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Full Length Research Paper

Study of using the bacterium *Bacillus thuringiernsis israelensis* in microbial control of *Musca domestica vicina*, Diptera Muscidae (Muscidae, Diptera)

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The second instar larvae of *Musca domestica* were treated with *Bacillus thuringiensis israelensis,* (B.t.i.) at concentrations of 0.5, 1.0, 1.5 and 2.0% under laboratory conditions. Cumulative mortality percentage increased gradually with the increase in B.t.i concentrations and this is represented by straight regression lines indicating homogenity. The LC_{30} and LC_{50} were 0.87 and 1.305%, respectively. The tested concentrations indicate significant prolongation in larval duration compared to control. There was an inverse relation between the concentration and pupation percentage and an increase in pupal duration. An insignificant decrease in percentage of adult emergence was observed. Sex ratio was not affected. 2.0% Concentration of B.t.i. caused the greatest reduction in female fecundity (964.5 eggs/female), and the hatchability of eggs also decreased. The pupae and adults appeared malformed when treated with B.t.i. as a 2nd larval instar.

Key words: Housefly, larvicide, bioassay, mortality, LC_{30}, fecundity, fertility, malformation

INTRODUCTION

The house fly *Musca domestica vicina* (Muscidae: Diptera) is considered as one of the most major and medical pest in all tropical and subtropical parts of the world (Cohen et al., 1991). The chemical control methods by insecticides are currently used in spite of their power of contamination, votalization, and bioaccumulation (Moon, 2002). The different groups of insecticides are considered the main factors affecting the ecosystem. From this point of view, it is necessary to minimize the applications of insecticides which is considered as the main source of environmental pollution and affected the human health. There are many different insect species that have been successfully controlled by microbial agents (Hogsette, 1999). The bacterium, *Bacillus thuringienis israelensis* (B.t.i.) is considered highly beneficial for its specific activity. Several studies have shown the toxicity of endotoxins from various B.t. strains against *M. domestica* (Indrasith et al., 1992; Hodgman et al., 1993; Lonc et al., 1997; Johnson et al., 1998; Zhong et al., 2000; Labib and Rady, 2001; Padmanabhan et al., 2005; Luga et al., 2008). The present investigation aimed to evaluate the efficiency of this biocide at different concentrations against 2nd larval instar of *M. domestica,* as well as to evaluate the effects of these concentrations on some biological parameters (Figure 1 to 10).

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MATERIALS AND METHODS

Test insects

Sources of colony

Adults susceptible strain of house fly *M. domestica vicina* used in the present study were obtained from a well established colony originated from the Biology Department, Faculty of Science for girls, King Abdul Aziz University.

Rearing technique

Egg masses were used to maintain a colony in the laboratory under constant conditions of temperature and humidity (27 ± 2°C and 60

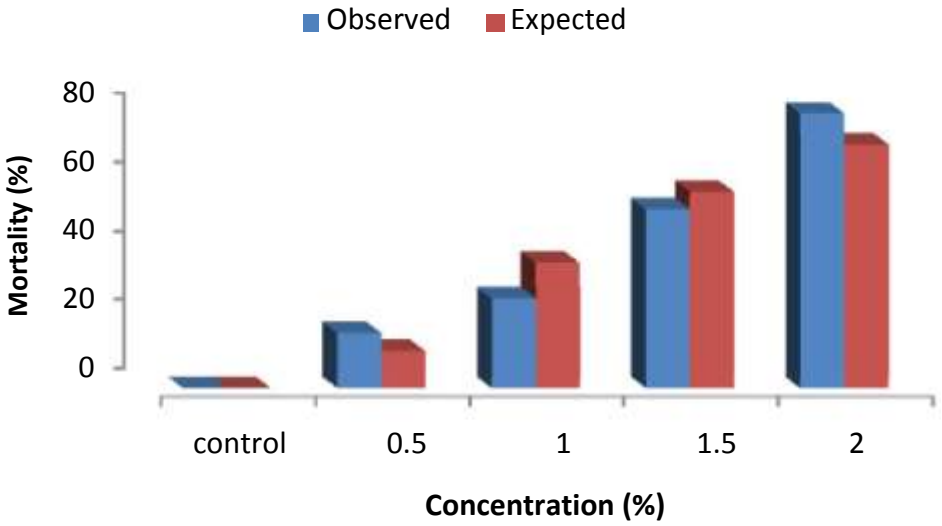

Figure 1. Susceptibility of 2nd instar larvae of *M. domestica* vicina to different concentration of *Bacillus thuringiensis israelensis* after 48 h of treatment.

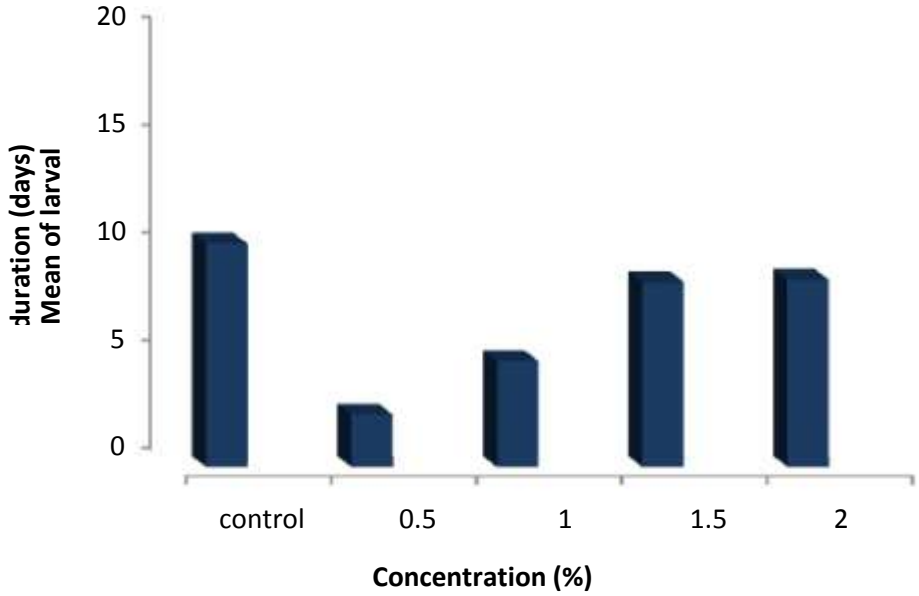

Figure 2. Effect of different concentrations of *Bacillus thuringiensis israelensis* on larval duration (days) of *M. domestica* treated as 2nd instar larvae.

±5% R.H.) Each egg mass was placed in a clean Petri dish (10 cm diameter), previously washed with 10% formalin solution to avoid any contamination according to a constant technique described by Lelwallen (1954). Full grown larvae were allowed to pupate in clean glass Petri dishes. Following emergence, the adults were provided with a piece of cotton soaked in 10% sugar, 2% milk solution as a source of food.

Source of the bacterial pathogen

The bacterium *B. thuringiernsis israelensis* was chosen as a pathogen for this study because of its wide use in biological control. The powder was obtained from Valent Biosciences, U.S.A.

Susceptibility of house fly to the bacterial pathogen

Experimental larvae

Newly moulted 2nd instar larvae of house fly were segregated from the stock colony in clean glass Petri dishes (10 cm diameter) and starved for about 24 h.

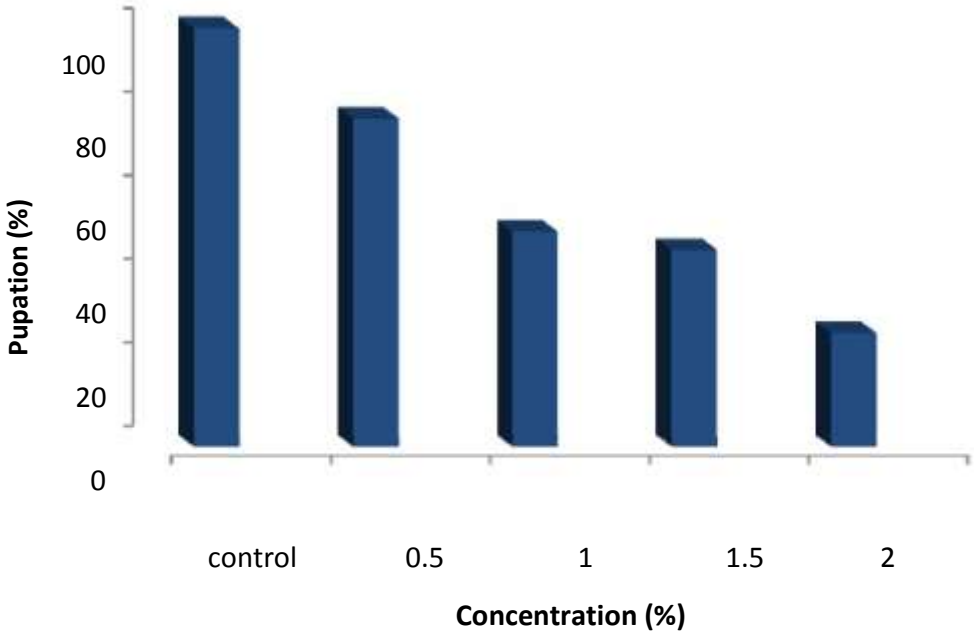

Figure 3. Effect of different concentrations of *Bacillus thuringiensis israelensis* on the percentage of pupation of *M. domestica* treated as 2nd instar larvae.

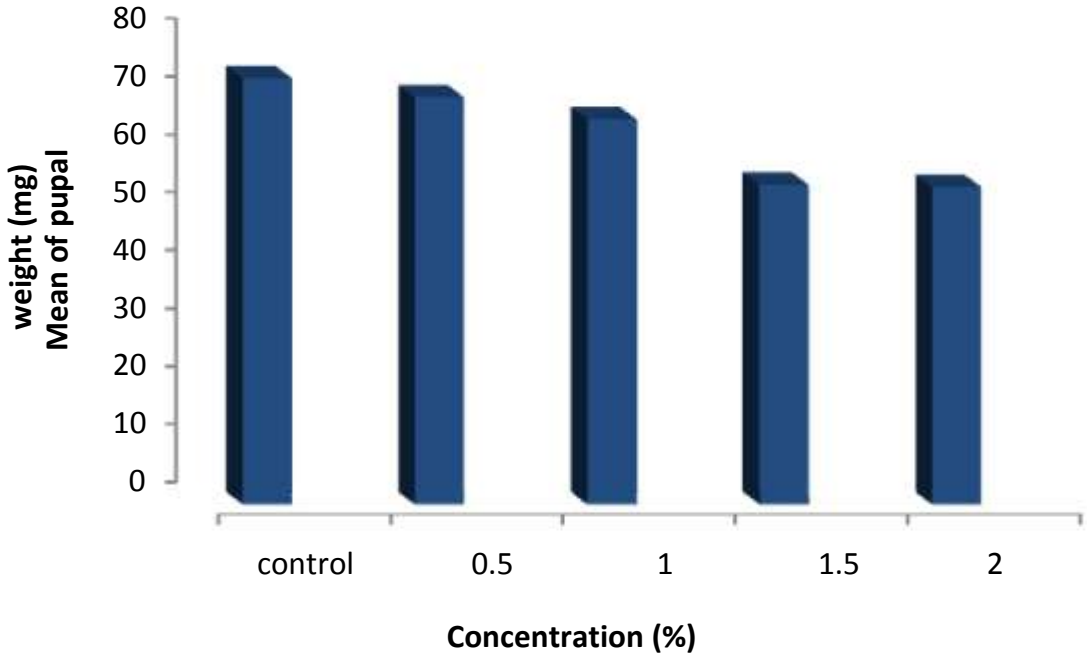

Figure 4. Effect of different concentrations of *Bacillus thuringiensis israelensis* on pupal weight (mg) of *M. domestica* treated as 2nd instar larvae.

Treatment technique

Four concentrations (0.5, 1.0, 1.5, and 2.0%) of the bacterial pathogen were used. Fifty of the starved larvae were distributed in five replicates, were used for each concentration to feed for 48 h on treated larval media under constant laboratory conditions (27 ± 2% and 60 ± 5% R.H). The same technique described before was used except that the control larvae were allowed to feed on untreated media.

Biological studies

Final mortality percentage of treated and control larvae were recorded 48 h post treatment. Daily inspections were carried out until emergence occurred and the number of individuals (larvae, pupae, and adults) were recorded or each concentration. The larval

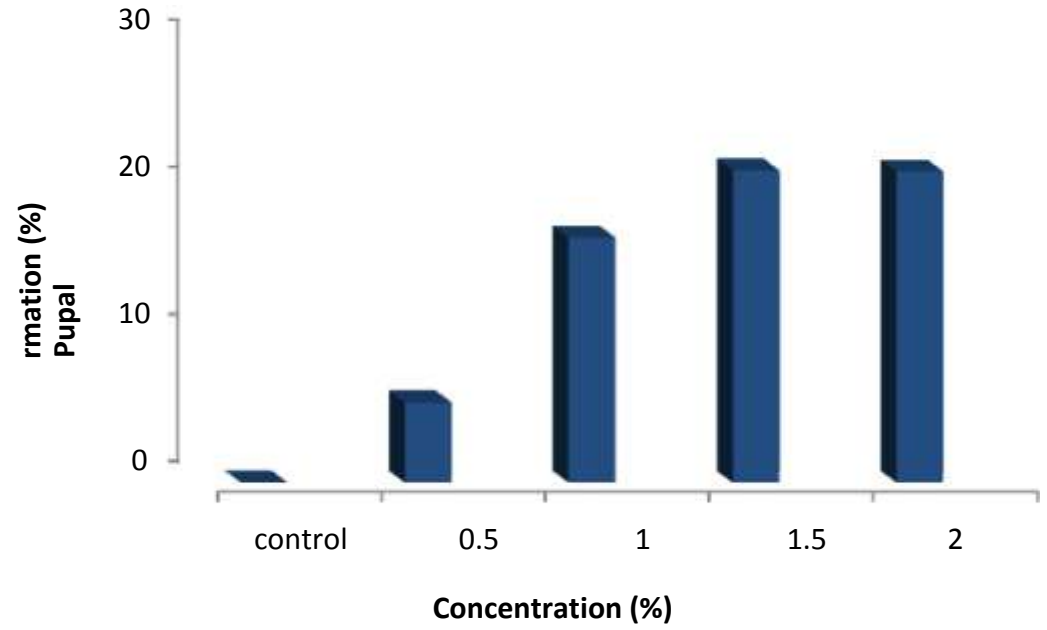

Figure 5. Effect of different concentrations of *Bacillus thuringiensis israelensis* on the percentage of pupal malformation of *M. domestica* treated as 2nd instar larvae.

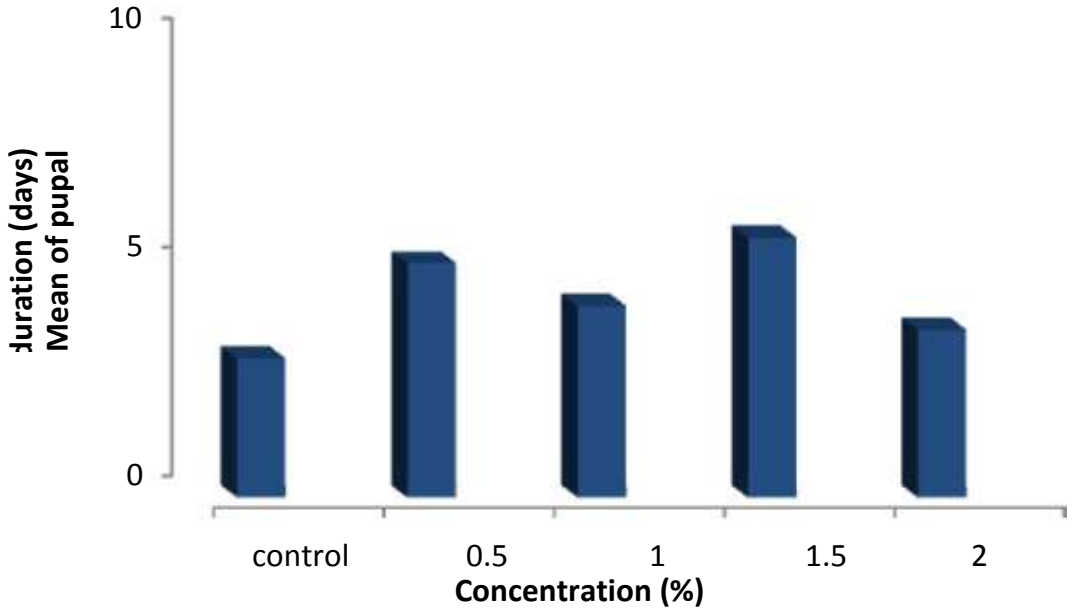

Figure 6. Effect of different concentrations of *Bacillus thuringiensis israelensis* on pupal duration (days) of *M. domestica* treated as 2nd instar larvae.

duration, pupation, pupal duration, pupal weight and pupal malformation were calculated. Also, adults emergence, longevity, fecundity, fertility and malformation were recorded.

Statistical analysis

Data were expressed as mean ± standard error; the statistical significance of differences between individual means was determined by student "t" test for paired observations. The level of significance of each experiment was stated to be non significant ($P< 0.05$) and highly significant ($P< 0.05$). The percentage reduction was calculated according to Khazanie (1979). The corrected mortality percentage was statistically computed according to Finney (1972), from which the corresponding concentration Probit lines (Ld –p line) of the 2nd instar larvae were estimated, in

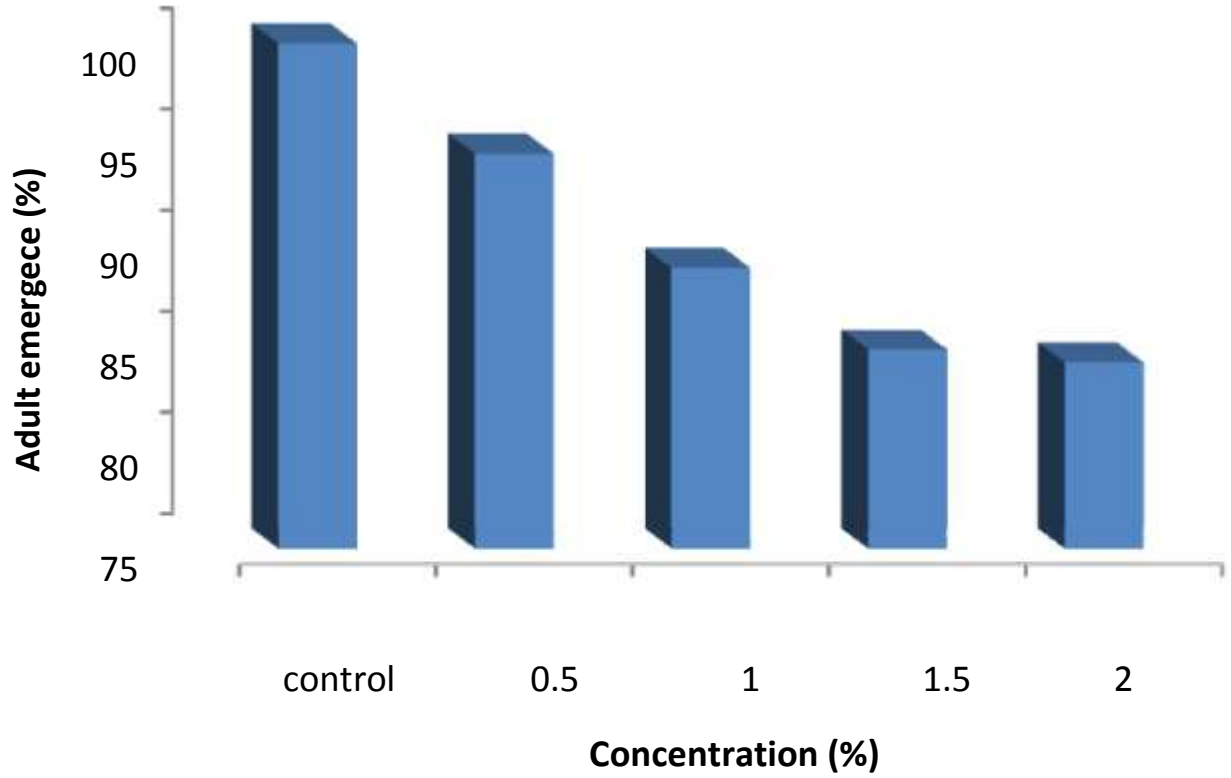

Figure 7. Effect of different concentrations of *Bacillus thuringiensis israelensis* on the percentage of adult emergence of *M. domestica* treated as 2nd instar larvae.

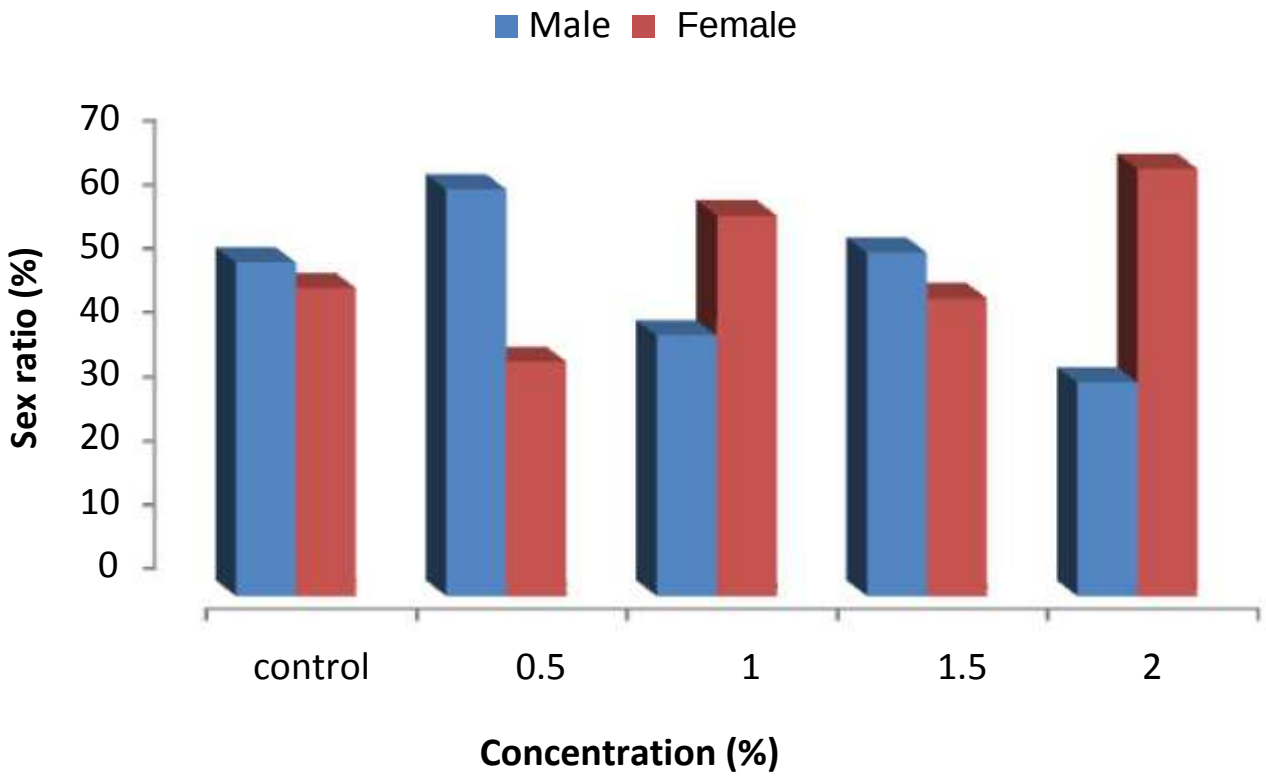

Figure 8. Effect of different concentrations of *Bacillus thuringiensis israelensis* on the percentage of sex ratio (male, female) of *M. domestica* treated as 2nd instar larvae.

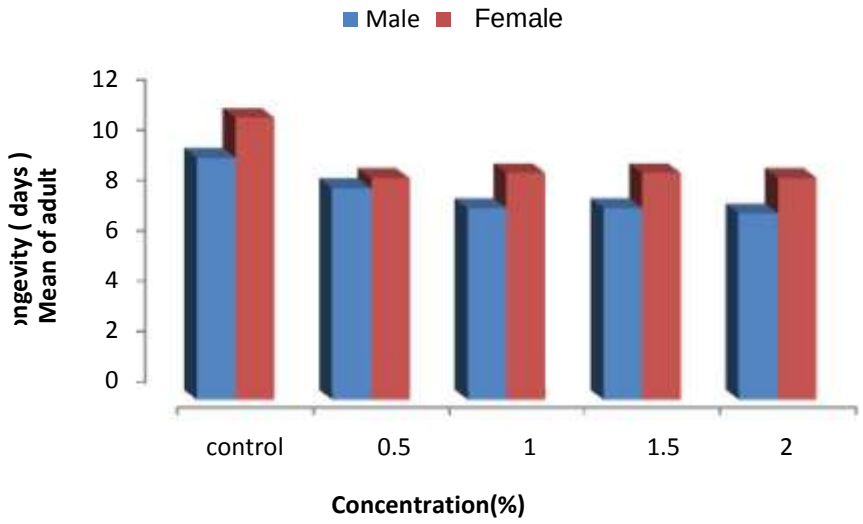

Figure 9. Effect of different concentrations of *Bacillus thuringiensis israelensis* on adult longevity (days) of *M. domestica* treated as 2nd instar larvae.

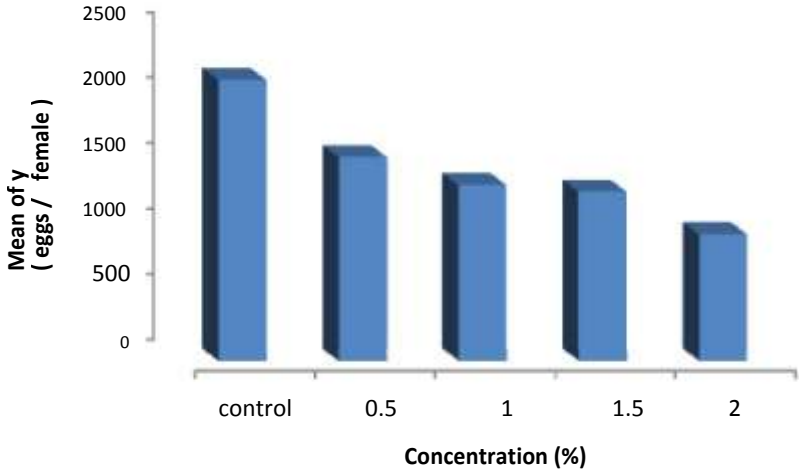

Figure 10. Effect of different concentrations of *Bacillus thuringiensis israelensis* on fecundity (eggs / female) of *M. domestica* treated as 2nd instar larvae.

addition to determine 30 and 50% mortalities and slope value of the tested material (Figures 11 and 12).

RESULTS

Susceptibility of 2nd larval instar of *M. domestica vicina* to different concentrations of *B. thuringiernsis israelensis* after 48 h of treatment

Data presented in Table 1 summarized the efficacy of B.t.i at different concentrations (0.5, 1.0, 1.5, and 2.0%) against the 2nd larval instar larvae *M. domestica*. It is clear that the pathogen affected the percentage of observed larval mortality, increasing gradually with the increase in concentration. The response of larvae to different concentrations by straight regression lines indicating homogeneity. The LC_{30} and LC_{50} were 0.87 and 1.305, respectively. Figure 11 and 12

Impact of the tested bioinsecticide (B.t.i) at different concentration on some biological attributes of 2nd instar larvae of *M. domestica vicina*

The latent effects of the tested bioinsecticides on the 2nd instar larvae of *M. domestica vicina* are shown in Tables 2 and 3. Larval duration, pupal duration, pupal percentages, pupal weight, adults emergence percentage, longevity of adults, number of eggs laid/ female

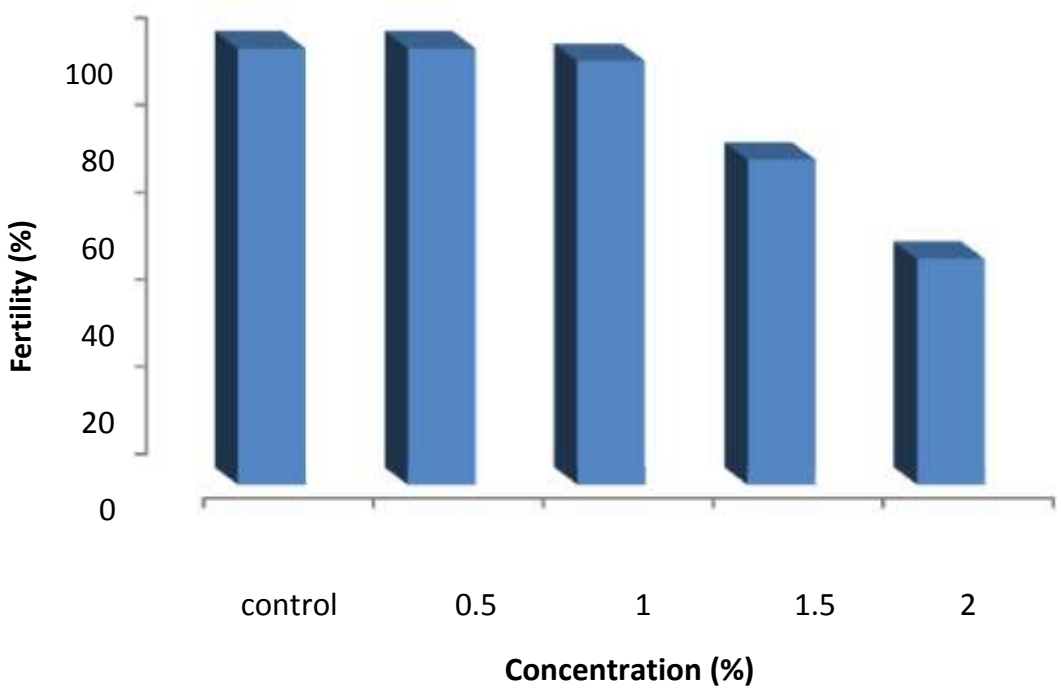

Figure 11. Effect of different concentrations of *Bacillus thuringiensis israelensis* on the percentage of fertility of *M. domestica* treated as 2nd instar larvae.

Table 1. Susceptibility of 2nd instar larvae of *M. domestica* vicina to different concentration of *B. thuringiensis israelensis* after 48 h of treatment.

Concentration (%)	Observed % of mortality	Expected % of mortality
Control	-	-
0.5	16	10.745
1.0	26	36.525
1.5	52	57.125
2.0	80	70.934

Fifty larvae were used for each concentration. P-value = 0.0017, slope of the regression line = 2.976, LC_{30} = 0.87, LC_{50} = 1.305.

(fecundity), hatchability percentage (fertility) and morphological malformations of pupae and emerged adults were investigated and recorded. Data in Table 2 showed that, all treatments caused significant prolongation in the total larval duration compared to control. The duration period ranged between 2.45 ±0.251 and 8.68 ± 0.158 days at concentrations of 0.5 and 2.0% respectively. Also, data clearly indicated that there was an inverse relationship between different concentrations and the pupation percentages, this criterion was 78.57 and 27.14% at concentrations of 0.5 and 2.0% respectively as compared with 100% in the check experiment. As well as all tested concentrations induced significant reduction in pupal weight. Also, the duration periods of pupal stage were increased as affected by treatment, this increase ranged between 3.12 and 5.68 days.

From our results presented in Table 3, it was clear that the percentage of adults emergence was insignificantly decreased with an increase in applied concentrations, it reach to 84.21 for 2.0% as compared with 100% in the check experiment. As for sex ratio, it was remarkable that the sex ratio was directed to the female side. Also, there was a significant reduction in the percentage of adult longevity of both sexes (8.8 ± 1.02) days for 2.0% as compared to 11.2 ± 0.37 days in the control group. The total number of eggs laid/ female were significantly reduced as compared to control ($P < 0.01$), these effects were increased with increasing the concentration. Concerning the effect of larval treatment at different concentrations on the fertility of eggs laid by the resulted females (Table 3) it is clear from the obtained results that, the hatchability percentage of *M. domestica* eggs laid by flies resulted from treated 2nd instar larvae ranged from

Table 2. Latent effects of different concentrations of *Bacillus thuringiensis israelensis* on some biological aspects of immature stages of *M. domestica.*

Concentration (%)	Larval duration (days) Means ± SE	+ or – (%)	Pupation (%)	+ or – (%)	Pupal weight (mg) Means ± SE	+ or – (%)	Pupal malformation (%)	Pupal duration (days) Means ± SE	+ or – (%)
Control	10.36 ± 0.127	-	100	-	73.5±8.85	-	0	3.04 ±0.115	-
0.5	2.45 ± 0.251**	20.17	78.57	-21.43	70.2 ±12.80	-1.21	5.46	5.12 ±0.119	0.81
1	4.92 ± 0.363 **	44.02	51.43	-48.57	66.4 ± 10.01*	-2.60	16.67	4.19 ±0.337**	23.72
1.5	8.58 ± 0.666**	79.34	47.14	-52.86	55.1 ±12.20**	-6.73	21.21	5.68 ±0.861**	73.40
2	8.68 ± 0.158 **	80.31	27.14	-72.86	54.7 ± 12.20*	-6.87	21.15	3.67 ±0.836**	40.12

Data expressed as Mean ± Standard Error (S.E). *Significant (P< 0.05); ** Highly significant (P< 0.01); + or – (%): $\frac{\text{Treated-Control}}{\text{Control}} \times 100$ = Percentage of increase or decrease as compared to the control

Table 3. Latent effects of different concentrations of *Bacillus thuringiensis israelensis* on some biological aspects of adult stage of *M. domestica* treaded as 2nd larval instar.

Conc. (%)	Adult emergence (%)	+ or – (%)	Sex ratio (%)		Adult longevity (days) means ± SE				Fecundity (eggs/ female) means ± SE	+ or – (%)	Fertility (%)	+ or – (%)	Adult malformation (%)	+ or – (%)
			Male	Female	Male	+ or – (%)	Female	+ or – (%)						
Control	100	-	52	48	9.6±0.51	-	11.2 ±0.37	-	2142.00 ±159.80	-	100	-	4	-
0.5	94.55	-5.46	63.46	36.54	8.4 ±0.40*	-12.50	8.8 ±0.49**	-21.43	1552.50 ±157.80**	-27.52	100	0	17.31	332.75
1	88.89	-11.11	40.63	59.38	7.6 ±0.68**	-20.83	9.0 ±1.18**	-19.64	1339.80 ±40.03**	-37.45	97.12	-2.88	21.88	447.00
1.5	84.85	-15.15	53.57	46.43	7.6 ±1.30**	-20.83	9.0±0.55**	-19.64	1289.75 ±40.03**	-39.79	74.49	-25.51	28.57	614.25
2	84.21	-15.79	33.33	66.67	7.4 ±1.21**	-22.92	8.8 ±1.02**	-21.43	964.50 ±111.06**	-54.97	51.70	-48.30	37.50	837.50

Data expressed as Mean ± Standard Error (S.E). *Significant (P< 0.05), ** Highly significant (P< 0.01). + or – (%): $\frac{\text{Treated-Control}}{\text{Control}} \times 100$ = Percentage of increase or decrease as compared to the control

51.70 to 97.12% at the concentrations of 2.0 and 1.0%, the hatchability percentage of deposited eggs was not affected by treatment (0.5%).

Malformed individuals (pupae and adults) were observed, high concentrations induced dwarf larvae and prepupae with dark colour of the whole body positively directed with dose. It is clear that the abnormalities extended to the resulted pupae, they were characterized by the following; small size directed downward head capsule, undifferentiated body segments and the puparum was fully separated and all these malformations of pupae were concentration dependent. Flies of *M. domestica* resulted from treated 2nd instar larvae which showed also varying degrees of deformities as a side effect of bioinsecticide at different concentrations. The deformities include the attachment of pupal exuvium and the fly failed to emerge, enlargement in the abdominal region, severe shrinkage of appendages especially wings. Wings were folded and extremely reduced in size.

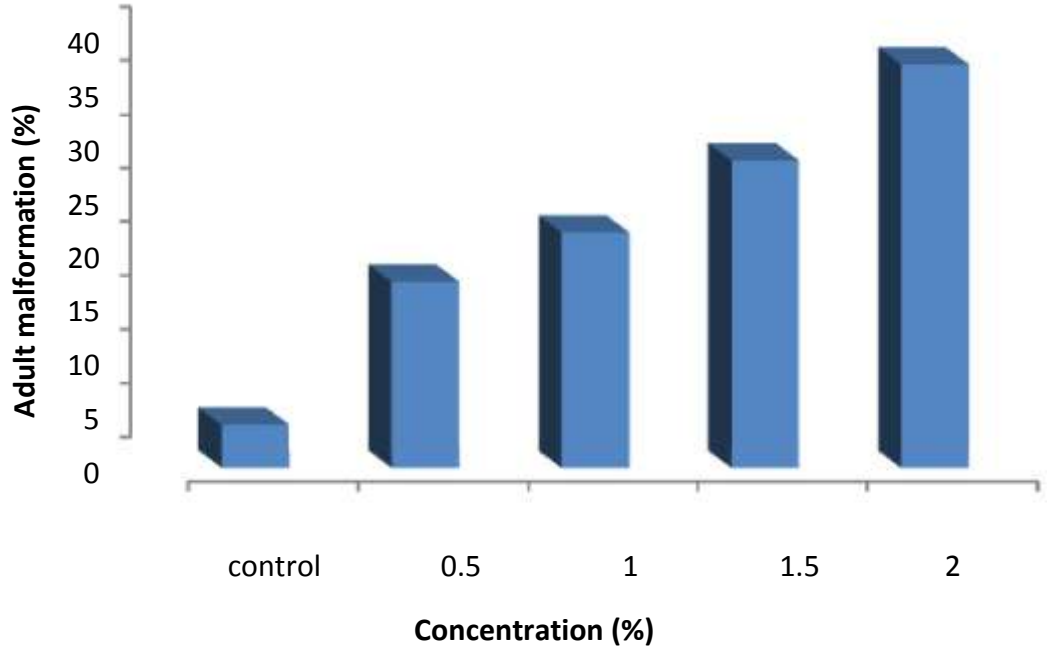

Figure 12. Effect of different concentrations of *Bacillus thuringiensis israelensis* on the percentage of adult malformation of *M. domestica* treated as 2nd instar larvae.

Finally, the resulted adults appeared unable to fly.

DISCUSSION

The biocides *(B. thuringiernsis)* used in the present study caused considerable toxic effects against 2nd instar larvae of *M. domestica vicina*. Our results clearly indicated that, the different applied concentration of the bioinsecticides clearly affected the percentage of larval mortality and the response of larvae is represented by straight regression lines indicating homogeneity. The present conclusion was in harmony with Gharib and Wyman (1991), El Zoghbey and Attalla (2003), Wang and Jall (2005).The obtained results showed that, there was an inverse relationship between the different concentrations under investigation and the pupation percentage, pupal weight (Ayanta et al., 1999; Attala et al., 2003; Dotton et al., 2003; Koja et al., 2006). Moreover, B.t.i showed an increase in the percentage of malformed pupae and adults. The reason of malformations may be due to the reduction in proteins, transaminase enzymes, carbohydrate hydrolyzing enzymes and lipids and these results and observations are in agreement with those of Attalla et al. (2003).

Adult mortality and longevity of both male and female was significantly decreased, these may be due to the latent toxic effects of the tested material, and this was agreed with that obtained by Mohamed et al. (2005), Koja et al. (2006) and Younes et al. (2008). The mean number of deposited eggs per female significantly decreased after the treated 2nd instar larvae of *M. domestica* with tested material at different concentrations due to the inhibition of protein contents and its synthesis, which is necessary for the nutrition of eggs (El- Halim, 1993; Tawfik et al., 2002; El Bandary, 2004). Hatchability percentage (fertility of the deposited eggs decreased after treatments at different concentrations). These results are in harmony with those obtained by Sondos et al. (2000), El Gemiey (2002), Omar (2003) and Narayanan (2004). It may be possible in this instance to control flies by the use of this bacterium which incorporate spores and crystals of the appropriate strain of B.t.i. (Wang et al., 2009; Wirth et al., 2010; Fernandez et al., 2010).

REFERENCES

Ajanta C, Kaushik NC, Gupta GP, Chandra A (1999). Studies of *Bacillus thuringiernsis* on growth and development of *Helicoverpa armigere* Hiibner. Ann. Plant. Protection. Sci., 7(2): 154-158.

Attala FA, Shoeb MA, Ablas MST (2003). Evaluation of Agerin a commercial formulation of *Bacillus thuringiernsis* against certain insect pests of cabbage. Egyptian. J. Biol. Pest. Control., 13(112): 115-117.

Cohen D, Green M, Block C, Slepon R, Ambar R, Wasserman SS, Lavine MM (1991). Reduction of transmission of shigellosis by control of houseflies (*Musca domestica*). Lancent., 337: 993- 997.

Dutton A, Klein H, Romeis J, Bigler F (2003). Prey mediated effects of *Bacillus thuringiernsis* spray on predator *Chrysoperla earnea* in maize. Biol. Control., 26(2): 209-215.

El Bandary FE, Al-Khaalaf AA (2004). Laboratory studies of two commercial formulations of *Bacillus thuringiernsis* (Berliner) for activity *against 1st larval instar of Spodoptera littoralis* (Boisd.) Lepidoptera: Noctuidae). Ann. Agric. Sci. Moshtoher., 42(3): 1395-1404.

El-Gemeiy HM (2002). Impact of two formulations of *Bacillus thuringiernsis* var. *kurstaki* on the life table parameters of the spiny

ballworm *Earias insulana* (Boisd.) Ann. Agric. Sci.Moshtohor., 40(3): 1817-1824.

El–Halim SMA (1993). Bioactivity of Dipel 2X, a commercial preparation of *Bacillus thuringiernsis* Berliner against the cotton leafworm *Spodoptera littoralis* (Boisd.) .Egypt. J. Agric. Res., 71(1): 175-183.

El-Zoghbey AA, Attalla FA, Mesbah AH (2003). Effect of two biocides in controlling *Cassida Vittata* (vill.) and *Spodoptera littoralis* (Biosd.) infesting plants. Ann. Agric. Sci, Moshtohor., 41(1): 339-346.

Fernandez-Luna MT, Tabashnik BE, Lanz-Mendoza H, Bravo A, Soberon M, Miranda-Rios J (2010). Single concentration tests show synergism among *Bacillus thuringiensis* subsp. *Israelensis* toxins against the malaria vector mosquito *Anopheles albimanus.* J. Invertebr. Pathol., 104(3): 231 -3 .

Finney DJ (1972). Probit analysis. A. statistical treatment of the sigmoid response curve 7th Ed., Cambridge Univ. press, England.

Gharib AH, Wyman JA (1991). Food consumption and survival of *Trichoplusia ni* (Lepidoptera: Noctuidae) larva following intoxication by *Bacillus thuringiernsis* var. *kurstaki* and var. *thuringiernsis*. J. Econ. Entomol., 89(2): 436-439.

Hodgman TC, Ziniu Y, Ming S, Sawyer T, Nicholls CM, Ellar DJ (1993). Characterization of a *Bacillus thuringiensis* strain which is toxic to the housefly *Musca domestica .* FEMS. Microbiol. Lett., 114: 17- 22.

Hogsette JA (1999). Management of ectoparasites with biological control organismis . Internat. J. Parasitol., 29: 147- 151.

Indrasith LS, Suzuki N, Ogiwara K, Asano S, Hori H (1992). Activated insecticidal crystal proteins from *Bacillus thuringiensis* seovars killed adult houseflies. Lett. Appl. Microbiol., 14: 174 – 177.

Hohnson C, Bishop AH, Turner CL (1998). Isolation and activity of strains of *Bacillus thuringiensis* toxic to larvae of the housefly (Diptera: Calliphoridae). J. Invertebr. Pathol., 71: 138- 144.

Khazanie R (1979): Eementary statistics (Good Year Publishing Co; California, U.S.A, 488P)

Koja SMT, Rezk GN, Madiha, Hanafy HEM (2006). Effect of *Bacillus thuringiernsis* (Berliner) commercial formolutions against the spiny ballworm *Earias insulana* (Boisd.) Ann. Agric. Sci. Cairo., 51(1): 261-269.

Labib IM, Rady M (2001). Application of *Bacillus thuringiensis* in poultry houses as a biological control agent the housefly, *Musca domestica sorbons.* J. Egypt. Soc. Parasitol., 31(2): 531 – 544.

Lelwallen LL (1954). Biological and toxicological studies of the little house fly. Econ. Entomol., 47: 1137 – 1141.

Lonc E, Lecadet M, Lachowicz TM, Panek E (1997). Description of *Bacillus thuringiensis wratislaviensis* (H-47), a new serotype origination from Wroclaw (Poland), and other *Bt* soil isolates from the same area. Lett. Appl. Microbiol., 24: 467 – 473.

Luga R, Alberto S, Ignazio F (2008). Immature housefly (*Musca domestica*) control in breeding sites with a new *Brevibacillus laterosporus* formulation. Environ. Entomol., 37(2): 505 – 509.

Mohammed EHM, Abd El- Haleem SM, El- Husseini MM (2005). Efficacy and residual effect of *Bacillus thuringiernsis* against larvae of the cotton leaf worm*, Spodoptera littoralis* (Biosd.) in Egyptian clover fields. Egyptian. J. Biol. Pest. Control. 15 (1/2): 81-83.

Moon RD (2002). Muscid flies, In. Mullen G, Durden L, (Eds). Medical and veterinary entomology. Academic, London, United Kingdom. pp. 279-301.

Narayanan K (2004). Insect defense: its impact on microbial control of insect pests. Curr. Sci., 86(6): 800 - 814.

Omar NAM (2003). Impact of *Bacillus thuringiernsis* on some biological, histological and physiological aspects of *Galleria mellonella L*. as a susceptible host. Ph. D. Thesis Fac. Agric. Cairo Univ.

Padmanabhan V, Prabakaran G, Paily KP, Balaraman K, (2005). Toxicity of a mosquitocidal metabolite of *Pseudomonas fluorescens* on larvae and pupae off the housefly, *Musca domestica.* Indian. J. Med. Res., 121: 116 – 119.

Sondos Mohamed A, Bader NA, El Hafez AA (2000). Efficacy of two formulations of pathogenic bacteria *Bacillus thuringiernsis* against the first instar larvae of *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hfn.) (Lepidoptera: Noctuidae). Egyptian. J. Agric. Res., 78(3): 1025-1040.

Tawfik SM, Farghali AA, Sokar A, Abd- El-Wahab IS, (2002). Biochemical studies of Dipel 2X and abamectin on the 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.). Egypt. J. Appl. Sci., 17(3): 371-386.

Wang LY, Jael Z (2005). Sublethal effects of *Bacillus thuringiernsis* H-14 on the survival rate, longevity, fecundity and f1 generation developmental period of *Aedes aegypti*. Denque- Bull., 29: 192-196.

Wang Y, Jin X, Zhu A, Chu F, Yang X, Ma Y (2009). Expression pattern of antibacterial genes in the *Musca domestica.* Sci. China. C. Life. Sci. 52 (9): 823 – 30 .

Wirth MC, Walton WE, Federici BA (2010). Evolution of resistance to the *Bacillus sphaericus* Bin toxin is phenotypically masked by combination with the mosquitocidal proteins of *Bacillus thuringiensis* subspecies *israelensis* . Environ. Microbiol., 12(5): 1154 – 60.

Younes MWF, El-Sayed YA, Hegazy MMA (2008). Effect of *Bacillus thuringiernsis* var. Kurstaki on some biochemical parameters of the cotton leafworm *Spodoptera littoralis* (Boisd.). 4th Int. Conf. Appl. Entomol. Fac. Sci. Cairo. Univ.

Zhong C, Ellar DJ, Bishop A, Johnson C, Lin S, Hart ER (2000). Characterization of a *Bacillus thuringiensis* delta-endotoxin which is toxic to insects in three orders. J. Invertebr. Pathol., 76: 131-139.