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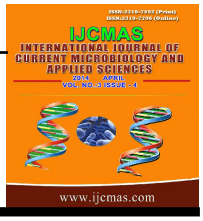


Original Research Article

Study of combined effect of locally isolated *Bacillus thuringiensis* and Turmeric powder on Red Flour Beetle (*Tribolium castaneum*)

Kausarmalik* and RizwanaRiasat

Department of Zoology, Lahore College for Women University, Lahore, Pakistan
******Corresponding author*

A B S T R A C T

Bacillus thuringiensis crystal proteins are spore-forming toxins used as insecticides around the world also known as a biopesticides. Crystal toxin proteins from the Gram-positive rod shaped bacterium, *B. thuringiensis*, 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. Turmeric powder is used as repellent against the pests. Turmerones and arturmerone are the components which acts as insect repellent in turmeric. The red flour beetle (*Triboliumcastaneum*) (Coleopteran: Tenebrionidae) is a common pest of wheat flour and is found worldwide. The present research is based on determining the effect of turmeric powder and three *Bacillus thuringiensis* isolatesalone and in combination against *Triboliumcastaneum*(adults and larvae). *B. thuringiensis*isolates were cultured on different media by applying microbial techniques, upto sporulation stage. The bio-toxicity was analyzsed by SPSS software, determined that larvae are more susceptible and showed significant results as compared to adults. Percentage mortality of each bioassay was determined. Synergistic effect was observed in the form of highest mortality percentage of pest, both in case of *B. thuringiensis* isolates and *B. thuringiensis*isolatesplus turmeric powder. All elements whenever are combined together, proved to be useful for the control of stored product pests.

Keywords

Bacillus thuringiensis ; biopesticides; *Tribolium castaneum*.

Introduction

Stored grains insect pests have been damaging our economy by infesting agricultural stored products. These are responsible for annual loss of 10-40% in the stored grains worldwide. Various synthetic insecticides have been used to protect stored grains and other agricultural products from insect infestation. But insects have acquired resistance against most of these pesticides (Zettler and Cuperus, 1990; Jemere*et al.*, 1995). For this purpose, many plant products have been evaluated for their toxic properties against different stored pests (Su, 1990, Mukherjee and Joseph, 2000) especially in the form of essential oils (Shaaya and Kostyukovsky, 1991; Ngamo*et al.*, 2007). *Triboliumcastaneum*is major pest of wheat

grains flour (Howe,1965). In the present study, laboratory studies reported on the toxic effect of turmeric powder plus locally isolated *B.t* (previous research students) against stored grains products pest, *T.castaneum.*

Tribolium castaneum is generally known as red flour beetle attacked the stored grain products like nut, flour, meal pasta, spices, beans, cereals, seeds, chocolate, cake mix and also museum specimens (Via, 1999; Weston and Rattlingourd, 2000). Red flour beetles are not able for stinging and biting but they have chewing mouthparts. These beetles may produce allergic responses but do not spread disease (Alanko*et al*., 2000). These beetles are of two types *Tribolium castaneum* and *Tribolium confusum* and both are crucial pests in home as well as in grocery stores. The confused flour beetle gets this name because at first glance it appears to be very similar to red flour beetle (Walter, 1990).

In 1909, Shigetane Ishiwata was first discovered the bacterium *Bacillus thuringiensis*(*B.t.*), and after ten years it was rediscovered by Berliner. From infected larvae of *Anagasta kuehniella*, this *B.t.* was isolated and this discovery led to the establishment of microbial pesticide or insecticides (Aizawa and Shigetane, 2001). *Bacillus thuringiensis*is rod shaped gram positive, spore forming bacteria and it produces delta endotoxins called the crystalline proteins at the time of its inactive phase of growth. At the end of sporulation, after breakdown of the cell wall the crystal is free to the environment (Schnepf*et al*., 1998).

Bacillus thuringiensis has 34 distinguished subspecies. Cry (crystal delta-endotoxins) and Cyt (cytolysins) are two common groups of insecticidal crystal proteins. The cry genes have been divided into four classes named CryI, CryII, CryIII and CryIV. Cyt genes have been classified into two classes (Hofte and Whiteley, 1989). The Cry proteins contain minimum 50 subgroups with 200 members (Bravo, 2007). Cry toxins binds to particular receptors on cells, in the insect midgut whereas, Cyt toxins does not recognize specific binding site (Lereclus*et al*., 1993). Cyt genes have shown action against dipteran and coleopteran pests as well as shemipterans (true bugs) and dictyopterans (roaches and termites) (Frutos*et al*., 1999). Different steps are involves in the mode of action of *Bacillus thuringiensis* and directly causes the mortality of insects, different strains follows same mode of action which were isolated from the toxins. After the ingestion of delta-endotoxins crystals, they break up in the midgut of insect, releasing the protoxins. This are proteolytically changed into fragments and then binds to the cells of midgut epithelium. The activated protein disturbs the osmotic equilibrium of these cells by creating pores in the cell membrane causing the cell to lyse (Van Rie*et al*., 1992). The insect stops feeding and the gut become paralyzed; and after a few hours of ingestion mainly insects will die (Marrone and Macintosh, 1993).

Insecticidal activity of *Bacillus thuringiensis* mainly dependent on Cry proteins and it differ with the type of insect. *B.t.* is also being used as biological pesticide against various insects belongs to the order like Diptera, Coleoptera, Lepidoptera and Homoptera (Cannon, 1993). Naturally occurring pesticides are very helpful alternative of chemical insecticides which pollute the environment therefore insecticides derived from the *Bacillus thuringiensis* are attaining worldwide value. Biological pesticides

are used for the control of pests in public health, forestry and agriculture (Entwistle*et al*., 1993). Proteins from the family *cry1A* and *cry2A* are very active against the lepidopteran's larvae these are gaining agronomic importance worldwide. These proteins are also noxious against the spruce budworms (Van Frankenhuyzen *et al*., 1993). For insect control especially for the members of Noctuidae family and lepidopteran's larvae, crystal protein Cry9Ca1 was also found (Lambert *et al*., 1996).

Chemical insecticides are helpful to maintain the economic losses and effective method for controlling pests, as it is inexpensive (Wadleigh*et al*., 1991). Synthetic insecticides are used for the control of insects in food products widely but on the other hand it was found that it affects the humans so it became necessary to lessen the use of insecticides in food products (Aldryhim, 1993). Fumigants and synthetic insecticides are extensively used for the control of insect pests in stored food products, which leads to hazards such as pest environmental disturbances, resurgence, and toxic effects on non-target organisms, pest resistance to pesticide and increasing costs of application (Isman, 2006). Fumigation plays very significant role for the eradication of insect pests in stored products, (Zettler and Arthur, 2000). In Pakistan, with fumigants like phosphine the control of *Triboliumcastaneum*is being successful (Rehman, 2000).

Insecticides used for the purpose of controlling pests can be categorized as organophosphates (chlorpyrifos and chlorpyrifosmethyl), insect growth regulators (IGRs) (*S*-hydroprene), pyrethroids (cyßuthrin, fenvalerate, and deltamethrin), carbamates (bendiocarb) and botanicals (pyrethrins) (Subramanyam*et al*., 1993). For the control of indoor and agricultural pests pesticides are being used (Garey and Wolff, 1998). In China, for control of insects one of the most accepted synthetic pesticides called pyrethroid is used (Bloomquist, 1996).

Plant extracts comprise a rich source of bioactive chemicals which is being used for the control of pests (Wink, 1993). It affects the insects in different ways; they might interrupt important metabolic pathways and cause death

Materials and Methods

Rearing of insect

Adults of *Triboliumcastaneum*were collected from wheat flour, semolina, cereals, rice, etcand introduced in glass jars of 300 ml capacity. Each jar was filled $1/4^{th}$ with sterilized mixture of semolina and some quantity of yeast extract as diet of *T. castaneum* and 50 adults were released in each jar. The semolina and yeast extract was used as diet for *T. castaneum*. The culture medium was placed at 60°C for three hours in an oven so that maximum reduction of contamination should be achieved. The larvae of *T. castaneum*were reared at 30±1°C and 60±5% relative humidity (Saleem and Shakoori, 1984). Every third day, to obtain enough larvae for experimentation, the new diet were added.

Microbiological techniques for growth of *B.t.*

Preparation, Sterilization and Pouring of Media

Shaf1, Sd1 and Sid1 *Bacillus thuringiensis*isolates were grown on Petri

plates containing T3 medium. The composition of T3 medium was 1.5 g of yeast extract, 15g of agar/liter, 3g of tryptone, 2g of tryptose, 0.005g of $MnCl_2.2H_2O$, and 2.5 ml of 1M phosphate buffered saline (pH 6.9). All these chemicals were dissolved in distilled water. All the glass wares, test tubes, petridishes, flasks, beakers, pipettes etc were sterilized at 121°C for 3 hours in an oven. Media was also sterilized at 121°C for 20 minutes in autoclave. After the media was autoclaved, it was kept for few minutes at room temperature to cool the flask and then poured it into the petri-plates. After pouring the plates were placed in the refrigerator to solidify the media for few minutes. All solidify poured plates were kept in incubator for 24 hours at 37°C to check the contamination.

Streaking of plates

First of all, label the bottom of the petriplate such as Shaf1, Sd1 and Sid1. Then took an inoculating loop and sterilized it by dipping in alcohol and then with flame and make sure that it became red hot. It was then cooled. After that a loop full of bacterial culture was taken and streaked it in a zigzag manner on the solidify plates. All this was done in laminar flow hood to avoid the contamination. Then the plates were kept in incubator at 30°C for 3 days.

Growth of bacteria on plates

After 3 days the bacterial growth was checked and growth was collected with the help of the inoculating loop in falcon tubes which also labeled as Shaf1, Sd1 and Sid1. Then distilled water was added in all the falcon tubes containing the *B. thuringiensis* and shaked it well. All the growth of *B. thuringiensis* was stored in the falcon tube and placed it in freezer so that it was further used in the *B.t.* bioassay.

Collection of pellets of *B. thuringiensis*

B. thuringiensis was stored in falcon tubeswas added in the centrifuge tubes according to their labeled as Shaf1, Sd1 and Sid1. After centrifugation at 3000 rpm to obtain the pure pellets of these three *B.t.* isolates, for bioassay.

Preparation of glycerol stock

For the preparation of glycerol stock 0.5ml of bacterial cultural of all three *B.thuringiensis* was added in sterile labeled eppendorf tubes separately. After that 300µl glycerol was added in each tube. By vortexing mixed them well and stored at -80°C. When required for use, defrost the stock and the bacterial colonies were obtained when small amount was again streaked on agar solidified plates.

Staining and biochemical characterization of *B. Thuringiensis*

Endospore staining and Gram staining as well as biochemical tests like starch hydrolysis were performed for the *B.t.* isolates (Karnataka, 2009).

Growth of *B.t.*in 7% Sodium Chloride

In this test,7% (w/v) trypticase salt was required. Inoculated the test tubes which contained LB medium and designated isolates. After incubation at 37°C for 14 hours the turbidity was observed.

Insect Bioassay

The toxicity of three *B. thuringiensis* isolates combined and turmeric powder was checked individually as well as in combined form on the second instar larvae of *Triboliumcastaneum* and newly formed

adults as well. At high concentration bioassay was first performed of all these and then checked the mortality of larvae and adults and after that other concentrations were adjusted. Each bioassay was performed in triplicated form and repeated three times. After three days, mortality was observed and LC_{50} was counted by probit analysis SPSS programme.

Control group

20 newly formed adults of *T. castaneum* were taken from the jars to carried out bioassay and placed it in vials which already had diet of *T. castaneum*like 0.2g of yeast extract and 0.8g of semolina as per gram in each glass vial. Vial is covered with its cap and placed it at 28°C and 70% relative humidity (RH) in incubator. The same procedure is done for the second in star larvae bioassay. In control group, mortality was also checked as there was no mortality in control group so that it was compared with experimental or treated groups.

Insecticidal plant bioassay

Theselected concentration ofturmeric powder 0.125, 0.25, 0.5 and 1g were mixed as per gram diet of red flour beetle. Each concentration had three replicate and introduced 20 larvae in each glass vial with the help of camel brush. The control sample was maintained without the turmeric powder. At regular interval of 24 hours the mortality data was checked for seven days. Same set of bioassay was carried out for adults.

Combined *B.t.* bioassay

Before the bioassay of three *B. thuringiensis*isolates such as Shaf1, Sd1 and Sid1 was carried, the collected pellet of each *B.t.* isolate in falcon tubes were mixed with distilled water and centrifuged them at 3000 rpm for 5 min. This centrifugation is repeated three times in centrifugation tubes till all the media was washed away and the supernatant was discarded. In the bottom of the tubes, the pellets were obtained which were dried in an oven.The dried pellet of each isolates was taken and crushed them in pestle mortar separately to form the fine granules like powder. The concentrations of *B.t.* isolateof Shaf1 were adjusted like 0.11g, 0.23g and 0.45g and concentration of *B.t.* of Sd1 were adjusted like 0.087g, 0.17g, 0.35g and concentration of *B.t.* of Sid 1 were adjusted like 0.0875g, 0.17g and 0.35g, for larvae. Three different combined concentrations were prepared in separate glass vials like first concentration was 0.11g Shaf1 *B.t*, 0.087g Sd1 *B.t*, and 0.087g Sid1 mixed them with per gram diet i.e. 0.8 gram semolina and 0.2 gram of yeast extract. These concentrations were added in glass vials and introduced 20 second instar larvae in each vial separately. Likewise second combination of *B.t* isolates was made 0.23g Shaf1 *B.t*, 0.17g Sd1 *B.t* and 0.17g Sid1 *B.t.* mixed them with per gram diet i.e. 0.8 gram semolina and 0.2 gram of yeast extract. These concentrations were added in glass vials and introduced 20 second instar larvae in each vial separately. 20 larvae were introduced in each vial and third combined concentration was 0.45g Shaf1 *B.t*, 0.35g Sd1 *B.t.* and 0.35g Sid1 *B.t.* mixed in 1g diet. For each combined concentration the bioassay was carried out for larvae. The concentrations of Shaf1 *B.t.* isolates selected for adult bioassay was (0.13, 0.26 and 0.53g) and of Sd1 was (0.10, 0.20 and 0.40g) and Sid1 was (0.16, 0.32 and 0.62g). Mixed the first concentrations of all the *B.t.* isolates 0.13, 0.10 and 0.16 (Shaf1, Sd1 and Sid1)

respectively. Similarly second and third concentration was made.*B.t*. showed its effectiveness or toxicity in three days and results were noted for seven days so the mortality of larvae and adults was observed and percentage mortality for each day was calculated by using percentage formula.

Combined bioassay

Bioassay was carried out in combination with turmeric powder, three combined *B.t.* isolates (Shaf 1, Sd1 and Sid1). Three different combined concentrations were prepared in separate glass vials like first concentration was 0.125g turmeric powder, 0.11g Shaf1 *B.t,* 0.087g Sd1 *B.t* and 0.087g Sid1 *B.t,* as along with 1 gram of diet. 20 larvae were introduced in each vial. Total 60 larvae were used in each combined concentration bioassay. Likewise second concentration was made 0.25g turmeric powder, 0.23g Shaf1 *B.t,* 0.17g Sd1 *B.t* and 0.17g Sid 1 *B.t,* in 1gm diet and third concentration was 0.5g turmeric powder, 0.45g Shaf1 *B.t,* 0.35g Sd1 *B.t* and 0.35g Sid 1 were mixed in 1gm of diet. For each combined concentration, the bioassay was carried out for larvae. The bioassay was also in triplicate form. The mortality rate was checked after every 24 hours for seven days. The combined %age mortality was calculated and compared it with individual mortality of larvae. Combined bioassay for adult was 0.125g turmeric powder, Shaf1*B.t.* 0.13g, Sd1*B.t.* 0.10g and Sid1*B.t.* 0.26g mixed all these concentrations with 1g diet to prepared first combined concentration. Likewise second concentration was made 0.25g turmeric powder, 0.26g Shaf1 *B.t,* 0.20g Sd1 *B.t* and 0.32g Sid 1 *B.t,* in 1gm diet and third concentration was 0.5g turmeric powder, 0.53g Shaf1 *B.t,* 0.40g Sd1 *B.t* and 0.62g Sid1 were mixed in 1gm of diet. Similarly the combined percentage mortality of adults was calculated and compared with individual mortality of turmeric powder and *B.t* isolates.

Statistical analysis

The individual effect of turmeric powder, combined effect of three *B.t*. isolates and combined effect of turmeric powder plus *B.t.* isolates were observed. Mortality rate was analysed by SPSS (probit) Programme and $LC_{50}$was calculated for turmeric powder after three and seven days and percentage mortality was calculated.

Results and Discussion

The present study was conducted to check the individual as well as combined insecticidal effect of *Bacillus thuringiensis* isolates along with turmeric powder against the *Triboliumcastaneum* larvae and adults. The mortality of these insects was observed and the LC_{50} value was calculated by SPSS (Probit) Programme.

Selection of *Bacillus thuringiensis* isolates

Three *Bacillus thuringiensis* isolates of previous research students were selected, and grown on T3 medium.

Confirmation of *B. thuringiensis*

After the selection and growth of *B.t*. isolates the further step was the confirmation of the bacteria. The growth on the T3 medium plates gave the colony morphology of *B.t* isolates. Like off white colour, rich and dry growth, entire margin. Moreover, for further confirmation the Gram's staining and endospore staining, were performed.

Gram's Staining

In the Gram's staining, first of all the slides were labeled as Shaf1, Sd1 and Sid1 and then slides were prepared and observed under the microscope at 100X with oil immersion, Gram positive, rod shaped and purple coloured bacteria were seen which confirmed it as *B.thuringiensis.*

Biochemical tests

For the confirmation of *B.t.* isolates, starch hydrolysis test was performed. The bacterial colony was streaked on the potato starch plate and incubated for 4 days after streaking. When starch was hydrolysed showed the formation of clear zone and the unchanged starch appeared in colour blue black when iodine solution was flooded on the plates. *B.t.* isolates showed turbidity after 14 hours, in the sodium chloride test.

Endospore staining

The crystals and vegetative cells were seen in deep pink colour and spores were in green colour under 100X power of microscope with oil immersion which also proved that it was *B. thuringiensis.*

Bioassay Results for *Triboliumcastaneum* larvae

The bioassays were carried out to check the individual effect of turmeric powder as well as combined effect of three *Bacillus thuringiensis* isolates, and combined effect of *B.t.* isolates and turmeric powder against *Triboliumcastaneum* larvae.

Turmeric powder

Turmeric powder was proved to be very toxic against *T. castaneum* larvae. The percentage mortality after 1st, 2nd and 3rd day for 0.125g was 16.6, 13.3 and 10% .For 0.25g was 20, 16.6, and11.6% . And for 0.5g powder was 25, 20 and 13% and for fourth concentration was 30, 18.3 and 16.6 for 1st, 2nd and third day respectively. And the LC_{50}, after three days at 0.125, 0.25, 0.5 and 1g was found to be 0.36 g/g . The mortality of *T. castaneum* larvae was high after 1st day of treatment and then the rate was dicreased as the days passed and the mortality was increased as the concentration of turmeric powder was increased. At 1.00g the highest mortality was observed that was 39 out of 60

The percentage mortality at four different concentrations was after 4th day of treatment 8.3, 10, 11.6 and 13.3% and after 5th day was 5, 8.3, 10 and 11.6. 6th day was 3, 6, 6 and 10% and 7th day was 1.6, 3, 5 and 0% . And the LC_{50} at 0.125, 0.25 and 0.5 and 1g was found to be 0.23 g/g after seven days.

The mortality of *T. castaneum* larvae was high after 4th day of treatment than after 5th, 6th and 7th day but less than first three days and the mortality was increased as the concentration of turmeric powder was increased. At 1.00g the highest mortality was observed.

***Bacillus thuringiensis* isolates combined of LC_{50}**

First of all combined effect of *Bacillus thuringiensis* isolates of Shaf1, Sd1and Sid1 was checked with $LC_{50}$of three *B.t* .isolates against larvae. The LC_{50} of these isolates was 1.8, 1.50 and 1.48g (Shaf1, Sd1and Sid1) respectively. These LC_{50} was combined with 1g of diet. First day high mortality rate was observed that was 41.6% then percentage of mortality was

decreased with day passed. Then was 21.6, 15, 10, 8.3, 3.3 and 1.6% for 2nd, 3rd, 4th, 5th, 6th and 7th day respectively.

Bacillus thuringiensis isolates combined bioassay

Three different concentrations of *B.t* .isolates Shaf1 (0.11, 0.23, 0.45), Sd1 (0.087, 0.17, 0.35) and Sid1 (0.087, 0.17, 0.35) were selected. The mortality was enhancing by increasing concentration and mortality decreased with the passage of time. At first concentration of three *B.t.* isolates the mortality for first day was 16.6% and 15, 13, 11.6, 10, 6.6 and 1.6 for 2nd, 3rd, 4th, 5th, 6th and 7th day respectively. For second concentration mortality was 20, 16.6, 13.3, 11.6, 3.3 and 1.6% for 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day respectively. For third concentration was 21.6, 20, 15, 15, 11.6, 5 and 1.6% for 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day respectively.

Combined bioassay of turmeric powder and *B.t.* isolates for larvae

In the combined bioassay the mortality of the larvae was high as compared to individual bioassay of turmeric powder and combined *Bacillus thuringiensis* isolates.When the bioassay was carried out for seven days it was observed that mortality rate of larvae was increased when the different concentrations of *Bacillus thuringiensis* isolates and turmeric powder was mixed. The percentage of mortality was also high when the concentrations were mixed as compared to individual concentration of each one.

Bioassay results for _Tribolium castaneum_ adult

Bioassay was carried out in the same way for newly emerged *T. castaneum* adult. The concentrations of turmeric powder were same as in case of larvae but the concentrations of *Bacillus thuringiensis* isolates as Shaf1, Sd1 and Sid1were different. The mortality rate and LC_{50} were different because the small size of larvae, it showed higher mortality than that of adults.

Turmeric powder

When the four different concentrations of turmeric powder was applied to *T. castaneum* adults then for first concentration percentage mortality was 10, 6.6, and 5%. For second concentration was 15, 11.6, and 10%. For third concentration was 20, 18.3, and 15%. Fourth concentration gave the percentage mortality was 23.3, 20 and 16.6%. All these percentage mortality was calculated after 1st, 2nd and 3rd day respectively for all four concentrations and combined LC_{50} was 0.527 g/g at 0.125, 0.25, 0.5 and 1g after three days. The mortality of adults decreased with the passage of time and according to concentrations the mortality rate was increased as the highest mortality was observed that was 36 out of 60 at high concentration of 1g after three days.

After fourth day, the percentage mortality were 5, 8.3, 13.3 and 13.3 at first, second, third and fourth concentrations respectively. In the same way, the percentage mortality for 5th day was 3.3, 5, 8.3 and 10%. And of 6th and 7th days were 1.6, 1.6, 5, 8.35 and 1.6, 3.3, 5, 6.6 respectively .The combined LC_{50} was 0.44 g/g at 0.125, 0.25, 0.5 and 1g after seven days .The mortality decreased with passage of time. The mortality was enhanced as the concentration increased.

Bacillus thuringiensis isolates combined

The percentage mortality at three LC_{50} combined concentrations of Shaf1, Sd1 and Sid1 *B.t* isolates was noted as 36.6, 23.3, 10, 6.6, 5, 3.3 and 1.6 % after 1st, 2nd, 3rd, 4th, 5th, 6th and 7th days respectively. The percentage of mortality of adults was decreased after each day.

Bacillus thuringiensis isolates combined for selected concentrations

Three different concentrations of *B.t* isolates Shaf1 (0.13, 0.26, 0.53g), Sd1 (0.10, 0.20, 0.40g) and Sid1 (0.16, 0.32, 0.62) were selected. The mortality was enhancing by increasing concentration and mortality decreased with the passage of time. At first concentration of three *B.t* isolates the mortality for first day was 13.3% and 11.6, 8.3, 5, 1.6, 1.6 and 0% for 2nd, 3rd, 4th, 5th, 6th and 7th day respectively. For second concentration mortality was 15, 15, 11.6, 8.3, 3.3, 5 and 1.6% for 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day respectively. For third concentration was 20, 16.6, 15, 11.6, 6.6, 6.6 and 1.6% for 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day respectively .

Combined bioassay for *Triboliumcastaneum* adults

In the combined bioassay the mortality of the adults was high as compared to individual bioassay of turmeric powder, *Bacillus thuringiensis* isolates. When the bioassay was carried out after 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day it was observed that mortality rate of adults was increased when the different concentrations of *Bacillus thuringiensis* isolates, and turmeric powder were mixed. When the concentrations were mixed the percentage of mortality was also high as compared to individual concentration of each one .

To check the accuracy of results, all the bioassays were carried out in triplicate form and every bioassay was repeated three times.

In the light of above study it is concluded that *Triboliumcastaneum* is a destructive pest of different stored products especially for grain foods. For the eradication of this insect and for the safe storage of food combined insecticidal effect of three *Bacillus thuringiensis* isolates along with the turmeric powder were checked. It was concluded that isolates of *B. thuringiensis,* biopesticide proved to be effective bacteria against the *T. castaneum*as it gave high mortality against the larvae and adults. Turmeric which was in the form of powder also showed its biopesticidal activity against the red flour beetles, as the response was slow. The combined insecticidal effect of turmeric powder plus three *B. thuringiensis* isolates was more effective as compared to individual effect of turmeric powder and combined *Bacillus thuringiensis* isolates. When all the concentrations of each were combined, it gave higher mortality to *T. castaneum* larvae and adults. Microbial insecticides which were also known as biological control agents were very useful as compared to synthetic chemicals because they were used for the control of different pests but they are non toxic to humans, mammals and other wildlife. These microbial insecticides were proved to be important tools for insect management, not only for stored product but for garden and lawn pests, agricultural and forest pests.

Table.1 Gram's staining and endospore staining of Shaf1, Sd1, Sid1 *Bacillus* Table 2: Mortality of the *Triboliumcastaneum* larvaeafter 1st, 2nd and 3rd day of turmeric powder treatment

Time Interval	No. of Insects Used in Experiment	Concentration (gm)	Mortality Observed (Dead/Total)	Percentage mortality for each concentration %
1st Day	60	Control	0	0
	60	0.125	10	16.6
		0.25	12	20
		0.5	15	25
		1	18	30
2nd Day	60	0.125	8	13.3
		0.25	10	16.6
		0.5	12	20
		1	11	18.3
3rd Day	60	0.125	6	10
		0.25	7	11.6
		0.5	8	13.3
		1	10	16.6

Table.3 LC_{50} valueof turmeric powder on *Triboliumcastaneum*larvae after three days

Concentration (gm)	No. of Insects used in experiments	Mortality Observed	LC_{50} (g/g)
0.125	60	24	
0.25	60	29	
0.5	60	35	
1	60	39	0.36

Fig.12 Effect of different concentration ofturmeric powder on the mortality of *Triboliumcastaneum*larvae after 1st, 2nd and 3rd day of treatment

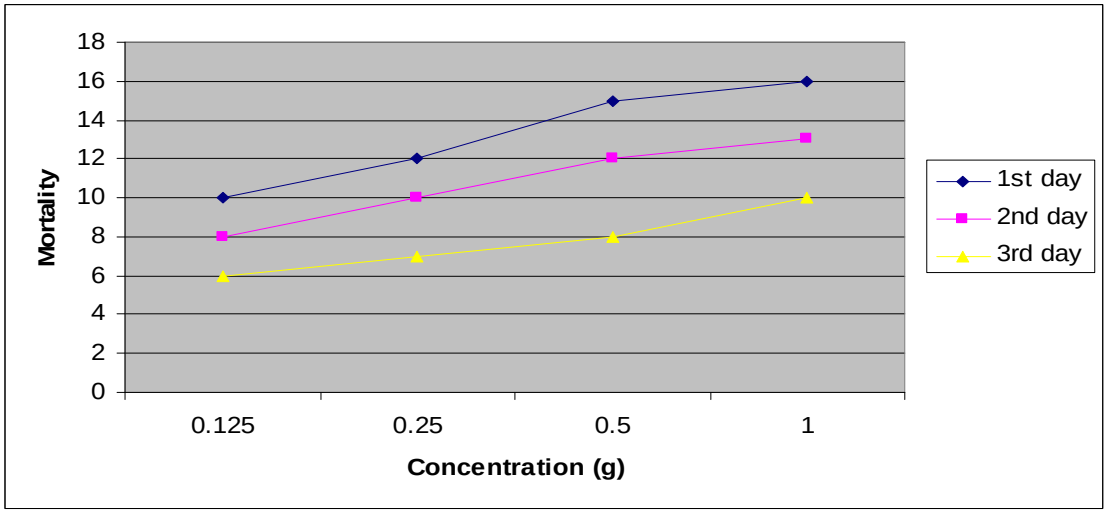


Table.9 Mortality of the*Triboliumcastaneum* adults after 1st, 2nd and 3rd day of turmeric powder treatment

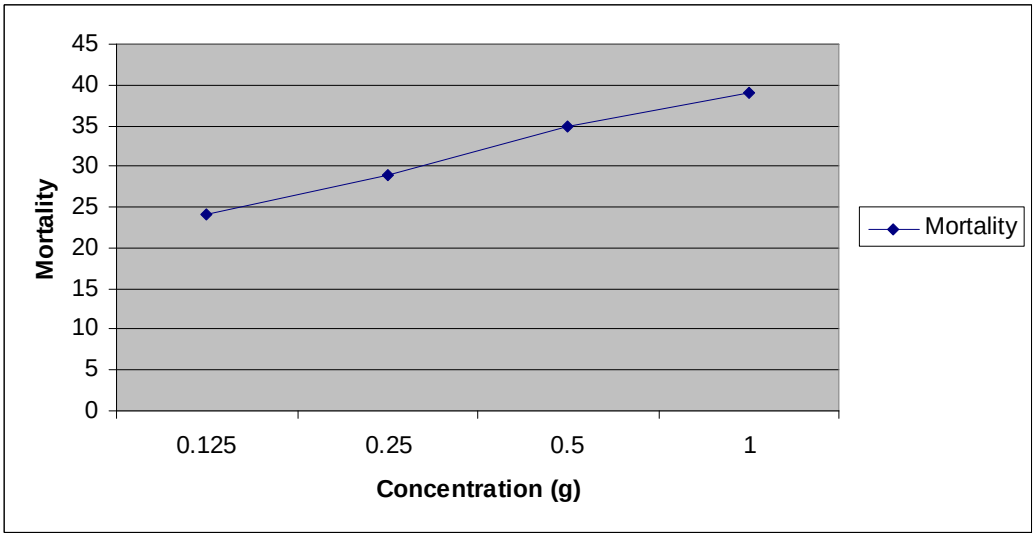
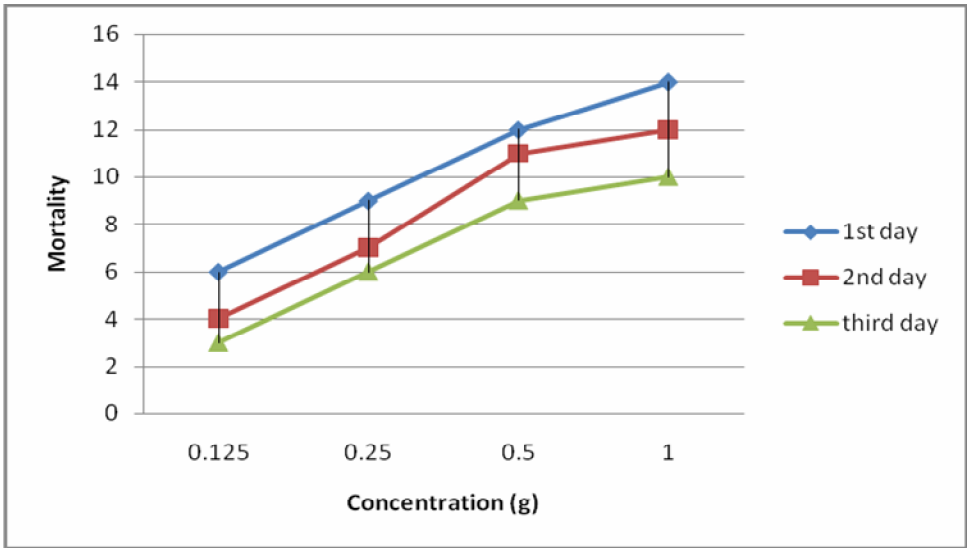


Table.10 LC_{50} values of turmeric powder on *Triboliumcastaneum*adults after three days

Time Interval	No. of Insects Used in Experiment	Concentration (gm)	Mortality Observed (Dead/Total)	Percentage mortality for each concentration %
1st Day	60	Control	0	0
	60	0.125	6	10
		0.25	9	15
		.5	12	20
		1	14	23.3
2nd Day	60	0.125	4	6.6
		0.25	7	11.6
		0.5	11	18.3
		1	12	20
3rd Day	60	0.125	3	5
		0.25	6	10
		0.5	9	15
		1	10	16.6

Concentration (gm)	No. of Insects used in experiments	Mortality Observed	LC50 (g/g)
0.125	60	14	
0.25	60	19	
0.5	60	32	
1	60	36	0.527



Isolates	Gram's staining	Cell arrangement	Endospore staining	
			Spores	Vegetative cell
Shaf1 Sd1 Sid1	Gram positive	Single and Chain	Green	Pink

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