

PERFORMANCE ESTIMATION AND SYNERGETIC ROLE OF CAFFEINE IN INCREASING EFFICACY OF *Bacillus thuringiensis* var. *kurstaki* ON *Plodia interpunctella* HÜBNER (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

The Indian meal moth, *Plodia interpunctella* (Hübner) is a widespread and serious pest of stored products. The aim of this study was to evaluate the effectiveness of *Bacillus thuringiensis* var. *kurstaki*, and its synergistic effect with caffeine, in the control of *P. interpunctella*. The minimum and maximum lethal concentrations (LC) of *B. thuringiensis* for first instar larvae control, respectively were 65 ppm and 7500 ppm. The LC₅₀ for the first instar stage of larvae was 637.87 ppm. When first instar larvae are fed a diet which contained LC₅₀ of both *B. thuringiensis* and caffeine, an increased synergistic interaction in terms of mortality was found. The mixture of minimum effective concentrations of 65 ppm bacteria and 4000 ppm of caffeine have resulted in 95% mortality after 144 h, whereas caffeine and bacteria alone caused 10% and 20% mortality, respectively.

Key words: Stored products, LC₅₀, concentration, mortality.

ABSTRAK

Indian meal moth, *Plodia interpunctella* (Hübner) mempunyai taburan meluas dan merupakan antara spesies utama perosak simpanan. Tujuan utama kajian ini adalah untuk mengkaji keberkesanan *Bacillus thuringiensis* var. *kurstaki*, dan kesan sinerginya ke atas kafein dalam mengawal *P. interpunctella*. Nilai maksimum dan maksimum kepekatan kematian (LC) *B. thuringiensis* dalam mengawal instar pertama adalah 65 ppm dan 7500 ppm, masing-masing. LC₅₀ untuk peringkat instar pertama adalah 637.87 ppm. Kesan interaksi sinergi dari sudut

kematian ditentukan apabila larva diberikan diet yang mengandung LC₅₀ *B. thuringiensis* dan kafein. Campuran minimum untuk kepekatan adalah 65 ppm bakteri dan 4000 ppm kafein memberikan 95% kematian selepas 144 jam rawatan, manakala kafein dan bakteri setiap satu memberikan 10 dan 20 peratus kematian, masing-masing.

Kata kunci: Produk simpanan, LC₅₀, kepekatan, kematian.

INTRODUCTION

The Indian meal moth, *Plodia interpunctella* (Hübner) has been called the number one pest insect in storehouse situations (Hassan et al. 2020). These moth commonly find ways into homes, either from outdoors or through purchased food goods and then easily spread into cereals, pasta, pet food, dried foods, nuts, grains and other dried goods. Adult female Indian meal moth uses her antennae to locate food and will then lay eggs singly or in groups on or very near that food. Within two to fourteen days, the larvae hatch and are so small at this point that they can easily enter pinholes in food packages, and will begin to feed within a few hours of hatching (Javi et al. 2004; Na & Ryoo 2000). The larvae are the only stage of this insect that consumes food, and will utilize many different types of stored products, especially nuts and dried fruits. The adult stage does not feed. Larvae will molt between five to seven times (Nouri-Ganbalani et al. 2016), depending on food source, temperature, and other environmental conditions, with the larval stage lasting from two weeks to as long as forty weeks.

Food source is an important factor dictating the biological parameters of *P. interpunctella* (Subramanyam & Hagstrum 1993). The use of microbial insecticides, such as entomopathogens, are effective non chemical substitute's forte control of crop pests (Heimpel 1967). *Bacillus thuringiensis* is actually a group of closely related soil microbes, with each type of microbe producing a different kind of protein that is toxic to specific groups of insects. When insects feed on the protein, the protein changes shape and attaches to the insect gut wall, creating holes in it. The larvae then stop feeding and eventually die. In contrast, when humans consume the same proteins, the protein does not change shape and cause no harm. *Bacillus thuringiensis* is found in over 180 pest control products which generally contain a combination of Bt toxins with a diverse range of compounds for specific target insects. For example, Bt *kurstaki* products are used on garden plants to control caterpillars. Other strains, such as Bt *tenebrionis*, Bt *israeliensis* and Bt *aizawai* are known to target coleopteran and/or dipteran insects (Baum et al. 1999; Hurst et al. 2007; Kaur 2000). Plant alkaloids are one of the largest groups of secondary plant metabolites, which encompass neuroactive molecules such as caffeine (1, 3, 7-trimethylxanthine, C₈H₁₀N₄O₂). This compound is a purine alkaloid naturally found in coffee plants, tea leaves, cocoa beans, cola nuts and other plants (Nawrot et al. 2003). Caffeine can be an effective inhibiting agent on the nervous system of insects as well as deterring their predators (Yang & Stöckigt 2010). The individual effects of Bt have been studied frequently on numerous pest insects, although there are few studies on the combined synergistic effects of Bt and caffeine. Results of studies on the effects of Bt and caffeine on first-instar larvae of *P. interpunctella* under laboratory conditions are provided here.

MATERIALS AND METHODS

Sample Rearing

Eggs and pupae of *P. interpunctella* were collected from dried fruits. Rearing was performed on almond and dried berry under laboratory conditions 27±2 °C, 70±5% relative humidity, and photoperiod 11:13 (Light: Dark). Four generations were reared, with fifth generation larvae

used for testing. Thirty first instar larvae were put in containers and were covered with net cloth. First instar larvae were used as that stage is more susceptible to control measures than subsequent instars (Askari Seyahoei et al. 2018). Food material (artificial diet) was put in dishes within the container with supply refreshed once every three days. Artificial food ration mixes contained 800 grams wheat bran, 160 grams yeast, 200 ml glycerin and 200 ml honey (Sait et al. 1997).

***Bacillus thuringiensis* var. *kurstaki* Preparation**

In order to investigate pathogenicity, a commercially available *B. thuringiensis* var. *kurstaki* was utilized from the Tehran plant disease and pest research Institute. The commercial product was first cultured in Neutrinite agar medium to ensure its reproducibility. The experimental tube containing agar medium and Bt. var. *kurstaki* product was incubated at 30 ± 2 °C. Colonies of bacterium propagated after 24 hours and sampling was performed from this medium after four days. Preparation and staining of the sample were done by first pouring a drop of distilled water onto a glass slide, spreading the bacterial sample on the slide with a needle, and subsequent warming on an alcoholic light until dry. Lactofenene stain was added to the dry slide and washed for one minute. Bacteria were then identified using a microscope.

Preparation of Bacterial Concentrations

Varying concentrations of bacterium were prepared following Morad Eshaghi and Pour Mirza (1974), and selected for testing based on logarithmic distances between minimum and maximum dosages. 20 ml bacteria concentrations were mixed with distilled water and sprayed onto 20 grams of artificial food. Fifteen larvae were introduced to the food for 72-96 hours. This test was carried out using eight treatments and three replications in a randomized design experiment. Bacteria concentrations were determined using methodologies provided by Morad Eshaghi and Pour Mirza (1974).

Synergetic Effect of Caffeine

Merck Company caffeine used in these tests was prepared by the Ilam School of Medicine.

Experimentation of the Synergetic Effect of Caffeine Together with Bacteria

First instar larvae were exposed to artificial food with varying caffeine and bacteria concentrations, with larval mortality rate counted every 24 hours. Tests were carried out using nine treatments and four replications in a complete randomized design.

1. Pure B.t. (effective concentration minimum: 65 ppm)
2. Caffeine (1000 ppm) + B.t. (effective concentration minimum: 65 ppm)
3. Caffeine (2000 ppm) + B.t. (effective concentration minimum: 65 ppm)
4. Caffeine (3000 ppm) + B.t. (effective concentration minimum: 65 ppm)
5. Caffeine (4000 ppm) + B.t. (effective concentration minimum: 65 ppm)
6. Caffeine (5000 ppm) + B.t. (effective concentration minimum: 65 ppm)
7. Caffeine (6000 ppm) + B.t. (effective concentration minimum: 65 ppm)
8. Caffeine (7000 ppm) + B.t. (effective concentration minimum: 65 ppm)
9. Control treatment (distilled water)

Effects of Different Caffeine Concentrations on Larvae of *P. interpunctella*

Varying concentrations of caffeine were tested on first instar larvae of *P. interpunctella*. Larvae were exposed to artificial food with varying caffeine concentrations, with larval mortality rate counted every 24 hours. This test was carried out using eight treatments and four replications in a randomized design.

1. Caffeine (1000 ppm)
2. Caffeine (2000 ppm)
3. Caffeine (3000 ppm)
4. Caffeine (4000 ppm)
5. Caffeine (5000 ppm)
6. Caffeine (6000 ppm)
7. Caffeine (7000 ppm)
8. Control treatment (distilled water)

Effect of Caffeine Repelling

A filter paper was divided into two equal parts, with one side submerged in the control treatment and the other side submerged in a caffeine concentration (tests were replicated using seven different caffeine concentrations). After drying, the two sides of filter paper were glued to each other. Twenty first instar larvae of *P. interpunctella* were then placed in the middle of the filter paper. Numbers of larvae were counted every 6 hours in the control treatment and, according to the formula $PR = 2 (C-50)$, where C equals the number of insects per control treatment, the replant percentage could be calculated.

Effect of Bacteria Feeding Time Period on First Instar Larvae Mortality

Concentration for this test was 640 ppm, with a sampling duration of 24 hours continuing for 144 hours. This test was performed using nine treatments and four replications (every replication with 15 individuals of larvae) in a randomized design.

Effect of Caffeine Feeding Time Period on First Instar Larvae Mortality

Concentration for this test was 4000 ppm, with a sampling duration of 24 hours, continuing for 144 hours. This test was performed using nine treatments and four replications (every replication with 15 larvae) in a randomized design.

The Duration Simultaneous Effects of Feeding Bacteria and Caffeine in Larval Mortality

This Experiment was performed with a caffeine concentration of 4000 ppm and a bacterial concentration of 65 ppm, in a completely randomized design with 9 treatments and 4 replications, each with 10 first instar larvae.

Data Analysis

The Data were analyzed using SAS 6 and Probit programs. Duncan's test and Excel software were employed to compare the means and plot graphs, respectively.

RESULTS

Effect of Different Bacteria Concentrations and Determining of Bacteria LC₅₀

Minimum and maximum bacteria concentrations were recorded in primary tests and then, according to logarithmic distance, their middle concentration was measured (Table 1). Mortality percentages were 1.6, 10, 20, 43.33, 55, 66.66, 80, 88.33 and 91.66 in concentrations 0, 65, 143.43, 316.44, 698.21, 1540.56, 3340.14, 7500 and 16548.26 ppm, respectively. The minimum and the maximum concentrations were 65 and 7500 ppm, respectively. LC₅₀ was 637.87 ppm. There was not any significant difference between treatments, but there was significant difference between 7500 and 16548.26 ppm concentrations (Figure 1; Table 2).

Table 1. Measuring of LC₅₀ of different bacteria concentrations

Substance	Insect number	LC ₅₀	Confidence interval (95)	Slope±SE	df	x ²
B.T.	60	637.87	482.128-830.865	1.119±0.939	6	3.205

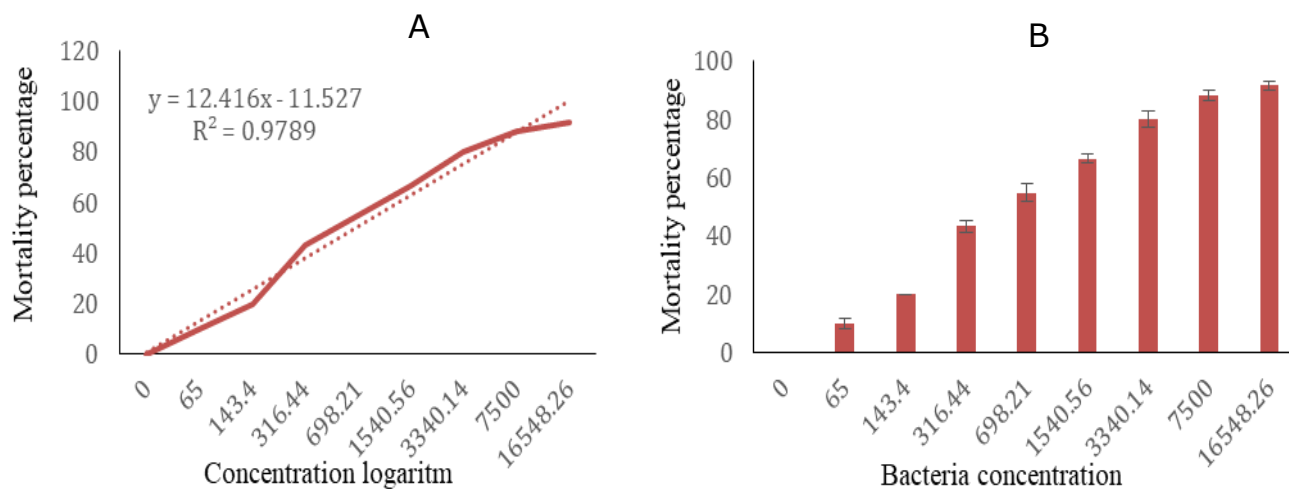


Figure 1. A) Concentration logarithm and mortality percentage of varying bacterial concentrations on first instar larvae of *P. interpunctella*. B) Effect of varying bacterial concentrations on larvae of *P. interpunctella*

Table 2. Variance analysis of effect of different bacteria concentrations on larvae of *P. interpunctella*.

Source	SS	MS	Df	F
Treatment	38024.69	4753.07	8	320.83**
Error	399.99	14.81	27	
Total	338241.69	35		

Cv: 7.585

Effect of Varying Caffeine Concentrations on First Instar Larvae of *P. interpunctella*

In this experiment, mortality percentages were 0, 5, 12.5, 15, 20, 27.5, 27.5 and 27.5% in the control treatment and with caffeine concentrations from 1000-7000ppm, respectively. LC₅₀ was 18098 ppm (Table 3). There was significant difference between treatments, but there was not a significant difference between 5000, 6000 and 7000 ppm concentrations, so a 5000 ppm concentration was used (Figure 2; Table 4).

Table 3. Measuring of LC₅₀ of differing caffeine concentrations

Substance	Insect number	LC ₅₀	Confidence interval (95)	Slope±SE	df	x ²
Caffeine	40	18098	9816.1-0.14969	1.2624±0.370	5	0.524

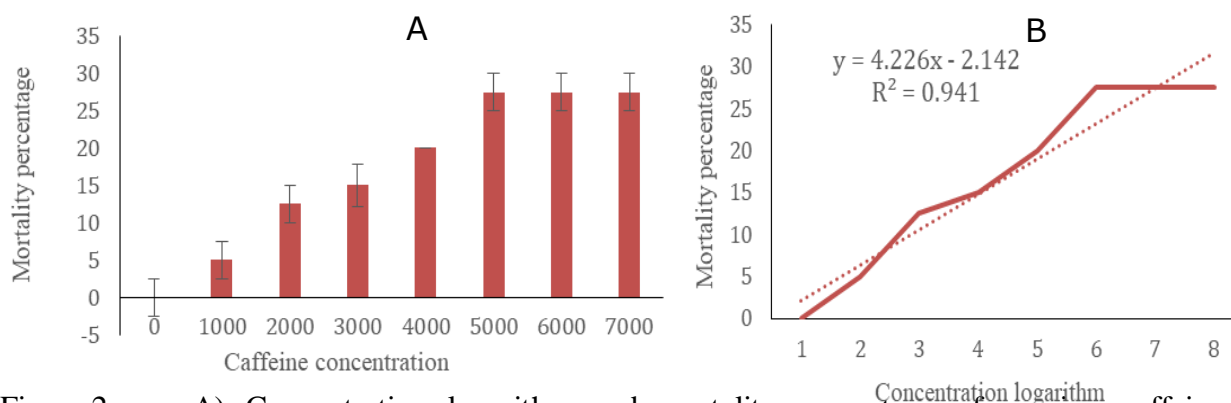


Figure 2. A) Concentration logarithm and mortality percentage of varying caffeine concentrations on first instar larvae of *p. interpunctella*. B) Mortality percentage of varying caffeine concentrations on larvae of *p. interpunctella*

Table 4. Variance analysis of effect of varying coffee concentrations on larvae of *P. interpunctella*.

Source	SS	MS	Df	F
Treatment	7	76/835	10/976	17/86**
Error	24	14/753	0/614	
Total	31	91/589		

Cv: 14/581

Effect of Feeding Time on First Instar Larvae Mortality

In this experiment, feeding time required to cause 50% mortality in first instar larvae was computed, using a concentration of 640 ppm bacteria (Table 5). Larvae were counted in 0, 24, 48, 72, 96, 120 and 144 hours, respectively. Comparison of mortality percentage showed that the highest mortality rate was 144 hours, but since there was not any significant difference between 120 and 144 hours, 120 hours was considered for further test. Probit analysis showed the time required for 50% mortality in first instar larvae was 38061.1 minutes, or approximately 63.435 hours after feeding (Figure 3; Table 6 & 7).

Table 5. Effects of differing bacterial concentrations on larvae of *P. interpunctella*.

Treatment	X±Se	Group
0	0±0	F
24	886/2±000/15	E
48	886/2±000/35	D
72	0±000/50	C
96	886/2±000/65	B
120	886/2±000/85	A
144	886/2±000/85	A

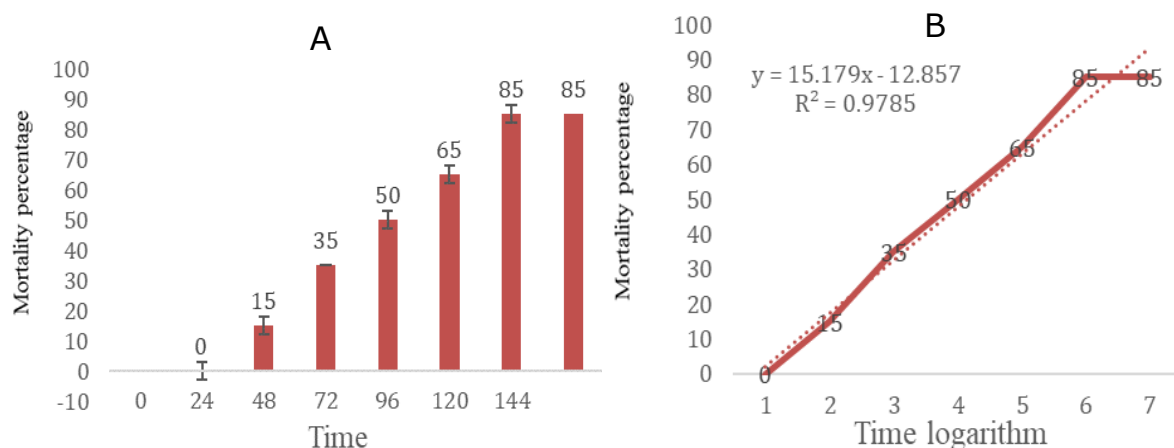


Figure 3. A) Time logarithm and mortality percentage of 640 ppm bacteria concentration in different time durations on first instar larvae of *P. interpunctella*. B) Mortality percentage in different time durations on first instar larvae of *P. interpunctella*

Table 6. Measuring of LT_{50} of bacteria 640 ppm concentration

Substance	Insect number	LC_{50}	Confidence interval (95)	Slope±SE	df	χ^2
Bacteria	40	63.435	53.826-73.247	2.796±0.370	4	2.521

Table 7. Variance analysis of effect of different bacteria concentrations on larvae of *P. interpunctella*.

Source	SS	MS	Df	F
Treatment	6	26371/42	4395/238	184/60**
Error	21	500/000	23/809	
Total	27	26871/428		

Cv: 10.195

In this experiment, 4000 ppm concentration of caffeine was used, which caused 20% mortality in first instar larvae. Larvae were counted in 0, 24, 48, 72, 96, 120, 144 (Table 8) hours. Mortality rates were 0, 7.5, 10, 12.5, 15, 20 and 20, respectively. The lowest mortality rate occurred in 24 hours and the highest mortality rate occurred after 144 hours (20%) (Figure 4; Table 9). There was not any significant difference between 120 and 144 hours, so a time of 120 hours was considered.

Table 8. Effects of differing coffee concentrations on larvae of *P. interpunctella*

Treatment	$X \pm Se$	Group
0	0/000±0	D
24	2/500± 7/500	C
48	10/000±0	CB
72	12/500±2/500	CB
96	15/000±2/886	AB

120	20/000±0	A
144	20/000±0	A

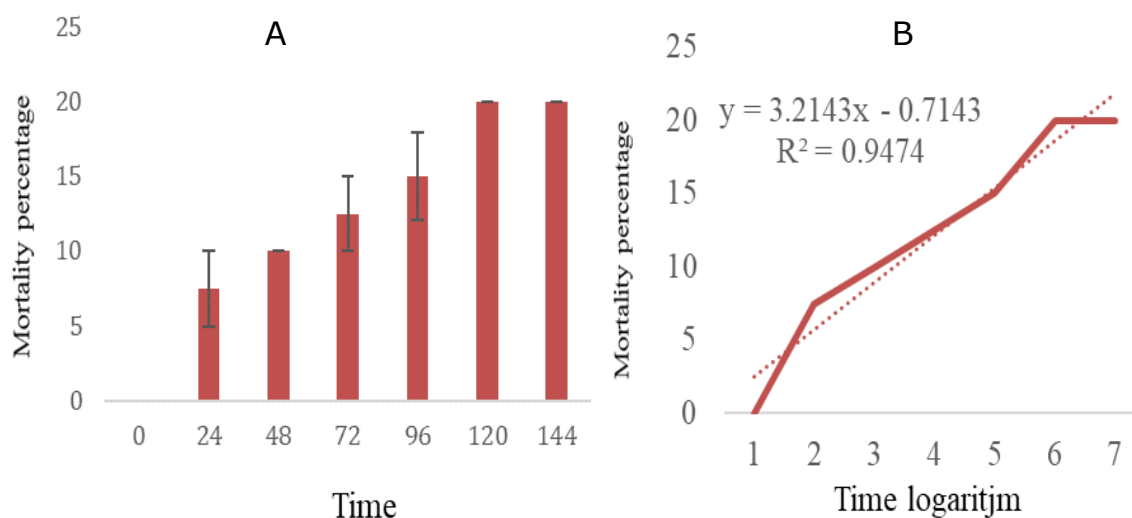


Figure 4. A) Time logarithm and mortality percentage of 4000 ppm concentration of Caffeine on first instar larvae of *P. interpunctella*. B) Mortality percentage of 4000ppm concentration of caffeine on first instar larvae of *P. interpunctella*

Table 9. Variance analysis of effect of different caffeine concentrations on larvae of *P. interpunctella*.

Source	SS	MS	Df	F
Treatment	6	42/523	7/0872	19/30**
Error	21	7/713	0/3672	
Total	27	50/236		

Cv: 12.40

Effect of Time of Feeding from 4000 ppm Concentration of Caffeine + 65 ppm Bacteria Concentration on First Instar Larvae Mortality rate

In this experiment, a concentration of 4000 ppm concentration of caffeine caused 20% mortality in first instar larvae accompanied by a 65 ppm bacteria concentration. This was the least lethal concentration used. Larvae were counted in 0, 24, 48, 72, 96, 120, 144 hours (Table 10). Mortality rates were 0, 27.5, 45, 65, 80, 92.5 and 92.5, respectively. The lowest mortality rate occurred in 24 hours and the highest mortality rate occurred after 144 hours (90%). There was significant difference between different treatments (Figure 5, Table 11).

Table 10. Effect of time duration of 4000 ppm concentration of caffeine plus 65ppm bacteria concentration on larvae of *P. interpunctella*

Treatment	X±Se	Group
0	0.0±0.0	E
24	27.50±2.50	F
48	45.0±2.886	D

72	65.0±2.886	C
96	80.0±0.0	B
120	90.0±0.0	A
144	90.0±0.0	A

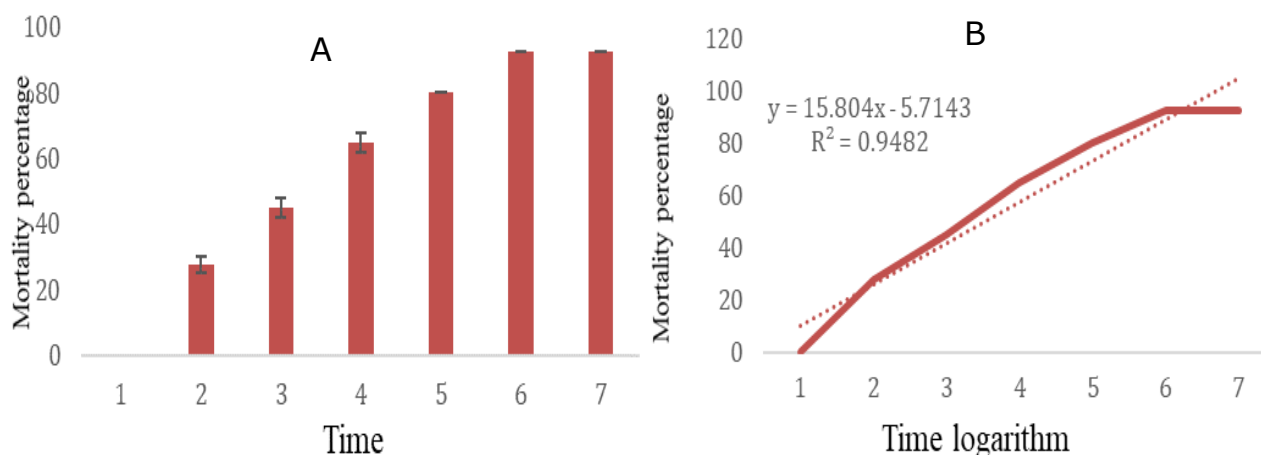


Figure 5. A) Time logarithm and mortality percentage of 4000 ppm concentration of Caffeine accompanied by 65 ppm bacteria concentration in different time durations on first instar larvae of *P. interpunctella*. B) Effect of time period of feeding from 4000 ppm concentration of caffeine plus 65 ppm bacteria concentration on first instar larvae mortality

Table 11. Variance analysis of effect of 4000ppm concentration of Caffeine accompanied by 65 ppm bacteria concentration in different time durations on first instar larvae of *P. interpunctella*.

Source	SS	MS	Df	F
Treatment	6	28135/714	4689/285	358/0**
Error	21	275/000	13/095	
Total	27	28410/714		

Cv: 10.196

Effects of Varying Caffeine Concentrations with the Lowest Bacteria Concentration on First Instar Larvae Mortality rate

In this experiment, concentrations of 1000 + 65, 2000 + 65, 3000 + 65, 4000 + 65, 5000 + 65, 6000 + 65 and 7000 + 65 of caffeine/bacteria respectively, were used (Table 12). Larvae were compared with control treatment, 65 ppm bacteria concentration and varying concentrations of caffeine accompanied with a 65 ppm bacteria concentration. Mortality rates were 0, 10, 30, 35, 75, 90, 95, 95 and 95 respectively. There was significant difference between different treatments (Figure 6; Table 13).

Table 12. Effect of time duration of different concentrations of Caffeine + 65 ppm bacteria concentration on larvae of *P. interpunctella*

Treatment	X±Se	Group
0	0.0±0.0	E
65	10.0±0.0	DE
65+1000	20.0±7.071	D
65+2000	55.0±5.0	C
65+3000	72.0±2.50	B
65+4000	92.05±2.50	A
65+5000	97.50±2.50	A
65+6000	95.0±2.886	A
65+7000	95.0±2.886	A

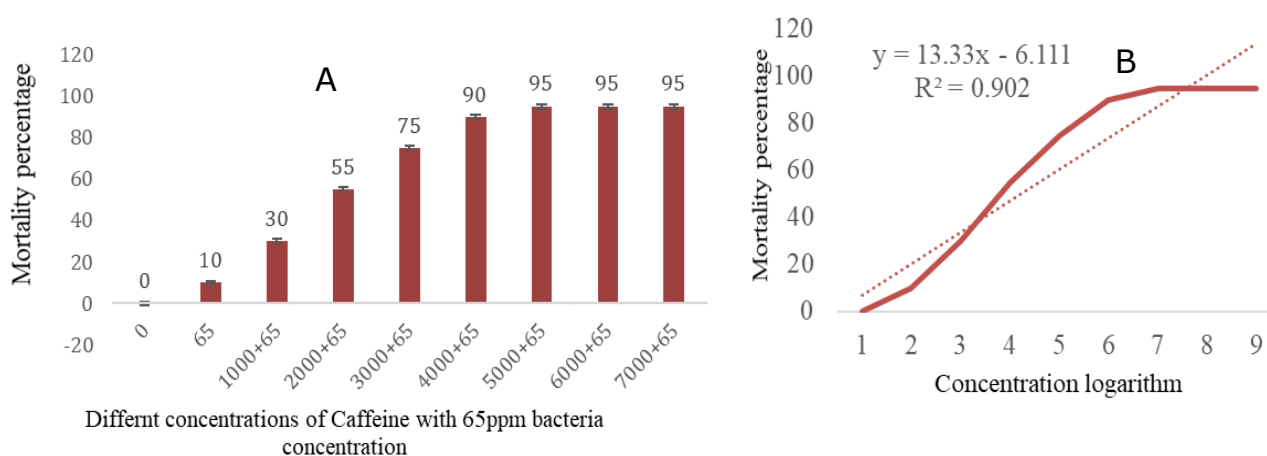


Figure 6. A) Time logarithm and mortality percentage of different concentrations of caffeine accompanied with 65ppm bacteria concentration on first instar larvae of *P. interpunctella*. B) Mortality percentage of varying concentrations of caffeine accompanied with 65ppm bacteria concentration on first instar larvae of *P. interpunctella*

Table 13. Variance analysis of effect of caffeine accompanied with 65 ppm bacteria concentration on first instar larvae of *P. interpunctella*.

Source	SS	MS	Df	F
Treatment	8	51172/222	6396/527	130/34**
Error	27	1325/000	49/074	
Total	35	52497/222		

Cv: 11.729

Survey of Effect of Caffeine Repellency on First Instar Larvae Mortality rate

In this experiment, caffeine concentrations of 0, 1000, 2000, 3000, 4000, 5000, 6000 and 7000 ppm were used (Table 12). Replant percentage was 0, 0, 0, 0, 0, 30, 57.5 and 77.5, respectively (Figure 7).

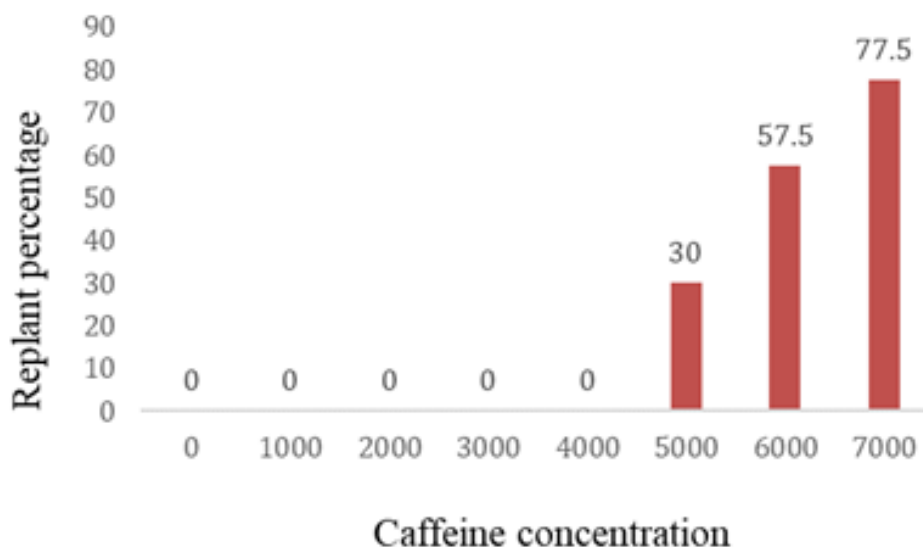


Figure 7. Replant percentage of different concentrations of caffeine on first instar larvae of *P. interpunctella*

DISCUSSION

Bacillus thuringiensis strains, as one of the most common biological agents, are currently using in more than 180 pesticide products in crops and ornamental plants (Burgess 2001; Hajjaliloo et al. 2017; Phillips et al. 2000), but this potency can be reinforced alongside using a synergist (Nouri-Ganbalani et al. 2016). Aim to protect natural enemies, some plant species such as have this ability to produce substances which are derived from their secondary metabolism with some specific properties against herbivore insects (Bernhard et al. 1997; Isman 2006), such as caffeine in coffee, glucosinolates in Brassicaceae, psoralen in celery or even nicotine in tobacco that are causing repulsive, metabolic dysfunctions, or have a toxic effect leading to the death of the insect (Siegwart et al. 2015). In this study, results show that the least and the most effective concentration of bacteria and caffeine for killing 10% of first instar larvae of *P. interpunctella* were 65 and 7500 ppm, respectively. Also, LC_{50} obtained in bioassay tests was 637.87 ppm. LT_{50} of 640 ppm concentration of bacteria was 63.435 hours. After 24 hours of feeding, mortality was seen in treatments. The least and the most effective concentration of Bt. and caffeine was 50 and 7500 ppm, respectively, in a previous study by Modares Najafabadi et al. (1998). Ferro and Lopez (1995) demonstrated that LC_{50} for first instar larvae of *Leptinotarsa decemlineata* (Say) (Coleoptera, Chrysomellidae) was 178.79 ppm, differing because of insect biotype, different formulations of bacteria, and overall laboratory conditions. Surveys of the effect of caffeine on larvae of *P. interpunctella* showed that the minimum and the maximum concentration (1000 and 7000 ppm) had 5 and 27.5% mortality, respectively, and time duration of feeding from caffeine after 120 hours had 20% mortality. These results demonstrate that caffeine alone needs significant time to show effects, but in the case of caffeine with bacteria, larval mortality occurred more rapidly during treatments. These results were observed to some extent on *Leptinotarsa decemlineata*. (Javi et al. 2004). It is also important to note that younger larvae are more sensitive to BT bacteria and the synergist factors on their effect (Lang et al., 2019).

As the world's population grows, humans have begun to manipulate ecosystems to prepare food, upsetting this balance of nature. Humans use strong chemical toxins to produce

more products. The fact that it kills soil microorganisms has negligible negative effects on the plant itself, which reduces the yield and growth of products that enter the human food chain through adipose tissue in animal nutrition. Another major problem with the use of these pesticides is the lack of resistance to insecticides, which is a major problem for agriculture and the control of disease vectors. Resistance cases have been reported since the 1950s, although they have been investigated in recent years. Occurrence overlapping between genetic and biochemical factors that potentially caused cross-resistance could provide the main threat to effective control of pests. Natural enemies and other biological pest control agent cause the least damage to the environment (Fallahzadeh et al. 2020; Heidari Latibari et al. 2020). Thus, the identification of agents that can have a synergist role on biological agents effect, can provide a new approach for designing new effective insecticides. Bio-insecticides contain a wide range of compounds and organisms which ensure their ability to plant protection. One would expect that the diversification of the molecular and biochemical targets in pests could limit the emergence of resistance (Regnault-Roger et al. 2002).

CONCLUSION

Therefore, pest control by biocidal insects based on insect pathogens can be relied on as one of the safest methods of pest control and management. Also the results of the present research demonstrate that the use of both Bt had caffeine combination can create a much more efficient effect to control *P. interpunctella*, especially on the first larvae stages.

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REFERENCES

- Askari Seyahooei, M., Mohammadi-Rad, A., Hesami, S. & Bagheri, A. 2018. Temperature and exposure time in cold storage reshape parasitic performance of *Habrobracon hebetor* (Hymenoptera: Braconidae). *Journal of Economic Entomology*. 111 (2): 564-569.
- Baum, J.A., Johnson, T.B. & Carlton, B.C.1999. *Bacillus thuringiensis*. Natural and recombinant bioinsecticide products. *Methods in Biotechnology*. 5: 189–209.
- Bernhard, K., Jarrett, P., Meadows, M., Butt, J., Ellis, D.J., Roberts, G.M., Pauli, S., Rodgers, P. & Burges, H.D. 1997. Natural isolates of *Bacillus thuringiensis*: Worldwide distribution, characterization, and activity against insect pests. *Journal of Invertebrate Pathology*. 70: 59-68.
- Burges, H.D. 2001. *Bacillus thuringiensis* in pest control: Now and the future. *Pest Outlook 2*: 90–97.
- Ferro, D.N. & Lopez, R. 1995. Larviposition response of *Myiopharus doryphora* to Colorado potato larvae treated with lethal and sublethal doses of *Bacillus thuringiensis* Berliner subsp. *tenebrionis*. *Journal of Economic Entomology*. 88 (4): 870-874.
- Hajjaliloo, S., Moravvej, G. & Heidari Latibari, M. 2017. Comparative study on the effect of *Bacillus thuringiensis* var. *tenebrionis* on adult and third instar larva of elm leaf beetle, *Xanthogaleruca luteola* (Mull) under laboratory and field conditions. 8th Conference on Biological Control in Agriculture and Natural Resources, Guilan University, Iran.
- Hasan, M. M., Chowdhory, S. A., Rahman, A. S., & Athanassiou, C. G. (2020). Development and diapause induction of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) at different photoperiods. *Scientific Reports*. 10 (1): 1-10.
- Heidari Latibari, M., Moravej, G., Ghafouri Moghaddam, M., Barahoei, H. & Hanley, G.A. 2020. The novel host associations for the aphid parasitoid, *Pauesia hazratbalensis* (Hymenoptera: Braconidae: Aphidiinae). *Oriental Insects* 54 (1): 88-95.
- Heimpel, A.M. 1967. A critical review of *Bacillus thuringiensis* var. *thuringiensis* Berliner and other crystalliferous bacteria. *Annual Review Entomology*. 1967 (12): 287–322.
- Hurst, M.R., Jones, S.M., Tan, B. & Jackson, T.A. 2007. Induced expression of the *Serratia entomophila* Sep proteins shows activity towards the larvae of the New Zealand grass grub *Costelytra Zealandica*. *FEMS Microbiol Letter*. 275: 160–167.
- Isman, M. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*. 51: 45–66.
- Javi, E., Safare Alizadeh, M.H. & Poormirza, A.A. 2004. Survey of pathogenic of *Bacillus thuringiensis* Berliner on different instar larvae of *Leptinotarsa decemlineata* (Say) (Col., Chrysomelidae) and effect of plant synergetics in increasing its efficiency in laboratory conditions. *Sciences and Techniques of Agriculture and Natural Sources*. 4: 187-198.

- Kaur, S. 2000. Molecular approaches towards development of novel *Bacillus thuringiensis* biopesticides. *World Journal of Microbiology and Biotechnology*. 16: 781–793.
- Lang, A., Lee, M., Dolek, M., Berchtold, J., & Otto, M. (2019). Laboratory tests with Lepidoptera to assess non-target effects of Bt maize pollen: analysis of current studies and recommendations for a standardised design. *Environmental Sciences Europe*, 31(1), 1-10.
- Modares Najafabadi, S.S., Shayesteh, N. & Safare Alizadeh, M.H. 1998. The survey of biology of *Plodia interpunctella* Hüb. in temperature, humidity and different food substance conditions and the using possibility of *Bacillus thurengiensis* Ber. and temperature for controlling of this pest. Thesis M.Sc. Urmia University.
- Morad Eshaghi, M.J. & Pour Mirza, A.A. 1974. Resistance survey of larvae different ages of *Plodia interpunctella* Hübner (Lep. pyralidae) against microbial insecticide. *Iran Entomologists Union Letter* 2 (1): 25-34.
- Na, J.H. & Ryoo, M.I. 2000. The influence of temperature on development of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried vegetable commodities. *Journal of Stored Products Research* 36: 125-129.
- Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A. & Feeley, M. 2003. Effects of caffeine on human health. *Food Additives and Contaminants* 20 (1): 1-30.
- Nouri-Ganbalani, G., Borzoui, E., Abdolmaleki, A., Abedi, Z. & George Kamita, S. 2016. Individual and combined effects of *Bacillus thuringiensis* and azadirachtin on *Plodia Interpunctella* Hübner (Lepidoptera: Pyralidae). *Journal of Insect Science*. 16 (1): 1-8.
- Phillips, T.W., Berbert, R.C. & Cuperus, G.W. 2000. Post-harvest integrated pest management. In Francis, F.J. (ed.). *Encyclopedia of Food Science and Technology*. 2nd Edition, pp. 2690-2701. New York: Wiley Inc.
- Regnault-Roger, C., Philogène, B.J. & Vincent, C. 2002. *Biopesticides D'origine Végétale* Paris: Editions Tec & Doc.
- Sait, S.M., Thompson, D.J., Harvey, J.A. & Hails, R.S. 1997. Factors affecting host selection in an insect host-parasitoid interaction. *Ecological Entomology*. 2: 225-230.
- Sieglwart, M., Graillot, B., Blachere Lopez, C., Besse, S., Bardin, M., Nicot, P.C. & Lopez-Ferber, M. 2015. Resistance to bio-insecticides or how to enhance their sustainability: A review. *Frontiers in plant science*. 6: 1-19.
- Subramanyam, B. & Hagstrum, D.W. 1993. Predicting development times of six stored product moth species (Lepidoptera: Pyralidae) in relation to temperature, relative humidity and diet. *European Journal of Entomology*. 90: 51-64.
- Yang, L. & Stöckigt, J. 2010. Trends for diverse production strategies of plant medicinal alkaloids. *Natural Product Reports*. 27: 1469–79.