



Research Article

Laboratory Evaluation of Selected Botanical and Microbial Formulations against Khapra Beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

Hassan Ali¹, Abu Bakar Muhammad Raza¹, Muhammad Zeeshan Majeed^{1*} and Muhammad Imran Hamid²

¹Department of Entomology, College of Agriculture, University of Sargodha, 40100 Sargodha, Pakistan; ²Department of Plant Pathology, College of Agriculture, University of Sargodha, 40100 Sargodha, Pakistan

Abstract | Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is an economically important stored grain and quarantine pest. It has developed considerable resistance to phosphine and other frequently used grain protectants. This situation necessitates looking for alternate biorational control options such as botanical and microbial insecticides. This *in-vitro* study assessed the anti-insect potential of four local plant extracts and two promising microbial formulations against 5th instar larvae of *T. granarium*. Toxicity bioassays revealed that the extracts of *Citrus reticulata* L. and *Solanum nigrum* L. were most effective against *T. granarium* causing significantly higher larval mortality (35 – 40%) than other botanical treatments. Similarly, the highest concentration (15%) of *C. reticulata* extract exhibited maximum repellency (88%) of larvae, followed by *Datura stramonium* (84%) and *Azadirachta indica* (79%) at 24 h post-exposure. Regarding bioassays with microbial insecticides, maximum mean mortality of larvae (*i.e.* 42.6 and 46.1%) were exhibited by the highest concentration of *Bacillus thuringiensis* (18000 CFUs mg⁻¹) and *Lecanicillium lecanii* (1.0 × 10⁹ conidia g⁻¹) recorded at 5th and 9th day of bioassay, respectively. Based on overall results, the local botanical extracts, particularly peel extract of *C. reticulata* and leaf extract of *S. nigrum* and microbial formulations of *B. thuringiensis* and *L. lecanii* are recommended to the indigenous farmers as biorational options for the management of *T. granarium* and other stored grain insect pests.

Received | September 25, 2021; **Accepted** | March 07, 2022; **Published** | March 12, 2022

***Correspondence** | Muhammad Zeeshan Majeed, Department of Entomology, College of Agriculture, University of Sargodha, 40100 Sargodha, Pakistan; **Email:** zeeshan.majeed@uos.edu.pk

Citation | Ali, H., A.B.M. Raza, M.Z. Majeed and M.I. Hamid. 2022. Laboratory evaluation of selected botanical and microbial formulations against khapra beetle *Trogoderma granarium* everts (Coleoptera: Dermestidae). *Pakistan Journal of Agricultural Research*, 35(1): 154-164.

DOI | <https://dx.doi.org/10.17582/journal.pjar/2022/35.1.154.164>

Keywords | Stored grain insect pests, *Trogoderma granarium*, Biorational pesticides, Insecticidal phytoextracts, *Bacillus thuringiensis*, *Lecanicillium lecanii*



Copyright: 2022 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop and an important constituent of human

diet all over the world. In Pakistan, it is the main staple grain crop being cultivated by 80% farmers. It contributes about 1.7 and 8.7% to GDP and value-added in agriculture, respectively. The area under

wheat cultivation in Pakistan was 8,825 thousand hectares and its production increased by 2.5% to 24.95 million tonnes over the last year production (GOP, 2019).

Nevertheless, per unit area wheat production in Pakistan is much less than other top wheat producing countries (Khaliq *et al.*, 2019). One of the reasons for this low production is the incidence of many insect pests and diseases which not only cause considerable damage to wheat crop in the field but also pose substantial qualitative and quantitative losses to wheat grains during storage. Most destructive insect pests of wheat and other cereal grains are *Sitotroga cerealella*, *Tribolium castaneum*, *Tribolium confusum*, *Sitophilus oryzae*, *Ryzopertha dominica* and *Trogoderma granarium* accounting for 10–40% post-harvest losses in Pakistan (Ahmad *et al.*, 1992; Ashfaq *et al.*, 2001; Ahmedani *et al.*, 2011; Manzoor *et al.*, 2020).

Khapra beetle (*T. granarium*) is one the most destructive stored grain and quarantine pests in tropical and subtropical regions of the world including Pakistan (Ahmedani *et al.*, 2007a; Day and White, 2016; Athanassiou *et al.*, 2019). Larvae of this beetle cause heavy losses to wheat and other stored grains ranging from reduction in nutritional value and weight to rendering produce unfit for human consumption (Ahmedani *et al.*, 2007a; Honey *et al.*, 2017). Farmers and state storage facilities in Pakistan region rely exclusively on the use of different synthetic chemicals such as permethrin, deltamethrin, spinosad, indoxacarb and fumigants such as aluminum phosphide, sulphonyl fluoride and methyl bromide for the control of *T. granarium* and other stored grain insect pest infestations (Khan, 2020; Wakil *et al.*, 2021).

Synthetic chemicals are used for the management of different insect pests. These pesticides are still used in developing countries for the control of stored grain insect pests (Koureas *et al.*, 2012; Wasala *et al.*, 2016). Extensive use of synthetic insecticides such as of methyl bromide and phosphine has resulted in development of resistance in stored grain insect pests (Benhalima *et al.*, 2004; Pimental *et al.*, 2007). Indiscriminate and wide use of these synthetic grain protectants have also resulted in several residual effects and ecological consequences such as development of insect pest resistance, pest resurgence and human health hazards (Satya *et al.*, 2016). For instance, populations of *T. granarium* have been reported from all over the world

to develop resistance against phosphine and other extensively used grain protectants (Ahmedani *et al.*, 2007b; Honey *et al.*, 2017; Riaz *et al.*, 2018; Yadav *et al.*, 2020; Wakil *et al.*, 2021).

Aforementioned residual effects and problems manifested by the extensive use chemical grain protectants necessitate searching for biorational and safe alternatives for stored grain pest management such as phytoextracts and entomopathogenic formulations. In this context, this study was aimed to assess the comparative toxicity of acetonic extracts of some selected local plants and two promising microbial insecticides against 5th instar larvae of *T. granarium* under laboratory conditions.

Materials and Methods

Bioassays regarding the comparative evaluation of biorational insecticides comprising of four local plant extracts and commercial formulations of entomopathogenic bacterium and fungus against *T. granarium*, were conducted in Laboratory of the Department of Entomology, College of Agriculture, University of Sargodha (Punjab, Pakistan).

Collection and rearing of *T. granarium*

Mixed population of *T. granarium* adults and larvae was collected from grain market godowns of district Sargodha (Punjab, Pakistan) and this culture was taken in the laboratory for rearing in 1.0 L glass jars (11 × 5 cm) containing 500 g sterilized wheat grains and covered with fine muslin cloth. Culture was reared for at least 3rd generation at 30 ± 2°C temperature and 65 ± 5% relative humidity. The newly emerged adults were shifted into other jars to get a homogeneous population.

Collection and preparation of plant extracts

Leaves of three local plant species namely datura (*Datura stramonium* L., Solanaceae), black night shade (*Solanum nigrum* L., Solanaceae) and neem (*Azadirachta indica* A. Juss., Meliaceae) and peels of fresh fruits of sweet orange (*Citrus reticulata* L. cv. Feutrell's early; Rutaceae) were collected and shade-dried for three days at room temperature (28°C), and then were grinded to make fine powder using heavy duty electric grinder. Botanical extractions were done using Soxhlet apparatus (DH.WHM-12393, Daihan Scientific, South Korea) using an already described protocol (Majeed *et al.*, 2020). In brief, apparatus

thimble was filled with about 50 g of powdered plant material and was extracted using 500 mL of pure acetone. Crude extracts were further purified using rotatory vacuum evaporator (Daihan Scientific Co., Ltd., South Korea) maintained at 60 °C. Final botanical extracts were stored in dark glass hermetic vials in refrigerator at 4 °C until their utilization in downstream experiments.

Toxicity bioassays with botanical extracts

Standard filter paper-dip method was used for assessing the toxicity of three different concentrations (*i.e.*, 5, 10 and 15%) of botanical extracts against *T. granarium* 5th instar larvae (Hanif *et al.*, 2015). Whatman No.1 filter paper discs (90 mm dia) were dipped in each botanical concentration for 10 s and were air-dried for 5 min and placed in sterilized glass Petri-plates (90 mm dia). In control treatments, pure acetone alone was used. Ten 5th instar *T. granarium* larvae were released in each Petri-plate with fine tipped camel hair brush, and plates were covered with lid to prevent the escape of insects. All Petri-plates were placed in an incubator at 30±2°C temperature and 65±5% relative humidity. Experimental design was completely randomized with five replications per treatment. Larval mortality data were calculated 24, 48 and 72 h post-exposure. Percent corrected mortality was calculated using Abbott formula (Abbott, 1925) as follows;

$$\text{Percent corrected mortality} = \frac{Mt - Mc}{100 - Mc} \times 100$$

Where; Mt is the number of dead insects in the treatments and Mc is the number of dead insects in the control.

Repellency bioassay with botanical extracts

Repellency potential of botanical extracts was determined using standard filter paper-disc method (Khan *et al.*, 2019). In this experiment, Whatman No. 1 filter paper discs (90 mm dia) were cut into two halves. Half disc was treated with different concentrations (5, 10 and 15%) of plant extracts, while half was treated with pure acetone (control). Both were subjected to air dry for 5 min and were rejoined and placed in Petri-dishes (90 mm dia). Ten uniform sized 5th instar larvae of *T. granarium* were released in each Petri-plate. After covering with glass lids, all Petri-plates were placed in an incubator at controlled conditions as described above. Experimental design was completely randomized with five replications per treatment. Data of total number of insects present on

treated and untreated filter paper halves were recorded at 24, 48 and 72 h post-exposure. Percent repellency (PR) was calculated as described by Asawalam *et al.* (2006) using following formula:

$$\text{Percent repellency} = \frac{N_c - N_t}{N_c + N_t} \times 100$$

Where; N_c is the number of insects on the control (untreated) filter paper half and N_t is the number of insects on treated filter paper half.

Toxicological evaluation of selected entomopathogens against *T. granarium* larvae

Commercial formulations of *B. thuringiensis* subsp. *kurstaki* (MCC 0089) (Lipel[®]; 18000 CFUs mg⁻¹ WP) and *Lecanicillium lecanii* (MCC 0058) (Mealikil[®]; 1.0 × 10⁹ conidia g⁻¹ SP) were procured from AgriLife[™], Hyderabad, India. Three concentrations of *Bt* (*i.e.*, 18000, 9000 and 4500 CFUs mg⁻¹) and *L. lecanii* (*i.e.*, 1.0 × 10⁹, 1.0 × 10⁸ and 1.0 × 10⁷ conidia g⁻¹) were bioassayed against 5th instar larvae of *T. granarium* using standard diet-mix (Khaliq *et al.*, 2019) and topical spray (Sagheer and Sahi, 2019) bioassays, respectively. Double-distilled sterilized water was used in control treatments.

In diet-mix bioassays, 50 g of wheat grains were thoroughly treated with different concentrations of *Bt* formulation (Lipel[®]) as mentioned above and were exposed to 15 uniform sized 5th instar larvae of *T. granarium* in 1.0 L sterilized glass jars. Jars were incubated in an environment chamber at 28 ± 2°C temperature, 65 ± 5% relative humidity and at a photoperiod of 14 h light: 10h dark. Larval mortality data were recorded at 1, 2, 3, 4 and 5 days post-exposure and were corrected according to Abbot's formula (Abbot, 1925) as mentioned above. In *L. lecanii* entomopathogenicity bioassay, 15 uniform larvae were released in sterilized glass Petri-plates (90 mm dia) and were treated by different concentrations of *L. lecanii* using hand atomizer spray bottle and Petri-plates were incubated under controlled conditions as described above. Data regarding larval mortality were recorded at 1, 3, 5, 7 and 9 days of exposure to fungal concentrations and were corrected according to Abbot's formula (Abbot, 1925) as described above.

Statistical analysis

Apart from graphical presentation, data regarding corrected percent mortality and repellency of *T. granarium* larvae were subjected to factorial analysis

of variance (ANOVA) and the treatment means were compared using Tukey's HSD post-hoc test at 95% level of significance. Statistical software Statistix® Version 8.1 was used for statistical interpretation of data.

Results and Discussion

Present study was carried out to determine the efficacy of acetone extracts of four selected indigenous plant species (*D. stramonium*, *S. nigrum*, *A. indica* and *C. reticulata*) and two promising commercial formulations of microbial insecticides (*B. thuringiensis* and *L. lecanii*) against 5th instar larvae of khapra beetle *T. granarium*, one of the most damaging primary pests of stored grains. Toxicity and repellency effects of botanical extracts were tested for 3 days post-exposure, while pathogenicity potential of *B. thuringiensis* and *L. lecanii* were recorded for 5 and 9 days post-exposure, respectively.

Response of *T. granarium* larvae to botanical extracts

Larvae of *T. granarium* were exposed to three

concentrations (5, 10 and 15%) of botanical extracts and larval mortality was recorded at 24, 48 and 72 h post-exposure. Mortality response of *T. granarium* was concentration and time dependent and increased along with the increase of these factors. Overall factorial analysis of variance results revealed significant effect ($P \leq 0.05$) of botanical treatments, their concentrations, time intervals and their interactions on the mean mortality of larvae (Table 1). Similar trend of effectiveness was observed in case of individual time intervals (Table 2).

At 24 h, 15% extracts of *S. nigrum* and *A. indica* exhibited maximum mortality significantly higher from other all treatments, while at 48 h, the highest concentration of all four botanical extracts caused maximum mortality without any significant difference (Figure 1). However, at 72 h post-exposure, *C. reticulata* appeared as the most effective extract followed by *S. nigrum* causing up to 35–40% larval mortality and both were significantly higher than other

Table 1: Overall factorial analysis of variance (ANOVA) comparison regarding the mortality of 5th instar larvae of *Trogoderma granarium* bioassayed against different botanical extracts.

Source	DF	SS	MS	F-value	P-value
Treatment	3	1117.2	372.41	8.13	< 0.001***
Concentration	2	6453.3	3226.67	70.40	< 0.001***
Time	2	8230.0	4115.00	89.78	< 0.001***
Treatment×Concentration	6	764.4	127.41	2.78	0.0138*
Treatment×Time	6	1441.1	240.19	5.24	0.0001***
Concentration×Time	4	936.7	234.17	5.11	0.0007***
Treatment×Concentration×Time	12	752.2	62.69	1.37	0.1879 ^{ns}
Error	144	6600.0	45.83		
Total	179	26295.0			
Grand mean/ CV		15.17 /44.64			

Asterisk symbols *, ** and *** indicate significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively and ^{ns} indicates non-significant effect (factorial ANOVA; HSD at $\alpha = 0.05$).

Table 2: Analysis of variance (ANOVA) comparison regarding the mortality of 5th instar larvae of *Trogoderma granarium* bioassayed against different botanical extracts.

Source	24 h				48 h			72 h		
	DF	MS	F-value	P-value	MS	F-value	P-value	MS	F-value	P-value
Treatment	3	183.89	8.49	0.0001***	86.11	10.33	0.003**	228.33	9.30	0.0023**
Concentration	2	3705.01	171.01	< 0.001***	1221.67	47.29	< 0.001***	4245.07	74.91	< 0.001***
Treatment × Concentration	6	27.22	6.26	0.0137*	26.11	6.01	0.0165*	78.33	5.38	0.0316*
Error	48	21.67			25.83			56.67		
Total	59									
GM / CV		14.80 / 28.13			21.83/ 23.28			46.50 / 28.41		

Asterisk symbols *, ** and *** indicate significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively (factorial ANOVA; HSD at $\alpha = 0.05$).

botanical treatments. In control treatment, larval mortality varied from negligible at 24 and 48 h to 4% at 72 h post-exposure (Figure 1).

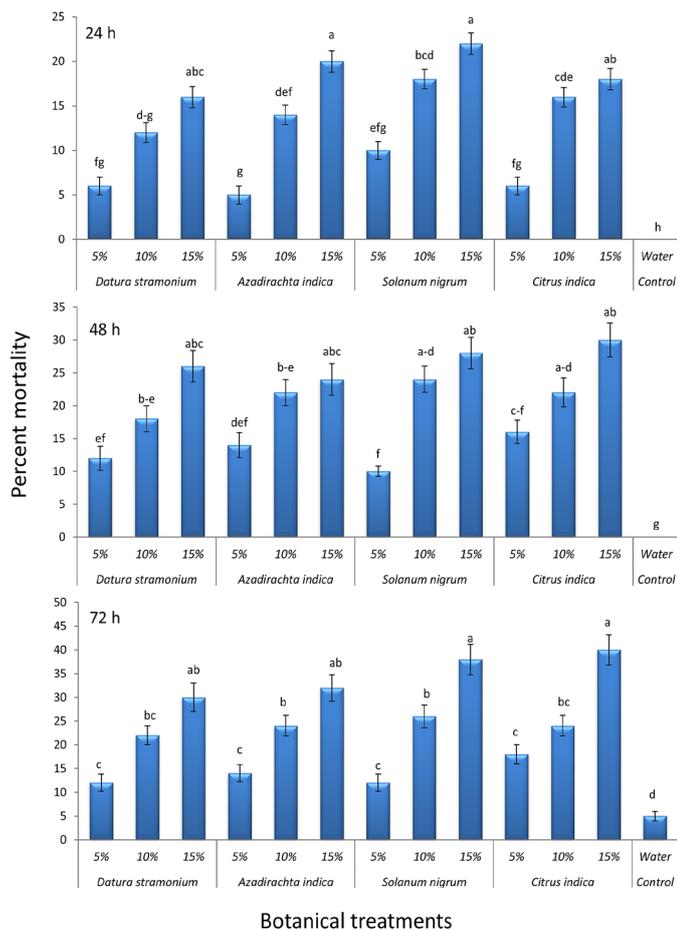


Figure 1: Percent repellency (mean ± SE; n = 5) of 5th instar larvae of khapra beetle *Trogoderma granarium* exposed to different concentrations of selected botanical extracts recorded at different time intervals. For each time interval, different alphabets at the bar tops indicate significant difference among the treatments (factorial ANOVA; Tukey's HSD at α = 0.05).

Repellency potential of botanical extracts against *T. granarium* larvae

Through filter paper disc-dip bioassay method, the repellency potential of the three concentrations (5, 10 and 15%) of botanical extracts was determined and the results of this bioassay revealed that all botanical concentrations caused considerable repellency of *T. granarium* larvae. The interaction of both factors (time and concentration) had non-significant impact on the percent repellency of larvae (Table 3). However, this repellent effect seemed positively and negatively dependent on botanical concentration and time intervals, respectively (Figure 2). Results of factorial analysis of variance for different time intervals revealed a significant effect (P ≤ 0.05) of botanical treatments and their concentrations, and their

interactions on the mean mortality of *T. granarium* larvae (Table 4). Highest concentration (15%) of *C. reticulata* extract exhibited maximum repellency (88%) of larvae, followed by *D. stramonium* (84%) and *A. indica* (79%) at 24 h post-exposure. However, this repellency decreased up to 60% for the same botanical concentrations at 72 h post-exposure (Figure 2).

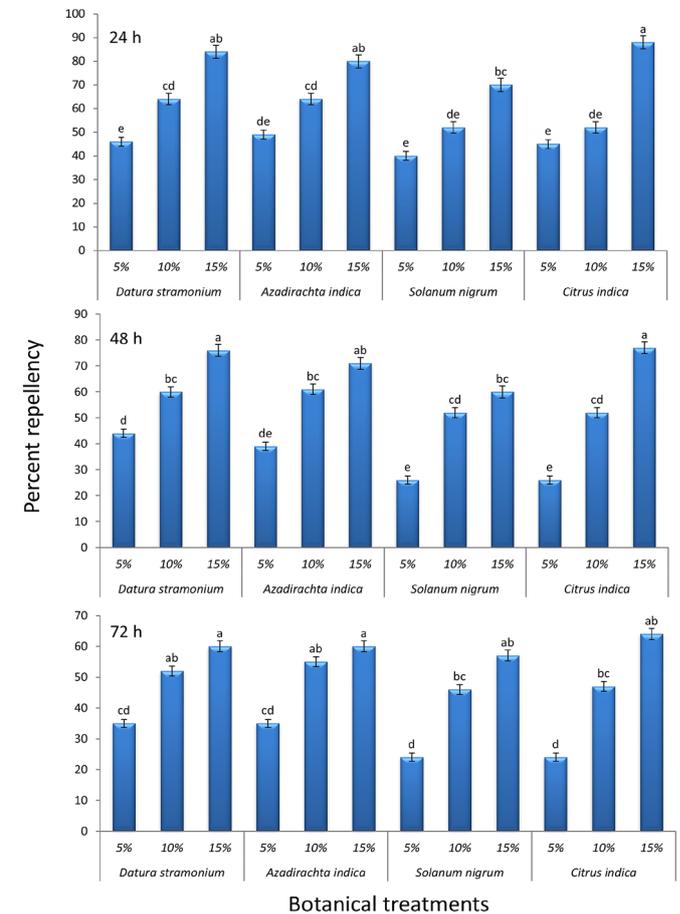


Figure 2: Percent repellency (mean ± SE; n = 5) of 5th instar larvae of khapra beetle *Trogoderma granarium* exposed to different concentrations of selected botanical extracts recorded at different time intervals. For each time interval, different alphabets at the bar tops indicate significant difference among the treatments (factorial ANOVA; Tukey's HSD at α = 0.05).

Efficacy of microbial insecticides against *T. granarium* larvae

Upon exposure of 5th instar larvae of *T. granarium* to different concentrations of entomopathogenic bacterium (*B. thuringiensis* subsp. *kurstaki*) and fungus (*L. lecanii*), mortality data of these bioassays up to 5th day and 9th day respectively were subjected to analysis of variance. Results revealed that both concentration and time factors exerted a significant effect on the mortality of larvae for both microbial treatments. However, the interaction of these factors was statistically significant (P ≤ 0.001) only in case of *B. thuringiensis*, while it was non-significant for

Table 3: Overall factorial analysis of variance (ANOVA) comparison regarding the repellency of 5th instar larvae of *Trogoderma granarium* bioassayed against different botanical extracts.

Source	DF	SS	MS	F-value	P-value
Treatment	3	3574.3	1191.4	26.01	< 0.001***
Concentration	2	36740.9	18370.4	400.98	< 0.001***
Time	2	6054.6	3027.3	66.08	< 0.001***
Treatment × Concentration	6	1023.0	170.5	3.72	0.002**
Treatment × Time	6	294.9	49.2	1.07	0.3815 ^{ns}
Concentration × Time	4	985.4	246.4	5.38	0.0001***
Treatment × Concentration × Time	12	603.5	50.3	1.10	0.3665 ^{ns}
Error	144	6597.2	45.8		
Total	179	55873.8			
Grand Mean / CV		54.11 / 12.51			

Asterisk symbols *, ** and *** indicate significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively and ^{ns} indicates non-significant effect (factorial ANOVA; HSD at $\alpha = 0.05$).

Table 4: Analysis of variance (ANOVA) comparison regarding the repellency of 5th instar larvae of *Trogoderma granarium* bioassayed against different botanical extracts.

Source	24 h				48 h			72 h		
	DF	MS	F-value	P-value	MS	F-value	P-value	MS	F-value	P-value
Treatment	3	380.89	6.91	0.001***	598.40	14.78	< 0.001***	310.44	7.41	< 0.001***
Concentration	2	6539.12	118.71	< 0.001***	7202.72	177.95	0.004**	5121.32	122.28	< 0.001***
Treatment × Concentration	6	115.41	4.10	0.0127*	138.92	3.43	0.007**	75.76	5.40	0.005**
Error	48	55.08			40.98			41.88		
Total	59									
GM / CV		61.33 / 12.10			53.86 / 11.81			47.13 / 13.73		

Asterisk symbols *, ** and *** indicate significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively (factorial ANOVA; HSD at $\alpha = 0.05$).

Table 5: Analysis of variance (ANOVA) comparison regarding the mortality of 5th instar larvae of *Trogoderma granarium* bioassayed against different microbial insecticides.

Source	DF	Bacillus thuringiensis			Lecanicillium lecanii		
		MS	F-value	P-value	MS	F-value	P-value
Concentration	3	1232.65	49.73	< 0.001***	3392.30	7.29	< 0.001***
Time	4	32156.57	548.49	0.03*	4491.80	97.50	0.007**
Concentration × Time	12	87.99	6.01	0.0001***	1134.30	3.32	0.0701 ^{ns}
Error	68	38.29			612.81		
Total	74						
GM / CV		20.53 / 10.20			21.08 / 37.59		

Asterisk symbols *, ** and *** indicate significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively and ^{ns} indicates non-significant effect (factorial ANOVA; HSD at $\alpha = 0.05$).

L. lecanii (Table 5). For both treatments, the mortality response of *T. granarium* was concentration and time dependent and increased along with the increase of these factors (Figure 3). Maximum mean mortality values of 42.6 and 46.1% were exhibited by the highest concentration of *B. thuringiensis* (18000 CFUs mg⁻¹) and *L. lecanii* (1.0 × 10⁹ conidia g⁻¹) recorded at 5th and 9th day of bioassay, respectively (Figure 3). Khapra

beetle (*T. granarium*) is an economical primary pest of stored grains in tropical and subtropical areas including Pakistan and has attained resistance against such commonly used chemicals as phosphine, methyl bromide and sulfuryl fluoride etc. (Ahmedani et al., 2007b; Honey et al., 2017; Riaz et al., 2018; Wakil et al., 2021). Therefore, this study was aimed to evaluate some promising biorational insecticidal treatments

including four local botanical extracts and two entomopathogenic formulations against 5th instar larvae of *T. granarium* under laboratory conditions.

Results of multi-factor ANOVA revealed that the extracts of *S. nigrum* (black nightshade) and *C. reticulata* (sweet orange) were most effective against *T. granarium* larvae exhibiting maximum mortality. Our results are consistent with some previous studies. Extracts of *S. nigrum* and other nightshade species are reported to constitute different alkaloids and are effective against many phytophagous insect pests (Rawani *et al.*, 2010; Carnot *et al.*, 2017) and plant diseases (Muto *et al.*, 2006). Spochacz *et al.* (2018a) showed the sublethal effects of glycol-alkaloids of *S. nigrum* fruits on the physiology and reproductive parameters of mealworm *Tenebrio molitor* which is also an important stored grain insect pest.

dominica (Abbas *et al.*, 2012). A study by Saeidi *et al.* (2011) reported significantly higher repellent effect of essential oils of *C. reticulata* and *C. aurantium* against stored grain insect pest *Callosobruchus maculatus*. However, one study showed that the essential oil of *C. reticulata* were less toxic to *T. granarium* and other stored grain insect pests as compared to other citrus species (Zia *et al.*, 2013).

A. indica (neem) is the most promising and leading botanical with well-known anti-insect and anti-microbial properties (Schmutterer, 1990; Benelli *et al.*, 2017). The essential oils and extracts of leaves, fruits and seeds of this plant have been demonstrated very effective against a wide number of insect pest species including *T. granarium* and other stored grain insect pests (Williams and Mansingh, 1996; Egwurube *et al.*, 2010; Kumar and Gupta, 2013; Chaudhary *et al.*, 2017). However, in this study, *A. indica* leaf extract showed less mortality as compared to *S. nigrum* and *C. reticulata*. One reason for this less relative toxicity of neem extract would be the wide use of neem leaves in the stored grains as a conventional practice to control insect pests (Egwurube *et al.*, 2010; Kumar and Gupta, 2013). Therefore, strain or population of *T. granarium* collected and used in this study might be already resistant against neem bio-constituents as shown Ganeshwari and Deole (2019) in spider populations in rice field. Similarly, the extracts of *C. reticulata*, *D. stramonium* and *A. indica* effectively and significantly repelled the larvae of *T. granarium* in repellency bioassay. Our results are consistent with the findings of many previous studies reviewed by Regnault-Roger (2012) and Spochacz *et al.* (2018b).

Our results regarding the effectiveness of *B. thuringiensis* and *L. lecanii* are also in line with the findings of many previous studies. Both of these entomopathogenic fungi have been demonstrated virulent and effective against different insect pests including stored grain insect pests including *T. granarium* (Ahmedani *et al.*, 2007c; Wakil *et al.*, 2014; Al-Hamdani *et al.*, 2018; Broumandnia and Rajabpour, 2020).

Conclusion and Recommendations

Based on overall results of the study, it is concluded that local botanical extracts, particularly acetone leaf extract of *S. nigrum* and peel extract of *C. reticulata* and microbial formulations of *B. thuringiensis* and *L.*

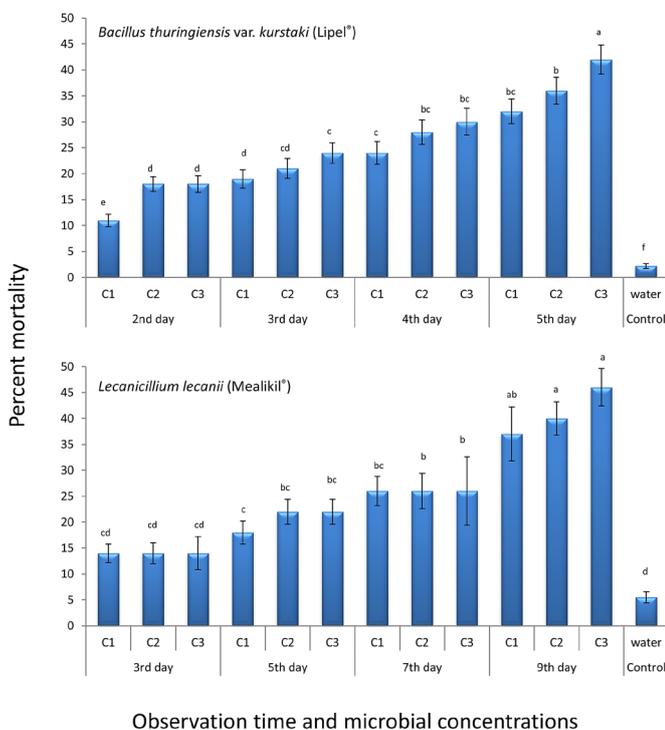


Figure 3: Percent mortality (mean ± SE; n = 5) of 5th instar larvae of khapra beetle *Trogoderma granarium* exposed to different concentrations of commercial microbial formulations recorded at different time intervals. For each formulation, different alphabets at the bar tops indicate significant difference among the treatments (factorial ANOVA; Tukey's HSD at $\alpha = 0.05$).

Similarly, *C. reticulata* extracts constitute certain limonoids such as limonin, nomilin and obacunone (Khalil *et al.*, 2003) capable of inhibiting insect moulting as demonstrated in *Culex* and *Aedes* mosquitoes (Jayaprakasha *et al.*, 1997; Bilal *et al.*, 2012), Asian corn borer *Ostrinia furnacalis* (Abrera *et al.*, 2015) and other stored grain insect pests such as *R.*

lecanii exhibited significant toxicity to *T. granarium*. Hence, these are recommended to the indigenous farmers as valuable tools for the management of khapra beetle and other stored grain insect pests.

Acknowledgments

Authors are grateful to Dr. Muhammad Tariq (PMAS-Arid Agriculture University, Rawalpindi, Pakistan) for providing microbial formulations of *B. thuringiensis* and *L. lecanii*.

Novelty Statement

This laboratory study assessed the insecticidal efficacy of local phytoextracts, particularly of *Citrus reticulata* peel and *Solanum nigrum* leave extracts, and of microbial formulations of *Bacillus thuringiensis* and against *Lecanicillium lecanii* against 5th instar larvae of khapra beetle *Trogoderma granarium*.

Author's Contribution

Abu Bakar Muhammad Raza: Conceived the research idea, designed the experiments and supervised the research work.

Hassan Ali: Conducted the bioassays, recorded data, wrote the initial manuscript draft.

Muhammad Zeeshan Majeed: Did the statistical analyses and prepared results.

Abu Bakar Muhammad Raza and Muhammad Zeeshan Majeed: Revised and proofread the final draft.

Muhammad Imran Hamid: Gave the technical support for experiments.

Conflict of interest

The authors have declared no conflict of interest.

References

- Abbas, S.K., A. Farooq, S. Muhammad, Y. Muhammad, A. Saeed and M. Wali. 2012. Insecticidal and growth inhibition activities of *Citrus paradisi* and *Citrus reticulata* essential oils against lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *World J. Zool.*, 7(4): 289-294.
- Abbott, W.S., 1925. A method of computing the effectiveness, of an insecticide. *J. Econ. Entomol.*, 18(2): 265-267. <https://doi.org/10.1093/jee/18.2.265a>
- Abrera, A.T., R.P. Abogado, M.T. Caasi-Lit and M.N. Manalo. 2015. Insect anti-feedant and growth-inhibitory activities of a limonoid from seeds of dalanghita (*Citrus reticulata* Blanco cv. Ladu) against larvae of the Asian corn borer (*Ostrinia furnacalis* (Guenee)). *Philip. Agric. Sci.*, 98(1): 7-14.
- Ahmad, F., M.R. Khan and M. Ahmad. 1992. Post-harvest losses of food grains and their contamination. *Pak. J. Entomol.*, 14(1-2): 103-112.
- Ahmedani, M.S., A. Khaliq and M.I. Haque. 2007c. Scope of commercial formulations of *Bacillus thuringiensis* Berliner as an alternative to methyl bromide against *Trogoderma granarium* Everts larvae. *Pak. J. Bot.*, 39(3): 871-880.
- Ahmedani, M.S., A. Khaliq, M. Tariq, M. Anwar and S. Naz. 2007a. Khapra beetle (*Trogoderma granarium* Everts): A serious threat to food security and safety. *Pak. J. Agric. Sci.*, 44(3): 481-493.
- Ahmedani, M.S., M.I. Haque, S.N. Afzal, M. Naeem, T. Hussain and S. Naz. 2011. Quantitative losses and physical damage caused to wheat kernel (*Triticum aestivum* L.) by khapra beetle infestation. *Pak. J. Bot.*, 43(1): 659-668.
- Ahmedani, M.S., N. Shaheen, M.Y. Ahmedani and M. Aslam. 2007b. Status of phosphine resistance in khapra beetle, *Trogoderma granarium* (Everts) strains collected from remote villages of Rawalpindi district. *Pak. Entomol.*, 29(2): 95-102.
- Al-Hamdani, A.H.A., M.K. Mubasher and M.K. Ihraheem. 2018. Effect of bacterial toxin for bacteria, *Bacillus thuringiensis* (Berliner) in some life stages of grain beetle (Kahpra) *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). *Biochem. Cell. Arch.*, 18(2): 2293-2297.
- Asawalani, E.F., S.O. Emosairue and A. Hassan. 2006. Bioactivity of *Xylopiia aetiopica* (Dunal) A. Rich essential oil constituents on maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Elect. J. Environ. Agric. Food Chem.*, 5(1): 1195-1204.
- Ashfaq, M., M.A. Saleem and F. Ahmad. 2001. Safe storage of food grains (in Urdu). *Pak Book Empire, Lahore, Pakistan*. pp. 250.
- Athanassiou, C.G., T.W. Phillips and W. Wakil. 2019. Biology and control of the khapra beetle,

- Trogoderma granarium*, a major quarantine threat to global food security. *Annu. Rev. Entomol.*, 64: 131-148. <https://doi.org/10.1146/annurev-ento-011118-111804>
- Benelli, G., A. Canale, C. Toniolo, A. Higuchi, K. Murugan, R. Pavela and M. Nicoletti. 2017. Neem (*Azadirachta indica*): towards the ideal insecticide? *Nat. Prod. Res.*, 31(4): 369-386. <https://doi.org/10.1080/14786419.2016.1214834>
- Benhalima, H., M.Q. Chaudhry, K.A. Mills and N.R. Price. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. *J. Stored Prod. Res.*, 40(3): 241-249. [https://doi.org/10.1016/S0022-474X\(03\)00012-2](https://doi.org/10.1016/S0022-474X(03)00012-2)
- Bilal, H., W. Akram and S. Ali-Hassan. 2012. Larvicidal activity of citrus limonoids against *Aedes albopictus* larvae. *J. Arthropod-Borne Dis.*, 6(2): 104-111.
- Broumandnia, F. and A. Rajabpour. 2020. Efficacies of some isolates of *Lecanicillium lecanii* to control *Tribolium castaneum* (Col., Tenebrionidae). *J. Plant Dis. Prot.*, 127(5): 625-631. <https://doi.org/10.1007/s41348-020-00324-y>
- Carnot, A.C., T.T.E. Alvarest, O. Justin, A. Zachée and M.T. Fabrice. 2017. Effect of culture density of black nightshade (*Solanum nigrum*) on the insect infestation. *Plant*, 5: 19-26.
- Chaudhary, S., R.K. Kanwar, A. Sehgal, D.M. Cahill, C.J. Barrow, R. Sehgal and J.R. Kanwar. 2017. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. *Front. Plant Sci.*, 8: 610. <https://doi.org/10.3389/fpls.2017.00610>
- Day, C. and B. White. 2016. Khapra beetle, *Trogoderma granarium* interceptions and eradications in Australia and around the world. SARE Working Paper 1609. Crawley: School of Agricultural and Resource Economics, U.K.
- Egwurube, E., B.T. Magaji and Z. Lawal. 2010. Laboratory evaluation of neem (*Azadirachta indica*) seed and leaf powders for the control of khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae) infesting groundnut. *Int. J. Agric. Biol.*, 12(4): 638-640.
- Ganeshwari, S.K. and S. Deole. 2019. Biosafety evaluation of essential oils and chemical insecticides on population of coccinellids in rice field. *J. Entomol. Zool. Stud.*, 7(4): 168-171.
- Government of Pakistan. 2019. Economic Survey 2019. Finance Division, Economic advisor's wing, Islamabad.
- Hanif, C.M.S., M.S. Mansoor-ul-Hasan, S. Saleem, K. Ali and S. Akhtar. 2015. Comparative insecticidal effectiveness of essential oils of three locally grown plants and phosphine gas against *Trogoderma granarium*. *Pak. J. Agric. Sci.*, 52(3): 709-715.
- Honey, S.F., B. Bajwa, M.S. Mazhar and W. Wakil. 2017. *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae), an alarming threat to rice supply chain in Pakistan. *Int. J. Entomol. Res.*, 5(1): 23-31.
- Jayaprakasha, G.K., R.P. Singh, J. Pereira and K.K. Sakariah. 1997. Limonoids from *Citrus reticulata* and their moult inhibiting activity in mosquito *Culex quinquefasciatus* larvae. *Phytochemistry*, 44(5): 843-846. [https://doi.org/10.1016/S0031-9422\(96\)00589-4](https://doi.org/10.1016/S0031-9422(96)00589-4)
- Khalil, A.T., G.T. Maatooq and K.A. El Sayed. 2003. Limonoids from *Citrus reticulata*. *Z. Naturforsch.*, 58(3-4): 165-170. <https://doi.org/10.1515/znc-2003-3-403>
- Khalique, T., D.S. Gaydon, M.J.M. Cheema. and U. Gull. 2019. Analyzing crop yield gaps and their causes using cropping systems modelling—A case study of the Punjab rice-wheat system, Pakistan. *Field Crop. Res.*, 232: 119-130. <https://doi.org/10.1016/j.fcr.2018.12.010>
- Khan, H.A.A., 2020. Susceptibility to indoxacarb and synergism by enzyme inhibitors in laboratory and field strains of five major stored product insects in Pakistan. *Chemosphere*, 257: 127167. <https://doi.org/10.1016/j.chemosphere.2020.127167>
- Khan, S.A., M.H. Ranjha, A.A. Khan, M. Sagheer, A. Abbas and Z. Hassan. 2019. Insecticidal efficacy of wild medicinal plants, *Dhatura alba* and *Calotropis procera*, against *Trogoderma granarium* (Everts) in wheat store grains. *Pak. J. Zool.*, 51(1): 289-294. <https://doi.org/10.17582/journal.pjz/2019.51.1.289.294>
- Koureas, M., A. Tsakalof, A. Tsatsakis and C. Hadjichristodoulou. 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. *Toxicol. Lett.*, 210(2): 155-168. <https://doi.org/10.1016/j.toxlet.2011.10.007>
- Kumar, A. and S. Gupta. 2013. Repellent and

- antifeedant activity of neem and amla against two stored grain pests viz. *Tribolium castaneum* and *Trogoderma granarium*. J. Entomol. Res., 37(4): 301-306.
- Majeed, M.Z., M. Afzal, M.A. Riaz, K.S. Ahmed, M. Luqman, M.Z. Shehzad and S. Wahid. 2020. Comparative toxicity of phyto-extracts of indigenous flora of Soone valley against some insect pests of agricultural and urban importance. Punjab Univ. J. Zool., 35(2): 239-253. <https://doi.org/10.17582/journal.pujz/2020.35.2.239.253>
- Manzoor, A., F. Tanwir, S. Akhtar and B. Shahbaz. 2020. An assessment of knowledge level and training gap of the farmers regarding post-harvest losses of wheat in Punjab, Pakistan. J. Pure Appl. Agric., 5(1): 99-106.
- Muto, M., V. Mulabagal, H.C. Huang, H. Takahashi, H.S. Tsay and J.W. Huang. 2006. Toxicity of black nightshade (*Solanum nigrum*) extracts on *Alternaria brassicicola*, causal agent of black leaf spot of Chinese cabbage (*Brassica pekinensis*). J. Phytopathol., 154(1): 45-50. <https://doi.org/10.1111/j.1439-0434.2005.01059.x>
- Pimentel, M.A.G., L.R.D.A. Faroni, M.R. Tótola, and R.N.C. Guedes. 2007. Phosphine resistance, respiration rate and fitness consequences in stored-product insects. Pest Manage. Sci., 63(9): 876-881. <https://doi.org/10.1002/ps.1416>
- Rawani, A., A. Ghosh and G. Chandra. 2010. Mosquito larvicidal activities of *Solanum nigrum* L. leaf extract against *Culex quinquefasciatus* Say. Parasitol. Res., 107(5): 1235-1240. <https://doi.org/10.1007/s00436-010-1993-9>
- Regnault-Roger, C., 2012. Botanicals in pest management. Integrated pest management, Abrol, DP, Shankar, U., Eds, pp. 119-132. <https://doi.org/10.1079/9781845938086.0119>
- Riaz, T., F.R. Shakoori and S.S. Ali. 2018. Phosphine-induced alterations in microsomal enzymes of a stored grain pest *Trogoderma granarium* collected from godowns of Punjab, Pakistan. Pak. J. Zool., 50(1): 1-4. <https://doi.org/10.17582/journal.pjz/2018.50.1.291.297>
- Saeidi, M., S. Moharrampour, F. Sefidkon and S. Aghajanzadeh. 2011. Insecticidal and repellent activities of *Citrus reticulata*, *Citrus limon* and *Citrus aurantium* essential oils on *Callosobruchus maculatus*. Integ. Protect. Stored Prod. IOBC/WPRS Bull., 69: 289-293.
- Sagheer, M. and S.T. Sahi. 2019. Bio-efficacy of entomopathogenic fungus *Beauveria bassiana* (Bals.) against *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst). Pak. J. Agric. Sci., 56(2): 429-434.
- Satya, S., N. Kadian, G. Kaushik and U. Sharma. 2016. Impact of chemical pesticides for stored grain protection on environment and human health. In: Proceedings of 10th international conference on controlled atmosphere and fumigation in stored products, Winn., Canada, pp. 92-97.
- Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol., 35(1): 271-297. <https://doi.org/10.1146/annurev.en.35.010190.001415>
- Spochacz, M., S. Chowański, K. Walkowiak-Nowicka, M. Szymczak and Z. Adamski. 2018b. Plant-derived substances used against beetles-pests of stored crops and food-and their mode of action: A review. Comparat. Food Sci. Food Saf., 17(5): 1339-1366. <https://doi.org/10.1111/1541-4337.12377>
- Spochacz, M., S. Chowański, M. Szymczak, F. Lelario, S.A. Bufo. and Z. Adamski. 2018a. Sublethal effects of *Solanum nigrum* fruit extract and its pure glycoalkaloids on the physiology of *Tenebrio molitor* (Mealworm). Toxins, 10(12): 504. <https://doi.org/10.3390/toxins10120504>
- Wakil, W., M.U. Ghazanfar and M. Yasin. 2014. Naturally occurring entomopathogenic fungi infecting stored grain insect species in Punjab, Pakistan. J. Insect Sci., 14(1): 182. <https://doi.org/10.1093/jisesa/ieu044>
- Wakil, W., N.G. Kavallieratos, M. Usman, S. Gulzar and H.A. El-Shafie. 2021. Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. Insects, 12(4): 288. <https://doi.org/10.3390/insects12040288>
- Wasala, W.M., C.A. Dissanayake, C.R. Gunawardhane, R.M. Wijewardhane, D.M. Gunathilake and B.M. Thilakarathne. 2016. Efficacy of insecticide incorporated bags against major insect pests of stored paddy in Sri Lanka. Proc. Food Sci., 6: 164-169. <https://doi.org/10.1016/j.profoo.2016.02.040>
- Williams, L.A. and A. Mansingh. 1996. The insecticidal and acaricidal actions of compounds from *Azadirachta indica* (A. Juss.) and their use in tropical pest management. Int. Pest Manage.

- Rev., 1(3): 133-145. <https://doi.org/10.1007/BF00130672>
- Yadav, S.K., C. Srivastava and S. Sabtharishi. 2020. Phosphine resistance and antioxidant enzyme activity in *Trogoderma granarium* Everts. J. Stored Prod. Res., 87: 101636. <https://doi.org/10.1016/j.jspr.2020.101636>
- Zia, S., M. Sagheer, A. Razaq, A. Mahboob, K. Mehmood and Z. Haider. 2013. Comparative bioefficacy of different citrus peel extracts as grain protectant against *Callosobruchus chinensis*, *Trogoderma granarium* and *Tribolium castaneum*. World Appl. Sci. J., 21(12): 1760-1769.