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Original Article

Pathogenicity aptness of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* against Saw-toothed Grain Beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae)

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Abstract

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) is one of the most destructive pests known to target foods that are stored. Due to cosmopolitan nature, it is practically certain to be present in any stored food. Excessive application of chemicals can interfere with natural pest control systems by developing resistance and requiring the use of stronger or higher concentrations of the chemicals. The present research was carried out to find a safe and environmentally sustainable substitute for synthetic insecticides to manage saw-toothed grain beetle. Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* for suppressing the saw-toothed grain beetle *Oryzaephilus surinamensis* was investigated by using varied fungal concentrations viz. $1x10^4$, $1×10^6$, $1×10^8$, $1×10^{10}$ and $1x10^{12}$ spores/ml in stored split chickpea. Findings of this study have shown that fewer F_1 adults appeared in split chickpea when the highest concentration of *B. bassiana* was applied as compared to *M. anisopliae.* Less inhibition percentage of saw-toothed grain beetle adults being found when *B. bassiana* was applied. Weight loss was more in split chickpea when treated with lowest concentration of *B. bassiana*. Adult saw-toothed grain beetles were found to be more susceptible towards *M. anisopliae* as compared to *B. bassiana*. The concentration of $1×10^{12}$ spores per ml of *B. bassiana* and *M. anisopliae* are suggested for management of *O. surinamensis* in stored split chickpea.

Keywords: *Oryzaephilus surinamensis, Beauveria bassiana*, *Metarhizium anisopliae,* Entomopathogenic, Fungi, Split chickpea

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Introduction

Saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) is important stored grain insect pest found worldwide (Hashem et al., 2012). Its diverse host range includes wheat, barley, sorghum, rice, corn, cornmeal, cornstarch, dried fruits, herbs, pasta, dried peas, spices, sugar and tea (Trematerra et al., 2016). Annually, billions of kilograms of pesticides are used to reduce yield losses (Pimentel, 2009). In response to the emergence of pesticide resistance and concerns over consumer health, environmental safety, alternatives to synthetic insecticides have been needed of the era (Kavallieratos et al., 2014; Ishaq et al., 2023). Microbial control is one of the emerging strategies (Gurr and You, 2016). Pathogens like bacteria, fungi and nematodes are used against various stored insect pests (Flinn and Scholler, 2012). The utilization of natural enemies and pathogens are more compatible than chemicals (Hassuba et al., 2024; Gurr and Kvedaras, 2010). Insect pathogens can be maintained for a more time period than beneficial insects and they can infect a variety of insect pest species (Lamp et al., 2007). Entomopathogenic fungi (EPF) *Beauveria bassiana* and *Metarhizium anisopliae* are potential substitutes to chemical pesticides (Kavallieratos et al., 2006) because they are eco-friendly, having even less mammalian toxicity and infect insects through contact. When entomopathogenic fungi penetrates through insect cuticle, the insect dies, the fungi are recycled in the corpses, adding more inoculum to the system (Thomas et al., 1997). The effectiveness of entomopathogenic fungi against insect pests has been investigated in the past, with encouraging outcomes (Mantzoukas et al., 2023) up to six months (Kim et al., 2019). According to Agrafioti and Athanassiou (2018) the saw-toothed grain beetle has well documented resistance to synthetic insecticides. Researchers are focusing on substitutes that are eco-friendly and have less mammalian toxicity (Batta and Kavallieratos, 2018). According to several studies, *B. bassaian* and *M. anisopliae* could be a potential alternative to insecticides against various numbers of insect pests of stored grains (Flinn and Scholler, 2012; Rumbos and Athanassiou, 2017; Batta and Kavallieratos, 2018; Wakil et al., 2021). The application of different strains of *M. anisopliae* and *B. bassiana*, on grains has increased the mortality of adult stages of the lesser grain borer, *Rhyzopertha dominica,* the granary weevil, *Sitophilus granarius*, the red flour beetle, *Tribolium castaneum,* and khapra beetle, *Trogoderma granarium*, etc. (Riasat et al., 2011; Kavallieratos et al., 2014; Wakil and Schmitt, 2015; Wakefield et al., 2010; Wakil et al., 2021). Keeping in view the aforesaid points, this research work was designed to investigate the effects of various fungal concentrations *B. bassiana* and *M. anisopliae*, against saw-toothed grain beetle for certain biological parameters.

Material and Methods

Oryzaephilus surinamensis culture was maintained on split chickpea in plastic jars covered with muslin cloth and tightened with rubber band. These jars were placed in incubator, which was set at 27±2°C and 70±5% RH in the Stored Product Laboratory, Entomology Department: PMAS-Arid Agriculture University Rawalpindi Pakistan. Male and female adults of saw-toothed grain beetle were identified by presence of spine like structure on meta-leg of male while absence in female (Mason, 2003). All management approaches were executed on desi chickpea variety namely, Bittal-2016 which was obtained from Pulse Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan. The reason of selecting this variety is that it has high yield potential of more than 4000 kg/ha (PAR, 2016). The entomopathogenic fungi *Beauveria bassiana* isolate (DEBI 005) and *Metarhizium anisopliae* isolate (DEMI 001) were collected from the Korean Agricultural Culture Collection (KACC), NAC, RDA, Suwon, Korea, 441-707. For the purpose of counting the conidia/spores per unit volume, cultures were first maintained in Potato Dextrose Agar (PDA) broth at 25±1 °C and two hundred rpm for 2 weeks. The conidia/spores were grown on PDA medium. Conidia/spores were counted with a hemocytometer after a 24-hour period (Pham et al., 2009). In each jar, 50g of split chickpea grains were placed. Ten pairs of saw-toothed grain beetles were released in jars for 5 days to allow mating and oviposition, then adults were removed and jars were shifted to incubator after applications of fungal concentrations of both fungi at $1x10^4$, $1x10^6$, $1x10^8$, $1x10^{10}$ and $1x10^{12}$ spores/ml. There were three replicates for each treatment and control. The effectiveness of entomopathogenic fungi against

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saw-toothed grain beetle were studied according to the following parameters after six weeks.

F_1 adult emergence

Newly emerging adults (F_1) were recorded in each container to assess the adult emergence of *O. surinamensis* caused by the use of various concentrations of both *Beauveria bassiana* and *Metarhizium anisopliae.*

Percent inhibition rate

The formula below was used to calculate the inhibition rate or percent suppression in the emergence of *O. surinamensis* (F_1).

$$\% IR = (C n - T n) / Cn \times 100$$

C_n = Emergence of adult (fresh) in jar (control)
T_n= Emergence of adult (fresh) in treated jar

Loss of weight (%)

Following formula was used to calculate the % weight loss of grains at the end of the experiment.

$$\text{Loss in weight (percent)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Statistical analysis

Experiments were designed in complete randomized design. The Duncan's Multiple Range Test (DMRT) was applied to compare mean values and standard error. Regression models were used to analyze the relationship of treatments regarding biological parameters of insects. Following equation was used;

$$y = a + bx$$

Whereas
y = Insects parameter
x = Fungal concentrations

linear regressions were used to check separate effects of every treatment regarding parameters of insects.

Results

F_1 adults emerged

In comparison to all other treatments of *B. bassiana*, the most number of F_1 progeny per jar (43.66) were found in the control. Population 19.33 and 18.33 showed the least difference when concentrations of 1×10^{10} and 1×10^{12} were used respectively. Additionally, there was a population difference between the two concentrations, with the highest concentration having 18.33 adults and the lowest concentration having 25.66 adults (Table 1).

Table 1. No. of F_1 adults emergence (Mean ± SE) / jar by *O. surinamensis* in split chickpea with various conc. of *M. anisopliae* and *B. bassiana.*

Sr. No	Conc (spores/ml)	*Beauveria bassiana*	*Metarhizium anisopliae*
1	$1x10^4$	25.66±0.33b	24.66±2.11bc
2	$1x10^6$	23.66±0.33cd	22.31±0.66de
3	$1x10^8$	20.34±0.00f	22.00±0.66e
4	$1x10^{10}$	19.33±0.66fg	18.33±0.33gh
5	$1x10^{12}$	18.33±0.66gh	17.66±0.66h
6	Control	43.66±0.66a	43.33±0.88a

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The least number of F_1 offspring were produced by the highest concentration of *M. anisopliae*, 1x10^{12} which was noticeably varied from all the other treatments aside from control. When concentrations 1x10^6 and 1x10^8 were used, no discernible difference between populations were observed as 22.31 and 22 adults per jar. The outcomes of distinct *B. bassiana* dilutions were validated using a linear regression model. The modelled equation (Y= -4.1714x + 35.095) showed that fungus dilution had negative effects on newly emerged adults. The intercept (a) and slope (b) remained on 35.095 & -4.17, respectively. As a result of the increased dilution of *B. bassiana*, the number of F_1 emerged dropped @-4.17. The (R^2) coefficient determination was 0.69, indicating that the independent variable influenced the dependent variable by 69 percent. The findings of diverse *M. anisopliae* concentrations were evaluated using a linear regression model, as shown in Figure. 1 The modelled equation (Y=-4.2286x + 34.905) showed that fungal concentration had a negative effect on the number of newly emerging adults. The values of the slope (b) and intercept (a) stayed -4.22 and 34.90, respectively. As a result, the number of F_1 emerged reduced @-4.22 as the concentration of *M. anisopliae* rose. The correlation value (R^2) was 0.71, suggesting that the various concentrations of entomopathogenic fungi had 71% effect on the F_1 adult emergence.

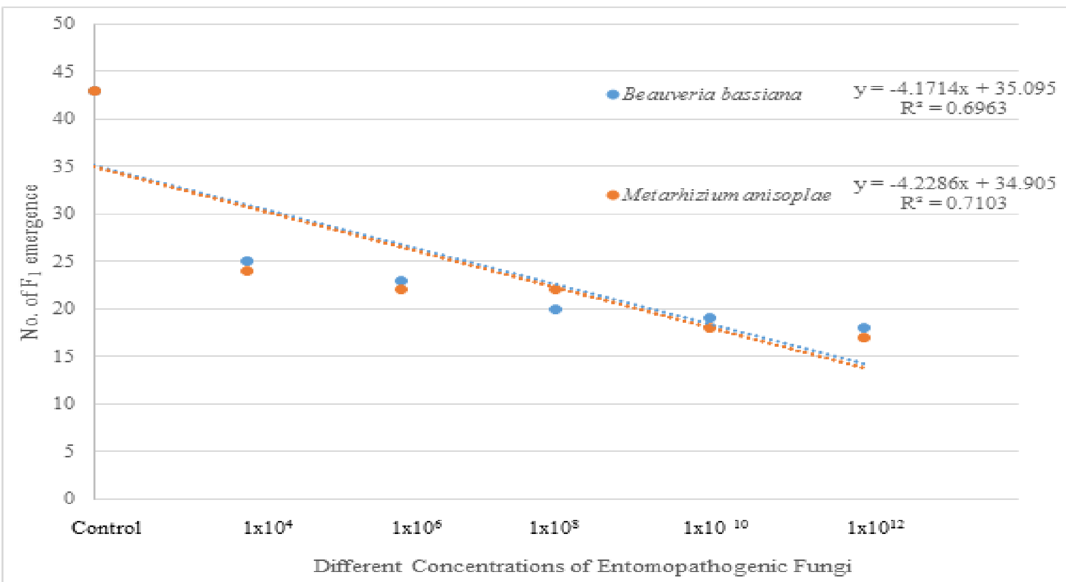

Figure 1. The F_1 adult emergence of the saw-toothed grain beetle towards varying concentrations of entomopathogenic *B. bassiana and M. anisopliae*

Inhibition rate

In the highest concentration of *B. bassiana* (1×10^{12}), the maximum percent inhibition rate was found in *O. surinamensis*, that was substantially different from all tested treatments. Within *B. bassiana* concentrations of 1×10^8 and 1×10^{10}, the percent inhibition was 39.00 and 44.66. Minimum inhibition rate was recorded in control. When 1×10^4 and 1×10^8 fungal concentrations of *M. anisopliae* was applied, the IR (%) was 40.33 and 41.33 was recorded respectively, which were substantially different from other applied treatments. The inhibition rate of *O. surinamensis* found to be minimum in the untreated group. Furthermore, the greatest percent IR was recorded at the maximum dose of *M. anisopliae* which was differed considerably from other tested treatments (Figure No. 2). The effects of different *B. bassiana* dilutions on percent inhibition rate were investigated using a linear regression model. As stated by the predicted equation (Y=8.2286x + 13.429), positive effects of fungal dilutions on inhibition rate of saw-toothed grain beetles were detected. The intercept (a) remained at 13.42, while the slope (b) remained at 8.22. As dilutions of fungi rose, rate of inhibition increased @ 8.22 percent. The determination coefficient was 0.75, indicating that treatments had a 75% influence on the rate of inhibition of *O. surinamensis.* (Figure 3).

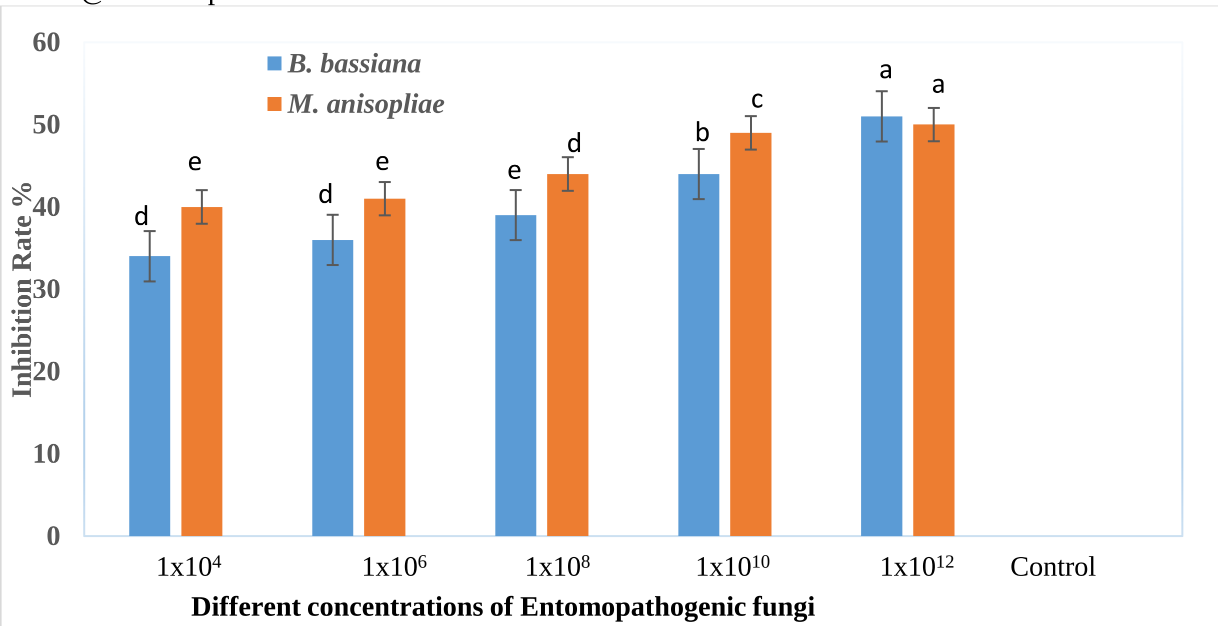

Figure 2. Inhibition rate (%) of saw-toothed grain beetle treated with varied concentrations of entomopathogenic *B. bassiana* and *M. anisopliae*

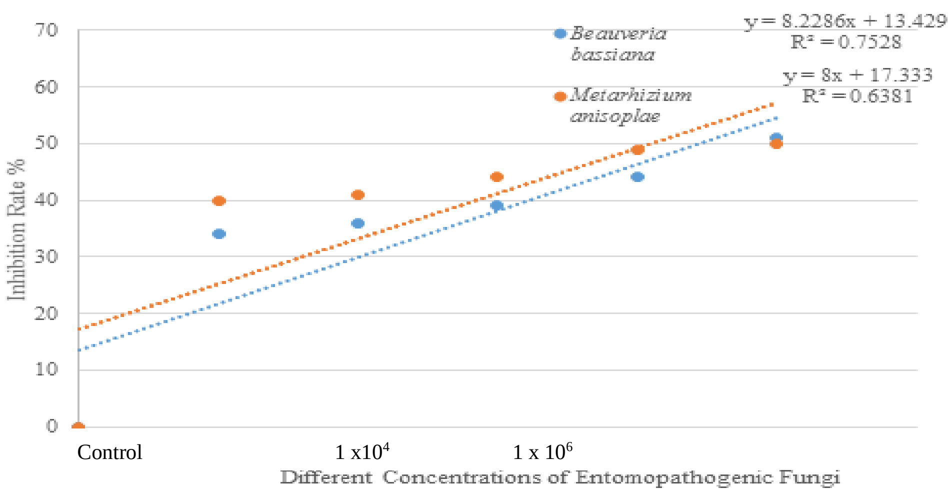

Figure 3. The model percent inhibition rate (Mean ± SE) of the saw-toothed grain beetles in split chickpea treated with diverse doses of entomopathogenic fungi *B. bassiana* and *M. anisopliae.*

The impact of various *M. anisopliae* doses on the inhibition rate was also investigated using a linear regression model (Figure 3). The modelled equation (Y=8x + 17.333) revealed a positive effect of fungal concentration on F_1 inhibition rate. The intercept (a) value stayed at 17.33, while the slope (b) value remained at 8. The inhibition rate increased by 8% as the fungal concentration increased. The coefficient of determination (R^2) was 0.63, indicating that concentrations had a 63% impact on the inhibition rate.

Weight loss (%) of split chickpea

Maximum reduction in weight was recorded in control. Twenty percent weight loss was reduced at the lowermost dose (1×10^4) of *Beauveria bassiana*. No significant percent weight reduction in the concentrations of *B. bassiana* 1×10^6 and 1×10^8 was observed. The maximum concentration of *B. bassiana*, i.e. (1×10^{12}), resulted in the lowest percent weight reduction (Figure 4). There was no significant difference in percent weight reduction when *M. anisopliae* concentrations of 1×10^6 and 1×10^8 were used. *O. surniamensis* showed the lowest percent weight loss (12%) with the highest dose of *M. anisopliae* (1×10^{12}). The modelled equation (Y= -6.2571x + 39.81) revealed that fungal dilutions had an unfavorable influence on feeding behavior of saw-toothed grain beetle. The results showed that weight loss caused by the saw-toothed grain beetle declined at a rate of -6.25 as *B. bassiana* dilution was applied. The determination co-efficient (R2) was 0.58, meaning that the percent weight loss was affected by fungal dilutions by 58 percent (Figure 5). Similarly, the linear regression model was employed to test the percent weight loss induced by saw-toothed grain beetles in response to varying fungal concentrations. The modelled equation (Y=-6.6857x + 39.714) showed that fungal concentrations had an unfavourable influence on feeding attributes of *O. surinamensis*. The values for the slope (b) and intercept (a) stayed at -6.68 and 39.71, respectively. Therefore, as *M. anisopliae* concentration increased, the rate of weight loss dropped was -6.68. The determination coefficient was 0.61 indicated that the percent weight loss was influenced by fungal concentrations by 61% (Figure 5).

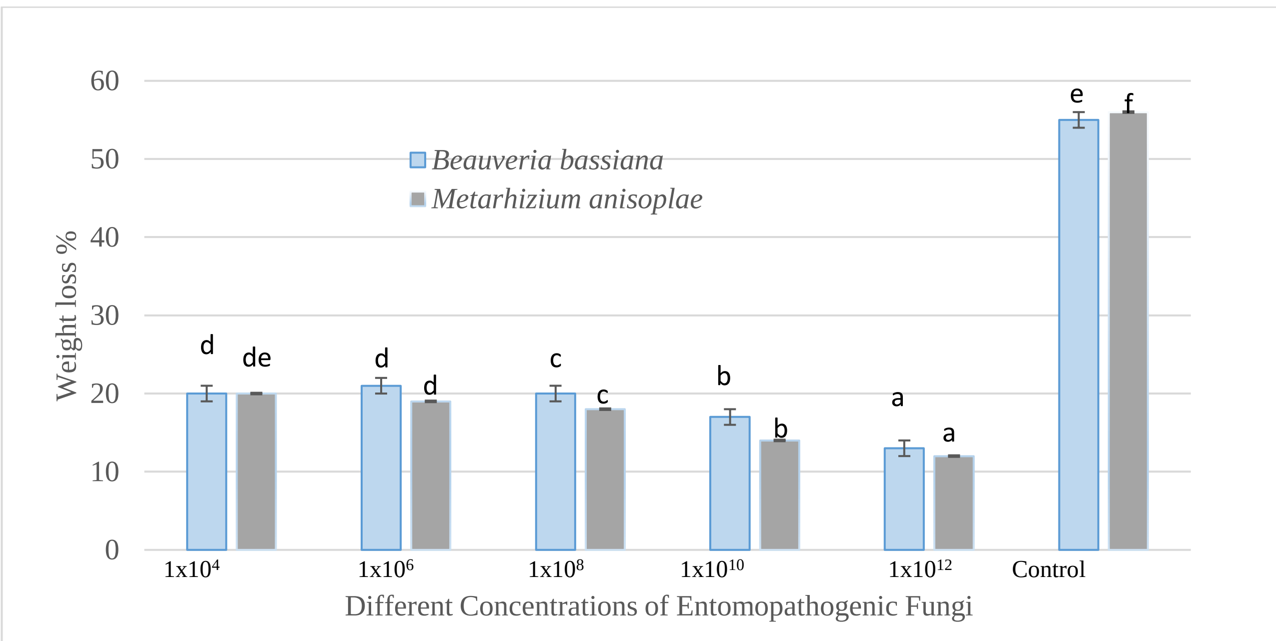

Figure 4. Weight loss (%) (Mean ± SE) in stored split chickpea with varied doses of *B. bassiana* and *M. anisopliae* against Saw-toothed grain beetle

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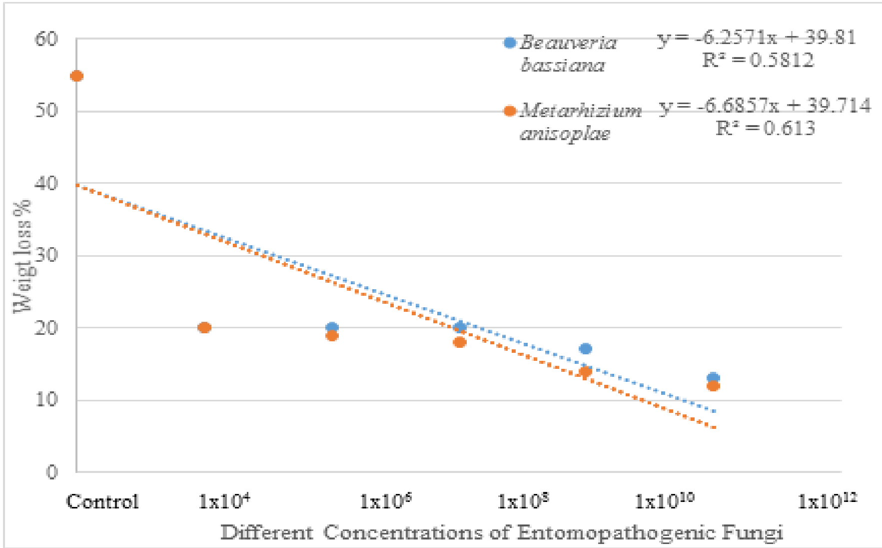

Figure 5. Trend of weight loss percent (Mean ± SE) by the saw-toothed grain beetle in split chickpeas treated with varied doses of entomopathogenic fungi *B. bassiana* and *M. anisopliae*

Discussion

Overall conclusions are in line with those of Abdel-Raheem et al. in 2015, who assessed that *M. anisopliae* was more effective against saw-toothed grain beetle than *B. bassiana*. As depicted from study that there were less number of F_1 adult emerged in case of *M. anisopliae* as compared to *B. bassiana.* Similarly, there was less inhibition rate was observed in case of *B. bassiana*. Inhibition rate is inversely proportional to F_1. Both the tested fungi were effective as both affected the emergence of progeny. Shaheen et al, 2016 employed fungus *Beauveria bassiana* as a bio-pesticide to control *Callosobruchus chinensis* on stored chickpea as grain protectant. Jassim et al. (1988) applied *Beauveria bassiana* at the rate of 1×10^3 spores/ml to stored date fruits and reduce the population of almond moth, *Cadra cautella.* Present study showed entomopathogenic fungi effected biological parameters of saw-toothed grain beetle which in line with study of Latifian et al., 2018. Ozdemir et al., 2020 found similar results when tested the effectiveness of two of these fungi against *C. maculatus*. The tested insect was exposed to various dilutions of each treatment, which were compared to the control and found that *M. anisopliae* is more effective. Radha, 2012 observed efficacy of two entomopathogenic fungi, *M. anisopliae* and *B. bassiana*, against *C. maculatus* and found effective. Mahdneshin et al., 2011 assessed the pathogenicity of *B. bassiana* and *M. anisopliae* versus *C. maculates* and observed that biological parameters were more affected by *M. anisopliae*. Iqbal et al., 2018 tested *Beauveria bassiana* and *Metarhizium anisopliae* as bio-control agents to manage stored pest in chickpea. Our study is in line with Iqbal et al., 2018, as there were less numbers of F_1 adult emergence in chickpea when *M. anisopliae* was applied as compares with *B. bassiana* for management of the pulse beetle *Callosobruchus chinensis.* Maximum Numbers of progeny F_1 recorded in control relative of other treatments. There exists negative impact of fungal concentrations of both the fungi on F_1 adult progeny and weight loss where as positive affects had been observed for inhibition rate. Overall conclusions are consistent with those of Iqbal et al., in 2018. The current study revealed that applications of *B. bassiana* and *M. anisopliae* could significantly reduce population of saw toothed grain beetle and weight loss of stored split chickpea.

Conclusions

The findings of this study will provide a baseline for bio-pesticide manufacturers and researchers to develop formulations of entomopathogens. Both the fungi *B. bassiana* and *M. anisopliae* were effective for the management of saw-toothed grain beetle. As a result, these data strongly support their usage as an

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effective biological control agent in the management of saw-toothed grain beetle in storage facilities. Future research will seek to determine the efficacy of the selected isolates under field situations.

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Contribution of Authors

Raza M: Collected the data, performed experiments and wrote manuscript.
Shaheen FA: Supervised the experiments, edited and approved the final manuscript.
Gulzar A & Maqsood A: Conceived idea, designed experiments and reviewed literature.
Ahmad SA: Conceived idea and designed the experiment, edited and approved the final manuscript.

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