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Study of Combined Pesticidal Effect of Black Onion Seed (*Nigella sativa*) and *Bacillus thuringiensis* on Red Flour Beetle (*Tribolium castaneum*)

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Abstract: *Bacillus thuringiensis*, commonly referred to as *B.t*. is a gram positive bacterium that produces insecticidal crystal proteins. This bacterium occurs naturally in the environment and has been used as a biological pesticide for more than 50 years. In order to manage the loss of stored grains and other agricultural products from insect infestation, many plant products have been evaluated for their toxic properties against different stored grain pests. In the present study, *B.t*. isolates from collected samples were grown on T3 (Tryptone, Tryptose, Yeast extract) media. Bio-toxicity was checked individually and by combination of Black onion seed (*Nigella sativa*) and *Bacillus thuringiensis*, against *Tribolium castaneum*. The LC_{50} of Black onion seeds powder (*N. sativa*) was 0.35 g/g against second instar larvae of *T. castaneum.* Toxicity was increased when this compound was combined with three isolates of *B.t.* (Shaf1, Sd1 and Sid1).The mortality data of *T. castaneum* was analysed by applying probit analysis programme, determined that larvae are more susceptible and show significant results as compared to adults.

Key words: Isolates · Biotoxicity · Assays · LC_{50}

INTRODUCTION

Our economy is continuously harmed by infestation of stored products brought by stored grain pests which are responsible for worldwide loss of 10 - 40% annually [1]. In tropical nations, humidity and temperature run high during summer season and these misfortunes reach to 50% [2-4]. In Pakistan, grain storage losses by insects have been evaluated to be 2.5 % in six months storage [5]. Liquid and gaseous insecticides are continuously being utilized for the control of stored product insects. These insecticides are harmful to mankind as they are disrupting environment due to the development of insect resistance [6,7]. Scientists are attempting to find new alternatives including many herbs and their extracts possessing insecticidal properties.

The red flour beetle, *Tribolium castaneum* (Herbst) belongs to the order Coleoptera and family Tenebrionidae. It can be a major pest for stored grain based items. This beetle might have occurred principally in rotting logs and under tree husks, feeding on insect eggs, pupae and animal detritus [8] yet milled grain items such as flour appear to be their preferred food [9]. Lifespan is dependent upon 3 years [10]. Larvae breed well in wheat items that have been processed or fine grained however in entire wheat parts the harm is restricted to the germ [11]. There is only chance for survival if appropriate food is promptly accessible.

Bacillus thuringiensis (*B.t*.) is a gram positive spore forming bacteria which was initially identified in 1901 by Shigetane Ishiwata [12]. It was rediscovered by Berliner [13] and recognized as microbial insecticide after the separation from *Anagastakuehniella*. This bacterium yields insecticidal crystal proteins named δ-endotoxin throughout the stationary stage of development and is discharged to the environment afterwards sporulation.

B.t. is dispersed worldwide. The soil has remained the primary living space. It is also being isolated from dead insect, stored grains, water etc. Products based on business scale are based on strains usually isolated from dead insects [14]. Examples are *Kurstaki* segregated from *A. kuehniella*; *tenebrionis*, separated from *Tenebrio monitor* larvae and *israelensis* separated from mosquitoes [15,16]. This bacterium is also known as entomopathogenic organism. An entomopathogenic organism is that which is greatly particular and successful

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against target organism. This control agent or entity must be accessible in formulations with sensible time span of usability and ought to remain innocuous to humans as well as non-target flora and fauna and *B.t.* fulfill all these requirements [17].

The arthropod pests areregulated by utilizing fumigants and residual chemical insecticides. Malathion has been used comprehensively for controlling red flour beetle, but now, it is low effective against this pest due to insect resistance [18]. Therefore, this has led to the usage of other biorational insecticides with different modes of action such as abamectin, deltamethrin and chlorpyrifos, which might bring about a proper control of the pest. It is reported that chlorpyrifos and deltamethrin had more viability contrasted with malathion against adult red flour beetle [19]. Abamectin is another expected insecticide that is known as antibiotic insecticide [20] and produced by the soil bacterium *Streptomyces avermitilis* [21]. It had more adequacy compared with indoxacarb, spinosad, buprofezin, polychlorinated petroleum hydrocarbon and azadirachtin against adult red flour beetle [22].

Aromatic plants are known for their healthful and medicinal aspects. These plants possess antiseptic and fungicidal properties, so being utilized in cleansers, scents, cosmetics and in addition in industries such as pharmacology and food flavoring [23,24]. It has been accounted for that that numerous sweet-smelling plant species and vital oils extracted from them, have insecticidal potential to ensure stored food products and crops from insect pests [25-27]. Materials of plant origin can likewise be adequate as ovipositiondetterents [28] and antifeedents [29].

The repellent activity of plant powders of *Argemonemexicane, Prosopisjuliflora, Tephrosiapurpurea*were tested against *T. castaneum.* In general, all the plant powders showed repellent activity except *P. juliflora* at 0.5 mg concentration. Highest repellent activity was observed in *T. purpurea*. *P. juliflora* however showed moderate activity at higher concentration. Thus powders of *T. purpurea* may prove to be a novel bio-treatment to protect the grains from the damages caused by *T. castaneum* [30].

In continuation of search for substances of plant origin with insecticidal effects, it was screened out root, leaf and stem of *Clerodendrumviscosum*, extracted into ethyl alcohol, ethyl acetate and chloroform has strong insecticidal and repellent effect against *T. castaneum* [31]. Alam *et al.* [32] conducted an investigation on other plant showed that residual film toxicity and repellent toxicity against both the larvae and adults of *T. castaneum*.

Efficacy of essential oils extracted from peel of various citrus species; *Citrus reticulata, Citrus sinensis, Citrus paradisi* and *Citrus grandis* were determined against the adults of *Callosobruchus chinensis* and *Tribolium castaneum* as well as against the larvae of *Trogodermagranarium* in order to find out safe alternatives of high toxics. The results revealed that the essential oil vapors showed variable toxicity to insects depending on concentration and exposure duration. Efficacy increased as the exposure time and concentration were increased for all insects [33]. In another study, The effect of volatile compounds of citrus reticulata peel essential oil was seen on the stored grain pest *Sitophilusoryze* and *Tribolium castaneum*. Results indicated that essential oil of *C. reticulate* showed toxic effect against these stored grain insect pests. The LC_{50} against the larva of *T. castaneum* was 18.733 µl at 48h exposure [34].

The focus of the present study was to check the individual as well as combined pesticidal effect of *Nigella sativa* and *B.thuringiensis* on Red flour beetle and to come up with the study which will give a new protocol to the pest management strategies. *Nigella sativa* also called black onion seed or kalongi, belongs to the family ranunculaceae. It is an annual herb of the ranunculaceae family. This spice is local to Europe, North Africa, South and South west Asia and has been generally utilized since old times as a paramount medicinal plant and flavor[35]. Active components of Black onion seeds are oxygenated and olefinicmonoterpenes, principally thymoquinone, α-thujene, p-cymene, thymohydroquinone and γ-terpinene[36].

Bioassays (organic tests) are the experimental strategies which can figure out the concentration of a pill, plant development factors and so forth. It is the determination of relative quality of a substance by looking at its impact on a target creature with that of a standard arrangement.

MATERIALS AND METHODS

Rearing of Insect: Rearing of *T. castaneum* was done by collecting adults from wheat flour, dry fruits, cereals, rice, semolina and so forth. These gathered adults were then presented in glass jars of 300 ml capacity. Each jar was filled 1/4th with sterilized mixture of diet consisting of 90% semolina and 10% yeast extract. Fifty adults of this beetle were introduced in each jar. Every third day, new diet was added to obtain enough larvae for experimentation.

The larvae were reared at 30±1 °C and 60±5% relative humidity. The 2nd instar larvae and newly emerged adults were collected and used in bioassays.

Microbiological Techniques for Growth of *B.t.*

Preparation of T3 Media: Three isolates of *B. thuringiensis*, Shaf1, Sid1, Sd1, were grown on T3 media with in petri plates. The composition of T3 medium is 1.5g yeast extract, 3g of tryptone, 2g of tryptose, 15g of agar/liter, 0.005 g $MnCl_2.2H_2O$ and 2.5ml of 1M potassium phosphate buffer (pH: 6.9). The mentioned quantities of tryptone, tryptose, yeast extract, $MnCl_2$ and potassium phosphate buffer were dissolved in distilled water using a magnetic stirrer. At the end, agar was added without shaking. The pH of media was adjusted and checked with the help of pH meter. Mouth of the flask containing media was closed with aluminum foil or tightly plugged with cotton plug.

Sterilization of Media and Apparatus: All the glass wares including, petridishes, flasks, beakers and pipettes etc. were sterilized at 121°C for 20 minutes in an autoclave. Media was also sterilized at 121°C for 20 minutes in autoclave. For complete sterilized environment air laminar flow hood was used.

Pouring of Media: Sterilized media was poured into the petriplates by filling them less than half of the plate and all was done in the laminar flow hood to avoid the contamination. The media was allowed to solidify at room temperature. Then solidify plates were kept in incubator at 37°C overnight to check the contamination.

Streaking of Plates: Each petriplate was labeled on the name of *B.t*. isolate such as Shaf1, Sid1 and Sd1. Then an inoculating loop was taken and sterilized by dipping in alcohol and then in flame until it became red hot. After that it was allowed to cool and a colony from bacterial culture was picked and streaked in a zigzag manner on the solidify media. Then the plates were kept in incubator at 30°C for 72 hours.

Bacterial Growth on Plates and Collection of Pellet: After three days, plates were taken out and bacterial growth was checked. The growth of *B.t.* was collected with the help of inoculating loop and distilled water. It was stored in falcon tubes which were also labeled and placed in freezer. *B. thuringiensis*stored in falcon tubes was then centrifuged at 3000 rpm for 10 minutes. To obtain pure pellets three times washing was given. The resulting pellets of *B.t.* isolates were then ready to be used in insect bioassays.

Preparation of Glycerol Stock: Glycerol stock was made for the future use. For the preparation of this stock 0.5 ml of bacterial culture of all three isolates of *B.t*. and 300 µl glycerol was added in each sterile labeled eppendorf separately and mixed them well by vortexing. These tubes were then stored at -20°C.

For confirmation of *B. thuringiensis*, Endospore staining and Gram staining as well as biochemical tests like starch hydrolysis were performed for the *B.t*. isolates [37, 38].

Insect Bioassay: To check the bio-toxicological effect of *Nigella sativa* (Kalongi) and *B. thuringiensis*, individual and combined bioassays were performed. The second instar larvae and newly emerged adults of *T. castaneum*were utilized within every bioassay. Each bioassay was performed in triplicate and repeated three times. In individual bioassay, three multiple concentrations were applied to get the LC_{50} value and then based upon the individual LC_{50} values, combined bioassay was performed.After seven days mortality was observed and analyzed by applying SPSS probit analysis programme.

Botanical Compound Bioassay: *Nigella sativa* or black onion seeds were ground to obtain a fine powder.Theselected concentrations ofblack onion seeds powder i.e. 0.2g, 0.4g and 0.8g were mixed per gram diet of red flour beetle. 20 second instar larvae were introduced in each glass vial with the help of camel brush. Each concentration was checked in triplicate. The control sample was maintained without the black onion seeds powder. At regular interval of 24 hours the mortality data was observed for seven days. Same set of compound bioassay was carried out for adults.

Combined *B.t.* Bioassay: Before the bioassay was carried out, pure pellets of three *B.t.* isolates i.e., Shaf1, Sd1 and Sid1 were dried and crushed in pastel mortar to obtain fine granules like powder. The concentrations of *B.t.* isolateof Shaf1 were adjusted like 0.11g, 0.23g and 0.45g, concentrations of *B.t.* of Sd1 were adjusted like 0.09g, 0.19g, 0.37g and concentrations of Sid 1 *B.t.* were adjusted like 0.09g, 0.19g and 0.37g, for larvae.

Initially combined concentration prepared in separate glass vial was 0.11g Shaf1 *B.t.*, 0.09g Sd1 *B.t.* and 0.09g Sid1 *B.t.* mixed per gram diet (0.8g semolina+ 0.2g yeast extract). The bioassay was performed in triplicate and 20 second instar larvae were presented in each vial separately. Likewise second combined concentration of *B.t.* isolates was made by taking 0.23g Shaf1 *B.t*., 0.19g Sd1 *B.t*. and 0.19g Sid1 *B.t*. mixed per gram diet (0.8g semolina+ 0.2g yeast extract) in three separate vials. 20 second instar larvae were introduced in each vial. Third combined concentration was 0.45g Shaf1 *B.t*., 0.37g Sd1 *B.t.* and 0.37g Sid 1 *B.t.* mixed in one gram diet. The number of second instar larvae presented was same as for first two combined concentrations i.e., 20.

The first combined concentration for newly emerged adults was 0.13g Shaf1+ 0.10g Sd1+ 0.16g Sid1 *B.t.* isolates. Similarly second (0.26g Shaf1+ 0.20g Sd1+ 0.32g Sid1) and third (0.53g Shaf1+ 0.40g Sd1+ 0.62g Sid1) concentrations were made. Mortality and percentage mortality both for larvae and newly emerged adults was noted and calculated for seven days.

Combined Bioassay (Three Combined *B.t.* isolates+ Botanical Compound): Combined bioassay was carried out in combination with Black onion seeds powder and three combined *B.t.* isolates (Shaf 1, Sd1 and Sid1). Three different combined concentrations were prepared in separate glass vials for larvae like first concentration was 0.2g Black onion seeds powder, 0.11g Shaf1 *B.t.,* 0.09g Sd1 *B.t.* and 0.09g Sid1 *B.t.* per gram diet along with 20 larvae in each vial. Likewise second concentration was made by taking 0.4g Black onion seeds powder, 0.23g Shaf1 *B.t.*, 0.19g Sd1 *B.t.* and 0.19g Sid 1 *B.t.,* in 1gm diet and third concentration was 0.8g Black onion seeds powder, 0.45g Shaf1 *B.t.*, 0.37g Sd1 *B.t.* and 0.37g Sid 1 mixed in 1gm of diet. For each combined concentration, the bioassay was carried out in triplicate. The mortality rate was checked after every 24 hours for seven days. The combined percentage mortality was calculated and compared it with individual mortality of larvae.

Combined bioassay for newly emerged adults was carried out in the same way. 0.2 g Black onion seeds powder, 0.13g Shaf1, 0.10g Sd1 and 0.26g Sid1 *B.t.* isolates were mixed in 1g diet to prepare first combined concentration. 20 newly emerged adults were introduced in each vial. Likewise second concentration was made by taking 0.4g Black onion seeds powder, 0.26g Shaf1, 0.20g Sd1 and 0.32g Sid 1 *B.t.* isolates in 1gm diet and third concentration was 0.8g Black onion seeds powder, 0.53g Shaf1, 0.40g Sd1 and 0.62g Sid1 *B.t.* isolates were mixed in 1g of diet. Similarly the combined %age mortality of newly emerged adults was calculated and compared with individual mortality of Black onion seeds powder and *B.t.* isolates.

Statistical Analysis: The individual effect of Black onion seeds powder, combined effect of three *B.t*. isolates and combined effect of Black onion seeds powder plus three *B.t.*isolates were observed both for larvae and adults. Death rate was analyzed by SPSS probit analysis programme and $LC_{50}$was calculated for Black onion seeds (Kalongi) powder after three and seven days and percentage mortality was calculated.

RESULT AND DISCUSSION

Black Onion Seeds Powder (Kalongi): Black onion seeds powder, *Nigella sativa*, has proved to be toxic against *T. castaneum* larvae. The percentage mortality after first day for 0.2 g/g was 6.6%. Then was 6.6, 5, 3.3, 0, 1.6 and 1.6% for 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day respectively. The percentage mortality after first day for 0.4 g/g was 13.3%. Then was 11.6, 10, 5, 5, 5 and 3.3% for 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day respectively. The percentage mortality after first day for 0.8 g/g was 16.6%. Then was 16.6, 15, 11.6, 11.6, 8.3 and 8.3% for 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day respectively.

The LC_{50}, after three days at 0.2g, 0.4g and 0.8g was found to be 0.82 g/g. The LC_{50}, after seven days at 0.2g, 0.4g and 0.8g was found to be 0.35 g/g (Table: 1). The LC_{50} was calculated by applying SPSS probit analysis programme.

The mortality rate of *T. castaneum* larvae was high after 1^{st} day of treatment and then the rate was decreased as the days passed and high mortality was observed at high concentration of black onion seeds powder. After three days the highest mortality was 29/60 at 0.8g/g. After seven days the highest mortality was 53/60 at 0.8g/g.

Combined *B. thuringiensis* Bioassay: Three different concentrations of *B.t.* isolates Shaf1 (0.11g, 0.23g, 0.45g), Sd1 (0.09g, 0.19g, 0.37g) and Sid1 (0.09g, 0.19g, 0.37g) were selected. The mortality was enhanced by increasing concentration and mortality rate was decreased with the passage of time. At first concentration of combined three *B.t.* isolates the mortality for first day was 15%. Then was 13.3, 11.6, 8.3, 3.3, 1.6 and 1.6% for 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day respectively. For second concentration mortality was

Table 1: LC_{50} value of Black Onion Seeds powder on *T. castaneum*larvae

	After three days		After seven days	
Concentration (Gram)	Total Mortality Observed	LC50 g/g	Total Mortality Observed	LC50 g/g
0.2	11	0.82	15	0.35
0.4	21		32	
0.8	29		53	

Table 2A: Mortality and %age mortality of *T. castaneum* larvae for one week of treatment with combined of three *B. thuringiensis* isolates (Shaf1, Sd1 and Sid1) and Black Onion seeds powder at first concentration

Time Interval	Concentration (gm)	Mortality Observed (Dead/Total)	Percentage mortality (%)
1st day	Control	0/60	0
	Black Onion seeds powder 0.2	4/60	6.6
	B.t isolates combined (0.11+0.09+0.09)	9/60	15
	Combined *B.t* isolates + Black Onion seeds powder	9/60	15
2nd day	Control	0/60	0
	Black Onion seeds powder 0.2	4/60	6.6
	B.t isolates combined (0.11+0.09+0.09)	8/60	13.3
	Combined *B.t* isolates + Black Onion seeds powder	9/60	15
3rd day	Control	0/60	0
	Black Onion seeds powder 0.2	3/60	5
	B.t isolates combined (0.11+0.09+0.09)	7/60	11.6
	Combined *B.t* isolates + Black Onion seeds powder	8/60	13.3
4th day	Control	0/60	0
	Black Onion seeds powder 0.2	2/60	3.3
	B.t isolates combined (0.11+0.09+0.09)	5/60	8.3
	Combined *B.t* isolates + Black Onion seeds powder	5/60	8.3
5th day	Control	0/60	0
	Black Onion seeds powder 0.2	0/60	0
	B.t isolates combined (0.11+0.09+0.09)	2/60	3.3
	Combined *B.t* isolates + Black Onion seeds powder	2/60	3.3
6th day	Control	0/60	0
	Black Onion seeds powder 0.2	1/60	1.6
	B.t isolates combined (0.11+0.09+0.09)	1/60	1.6
	Combined *B.t* isolates + Black Onion seeds powder	1/60	1.6
7th day	Control	0/60	0
	Black Onion seeds powder 0.2	1/60	1.6
	B.t isolates combined (0.11+0.09+0.09)	1/60	1.6
	Combined *B.t* isolates + Black Onion seeds powder	1/60	1.6

Table 2B: Mortality and %age mortality of *T. castaneum* larvae for one week of treatment with combined of three *B. thuringiensis* isolates (Shaf1, Sd1 and Sid1) and Black Onion seeds powder at second concentration

Time Interval	Concentration (gm)	Mortality Observed (Dead/Total)	Percentage mortality (%)
1st day	Control	0/60	0
	Black Onion seeds powder 0.4	8	13.3
	B.t isolates combined (0.23+0.19+0.19)	12/60	20
	Combined *B.t* isolates + Black Onion seeds powder	14/60	23.3
2nd day	Control	0/60	0
	Black Onion seeds powder 0.4	7	11.6
	B.t isolates combined (0.23+0.19+0.19)	10/60	16.6
	Combined *B.t* isolates + Black Onion seeds powder	11/60	18.3
3rd day	Control	0/60	0
	Black Onion seeds powder 0.4	6/60	10
	B.t isolates combined (0.23+0.19+0.19)	8/60	13.3
	Combined *B.t* isolates + Black Onion seeds powder	9/60	15

Table 2B: Continued

Time Interval	Concentration (gm)	Mortality Observed (Dead/Total)	Percentage mortality (%)
4th day	Control	0/60	0
	Black Onion seeds powder 0.4	3/60	5
	B.t isolates combined (0.23+0.19+0.19)	6/60	10
	Combined *B.t* isolates + Black Onion seeds powder	8/60	13.3
5th day	Control	0/60	0
	Black Onion seeds powder 0.4	3/60	5
	B.t isolates combined (0.23+0.19+0.19)	4/60	6.6
	Combined *B.t* isolates + Black Onion seeds powder	6/60	10
6th day	Control	0/60	0
	Black Onion seeds powder 0.4	2/60	3.3
	B.t isolates combined (0.23+0.19+0.19)	2/60	3.3
	Combined *B.t*isolates + Black Onion seeds powder	3/60	5
7th day	Control	0/60	0
	Black Onion seeds powder 0.4	2/60	3.3
	B.t isolates combined (0.23+0.19+0.19)	2/60	3.3
	Combined *B.t* isolates + Black Onion seeds powder	2/60	3.3

Table 2C: Mortality and %age mortality of *T. castaneum* larvae for one week of treatment with combined of three *B. thuringiensis* isolates (Shaf1, Sd1 and Sid1) and Black onion seeds powder at third concentration

Time Interval	Concentration (gm)	Mortality Observed (Dead/Total)	Percentage mortality (%)
1st day	Control	0/60	0
	Black onion seeds powder 0.8	10/60	16.6
	B.t. isolates combined (0.45+0.37+0.37)	14/60	23.3
	Combined *B.t.* isolates + Black onion seeds powder	16/60	26.6
2nd day	Control	0/60	0
	Black onion seeds powder 0.8	10/60	16.6
	B.t. isolates combined (0.45+0.37+0.37)	13/60	21.6
	Combined *B.t.* isolates + Black onion seeds powder	15/60	25
3rd day	Control	0/60	0
	Black onion seeds powder 0.8	9/60	15
	B.t isolates combined (0.45+0.37+0.37)	10/60	16.6
	Combined *B.t* isolates + Black onion seeds powder	12/60	20
4th day	Control	0/60	0
	Black onion seeds powder 0.8	7/60	11.6
	B.t isolates combined (0.45+0.37+0.37)	7/60	11.6
	Combined *B.t* isolates + Black onion seeds powder	10/60	16.6
5th day	Control	0/60	0
	Black onion seeds powder 0.8	7/60	11.6
	B.t. isolates combined (0.45+0.37+0.37)	6/60	10
	Combined *B.t.* isolates + Black onion seeds powder	8/60	13.3
6th day	Control	0/60	0
	Black onion seeds powder 0.8	5/60	8.3
	B.t. isolates combined (0.45+0.37+0.37)	3/60	5
	Combined *B.t.* isolates + Black onion seeds powder	0/60	0
7th day	Control	0/60	0
	Black onion seeds powder 0.8	5/60	8.3
	B.t isolates combined (0.45+0.37+0.37)	2/60	3.3
	Combined *B.t.* isolates + Black onion seeds powder	0/60	0

20, 16.6, 13.3, 10, 6.6, 3.3 and 3.3% for 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day respectively. For third concentration was 23.3, 21.6, 16.6, 11.6, 10, 5 and 3.3% for 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day respectively.

Combined Bioassay of Black Onion Seeds Powder and *B.t.* Isolates for Larvae: In the combined bioassay the mortality of the second instar larvae was high as compared to individual bioassay of Black onion seeds powder and combined *B. thuringiensis*isolates.When the bioassay was carried out for seven days it was observed that mortality rate of larvae was increased when the 2nd and 3rd concentrations of *B.t.*isolates and Black onion seeds powder was mixed. There was no significant difference when first concentration was applied (Tables: 2A, 2B and 2C).

Bioassay Results for *T. castaneum* Adults: Bioassay was carried out in the same way for newly emerged adults as was for 2nd instar larvae of *T.c*. But the concentrations of *B.t.* isolates as Shaf1, Sd1 and Sid1were different. No significant effect was observed.

Bacillus thuringiensis spores have potential to kill insects belonging to the orders Lepidoptera, Coleoptera and Diptera. In this study, *B.t.* acts as biological pesticide and shows combined pesticidal effect when three isolates of *B.t.* were combined with Black onion seeds*, Nigella sativa.* First individual bioassay of Black onion seeds powder for newly emerged adults and second instar larvae was carried out. The calculated LC_{50} after seven days was 0.35g/g, but no bio-toxicological effect of Black onion seeds (Kalongi) was seen against adults. Occasionallythe adult's behavior was noticeable. They became slow in motion during treatment, due to pungent odour of the compound used. The high death rate for larvae was probably due to small size of larvae. The death rate was high at first day and decreased with the passage of time and high mortality was observed at high concentration. In this research the isolates were combined in a specific ratio and synergistic effect was seen both against larvae and adults of *T. castaneum*.

This study shows that by combining different biodegradable ingredients (Botanical compound+ three *B.t*. isolates) synergistic results can be obtained and this approach can reduce the use of chemical insecticides which are threatening the environment.

Distinctive isolates of *B. thuringiensis* like Ndr1, Ndr3 and Ndr11 demonstrated its adequacy and ended up being extremely dangerous against the white grub larvae and 40% mortality was recorded [39]. In the present study 100% mortality of *T.c.* larvae was recordedwhen three isolates of this bacterium were utilized seven days later. Coleopterans were discovered to be exceptionally ruinous pests in agriculture and woodlands and *B.t.* strains own diverse delta-endotoxins which indicated their pesticidal movement against these pests and also the pests incorporated in the families like Chrysomelidae, Curculionidae, Tenebrionidae and Scarabeidae [40]. This bacterium has a potential requisition against more than hundred pests as the revelation of parasporal crystal protein turned out to be exceptionally harmful against numerous sucking insects like whitefly and aphids [41].

On the groundwork of exploratory studies byHaq, Usmani and Abbas [42] it was discovered that the mixture of some plant materials display fast and moderate activity, It change from types of plants, a few plants are remarkably dangerous while different plants are less successful. Mixture of plant powder ended up being extremely viable for the security of stored grains. Universally a few plants inferred insect sprays that can influence a constrained run of pests, however have non-toxic impact on non-focused living beings on the earth. In few cases, a lot of the plant derived material control repellant and insecticidal exercises against the pests of the stored food product and likewise affirm their adequacy as potent control operator [43]. The plant powder (*N. sativa*) utilized in this study hold the property to control the stored grain product insects which attack the space canisters and godowns. This is because of the slow release of active components and pungent odour of chosen plant.

Arrangement of synergism was exhibited by Benz[44]. As per him, there is supplemental synergism in chemicals and microorganisms. An arrangement of two viable parts, for example impact of *B. thuringiensis* and plant powder (*N. sativa)* might be expanded as it together prepare more greater impact than algebraic whole of the singular impact. In the present studies it was seen that a blend of *B.t.* and *N. sativa* (Black onion seed) was more successful in making the death of the larvae of the *T. castaneum* In the previous studies, Malik *et al*. [37] checked the Bio-toxicological effect of *B.t.* combined with *Saracaindica* and chemical insecticides viz., sodium citrate and Bifenthrin+cypermethrin against larvae and adults of red flour beetle. In another study, combined insecticidal effect of this bacterium along with boric acid, insecticide cypermethrin and plant leaves powder *Azadirachtaindica*, was checked against red flour beetle [37, 38].

CONCLUSION

Nigella sativa, Black onion seed can be cost effective for communities to control stored product insect pests i.e., *T. castaneum*. Combinations of *B.t.*isolates with different botanical compounds like Black onion seed in a specific ratio can be helpful in making bio-pesticide product which can be a new approach in pest management programme. The lethal concentration (LC_{50}) of Black onion seeds powder was 0.35 g/g against second instar larvae of *T. castaneum.* Results were remarkable in case of larvae. Toxicity was increased when this compound was combined with three isolates of *B.t.* (Shaf1, Sd1 and Sid1).Bio-toxicological effect was evaluated by combining three isolates of *B.t.* and Black onion seeds powder (Kalongi) against larvae and adults of *T. castaneum*. Mortality rate was high at high concentration and decreased with the passage of time.

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