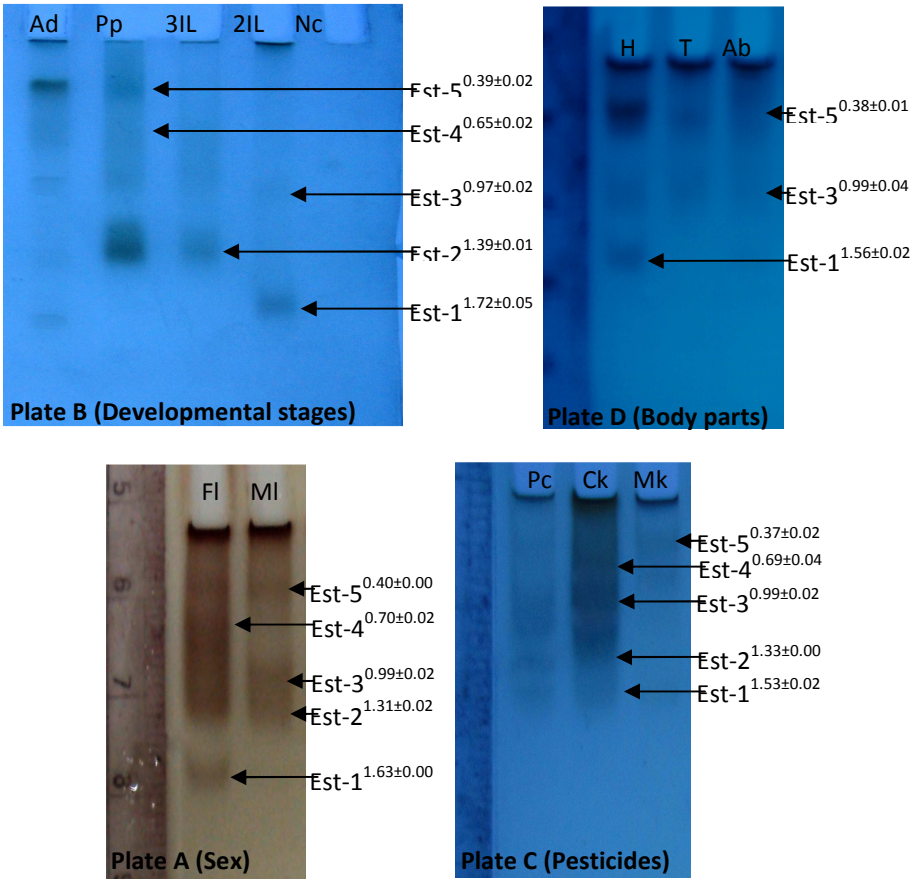


**Figure 3: Esterase isozyme variability of B. cucurbatae stained in both α and βnaphthyl acetates on 7.5% polyacrylamide gels (Plate A: Adult Female and Male; Plate B: Different life stages; Plate C: Insecticidal effects on esterases; Plate D: Different body parts). Ad, Pp, 3IL, 2IL, H, T Ab, Fl, Ml, Pc, Nc, Ck and Mk stands for Adults without considering male or female, Pupae, 3rd instar larvae, 2nd instar larvae, Head, Thorax, Abdomen, Female, Male, Positive control (with sample), Negative control (without sample), Cypermethrin killed adults and Malathion killed adults in order.**

**Body parts:** Altogether, three esterase bands (Est-1, Est-3 and Est-5) were found in different body parts of *B. cucurbatae.* All three bands were present in head, where as Est-1 was absent both in thorax and abdomen. Variation in the expression of these isozymes in different body parts was also observed in *B. dorsalis* and *B. tau*[38], *Oreochromis niloticus*[15] and in *Heteopneustes fossilis*[45]. Location and function of the

various esterases can vary from tissue to tissue and depend on the physiological demands of each system[46]. Relatively higher esterase activity was found in the anterior part of the body (head) that could be due to neuro-transmitting activity[47]. Many researchers observed frequently the high activity of esterases in the brain of different species[48].

**Male- female:** All five bands present in melon flies were found to be in female, but Est-1 was absent in male showing a sex specific variability. Certain band was found to be common in both male and female of *B. papayae* and *B. columbolae*[37], *B. dorsalis* and *B. tau*[38] that indicated a common function of this enzyme irrespective of sexual variation. Staining intensity of the bands indicated that esterase activity was higher in female than male resembling to horn fly[49] that may provide advantages to female against organophosphate resistance[50, 51]. Previous study on mosquito fish, *Gambusia affinis* showed that after certain stages of development, male and female individuals exhibited pronounced differentiation in the expression of non specific esterases[52]. Contrarily to this, no significant male female variation was observed in *Pediculus humanus capitis*[41].

**Pesticide:** In control group and cypermethrin killed adults, all five esterase bands were found, but the later group showed higher esterase activity than the previous one. Elevated esterase banding patterns were also found in resistant populations of *Schizaphis graminum*[53], *Myzus persicae*[54], *Bemisia tabaci*[55] and *Anopheles albimanus*[56]. On the other hand, malathion treated adults lack Est-2 and Est-3, other bands were also dully stained. In cypermethrin treated adult intensity of each band increased, but decreased in malathion treated adult indicating that the flies were more susceptible to malathion than cypermethrin[57]. Previous study on *B. dorsalis* and *B. tau* showed more or less same results[38]. Esterases play an important role in conferring or contributing to insecticide resistance in insects[58, 59, 60] which has been shown in *Myzus persiace*[61], *Culex quinquefasciatus* and *C. pipiens*[62], *Lucilia cuprina*[59] and *Musca domestica*[63]. When organisms were treated with insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase caused them to be shaky that might in turn result sudden death of the organism. The condition occurred due to low production of esterase or lack of gene that produced these isozymes. Moreover, the characteristics of each esterase isozyme could be determined by the addition of specific insecticide in the process of enzymatic staining of gels. Those esterases were inhibited by malathion (organophosphate) could be grouped as carboxylesterases[64] but, it was difficult to represent any straight forward conclusion regarding the biochemical properties of these isozymes and need further investigation.

In general, the level of insect esterase were found to be highly variable depending on the life stage, sex, tissue, hormones, strain, food, environmental conditions and numerous other factors[65].

## CONCLUSION

Knowledge of the life stages along with esterase variability and susceptibility to the insecticides of melon fly, *B. cucurbitae* will help us to prevent its infestation in many economically important fruits and vegetables, but it needs further experimentation in the field level.

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