

Full Length Research Paper

Efficacy of dichlorvos+tetramethrin, *Bougainvillea glabra*, potassium chloride and *Bacillus thuringiensis* on *Tribolium castaneum*

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The present research was based on determining the effect of dichlorvos + tetramethrin (3.1% emulsifiable concentrate, EC), *Bougainvillea glabra*, potassium chloride and Shaf I *Bacillus thuringiensis* alone or in combination against *Tribolium castaneum* (Jaculin Du val.) adults and larvae. *Bt* was isolated from samples, cultured on different media by applying microbial techniques and then crystal protein was isolated from bacteria. The bio-toxicity was analyzed by SPSS software and showed that larvae are more susceptible and showed significant results as compared to adults. Separate bioassays revealed that Shaf I *B. thuringiensis* was more toxic at all concentrations followed by an insecticide DDVP+tetramethrin, chemical potassium chloride and the least toxic was *B. glabra*. In the synergistic effect, highest mortality percentage was shown by the bioinsecticide, *B. thuringiensis*. All elements whenever combined together, proved to be useful for the control of stored product pests.

Key words: Dichlorvos (2-2, dichlorodiviny dimethyl phosphate), *Bacillus thuringiensis* (*Bt*), EC), Statistical Package of Social Sciences (SPSS), *T. castaneum*.

INTRODUCTION

Bacillus thuringiensis crystal proteins are pore-forming toxins used as insecticides around the world also known as a biological pathogen. Crystal toxin proteins from the Gram-positive rod shaped bacterium, *Bt*, are used extensively to control insect pests. These range from caterpillars (Lepidoptera) and beetles (Coleoptera) that infest crops to black flies and mosquitoes (Diptera) that transmit human diseases. The red flour beetle (*Tribolium castaneum*) (Coleopteran: Tenebrionidae) is a common pest of wheat flour and is found worldwide. *B. thuringiensis* has been used in developed countries to control target pest population in medicine, forest areas and agriculture. Many types of standardized formulations

are available commercially on spore-crystal combinations. The development of formulations with biodegradable property has replaced the use of chemical insecticide which threatens the environment (Garcia and Ninfa, 2009). *Tribolium castaneum* is commonly known as red flour beetle that attacked the store grain products like nut, beans, meal, flour, pasta, chocolate, spices, cereals, seeds, cake mix and also museum specimens (Via, 1999; Weston and Rattlingourd, 2000). Red flour beetles have mouthparts for chewing but they are not for stinging and biting. These beetles may bring forth allergic responses but do not spread disease (Alanko et al., 2000). The origin of red flour beetle is Indo-Australia and is also found in temperate regions, but will endure the winter in sheltered places where there is essential heat (Tripathi et al., 2001). The larvae and adults of *T.*

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castaneum prey on juvenile stages of rice moth, *Corcyra cephalonica*. Larvae and adults are big predators of eggs and pupae, so improving adult reproduction or larval development, thereby lessen competition for their descendants (Alabi et al., 2008).

As a model system, *T. castaneum* has been used for the study of environmental relationship, infestation, large brood sizes and short generation time. So, *T. castaneum* is widely used as an experimental insect in bioassay (Bucher et al., 2005).

A synergistic agent allows the insecticide to be more effective. *T. castaneum* collected from eight godowns and one silo was tested for their susceptibility to malathion, dichlorvos, fenitrothion, primiphos-methyl and phosphine. All strains were resistant against all the test insecticides in which malathion was more effective than DDVP (Rahman et al., 2007). Comparison was done between natural and five synthetic insecticides belonging to pyrethroids including bioallethrin, tetramethrin, resmethrin, allethrin and bioresmethrin. When all the insecticides were used individually against *T. castaneum* tetramethrin was least effective (Lloyd, 2003). There are currently no published studies in which tetramethrin and DDVP synergized efficacy has been determined for stored pests. Although a lot of studies have been done to determine the synergistic effect between pyrethroid and organophosphate, such combinations has proved to be very effective in controlling red flour beetles (Scott and Snodgrass, 2000).

Leaves of different plants like *Azadirachta indica*, *Ricinus communis*, *Bougainvillea glabra*, *Saraca indica* and *Eucalyptus* were evaluated as grain protectants against *T. castaneum*. After 45 days of storage, wheat grains were tested with these leaves powder by using 5% (by weight) which showed 78-76% repellency against red flour beetle (Haq et al., 2005). Potassium chloride is a weak chemical and show very low toxicity to adult *T. castaneum* but highly toxic to the larvae. Research was done to examine the pathogenicity of *Bt*, *S. marscens*, by using different chemicals like boric acid, sodium citrate and potassium chloride against termites and *Microcerotermes championi*. 1% boric acid and *Bt* was used against termites and 1% sodium citrate and potassium chloride with *S. marscens* was used against *M. championi*. Virulence was increased ranging from 1.5-1.8 for *Bt* and 1.3-1.6 for *S. marscens*. Boric acid is quite toxic to termites but sodium citrate and potassium chloride were nontoxic to *M. championi* (Khan, 2006).

The present research was based on the combination effect of *Bacillus thuringiensis*, chemical (potassium chloride), plant powder (*Bougainvillea glabra*) and insecticide (DDVP and Tetramethrin) to check their synergistic effects against *Tribolium castaneum*.

MATERIALS AND METHODS

Rearing of *T. castaneum*

For bioassay, adults and third instar larvae were collected in large amount. For rearing, about 100-200 adults of red flour beetle were added in glass jars. The jars were kept in the Zoology Research Laboratory of Lahore College for Women University, in an incubator at 37°C, with relative humidity of 75% for feeding and oviposition.

Collection of *Bacillus thuringiensis* samples

300 samples were collected from different areas of Lahore including bird droppings, soil and grain dust. Samples were placed in glass jars properly labeled. The jars were brought to the Zoology Research Laboratory of LCWU and placed in refrigerator at 4°C.

Isolation of *B. thuringiensis* from samples

For the isolation of *B. thuringiensis*, Lauria Broth medium was prepared by adding 10 g Tryptone, 5 g yeast extract, 5 g NaCl and 0.2 M sodium acetate in 1 L of distilled water. Subsequently, 10 ml of LB medium was taken in a beaker and 0.5 g of soil sample was added in the beaker. It was well shaken and placed in an incubator at 30°C for 4 h. After time completion, samples were filtered and heated at 80°C for 15 min. Then, samples were diluted and spread on LB agar plates by using spreader. Plates were incubated for overnight at 30°C. Colonies with *B. thuringiensis* like characteristics and morphology were picked and streaked on LB agar medium.

Microbial screening of *B. thuringiensis* samples

Shaf I *B. thuringiensis* cells were grown on Petri plates of T3 medium and it contained 2 g Tryptose, 3 g Tryptone, 1.5 g yeast extract, 0.005 g of $MnCl_2 \cdot 2H_2O$, 2.5 ml of 1 M potassium phosphate (pH 6.9) and lastly 15 g of agar was added. After that, the mouth of conical flask was covered with cotton plug and aluminum foil and it was then autoclaved. T3 media was poured in plates by filling them less than half. All poured plates were placed in an incubator for 24 h at 37°C. After that, Shaf I *B. thuringiensis* was streaked on the T3 media from the samples, labeled and then placed in an incubator for 72 h at 37°C. For bioassay, the growth of Shaf I *Bt* from T3 media was collected in falcon tubes filled with autoclaved distilled water and was placed in refrigerator at 4°C.

Biochemical characterization of Shaf I *B. thuringiensis*

A single colony of *Bt* was subjected to gram staining, endospore staining and tests for further identification.

In Gram staining method, *Bt* cells were taken on a sterilized slide with a drop of water, and smear was fixed by flame. Smear portion was flooded with Crystal violet stain after 15 s, and rinsed with water. Iodine stain was applied on smear and after 15 s, rinsed with water and after that, ethyl alcohol was applied evenly on the entire smear for decolorization, and quickly rinse with water. Finally, counter stain Safranin was applied on the smear after 15 s; smear was rinsed with water, was air dried and was observed under Camera Fitter Microscope. In endospore staining, *Bt* cells were taken on a sterilized slide with a drop of water and thin smear was

formed, and was fixed with flame. Patch of filter paper was put on the smear, then slide was placed on boiling water beaker and flooded with Malachite green stain at filter paper patch for 20 min. After that, stain was washed with water. Finally, counter stain Safranin was added on the smear, slide was air dried and observed under Camera Fitter microscope.

In this biochemical test, 7% (w/v) trypticase salt was used. LB broth medium in the test tubes were inoculated with Shaf I *Bt* strain. Shaf I *Bt* cells were streaked evenly across a potato starch plate and incubated at 30°C for 96 h. Afterward, plate was stained with iodine solution.

Bioassay

Estimated value of LC₅₀ used mostly in Bioassay is analyzed through probit from SPSS.

Bioassay of *Bougainvillea glabra*

B. glabra was collected from Bagh-e-Jinnah. Leaves were separated from the branches of the plant body and then air dried at room temperature for 7 to 14 days. Dried leaves were ground in electric grinder. After achieving fine plant powder, different concentrations were (1.5, 2.0, 2.5 g and Control) combined with 1 g diet containing semolina and yeast extract. *B. glabra* bioassays set was run in triplicate by introducing 20 adults in each vial, similarly 2nd instar larvae were introduced in each vial and repeated in triplicate with 1 g diet to determine mortality rate and LC₅₀ values up to 3 days.

Bioassay of potassium chloride

Potassium chloride was used in the evaluation of bioassays in three different concentrations (1.5, 2.0, 2.5 g and control). Bioassay set was run in triplicate by introducing 20 adults in each vial with 1 g diet consisting of semolina and yeast extract. Similarly, 2nd instars larvae were introduced in each vial and repeated in triplicate with 1 g diet to observe mortality rate and LC₅₀ values up to 3 days.

Bioassay of Tetramethrin+Dichlorvos

Three different concentrations were used (0.05, 0.15, 0.25% and control without insecticides but with acetone) against *T. castaneum* adults and larvae. Tetramethrin+DDVP were diluted in 10 ml acetone. From the stock solution of each concentration of insecticide % age was converted into ug/ml and very small quantity was used against adult and larvae. Insecticide when added into the vial was vaporized for few minutes due to their volatile nature so that insecticide runs all over the walls of vials. Bioassay set was run in triplicate by introducing 20 adults in each vial with 1 g diet consisting of semolina and yeast extract; similarly 2nd instars larvae were introduced in each vial and repeated in triplicate with 1 g diet to observe Mortality rate and LC₅₀ values up to 3 days.

Bioassay of Shaf I *Bacillus thuringiensis*

Shaf I *Bt* grown cultures from Petri plates were scraped and washed three times with distilled water and centrifuged at 3000 rpm. Shaf I *Bt* pellet was obtained, and collected growth of pure

culture was centrifuged three times. After that, *Bt* was mixed in 1 g diet of semolina and yeast extract, dried and then ground with pestle and mortar. Different concentrations were used (1.0, 1.5, 2.0 g and control without *Bt* but with agar). Bioassay set was run in triplicate by introducing 20 adults in each vial with 1 g diet consisting of semolina and yeast extract, and similarly 2nd instars larvae were introduced in each vial and repeated in triplicate with 1 g diet to observe mortality rate and LC₅₀ values up to 3 days.

Combined bioassay

In the combined bioassay, all the elements were mixed together and three different concentrations were formed and bioassay was done against adult *T. castaneum* and third instar larvae separately; first combination (1.5 g *B. glabra* powder, 1.5 g KCl, 0.05% Tetramethrin+DDVP and 1 g Shaf I *Bt*) second combination (2.0 g *B. glabra* powder, 2.0 g KCl, 0.15% Tetramethrin+DDVP and 1.5 g Shaf I *Bt*) and third combination (2.5g *B.glabra* powder, 2.5 g KCl, 0.25% Tetramethrin+DDVP and 2.0 g Shaf I *Bt*). Bioassay set was run in triplicate by introducing 20 adults in each vial with 1 g diet consisting of semolina and yeast extract. Similarly, 20 larvae were introduced in each vial and repeated in triplicate with 1 g diet to observe percentage mortality for 3 days separately and in combination.

Analysis of data

Results of bioassays were analyzed by using probit on SPSS 17.0 software. Lethal concentration (LC₅₀) values were observed at each day and of all three concentrations.

RESULTS

Collection of *B. thuringiensis* from samples

Of 300 samples collected, *B. thuringiensis* was isolated from bird droppings, grain dust and soil from various localities. Strain Shaf I was found in large amount.

Morphological and biochemical characterization of Shaf I *B. thuringiensis*

By growing on LB medium turbidity confirms the status of *Bt*. When *Bt* was streaked on T3 medium, it showed characteristic morphology. Grown colonies were white in color, sometimes dry, opaque, slippery, mucoid and smooth colonies (Figure 3).

After Gram and Endospore staining, the spores appeared green and produced central or ellipsoidal parasporal crystals and pink vegetative cells thus retained the purple color of crystal violet, confirming their status as Gram-positive rods in chains and single form (Figures 1 and 2, Table 1). In the 7% sodium chloride test, turbidity was observed after 14 days of incubation at

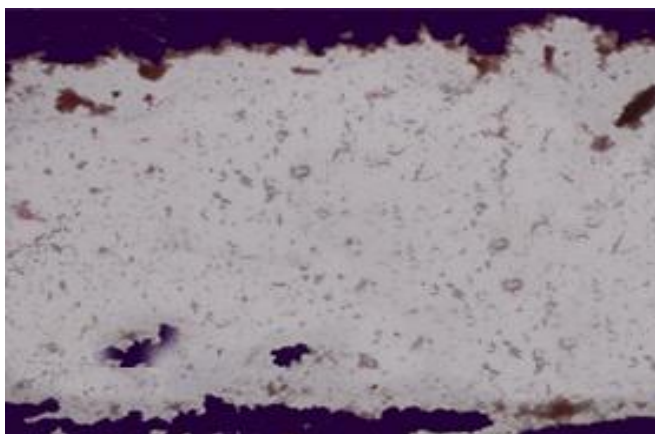


Figure 1. Gram staining results of rod shaped and Gram positive shaf I *Bacillus thuringiensis*.

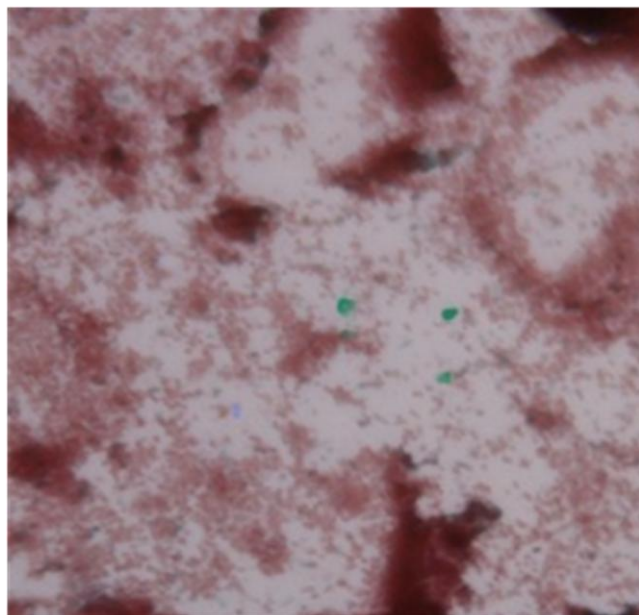


Figure 2. Endospore staining results of Shaf I *Bacillus thuringiensis*.

37°C. In starch hydrolysis test, starch appeared blue black and clear zone was indicated after hydrolysis.

Bioassay of adults

Bioassay or biological standardization is such type of scientific experiments which are used to measure the effect of a substance on test organism. *T. castaneum* is a



Figure 3. Petri plates are showing streaking results of Shaf I *Bacillus thuringiensis* colonies grown on T3 medium.

model organism for bioassay methods. Adults of *T. castaneum* have a characteristic potential to show its efficacy against different concentrations of Shaf I *B. thuringiensis*, *B. glabra*, potassium chloride, Tetramethrin and DDVP separately and in combination. At different concentrations, adults showed different mortality percentage, with regression equal or near to 1 after 3 days observations (Figures 4 to 6). By using probit in SPSS 17.0, LC₅₀ was counted according to days of the three concentrations.

Bioassay of Shaf I *B. thuringiensis*

The purpose of using bioassays based on an artificial diet is to provide worker, a rapid and standardized procedure for estimating activity of a microbial strain. At 1 g *B. thuringiensis*, 35% mortality was observed after 3 days. Similarly at 1.5 g *B. thuringiensis*, 41.6% and at 2.0 g *B. thuringiensis* 50% mortality was observed (Table 2). Regression line was equal or near to 1 (Figures 7 and 8). LC₅₀ was determined by probit analysis according to days in which at 24 h of all three concentrations, LC₅₀ value was 8.7 g/g artificial diet. However, at 48 h and 72 h, LC₅₀ values were 6.2 g/g and 8.2 g/g respectively (Table 3).

Bioassay of potassium chloride

At 1.5 g concentration, 25%, at 2.0 g 28.3% and at 2.5g 38.3% mortality was observed after 3 days (Table 4).

Table 1. Gram staining and endospore staining Shaf I of *Bacillus thuringiensis*.

Serial number	Isolate	Gram staining	Cell arrangement	Endospore staining	
				Spore	Vegetative cell
1	Shaf I	Gram positive	Single and Chains	Green	Pink

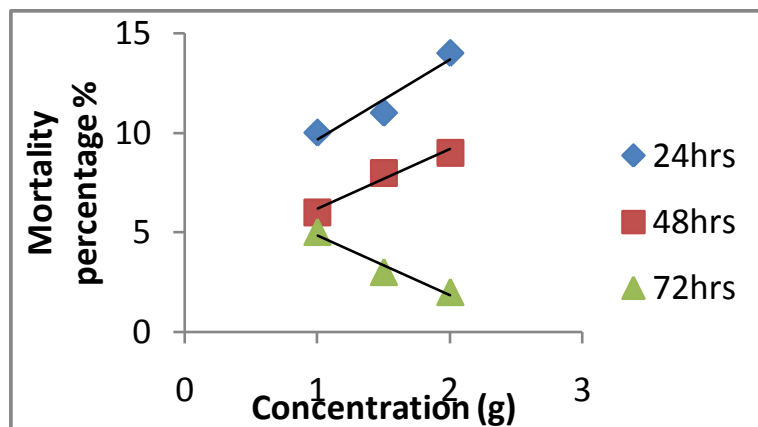


Figure 4. Regression lines of mortality percentage versus different concentration of Shaf I *B. thuringiensis* against adults of *T. castaneum* at different time interval.

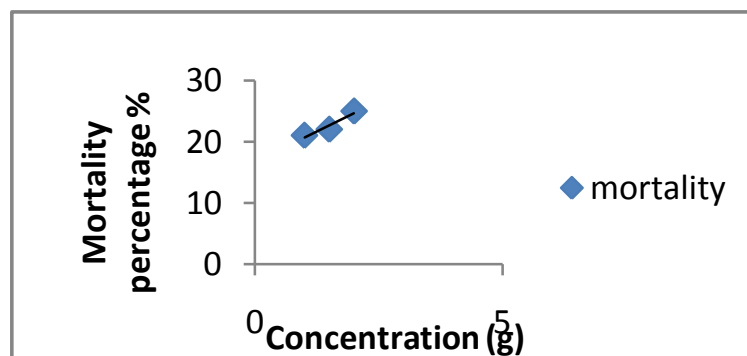


Figure 5. Regression lines of total mortality percentage versus different concentration of Shaf I *B. thuringiensis* against adults of *T. castaneum*.

Regression line was equal or near to 1 (Figures 9 and 10). LC_{50} value of the three concentrations at 24 h was 5.5 g/g artificial diet, at 48 h 6.0 g/g and at 72 h 10.9 g/g (Table 5).

Bioassay of *Bougainvillea glabra*

At all concentrations, no mortality was observed. *B. glabra*

enhanced the developmental period of adults *T. castaneum*. No statistics was applied for LC_{50} .

Bioassay of tetramethrin and DDVP

Synergistic effect of Tetramethrin and DDVP shows high magnitude against *T. castaneum* adults. At 0.05 g mortality was 45%, at 0.15 g 51.6% and at 0.25 g 58.3% mortality was observed (Table 6). Regression line was

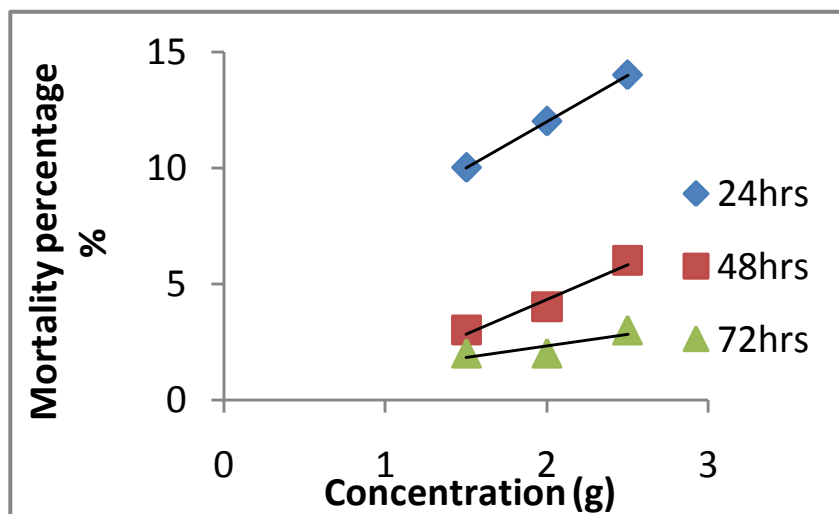


Figure 6. Regression lines of mortality percentage versus different concentration of potassium chloride against adults of *T. castaneum* at different time interval.

Table 2. Bioassay results of Shaf I *B. thuringiensis* at different concentrations showing percent mortality.

Serial number	Concentration (g)	Number of used adult	Time (h)			Percentage mortality (%)	R ² of all concentration
			24	48	72		
1	1.0	60	10	6	5	35	0.9
2	1.5	60	11	8	6	41.6	
3	2.0	60	14	9	7	50	
4	Control	60	0	0	0	0	

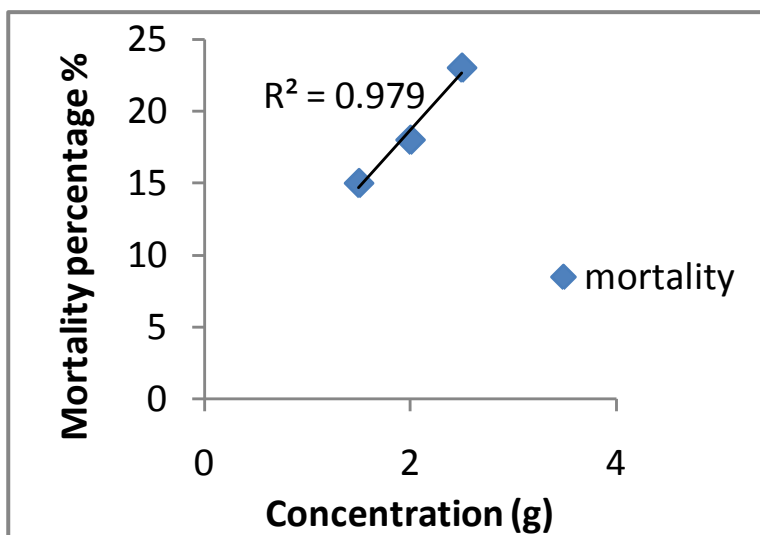


Figure 7. Regression lines of total mortality percentage versus 3 concentration of potassium chloride against adults of *T. castaneum*.

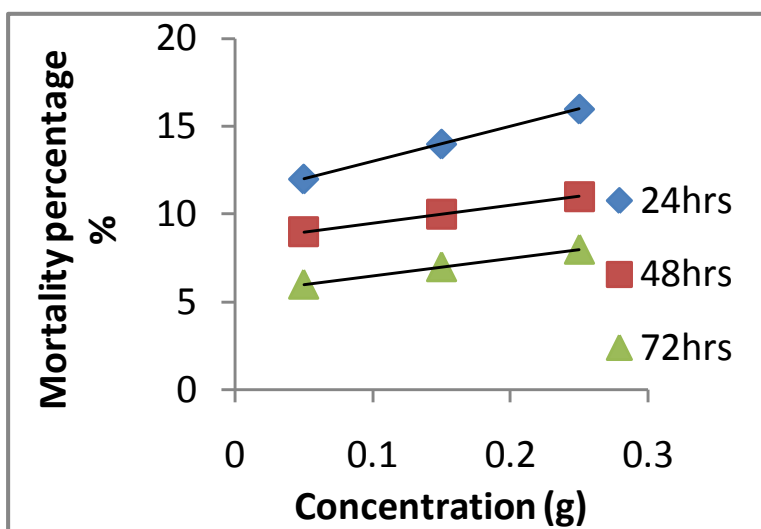


Figure 8. Regression lines of mortality percentage versus different concentration of Tetramethrin and DDVP against adults of *T. castaneum* at different time interval.

Table 3. Toxicity of Shaf I *B. thuringiensis* against *Tribolium castaneum* showing LC₅₀ value.

Serial number	Time (h)	Concentration (g)			LC ₅₀ (g/g)	LC ₅₀ value of all three concentration (g/g)
		1.0	1.5	2.0		
1	24	10	11	14	8.7	2.1
2	48	6	8	9	6.2	
3	72	5	6	7	8.2	

Table 4. Bioassay results of Potassium chloride at different concentrations showing percent mortality.

Serial number	Concentration (g)	Number of used adult	Time (h)			Percentage (%) mortality	R ² of al concentration
			24	48	72		
1	1.5	60	10	3	2	25	0.9
2	2.0	60	12	4	1	28.3	
3	2.5	60	15	5	3	38.3	
4	Control	60	0	0	0	0	

equal or near to 1 (Figure 11 and 12). LC₅₀ was counted for 3 days separately, at 24 h, LC₅₀ of three concentrations was 0.8. Similarly, at 48 h and 72 h, LC₅₀ was 1.6 and 1.5 (Table 7).

Percentage mortality was different for the three concentrations and mortality increased at higher concentration (Table 8).

Combine bioassay

In the combine bioassay, all the elements were mixed together and three different concentrations were formed and bioassay was done against adult *T. castaneum*.

Bioassay of larvae

Larvae of *T. castaneum* had a characteristic potential and showed its efficacy against different concentrations of *B. thuringiensis*, *B. glabra*, potassium chloride, Tetramethrin and DDVP separately and in combination.

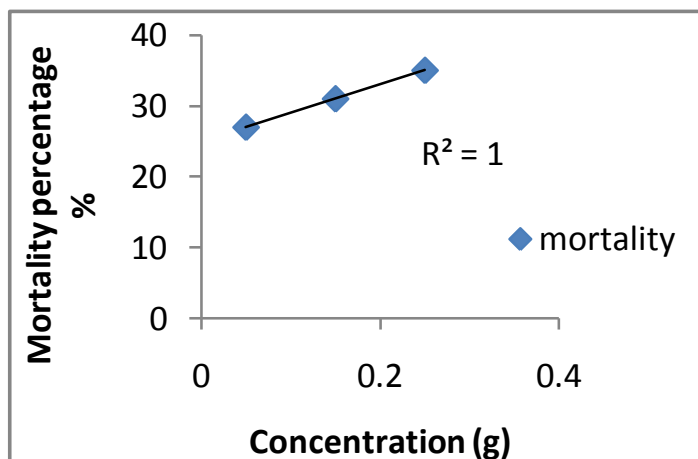


Figure 9. Regression lines of total mortality percentage versus 3 concentrations of Tetramethrin and DDVP against adults of *T. castaneum*.

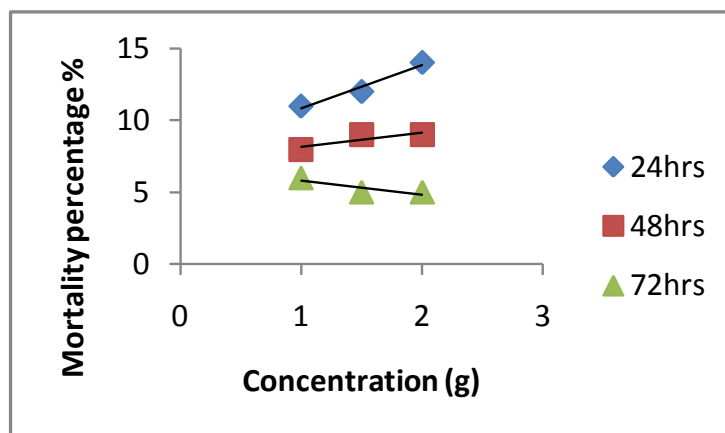


Figure 10. Regression lines of mortality percentage versus different concentration of Shaf I *B. thuringiensis* against third instar larvae of *Tribolium castaneum* at different time interval.

Table 5. Toxicity of potassium chloride against *T. castaneum* showing LC₅₀ value.

Serial number	Time (h)	Concentration (g)			LC ₅₀ (g/g)	LC ₅₀ value of all 3 concentrations (g/g)
		1.5	2.0	2.5		
1	24	10	12	14	5.5	3.3
2	48	3	4	6	6.0	
3	72	2	2	3	10.9	

Bioassay of Shaf I *B. thuringiensis*

At 1 g *Bt*, 40% mortality was observed after 3 days.

Similarly at 1.5 g *Bt*, 45% and at 2.0 g *Bt* 51.6% mortality was observed. (Table 9). Regression line was equal or near to 1 (Figures 13 and 14). LC₅₀ was determined by

Table 6. Bioassay results of Tetramethrin+DDVP at different concentrations showing percent mortality.

Serial number	Concentration (%)	Number used adult	Time (h)			Percentage mortality (%)	R ² of all three concentration
			24	48	72		
1	0.05	60	12	9	6	45	1
2	0.15	60	14	10	7	51.6	
3	0.25	60	16	11	8	58.3	
4	Control	60	0	0	0	0	

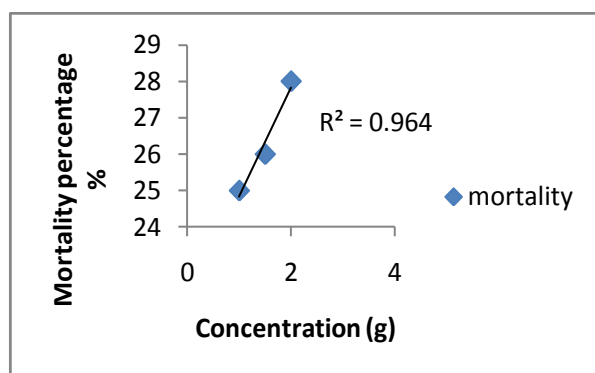


Figure 11. Regression lines of mortality percentage versus different concentration of Shaf I *B. thuringiensis* against third instar larvae of *Tribolium castaneum* at different time interval.

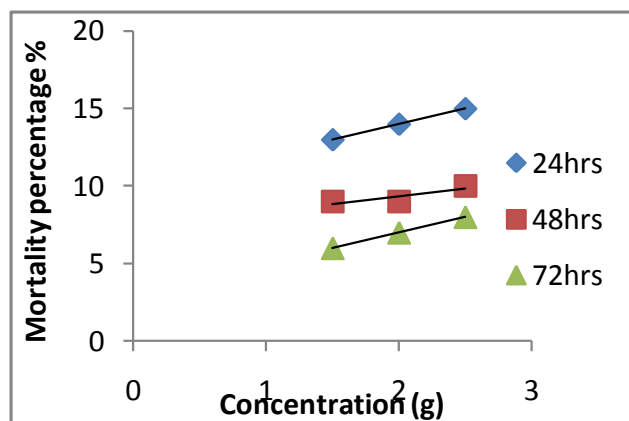


Figure 12. Regression lines of mortality percentage versus different concentration of Potassium Chloride against third instar larvae of *Tribolium castaneum* at different time interval.

probit analysis according to days in which at 24 h of all three concentrations LC₅₀ value was 6.2 g/g. However, at 48 and 72 h, LC₅₀ value was 8.7 and 8.2 g/g (Table 10).

Bioassay of potassium chloride

At 1.5 g, 45%, at 2.0 g 50% and at 2.5 g 55% mortality was observed after 3 days (Table 11). Regression line was equal or near to 1 (Figures 15 and 16). LC₅₀ value of the three concentrations at 24 h was 8.7 g/g at 48 h, the value was 9.2 g/g and at 72 h it was 9.0 g/g (Table 12).

Bioassay of *Bougainvillea glabra*

At all concentrations, no mortality was observed. No statistics was applied for LC₅₀.

Bioassay of Tetramethrin and DDVP

At 0.05 g mortality observed was 50%, at 0.15 g 56.6% and at 0.25 g it was 63.3%. (Table 13) Regression line was equal or near to 1 (Figures 17 and 18). LC₅₀ was counted for 3 days separately, at 24 h. LC₅₀ of the three concentrations was 0.6%. Similarly, at 0.15 g, it was 1.6% and at 0.25 g, 1.5% LC₅₀ was counted (Table 14).

Combine bioassay

In the combine bioassay, three different concentrations were formed and bioassay was done against third instar larvae of *T. castaneum*. Percentage mortality was different for the three concentrations and mortality increased at higher concentration (Table 15).

DISCUSSION

In this study, *B. thuringiensis* acted as biological insecticide and showed low mortality at lower concentration and high mortality at higher concentration. Regression was equal or near 1. The adult LC₅₀ value for all three concentrations was about 2.1 g/g while larvae showed 1.8 g/g value of LC₅₀. In both cases, by increasing the concentration, more number of insects

Table 7. Toxicity of Tetramethrin+DDVP against *T. castaneum* showing LC₅₀ value.

Serial number	Time (h)	Concentration (g)			LC ₅₀ (%)	LC ₅₀ value of all three concentration (%)
		0.05	0.15	0.25		
1	24	12	14	16	0.8	1.6
2	48	9	10	11	1.6	
3	72	6	7	8	1.5	

Table 8. Comparison of individual and combine effect of Shaf I *Bacillus thuringiensis*, Potassium chloride, *Bougainvillea glabra* and Tetramethrin+DDVP on *T. castaneum* larvae at 24, 48 and 72 h.

Time interval (h)	Concentration	Combine and individual concentration mixed with diet	Observed mortality (D/T)	Percentage mortality (%)
24	First concentration	Control	0/60	0
		KCL (1.5 g)	10/60	16.6
		<i>B.t</i> (1.0 g)	10/60	16.6
		<i>B. glabra</i> (1.5 g)	0/60	0
		Tet+DDVP (0.05%)	12/60	20
		Combine	32/60	53.3
48		Control	0/60	0
		KCL	3/60	5
		<i>B.t</i>	6/60	10
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	9/60	15
		Combine	18/60	30
72		Control	0/60	0
		KCL	2/60	3.3
		<i>B.t</i>	5/60	1.6
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	6/60	13.3
		Combine	13/60	21.6
24	Second concentration	Control	0/60	0
		KCL (2.0g)	12/60	20
		<i>B.t</i> (1.5g)	11/60	18.3
		<i>B. glabra</i> (2.0g)	0/60	0
		Tet+DDVP (0.15%)	14/60	23.3
		Combine	37/60	61.6
48		Control	0/60	0
		KCL	4/60	6.6
		<i>B.t</i>	8/60	13.3
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	10/60	16.6
		Combine	22/60	36.6
72		Control	0/60	0
		KCL	1/60	1.6
		<i>B.t</i>	0/60	0
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	7/60	11.6
		Combine	8/60	13.3

Table 8. Continued.

24	Third concentration	Control	0/60	0
		KCL (2.5 g)	15/60	25
		<i>B.t</i> (2.0 g)	14/60	23.3
		<i>B. glabra</i> (2.5 g)	0/60	0
		Tet+DDVP (0.25%)	16/60	26.6
48	Combine	Control	45/60	75
		Control	0/60	0
		KCL	5/60	8.3
		<i>B.t</i>	9/60	15
		<i>B. glabra</i>	0/60	0
72	Combine	Tet+DDVP	11/60	18.3
		Control	25/60	41.6
		Control	0/60	0
		KCL	3/60	5
		<i>B.t</i>	7/60	11.6
	Combine	<i>B. glabra</i>	0/60	0
		Tet+DDVP	8/60	13.3
		Control	18/60	30

Table 9. Bioassay results of Shaf I *B. thuringiensis* at different concentrations showing percent mortality.

Serial number	Concentration (g)	Number of used larvae	Time (h)			Percentage mortality (%)	R ² of all 3 concentration
			24	48	72		
1	1.0	60	11	8	5	40	0.9
2	1.5	60	12	9	6	45	
3	2.0	60	14	10	7	51.6	
4	Control	60	0	0	0	0	

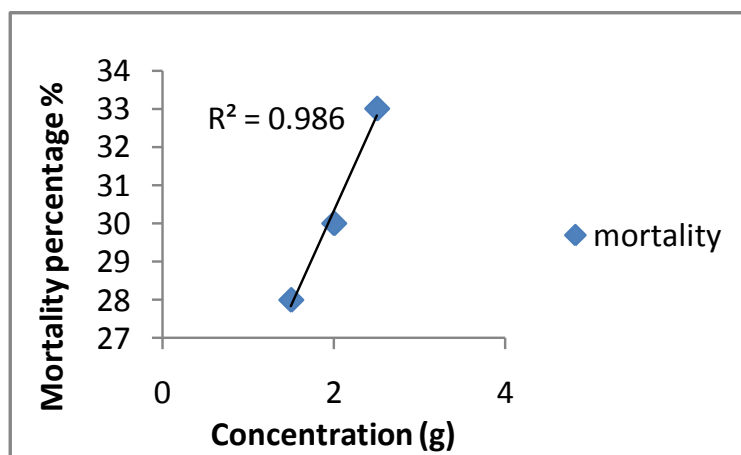


Figure 13. Regression lines of total mortality percentage versus 3 concentration of Potassium Chloride against third instar larvae of *Tribolium castaneum*.

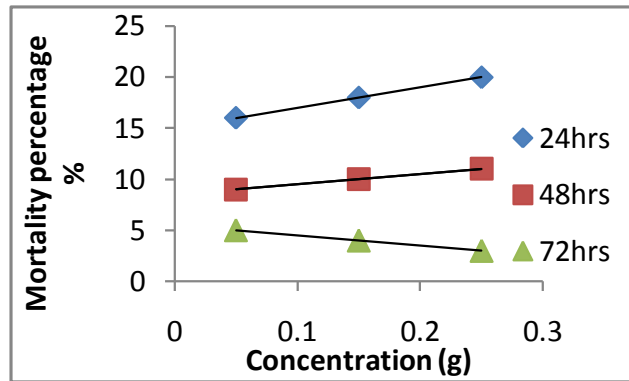


Figure 14. Regression lines of mortality percentage versus different concentration of Tetramethrin and DDVP against third instar larvae of *Tribolium castaneum* at different time interval.

Table 10. Toxicity of Shaf I *B. thuringiensis* against *Tribolium castaneum* showing LC₅₀ value.

Serial number	Time (h)	Concentration (g)			LC ₅₀ (g/g)	LC ₅₀ of all 3 concentration (g/g)
		1.0	1.5	2.0		
1	24	11	12	14	6.2	1.8
2	48	8	9	9	8.7	
3	72	6	5	5	8.2	

Table 11. Bioassay results of potassium chloride at different concentrations showing percent mortality.

Serial number	Concentration (g)	Number of used larvae	Time (h)			Percentage mortality (%)	R ² of all 3 concentration
			24	48	72		
1	1.5	60	13	8	6	45	0.9
2	2.0	60	14	9	7	50	
3	2.5	60	15	10	8	55	
4	Control	60	0	0	0	0	

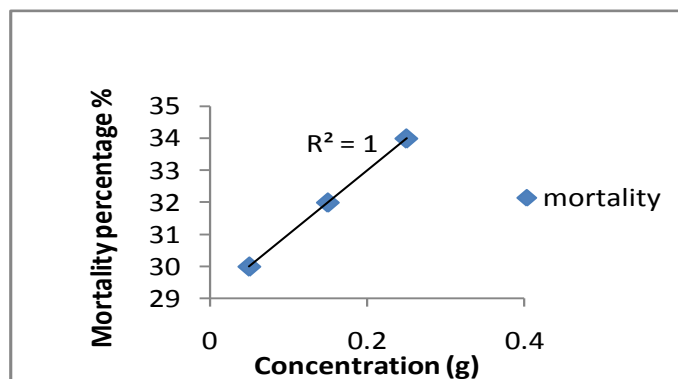


Figure 15. Regression lines of mortality percentage versus 3 concentrations of Tetramethrin and DDVP against third instar larvae of *Tribolium castaneum*.

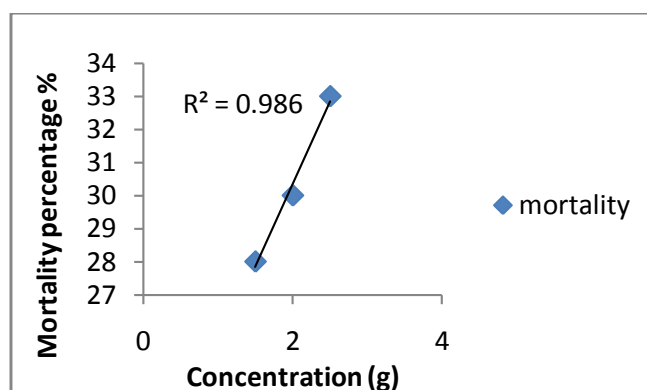


Figure 16. Regression lines of total mortality percentage versus 3 concentration of Potassium Chloride against third instar larvae of *Tribolium castaneum*.

Table 12. Toxicity of potassium chloride against *Tribolium castaneum* showing LC₅₀ value.

Serial number	Time (h)	Concentration (g)			LC ₅₀ (g/g)	LC ₅₀ of all three concentration (g/g)
		1.5	2.0	2.5		
1	24	13	14	15	8.7	1.3
2	48	8	9	10	9.2	
3	72	6	7	8	9.0	

Table 13. Bioassay results of Tetramethrin+DDVP at different concentrations showing percent mortality.

Serial number	Concentration (g)	Number of used larvae	Time (h)			Percentage mortality (%)	R ² of all 3concentration
			24	48	72		
1	0.05	60	16	9	5	50	1
2	0.15	60	18	6	4	56.6	
3	0.25	60	20	11	7	63.3	
4	Control	60	0	0	0	0	

died, while according to hours, less number of insects died; at first day high number of insects become dead as *Bt* was highly effective in the beginning, and is human safe, but on the 2nd and 3rd day, its efficacy becomes lower and it started degrading and so there were less number of insects. Larvae showed remarkable results. Bioassay of potassium chloride of both adults and larvae showed comparably different results in case of mortality percentage and LC₅₀. Third instar larvae were more susceptible to chemical and thus they showed greater percentage of mortality as compared to adult. Regression was equal or near to 1. The adult showed 3.3 g/g value of LC₅₀ of all the three concentrations, while larvae showed 1.3 g/g value of LC₅₀. In the case of adult, potassium chloride caused mortality over a longer period of time, but

in the case of larvae, mortality occurred within three days. LC₅₀ value of larvae was lower as compared to adult, which indicates that potassium chloride was toxic to larvae as compared to adult.

Bioassay of *B. glabra* for both adults and larvae showed similar results, no mortality was observed and insect were repelled by *B. glabra* leaves. *B. glabra* prolonged the developmental period of *T. castaneum*. Bioassay of Tetramethrin and DDVP showed strong efficacy against *T. castaneum* adults and larvae. Estimated value of LC₅₀ of adult was 0.2% while larvae showed 0.1% LC₅₀ value. When two insecticides work together in synergism, their tendency to kill insect become higher. Tetramethrin do not show residual effect but DDVP shows residual effect which may lasts for 3 to

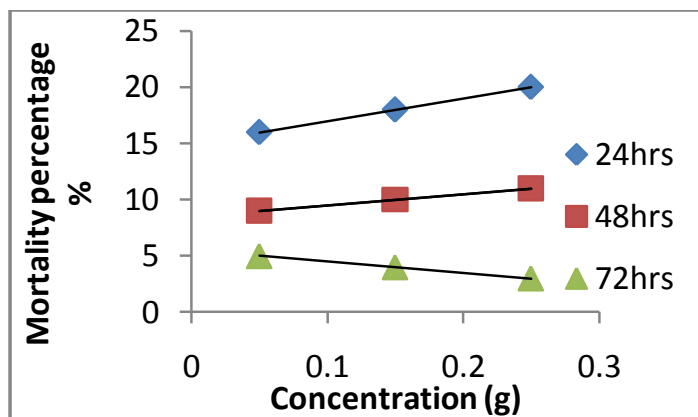


Figure 17. Regression lines of mortality percentage versus different concentration of Tetramethrin and DDVP against third instar larvae of *Tribolium castaneum* at different time interval.

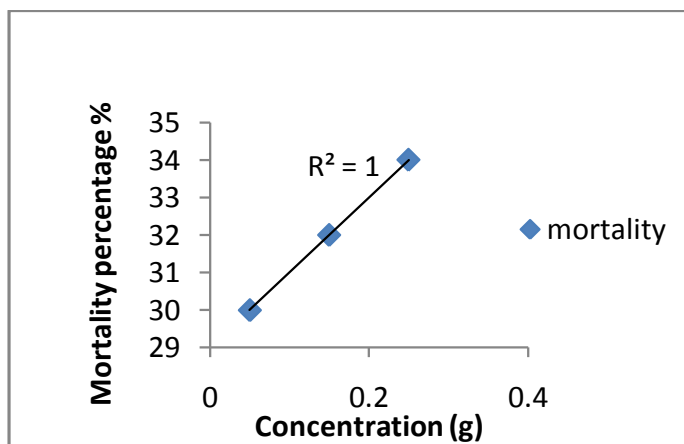


Figure 18. Regression lines of mortality percentage versus 3 concentrations of Tetramethrin and DDVP against third instar larvae of *Tribolium castaneum*.

Table 14. Toxicity of Tetramethrin+DDVP against *Tribolium castaneum* showing LC₅₀ value.

Serial number	Time (h)	Concentration (g)			LC ₅₀ (%)	LC ₅₀ of all 3 concentration
		0.05	0.15	0.25		
1	24	16	18	20	0.6	
2	48	9	10	11	1.6	
3	72	5	6	7	1.5	1.9

4 days. Both insecticides either used in low concentration or high concentration, killed adult and larvae of *T. castaneum* within 3-4 days or were effective for stored grain pests. As the concentration increased, more

number of insects died, but rate of mortality became low day after day. They are very useful for integrated pest management strategies.

In the combine bioassay, all the elements were mixed

Table 15. Comparison of Individual and Combine effect of Shaf I *Bacillus thuringiensis*, Potassium chloride, *Bougainvillea glabra* and Tetramethrin+DDVP on *Tribolium castaneum* larvae.

Time Interval (h)	Concentration	Combine and Individual Concentration mixed with diet	Observed mortality (D/T)	Percentage mortality (%)
24	First concentration	Control	0/60	0
		KCL (1.5 g)	13/60	21.6
		<i>B.t</i> (1.0 g)	11/60	18.3
		<i>B. glabra</i> (1.5 g)	0/60	0
		Tet+DDVP (0.05%)	16/60	26.6
		Combine	40/60	66.6
48		Control	0/60	0
		KCL	8/60	13.3
		<i>B.t</i>	8/60	13.3
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	9/60	15
		Combine	25/60	41.6
72		Control	0/60	0
		KCL	6/60	10
		<i>B.t</i>	5/60	8.3
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	5/60	8.3
		Combine	16/60	26.6
24	Second concentration	Control	0/60	0
		KCL (2.0 g)	14/60	23.3
		<i>B.t</i> (1.5g)	12/60	20
		<i>B. glabra</i> (2.0 g)	0/60	0
		Tet+DDVP (0.15%)	18/60	30
		Combine	44/60	73.3
48		Control	0/60	0
		KCL	9/60	15
		<i>B.t</i>	9/60	15
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	10/60	16.6
		Combine	28/60	46.6
72		Control	0/60	0
		KCL	7/60	11.6
		<i>B.t</i>	6/60	10
		<i>B. glabra</i>	0/60	0
		Tet+DDVP	6/60	10
		Combine	19/60	31.6
24	Third concentration	Control	0/60	0
		KCL (2.5 g)	15/60	25
		<i>B.t</i> (2.0 g)	14/60	23.3
		<i>B. glabra</i> (2.5 g)	0/60	0
		Tet+DDVP (0.25%)	0/60	0
		Combine	20/60	33.3
48		Control	49/60	81.6
		KCL	0/60	0
		<i>B.t</i>	10/60	16.6

Table 15. Continued.

72	<i>B. glabra</i>	10/60	16.6
	Tet+DDVP	0/60	0
	Combine	11/60	18.3
	Control	31/60	51.6
	KCL	0/60	0
	<i>B.t</i>	8/60	13.3
	<i>B. glabra</i>	7/60	11.6
	Tet+DDVP	0/60	0
	Combine	7/60	11.6

together at three different concentrations; in the case of adult percentage, mortality was low at first and second combination but high at third combination. While in the case of larvae, all three combinations showed significant results and high mortality rate was revealed. This is a new study in which combining together all the essential elements are very effective against *T.castaneum*. All elements are safe for humans and animals. Their synergistic effect cannot only control stored target pest but also non-target organisms. The present result can trace new strategies for international pest management programs.

The crystal proteins of *B.t* had insecticidal properties against different pests. One crystal protein may be effective for many different kinds of pests. Likewise boric acid is a chemical and cypermethrin an insecticide which have no residual effects and safe for humans and other mammals as well as for the whole environment. In godowns the spray of insecticide kept the grain products safe from the different stored product pests. Different kinds of formulations were made from these biopesticides which helped in for the control of not only stored product pests but for the control of other forest, agriculture and household pests.

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