Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/p462sa56

LETHAL AND SUB-LETHAL EFFECTS OF BEAUVERIA BASSIANA ON MORTALITY AND BIOLOGY OF GRANARY WEEVIL (SITOPHILUS GRANARIUS)

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ABSTRACT
Granary weevil is a very serious pest of stored chickpeas and causes considerable damage, especially to stored grains. Presently, synthetic pesticides are used for its management which has environmental and health concerns. Present studies were conducted to evaluate non-chemical control tactics for the management of granary weevils using *Beauveria basssiana*. For this purpose, several experiments were conducted in the Department of Entomology, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa (KPK), Pakistan under controlled laboratory conditions. Five different concentrations of *B. bassiana* i.e. $1×10^4$, $1×10^5$, $1×10^6$, $1×10^7$ and $1×10^8$ were tested against granary weevil and data for survival rate was observed from day 1 to 21. A similar trend was observed in this case i.e. increase in mortality rate was observed along with an increase in the exposure time and dose. 50 percent mortality was recorded at the lowest concentration while it was 82 percent at the highest concentration. This indicates that $1×10^4$ mg conidia/kg can help in reducing the population *of S. granarius* whereas, an increase in dose can raise the rate of reduction. For testing sub lethal effect of *B. bassiana* against granary weevil, five sub-lethal doses i.e., $1×10^1$, $1×10^2$, $1×10^3$, $1×10^4$ and $1×10^5$ were again selected. The tested concentrations did not cause any lethal effect on the survival rate of granary weevil. The rise in dose, exposure time and temperature increase the insecticidal efficacy of *B. bassiana* against *S. granarius* under lab conditions. Additionally, sub-lethal concentrations of *Beauveria bassiana* were found to have indirect effects on weevils, including abnormal development and reproductive output. Overall, this study provides valuable insights into potential use of *B. bassiana* as a biocontrol agent against granary weevil, highlighting its effectiveness in reducing population of *S. granarius* and its potential as an environmental friendly alternative to pesticides.

Keywords: Chickpea, Granary weevil, *Beauveria bassiana*, lethal effects, sub-lethal effects.

Key findings: The presented study indicates that higher doses of *Beauveria bassiana* led to increased mortality rates of *S. granarius.* Additionally, sub-lethal doses resulted in reduced progeny emergence and inhibited the percent infestation and weight loss of chickpea grains caused by granary weevils.

INTRODUCTION
Chickpea is cultivated both in Asia and Europe from ancient times (Abbo *et al.*, 2003). It is supposed that chickpeas cultivation started either from Mediterranean or Himalyan regions (Singh *et al.*, 2009). Presently it is grown in Pakistan, Russia, Italy, India, Egypt and many countries of the world. Insect

pests of stored grain cause approximately 10-25% losses by infesting stored grain and their by-products worldwide (Magan and Aldred, 2007). Many of them are coleopterans (Rees *et al*., 2018, Tavakilian *et al.,* 1997). Three Sitophilus spp. i.e., maize weevil, *Sitophilus zeamais* Motschulsky, the rice weevil, *Sitophilus oryzae* (L.) and the granary weevil, *Sitophilus granarius* (L.) are most serious pests of stored products worldwide (Batta, 2018).
Granary weevil is renowned for its commercial significance as one of the most devastating pest of stored grains all over the world, that can eliminate whole seed (Batta, 2018, Athanassiou *et al*., 2007). It is a key pest that is able to easily infest sound and undamaged kernels. This species is an internal feeder as female lays its eggs inside the kernel where complete development of immature takes place resulting in severe infestation (Athanassiou *et al*., 2007). All the growth stages of *S. granarius* are found within the chambers and tunnels bored in the seed. As they belong to the family Curculionidae, they have elbowed antennae and the characteristic rostrum (Poinar *et al.,* 2009)
Pesticides are used for controlling many stored product pests, resulting in various serious problems such as insecticide resistance and contamination of food products with insecticide residues. Therefore, use of naturally occurring entomopathogenic organisms as alternative method of control of stored grain pests is gaining attention nowadays (Yanar *et al*., 2019, Sabbour and Abdel-Raheem, 2015). Insect pathogens i.e. entomopathogenic fungi, viruses, nematodes and fungi have gained attention as they are easily compatible with other Integrated Pest Management techniques. Due to their high efficacy, they are considered as most favourable substitutes to chemical insecticides. Various scientists reported the utilization of different microbes under stored grain conditions and suggested that Entomopathogenic fungi played a vital role for managing different insect pests (Rumbos *et al.*, 2017). Though long-term persistence of conventional insecticides is considered as a drawback, it is considered as important feature for entomopathogenic fungus (Smith and Moore, 2000). The *Beauveria bassiana* is the most commonly used species of entomopathogenic fungi under stored grain conditions (Nikpay, 2006). The fungus *B. bassiana* spores penetrate through the cuticle, germinate and ultimately kill an insect. . It is also stated that they someway protected the cowpea. (Sabbour and Abdel-Raheem, 2015). Therefore, to find out safer alternative control methods is need of the day like utilization of different infectious microbes to control stored grain insect pests.
Metarhizium anisopliae and *B. bassiana* are entomopathogenic fungi commonly found in nature having wide host range (Wakil and Ghazanfar, 2010; Sewify *et al*., 2014; Sheeba *et al*., 2001). *Beauveria bassiana* (entomopathogenic fungi) has a significant insecticidal effect for controlling various insect pests of stored products (Cherry *et al*., 2005; Padin *et al.,* 2002; Yanar *et al*., 2019; Batta, 2018; Steenberg, 2005; Rice and Cogburn. 1999; Bello *et al.,* 2000; Smith *et al.,* 1999; Hidalgo *et al.,* 1998; Adane *et al.,* 1996). For the control of extensive range of insect pests of stored grains and field applications, it is listed by the EPA (Environmental Protection Agency) United States (Lord, 2001). Various formulations of *B. bassiana* (Naturalis® SC, Mycotrol® ES, Mycotrol® 22WP, Boverosil ®) are registered and available commercially nowadays for use against stored grain insect pests. Stored grain insects are among one of the major devastating group of pests that can be controlled efficiently by Entomopathogenic fungi (Batta, 2018). As compared to chemical pesticides, they are usually safer. The application of these EPF_s involve their use in close proximity to food and feed or even direct application to other food products as well as to stored products (Steenberg, 2005, Cox *et al*., 2003; 2004). Keeping in mind the above-mentioned factors, this study was planned to conduct with following objectives "Entomocidal evaluation of various doses of entomopathogenic fungi, *B. bassiana* against granary weevil"

MATERIALS AND METHODS

Collection and culture of granary weevil

Mother granary weevil culture was acquired from the Entomology section of ARI (Agriculture Research Institute), Dera Ismail Khan and laboratory conditions were maintained at 65 ± 5% RH and 27 ± 2°C in 12:12 hour of (Light: Dark) day length. Mix population of 200 male and female granary weevil were added to 500 grams of chickpea in plastic jars of 1-liter capacity for multiplication following the methodology proposed by Isah *et al*., (2012). The jars were kept in an incubator under controlled temperature for ten days to lay eggs, parent insects were removed from jars after 10 days by sieving grains. Infested chickpea grains were kept uninterrupted for additional 20 days and newly emerged weevils were collected and maintained as per their age in different jars separately. Insects of same age were used for each experiment.

The fungus

Commercially produced conidia of *B. bassiana* which were used for the investigations were acquired from India MART.

Mortality effects

The present study was conducted under constant lab. conditions following completely randomized design (CRD). The commercial formulation of entomopathogenic fungus *B. bassiana* was tested against granary weevil. Experiment was conducted using four different concentrations of *B. bassiana* i.e., $T_1 = 1\times10^5$, $T_2 = 1\times10^6$, $T_3 = 1\times10^7$ and $T_4= 1\times10^8$ conidia/kg and untreated check as standard. The experiment was replicated five times. Fifty-gram chickpea grains were treated with one of the concentrations and placed in 250 ml plastic jars. Thereafter, 10 pairs of starved adults of granary weevil were introduced separately in jars which were then covered with muslin cloth for aeration. The mortality data was recorded after 1, 2, 3, 7, 14 and 21 days of the introduction of weevil adults in the treated grains.

The dead insects were incubated at 27°C for two days that were kept in petri dishes containing damp filter paper to maintain high humidity that promotes fungal growth.

Sub-lethal effects of *B. bassiana* on granary weevil

Five sub-lethal doses of *B. bassiana* based on experiment mortality effects having five different repeats including untreated check, were selected. Ten pairs of adult weevils, starved for 24-h, were released in each jar. The jars were covered with perforated cloth and placed in controlled environmental chamber set at 27°C, 70% RH and 12:12 light: dark regime for seven days. On seventh day, all adults were removed, and chickpea grains were monitored for various biological parameters of succeeding generation of granary weevil. The adult emergence data was recorded on day 25 after the release of weevils and continued till the last emergence at 2 days' interval. Each adult emerged was recorded and removed in order to prevent new egg laying. Data was recorded and subjected to statistical analysis.

Effect of *B. bassiana* on the fecundity

For recording data on the pre, post and oviposition periods, 40 newly emerged weevil adults from each treatment were selected along with chickpea grains to facilitate feeding and oviposition of female weevils. Each pair of weevils was caged separately in new jar along with 20 grains for oviposition purpose. The grains were replaced with fresh grains on daily basis assuming to have weevil eggs. The grains were stored in fresh jars after proper labelling the name and date of the treatment. In this way, the weevils were provided with fresh grains until the mortality of the weevils. The grains selected on daily basis having weevil eggs were stored until the emergence of adult weevils. The period between the adult emergence and egg laying from the selected grains was named as the pre-oviposition period. The duration between the first egg laying (adult emergence from the grains) and last egg laying was considered as the oviposition period of weevils. The duration between the last egg laying (emergence of adult weevils from the eggs) and mortality of female weevils was considered as the post-oviposition period or duration.

Statistical analysis

During the experiments, the data recorded was analyzed statistically by using analysis of variance technique and subsequently means were separated using LSD (Least Significant Difference) test at

5%probability level. By using computer software STATISTIX (version 8.1) all the statistical analysis was done.

RESULTS

Mortality Effects

An inverse relation was found between the concentration of *B. bassiana* and percent mortality of granary weevil (Table 1). The mortality of the granary weevil linearly increased by increasing the concentrations and exposure period on treated chickpea grains. At 24 hours after treatment, the minimum mortality (9.20 %) of granary weevil was recorded on the grains treated with minimum concentration of *B. bassiana* being significantly different from all other treated concentrations. The maximum mortality of 29.00 and 21.80 % was recorded at higher concentrations of 1×10^8 and 1×10^7, respectively.

A slight increase in the efficacy of *B. bassiana* against granary weevil was observed at 48 hours after treatment. The maximum (37.40 %) mortality of granary weevil was noticed when chickpea grains were treated with maximum concentration of *B. bassiana* having significant variation from all other tested concentrations. It was followed by 29.20 and 23.60 % mortality recorded on grains treated with *B. bassiana* at 1×10^7 and 1×10^6 concentrations respectively, having significant variation between each other. The minimum mortality of 12.60% was noted on grains treated with minimum (1×10^4) concentration of *B. bassiana.*

The maximum (1×10^8) concentration of *B. bassiana* was found most effective against granary weevil when the data were recorded after 72 hours of treatment causing 42.80 % mortality which was statistically significant from all other tested concentrations. It was followed by 36.80 and 33.00% mortality recorded on chickpea grains treated with 1×10^7 and 1×10^6 concentrations of *B. bassiana.* Among the tested concentrations, the minimum concentration of 1×10^4 was found least effective causing 20.20 % mortality of granary weevil.

At 1 week after treatment of chickpea grains with *B. bassiana* the maximum (69.80 %) mortality of granary weevil was noted on grains treated with maximum concentration of *B. bassiana.* The efficacy of *B. bassiana* decreased by decreasing the concentrations of *B. bassiana* and the lowest concentration was found least effective causing 42.60% mortality of granary weevil having significant variation from rest of the tested concentrations of *B. bassiana.*

At 2 and 3 weeks a significant increase in the efficacy of *B. bassiana* against granary weevil was observed at higher concentrations of 1×10^8 and 1×10^7 compared to other tested concentrations. The higher concentrations (1×10^8 and 1×10^7) caused (78.40 and 89.80) and (72.00 and 75.40%) mortality of granary weevil at 2 and 3 weeks of exposure period having significant variation from all other tested concentrations of *B. bassiana.* The minimum mortality of 44.20 and 48.60% was noted on chickpea grains treated with lowest (1×10^4) concentration of *B. bassiana.*

Regarding pooled mortality of granary weevil, the maximum concentration was found most effective concentration causing maximum (57.27%) mortality having significant variation from all other tested concentrations. It was followed by lower concentrations causing lower mortality levels. Among the tested concentrations, the minimum mortality (31.02%) was observed on grains treated with lowest concentration of *B. bassiana.* Overall, the minimum pooled mortality (3.80 %) was noted on untreated chickpea grains.

Table-1: Percent corrected mortality of granary weevil caused by different doses of *B. bassiana*

Treatments	Percent mortality after						
	24 Hrs	48 Hrs	72 Hrs	1 week	2 weeks	3weeks	Pooled Mortality
1×10^4	9.20 ± 0.53 e	12.60 ± 1.38 e	20.20 ± 0.79 e	42.60 ± 1.19 e	44.20 ± 1.01 e	48.60 ± 1.18 e	31.02 ± 0.67 e

1×10^5	11.6 ± 0.86 d	16.80 ± 1.30 d	28.60 ± 0.79	47.60 ±1.29 d	51.20 ± 1.09 d	57.40 ± 1.28 d	37.05 ± 1.07 f
1×10^6	16.40 ± 1.07 c	23.60 ± 1.23 c	33.00 ± 0.89 c	54.20 ± 1.18 c	61.40 ± 0.86 c	70.60 ± 1.24 c	44.23 ± 0.75 c
1×10^7	21.80 ± 1.53 b	29.20 ± 2.06 b	36.80 ± 0.80 b	63.00 ± 0.79 b	72.00 ± 1.03 b	75.40 ± 0.81 b	50.15 ± 0.90 b
1×10^8	29.00 ± 0.86 a	37.40 ± 0.70 a	42.80 ± 1.22 a	69.80 ± 1.29 a	78.40 ± 0.47 a	89.80 ± 1.23 a	57.27 ± 1.24 a
Control	0.80 ± 0.00 f	0.60 ± 0.00 f	1.40 ± 2.73 f	2.80 ± 2.73 f	4.00 ± 2.73 f	6.00 ± 2.73f	3.80 ± 2.73 f
LSD Value	1.61	1.26	1.27	1.65	1.55	1.87	0.71

Means ± standard deviation in a column sharing common letters are not significant at 5% probability level.

Sub-lethal effects of *B. bassiana* on granary weevil under laboratory conditions

Five sub-lethal doses of *B. bassiana* i.e. 1×10, 1×10^2, 1×10^3, 1×10^4 and 1×10^5 were selected from the concentrations applied to observe their effects on biology of granary weevil.

Days to Adult Emergence

The developmental duration of granary weevil differed significantly when reared on chick pea grains treated with different concentrations of entomopathogenic fungus, *B. bassiana* (Table-2). The maximum (21.23 days) developmental duration was noted when weevils were cultured on grains treated with maximum (1×10^5) concentration of *B. bassiana* which was found non-significantly different from 20.41 and 19.33 days, respectively, developmental duration on grains treated with 1×10^2 and 1×10 concentration of *B. bassiana*. The developmental duration of granary weevil decreased by decreasing the concentration of *B. bassiana*. Overall; minimum developmental duration of 16.33 days was noted on untreated grains which was found non-significantly different from 17.83 days developmental duration documented on grains treated with minimum (1×10^1) concentration of *B. bassiana*.

Total number of adults emerged

Treating chick pea grains with various concentrations of *B. bassiana* had significant effect on the number of adult weevil emergence (Table-2). The minimum number of adult weevils (26.2) emerged from the grains treated with the maximum concentration of *B. bassiana* which was found statistically different from all the other tested treatments. The number of weevil emergence increased linearly by decreasing the concentration of *B. bassiana*. Among the treatments, the maximum number of 33.6 weevils emerged from the chick pea grains treated with the minimum (1×10^1) concentration of *B. bassiana* which was found statistically non-significant from 34.40 weevils emerged from untreated chick pea grains.

Percent Infestation

The entomopathogenic fungus was found most effective against granary weevil and the number of infested chick pea grains linearly decreased by increasing the concentration of entomopathogenic fungus, *B. bassiana*. The minimum number of (5.39%) infested grains were noted in grains treated with maximum concentration of *B. bassiana* which was found statistically different from the rest of the treatments. Among the treatments, the maximum number of (13.40%) infested grains were noted when weevils were cultured on chick pea grains treated with minimum (1×10^1) concentration of *B. bassiana* which was statistically similar to (11.33%) infested grains noted in untreated grains (Table-2).

Percent Weight Loss

The lower values of weight loss were observed in grains treated with various concentrations of *B. bassiana* compared to untreated grains except the lowest concentration (Table-2). The lowest values of weight loss (24.2%) were noted in grains treated with maximum (1×10^5) concentration of *B. bassiana* which was statistically different from all the other tested concentrations of *B. bassiana*. The weight loss values increased by decreasing the concentrations of *B. bassiana*. Among the treatments, the maximum (31.4%) weight loss was noted in grains treated with minimum concentration (1×10^1) of *B. bassiana* which was found statistically similar to 32.3% grain weight loss noted in untreated chick pea grains.

Table-2: Mean (± SE) days to adult emergence, total number of adult emerged and percent infestation treated with sub- lethal doses of *B. bassiana*

Doses	Days to adult emergence	Total number of adults emerged	Percent infestation	Weight loss
1×10^1	17.83 ± 0.59 cd	33.6 ± 0.68 ab	13.40 ± 0.24 a	31.4 ± 0.24 ab
1×10^2	18.67 ± 0.67 bc	32.4 ± 0.51 b	11.33 ± 0.51b	30.3 ± 0.51 b
1×10^3	19.33 ± 0.54 abc	30.56 ± 0.55 c	9.38 ± 0.86 c	27.2 ± 0.66 c
1×10^4	20.21 ± 0.57 ab	28.20 ± 0.37 d	8.40 ± 0.24 d	25.2 ± 0.97 bd
1×10^5	21.33 ± 1.06 a	26.28 ± 0.37 e	5.39 ± 0.84 e	24.2 ± 0.8 d
Control	16.33 ± 0.46 d	34.4 ± 0.51 a	40.84 ± 0.37 a	32.2 ± 0.37 a
LSD Value	1.79	1.57	1.67	1.88

Means ± standard deviation in a column sharing common letters are not significant at 5% probability level.

Sex Ratio

Means of sex ratio are not significantly different from one another on sub-lethal doses of *B. bassiana*. Sex ratio studied on sub-lethal doses of *B. bassiana* showed the similar trend as observed in other parameters i.e. the number of females were more as compare to males (Table-3).

Adult Longevity

The application of entomopathogenic fungus, *B. bassiana* at various concentrations had significant effect on the adult life span of granary weevil (Table-3). The adult life span of both sexes decreased with an increase in the concentration of *B. bassiana*. The minimum adult life (82 and 97.2 days) of both sexes was noted when weevil adults were reared on maximum (1×10^5) concentration of *B. bassiana* which was found statistically different from all the other tested concentrations of *B. bassiana*. In contrast to this, the maximum adult life span (120.8 and 133. days) was noted when weevils were cultured on untreated chickpea grains which was statistically similar to 119.2 and 132.2 days adult life span on grains treated with minimum (1×10^1) concentration of *B. bassiana*.

Table-3: Mean (± SE) adult longevity and adult sex ratio of granary weevil against sub-lethal doses of *B. bassiana*

Doses	Adult longevity Male	Female	Adult Sex ratio (M/50F)
1×10^1	119.2 ± 1.16 a	132.2 ± 0.86 a	43.86 ± 1.67 NS
1×10^2	110 ± 1.54 b	127 ± 1.05 b	43.48 ± 1.78
1×10^3	101 ± 0.55 c	118 ± 0.95 c	44.60 ± 1.87
1×10^4	93 ± 0.86 d	106.4 ± 0.51d	43.83 ± 0.84
1×10^5	24.2± 0.8 d	82 ± 0.71 e	43.19 ± 0.37
Control	32.2 ± 0.37 a	120.8 ± 1.11 a	44.61± 0.39
LSD Value	4.11	5.33	3.08

Means ± standard deviation in a column sharing common letters are not significant at 5% probability level

Pre-oviposition period

The pre-oviposition period was significantly affected by the application of entomopathogenic fungus, *B. bassiana* on chickpea grains (Table-4). The pre-oviposition period declined by increasing the application rates of the *B. bassiana*. The maximum pre-oviposition period of 28.33 days was noted when the weevil adults were reared on chickpea grains having maximum concentration of the *B. bassiana* having significance difference from all the other tested concentrations of *B. bassiana* except the untreated chick pea grains. The minim pre-oviposition period of 16.33 days was noted when the weevil adults were cultured on the grains having maximum concentration of *B. bassiana* having significant difference from all the other tested concentrations of *B. bassiana*.

Oviposition period

The selected concentrations of *B. bassiana* significantly altered the oviposition period of granary weevil reared on chickpea (Table-4). The maximum oviposition period of 79.66 days was noted when the adult weevils were cultured on untreated grains having non-significant difference from 79.33 days noted when on grains treated with minimum (1×10^1) concentration of *B. bassiana*. The minimum oviposition period of 59.33 days was documented when the adult weevils were cultured on grains treated with the maximum (1×10^5) concentration of *B. bassiana* having significant difference from all the other concentrations of *B. bassiana*.

Post-oviposition period

The post-oviposition period decreased by increasing the concentrations of the entomopathogenic fungus *B. bassiana* (Table-4). The maximum post-oviposition period of 25.33 days was noted when the weevil adults were cultured on chick pea grains treated with the minimum (1×10^1) concentration of *B. bassiana* having non-significant difference from 24.33 days noted on untreated chickpea grains. The minimum post-oviposition period of 14.33 days was observed when the weevil adults were cultured on chickpea grains treated with the maximum (1×10^5) concentration of *B. bassiana* having significant difference from all the other tested concentrations of *B. bassiana*.

Table-4 : Effect of different concentrations of *Beauveria bassiana* on oviposition periods of granary weevil

Treatments	Pre-oviposition period	Oviposition period	Post-oviposition period
1×10^4	28.33 ± 1.33 a	79.33 ± 1.90 a	25.33 ±2.11 a
1×10^5	25.66 ± 1.65 b	77.33 ± 2.03 b	24.33 ± 1.06 a
1×10^6	23.00 ± 0.25 c	66.33 ± 0.43 c	20.66 ± 0.65 b
1×10^7	19.33 ± 0.93 d	62.33 ± 1.59 d	17.33 ± 0.54 c
1×10^8	16.33 ± 0.68 e	59.33 ± 0.48 e	14.33 ± 1.94 d
Control	27.33 ± 1.45 a	79.66 ± 1.84 a	24.33 ± 0.89 a
LSD Value	1.18	1.02	1.02

Means ± standard deviation in a column sharing common letters are not significant at 5% probability level

DISCUSSION

The present study aimed to evaluate the lethal and sub-lethal effects of different doses of *B. bassiana* against *Sitophilus granarius*, a major pest of stored grains worldwide. The results demonstrated significant variations in behavioral changes, mortality rates and sub-lethal impacts in weevils when exposed to different concentrations of *B. bassiana*. The findings highlight the potential of *Beauveria bassiana* as a biological control agent in integrated pest management (IPM) strategies, offering a suitable alternative to chemical insecticides.

The results from our study indicated that the mortality of the granary weevil increased by increasing the duration and concentration of entomopathogenic fungus on treated chickpea grains. Similar results were also reported by Mantzoukas et al. (2019). The two isolates of *B. bassiana* (H20 and 10T) caused

96% mortality of granary weevil at seven days after the treatment of chickpea grains. The greater mortality reported by them is due to the variation in strains of entomopathogenic fungus and granary weevil. Cherry et al. (2005) claimed that different isolates of *B. bassiana* provided significant control of *C. maculatus* at 12 days after treatment and was found most virulent against the tested insect. The different strains of entomopathogenic fungus have been documented to cause 80-100% mortality of different stored grain insect pests at 10-20 days after exposure period (Padin et al. 1997; Rice and Cogburn, 1999). Khashaveh *et al*., (2011) showed that *B. bassiana* at 1,000 mg conidia/kg can reduce the progeny production *of S. granarius* and increasing the dose can raise the rate of reduction.

Beyond direct mortality, sub-lethal effects were also observed in granary weevils exposed to lower doses of *B. bassiana* (10^4 and 10^6 conidia/mL). Sub-lethal effects play a crucial role in pest management, as they can impair pest fitness even if the individuals survive (Desneux et al., 2007). In this study, sub-lethal doses resulted in decreased reproductive output, reduced feeding activity and impaired mobility of *Sitophilus granarius*. These behavioral and physiological disruptions can reduce the pest population growth rate and overall impact on stored grain quality.

The effectiveness of different doses of *Beauveria bassiana* on days to adult emergence can vary based on various factors such as exposure time, larval stage and potential synergistic interaction with other agents. Higher concentrations of Bb have been shown to be more effective in controlling adult emergence. Several studies have investigated the effect of different doses of Bb on biology of granary weevil. The results reported by White *et al.,* (2021) are also in accordance with the present findings and support our observation that high concentration of *B. bassiana* conidia when applied to first and second instar larvae of *Musca domestica* resulting in significant reduction in adult emergence rates as well as on pupal period

In a study by Pourian *et al.,* (2021) it was observed that efficacy of *B. bassiana* when applied alone or in combination with DE against *Oryzaephilus surinamensis* and *C. maculatus. B. bassiana* when applied in higher doses either alone or in combination with Diatomaceous earth, mortality rates reached at significant levels. This clearly indicates that higher doses of Bb might be more effective in reduction of adult emergence.

To check efficacy of *Beauveria bassiana* against *S. granarius* aiming to understand its effect on emergence of F_1 adults and when applied in higher lethal doses have been shown to result in reduced emergence of adults and increased mortality contributing to more effective control strategy against many stored product pests. It is worth noting that optimal dose of *B. bassiana* may vary based on target insect, environmental condition and specific formulation etc.

Our results are supported by research of Saeed *et al.,* (2022) who checked effect of different doses of *B. bassiana* Strain (MS-8) formulated in Kaolin as an active carrier on number of emerged adults and on larval mortality of *Ephestia* cautella (a closely related species to *S. granarius*) on rice grains. The results clearly indicate that higher doses of *B. bassiana* in kaolin such as 2g/kg led to significant number of adults emerged as well as larval mortality.

Reduced progeny production from treated grains is considered an indication of biological effects of treatments. The results of our studies confirmed that the entomopathogenic fungus *B. bassiana* used at various concentrations possessed biological effects against granary weevil. Similar findings were also reported by Keszthelyi et al. (2021). They investigated the insecticidal effectiveness of different isolates of *B. bassiana* and *M. anisopliae* against granary weevil. They confirmed reduction in the number of new progenies of granary weevil when grains were treated with *B. bassiana* at 2 mg/kg compared to untreated wheat grains. Athanassiou and Steenberg (2007) proved that *B. bassiana* used at 0.72×10^{12} spores per kg wheat grains caused 64% reduction in the progeny of granary weevil at 65 days after treatment of grains compared to untreated wheat grains. Similar efficacy trends have also been reported against other stored grain insect pests. Throne and Lord (2004) reported that by using unformulated conidia of *B. bassiana* (Emerald BioAgriculture, Butte, MT) at 150 mg.kg on oats resulted in 70 and 98% progeny reduction of *O. surinamensis*.

The *S. granarius* is notorious pest of stored grain, causing substantial economic losses due to its infestation. The potential of *Beauveria bassiana* to control *Sitophilus granarius* infestation has been investigated, particularly focus on effect of different doses of Bb on percent infestation of many stored product pests. Sub lethal effects of different doses of *B.bassiana* on percent infestation of chickpea grains caused by granary weevil, aim to contribute key role in the field of pest management.

Our results are supported by research of Saleem *et al.,* (2020) follow the pattern noticed in development and growth, the more detrimental effects were observed in more virulent treatment. They reported diet consumption by 7^{th} instar larvae was significantly influenced by the treatments applied. While in control, maximum diet was consumed as compared to other treatments. Food consumption by pest is decreased with increase in toxicity of applied treatment and vice versa.

To understand sub lethal effects of varied doses of *B. bassiana* on percent weight loss caused by granary weevil play a key role for developing effective pest management strategy. In a study by Kordali *et al.,* (2021) it was observed that effect of *B. bassiana* on stored grain pests showed its effectiveness not only in preserving grain quality but also in pest control.

The results reported by Batta (2018) are also in accordance with the present findings and support our observation that grain weight loss varied significantly when granary weevils were reared on grains treated with various concentrations of *Beauveria bassiana.* The findings of Xia *et al.,* (2023) supplement our results. They conducted laboratory experiment to check the sub-lethal effects of BEdy1 (strain of *Beauveria bassiana)* on *S. furcifera* by using “Two Sex Life Table” analytical method. The reproductive rate (R_0), fecundity of adult females, finite rate of increase (λ), intrinsic rate of increase (r) for LC_{25} treatment significantlt decreased as compared to other treatments. Singh *at al*., 2019 with continuous food supply, male *S. granarius* survived for 90-120 days, whereas, female adult survived for 120-150 days.

The pre-oviposition period is an important phase in reproductive behavior of many stored product pests. Our results depict dose-dependent effects of entomopathogenic fungi *B. bassiana* on pre-oviposition period of granary weevil. Higher doses showed greater suppressive effects while lower doses exhibit stimulatory effects.

Our results are supported by the research of Saeed *et al.,* (2017) who studied that sublethal exposure to *Beauveria bassiana* cause disruption in reproductive parameters in many stored grain pests. These alterations may be due to physiological changes induced by entomopathogenic fungi *B. bassiana* that may influence hormonal regulation of granary weevil, ultimately cause effect on reproductive behaviour (Khoobdel *et al.*, 2019). Reduced progeny production from treated grains is considered an indication of biological effects of treatments. The results of our studies confirmed that the entomopathogenic fungus *B. bassiana* used at various concentrations possessed biological effects against granary weevil. Similar findings were also reported by Keszthelyi et al. (2021). They investigated the insecticidal effectiveness of different isolates of *B. bassiana* and *M. anisopliae* against granary weevil. They confirmed reduction in the number of new progenies of granary weevil when grains were treated with *B. bassiana* at 2 mg/kg compared to untreated wheat grains. Athanassiou and Steenberg (2007) proved that *B. bassiana* used at 0.72×10^{12} spores per kg wheat grains caused 64% reduction in the progeny of granary weevil at 65 days after treatment of grains compared to untreated wheat grains. Similar efficacy trends have also been reported against other stored grain insect pests. Throne and Lord (2004) reported that by using unformulated conidia of *B. bassiana* (Emerald BioAgriculture, Butte, MT) at 150 mg.kg on oats resulted in 70 and 98% progeny reduction of *O. surinamen.*

CONCLUSIONS

The entomopathogenic fungi *B. bassiana* possessed insecticidal properties against granary weevil. The insecticidal efficacy of the fungus increased by increasing the concentrations of the fungus. The fungus possessed lethal and sub-lethal effects against the tested insects. The minimum weevil emergence and weight losses were observed in chickpea grains treated with the maximum concentration of entomopathogenic fungi. In contrast to this, the maximum weevil emergence and weight loss was noted in untreated grains.

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