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Research Article

Management of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) in Stored Chickpea Grains by using Entomopathogenic Fungi

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Abstract | *Callosobruchus chinensis* is one of the most destructive insect pests of chickpea in storages and renders the grains unfit for human consumption. In current studies, entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* were used as the bio-control agents against this economic pest and proved good alternatives to chemicals. Less number of eggs (2 per grain), more number of holes (8.3 per grain), less number of F_1 adults (20.3 per jar) and more number of days (7) to 100% mortality of *C. chinensis* adults were recorded when chickpea grains were treated with the highest concentration of *B. bassiana* as compared to that of *M. anisopliae*. These non-chemical control strategies will have significant contribution towards development of commercial formulations of bio-pesticides to be used against this grain beetle and other stored product insect pests.

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Introduction

Chickpea ranks 2nd in area under cultivation and 3rd in production among pulses in the world (CGIAR, 2017). In Pakistan, annual production of 0.5 million tons of dry seed is obtained from an area of around one million hectares (FAO, 2016). Under storages, severe post-harvest losses are noted due to pulse beetle, *Callosobruchus chinensis* attack (Zia et al., 2011) causing about 10% damage and rendering grains unfit for human consumption (Aslam, 2004). Grain protectants and fumigants are used since long for controlling stored grain insect pests but their injudicious and long-term use has resulted in development of resistance in insects. Their residual effects are further posing risks to human as well as environmental health (Rajendran and Sriranjini, 2008). Botanicals, although relatively are safe grain protectants (Bakkali et al., 2008) but they also have faced development of resistance issues inviting the other safer approaches like entomopathogens to manage insect pests (Jung and Kim, 2006).

Entomopathogenic fungi are considered safer than pesticides due to minimum risk involved and can be used directly on food items due to their entomopathogenic nature. They have also registration with Environmental Protection Agency, USA. The marketable packing of *B. bassiana* is now accessible like Botanigard, Boverosil and Mycotrol. These are

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registered against many insect pests of lab as well as field (Hidalgo, 1998). *M. anisopliae* previously categorized in *Hyphomycetes*, is previously identified as *E. anisopliae* and is existed in nature in all types of soils acting as parasite against insects (Correa et al., 2016).

The objective of the research encompassed investigating effectiveness of *B. bassiana* and *M. anisopliae* to manage *C. chinensis* in stored chickpea grains.

Materials and Methods

Infested samples of stored chickpea grains by pulse beetle were collected from different research stations including National Agriculture Research Council (NARC) Islamabad, Pakistan. *C. chinensis* culture was maintained in incubator at temperature of 30±2°C and 70±5 % relative humidity in the Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

For bioassays, chickpea cultivar NOOR-2009 was obtained from NARC, Islamabad. Agtoxin was applied for fumigation to kill already existing insect pests (Shaheen et al., 2006). Entomopathogenic fungi *Beauveria bassiana* isolate (DEBI 005) and *Metarhizium anisopliae* isolate (DEMI 001) were obtained from Korean Agricultural Culture Collection (KACC), NAC, RDA, Suwon, 441-707, Korea. Initially theses cultures were developed in Potato Dextrose Agar (PDA) at 25°C at 200 rpm for two weeks and then it was multiplied in Potato Dextrose Broth medium to count the number of conidia/spores per unit volume. The conidia/spore was grown on PDA medium. Later on, then spores/conidia were counted by haemocytometer after 24 hours interval (Tuan et al., 2009). Different concentrations of spores/conidia were developed and a quantity of 0.02 percent of Tween 80 in fresh water was also added. Different concentrations of both fungi were made to conduct experiments. For propagation of fungus, petri plates were shifted to incubator at appropriate temperature and humidity.

In each jar, 50g of chickpea grains were put in plastic jars. The jar was enclosed with muslin fabric and then shifted to incubator. Ten pairs of *Callosobruchus chinensis* were then shifted into each jar. Pairing of pulse beetle (P.B.) was done following Halstead (1963). Different concentrations viz. 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spores/ml of both fungi were applied in this experiment.

The pathogenicity of EPF against *C. chinensis* was studied according to the following parameters:

Number of eggs laid

Average number of eggs laid per grain was counted to calculate effect of applications on fecundity and oviposition of P.B. For this, ten (10) grains were chosen at random from every jar and eggs were counted. Average of these laid eggs was then taken for each jar.

Number of holes made

Newly emerged F_1 adults of *C. chinensis*: Newly emerged adults (F_1) were counted in each jar.

Inhibition rate (% IR): The percent decrease in emergence of *C. chinensis* (F_1) or inhibition rate was measured by using this formula:

$$\%\, IR = (C_n - T_n) / C_n \times 100$$

Where;
C_n = Fresh emerged adults in un-treated jar (control);
T_n= Fresh emerged adults in treating jar.

Number of days to 100 percent mortality of F_1 of *C. chinensis*: The number of days to 100 percent mortality of *C. chinensis* (F_1) was measured to find out the effects of applications on new appeared progeny.

Loss of weight (%)

The percent weight loss was measured by this formula at the end of experiments.

$$\text{Weight loss } (\%) = \frac{(\text{initial weight} - \text{weight of healthy and damaged grains})}{\text{initial weight}} \times 100$$

Percent damage

Percent damage of the cultivar was calculated. Healthy grains (without holes) were separated from the sieved samples and were used for percent damage calculations by using the following formula:

$$\text{Percent damage} = \frac{(\text{initial weight} - \text{weight of healthy grains})}{\text{initial weight}} \times 100$$

Results and Discussion

The results revealed different trends in different

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parameters which are explained as follows:

Number of eggs per grain

According to Table 1, eight eggs were found when chickpeas were exposed to lowest concentration (1×10^4) of *B. bassiana*. Number of eggs was higher in this concentration which was significantly different from all other concentrations. Concentrations 1×10^5 and 1×10^6 showed almost similar results with mean average of six eggs. Lowest number of eggs was observed in highest concentration (1×10^8), which was significantly different from all other concentrations. The highest number of eggs (15) was recorded in control. In case of *M. anisopliae,* lowest number of eggs (3) was seen in the highest concentration (1×10^8) which was significantly different to rest of concentrations. Highest number of eggs was recorded in control. However, results of concentrations i.e. 1×10^5 and 1×10^6 were not significantly different and same number of eggs was observed. On an average, nine eggs per grain were counted with application of lowest concentration of *M. anisopliae*.

Table 1: *Number of eggs laid by C. chinensis under different concentrations of B. bassiana and M. anisopliae application.*

Sr. No	Concentrations (Cells/ml)	Number of eggs per grain (Mean ± SE)	
		B. bassiana	M. anisopliae
1	1×10^4	8±0.57c	9±0.57d
2	1×10^5	6±0.57bc	8±0.57cd
3	1×10^6	6±1.15bc	8±0.57bc
4	1×10^7	4.3±0.33b	5.3±0.66ab
5	1×10^8	2±0.57a	3±0.57a
6	Control	15±0.57d	17.66±1.45e

Linear regression model was applied to verify outcomes of different concentrations of fungi (*B. bassiana*) regarding eggs number. The modeled equation (Y= -2.1743x + 14.493) revealed that treatments had dangerous effects on fecundity. The intercept (a) value remained 14.49 while slope (b) was -2.17. With alteration in dilution of fungi, number of eggs was also minimized by -2.17. Coefficient of determination of (R^2) was 0.83 which showed that treatments (independent variable) have 83% effect dependent variable. The R^2 furthermore verified correctness of model to anticipate result of fungi treatments on oviposition rate. Correspondingly linear regression model was also used to confirm results of various concentration of fungus (*M. anisopliae*) on number of eggs. The modeled equation (Y=-2.4114 x + 16.933) indicates that treatments had harmful effect over fecundity. The intercept (a) value remained 16.93 while slope (b) was -2.41. So, with the change in concentration of fungus, number of eggs was also reduced by -2.41. Coefficient of determination of (R^2) was 0.81 which displayed that treatments (independent variable) had 81% effect on the dependent variable. The R^2 further confirmed accuracy of model in studying efficacy of fungal treatments against oviposition rate of the beetle (Figure 1 and 2).

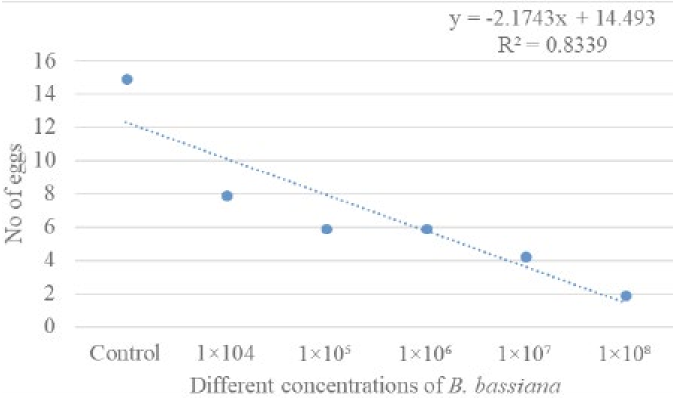

Figure 1: *Modeling trend for mean number of eggs laid by pulse beetle in response to different concentrations of B. bassiana.*

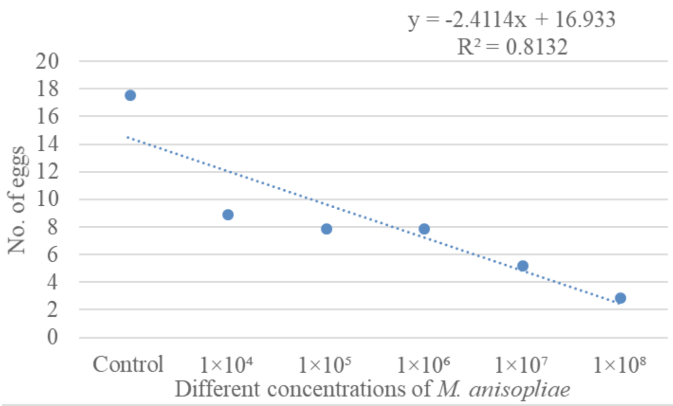

Figure 2: *Modeling trend for mean number of eggs laid by pulse beetle in response to different concentrations of M. anisopliae.*

Number of holes per grain

Minimum number of holes (1.3) per grain was observed with highest concentration (1×10^8) of *B. bassiana* which was significantly different from all other concentrations. With the lowest concentration (1×10^4 and 1×10^5), 06 and 5.3 holes were seen respectively which was not significantly different from each other. Highest number of holes (8.3) per grain were recorded in control where no dilution of *B. bassiana* was used. In case of *M. anisopliae,* more number of holes (3.6) per grain were recorded with lowest concentration (1×10^4) of all other concentrations. This concentration was not significantly different from increased concentration (1×10^5) which comes up with three number of hole. Highest numbers of holes (07) were obtained in control where no dilution of *M. anisopliae* was used. The minimum number of holes (1) was recorded in highest concentration of *M. anisopliae* with application rate of 1×10^8 (Table 2).

Table 2: *Number of holes made by C. chinensis under different concentrations of B. bassiana and M. anisopliae application.*

S. No	Concentrations (Cells/ml)	Number of holes per grain (Mean ± SE)	
		B. bassiana	M. anisopliae
1	1×10^4	6±0.57d	3.6±0.33d
2	1×10^5	5.3±0.33cd	3±0.00cd
3	1×10^6	4±0.00bc	2.33±0.33bc
4	1×10^7	3.6±0.33b	1.67±0.33ab
5	1×10^8	1.3±0.33a	1±0.57a
6	Control	8.3±0.88e	7±0.57e

Linear regression model was applied to verify outcomes of different concentrations of fungi (*B. bassiana*) regarding holes number. The modeled equation (Y= -1.2429x + 9.1) revealed that treatments had dangerous effects on fecundity. The intercept (a) value remained 9.1 while slope (b) was -1.24. So, with alteration in dilution of fungi, the number of holes also minimized @-1.24. Coefficient of determination of (R^2) was 0.95 which showed that treatments (independent variable) had 95% effect on the dependent variable. The R^2 furthermore verified the correctness of model to anticipate result of fungi treatments on larval development (Figure 3).

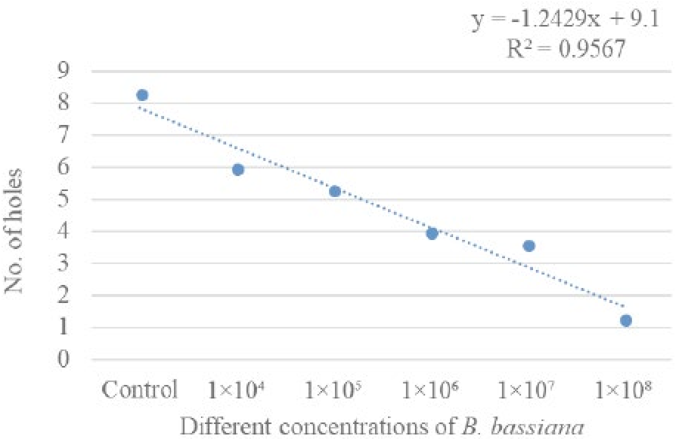

Figure 3: *Modeling trend for mean number of holes made by C. chinensis in response to different concentrations of B. bassiana.*

Correspondingly linear regression model was also used to confirm the results of various concentrations of fungus (*M. anisopliae*) on number of holes. The modeled equation (Y= -1.0417x + 6.746) showed that treatments had detrimental effects over fecundity. The intercept (a) value remained 6.74 while slope (b) was -1.0417. So, with the change in concentration of fungus, the holes number also reduced @-1.0417. Coefficient of determination of (R^2) was 0.84 which displayed that treatments (independent variable) had 84% effect on the dependent variable. The R^2 further confirmed the accuracy of model to expect result of fungal treatments on larval development (Figure 4).

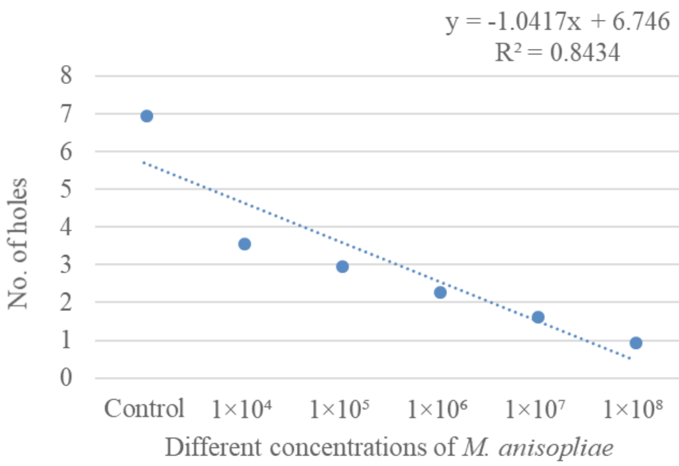

Figure 4: *Modeling trend for mean number of holes made by C. chinensis in response to different concentrations of M. anisopliae.*

F_1 adults emerged

Highest number of F_1 progeny emerged in control (42) as compared to all other treatments with *B. bassiana* (Table 3). Minimum difference was observed in population 24.3 and 22.3 where concentration 1×10^6 and 1×10^7 was applied respectively. There was also a difference of population in lowest concentration with 27 adults and highest concentration showing 20.33 adults. Lowest number of F_1 progeny emerged in highest concentration (1×10^8) of *M. anisopliae* which was significantly different with all other treatments except control. There was no significant difference in populations 24.33 and 24 adults where concentration 1×10^5 and 1×10^6 were applied.

It was revealed in Figure 5 that the outcomes of dissimilar *B. bassiana* dilutions were verified by means of linear regression model. Negative outcomes of fungi dilution on new appeared adults were reported as indicated by modeled equation (Y= -3.4653x + 39.563). The intercept (a) and slope (b) values remained 39.56 and -3.46, correspondingly. Consequently, as dilution of *B. bassiana* enhanced numbers of F_1 emerged decreased @-3.46. Determination coefficient (R^2) was 0.74 that indicated that independent variable has 74% influences on the dependent variable. The R^2 furthermore verifies correctness of model. Likewise results of dissimilar *M. anisopliae* concentrations were checked by means of linear regression model (Figure 6). Negative results for fungal concentration on the number of newly emerged adults were reported as shown by the modeled equation (Y= -3.1526x + 37.754). The intercept (a) and slope (b) value remained 37.75 and -3.15, correspondingly. Hence, as the concentration of *M. anisopliae* increased, number of F_1 emerged decreased @-3.15. Determination coefficient (R^2) was 0.59 which showed that independent variable had 59% influences on the dependent variable. The R^2 further confirmed the accuracy of model.

Table 3: *Number of F_1 adults emerged of C. chinensis in chickpea grains applied with different concentrations of B. bassiana and M. anisopliae.*

S. No	Concentrations (Cells/ml)	Number of F_1 adults emerged per jar (Mean ± SE)	
		B. bassiana	M. anisopliae
1	1×10⁴	27±0.57d	25.33±0.33b
2	1×10⁵	26.6±0.33cd	24.33±0.33b
3	1×10⁶	24.3±0.33bc	24±0.57b
4	1×10⁷	22.3±1.20ab	23.66±0.33b
5	1×10⁸	20.33±0.33a	21±0.57a
6	Control	42±1.15e	42±1.5c

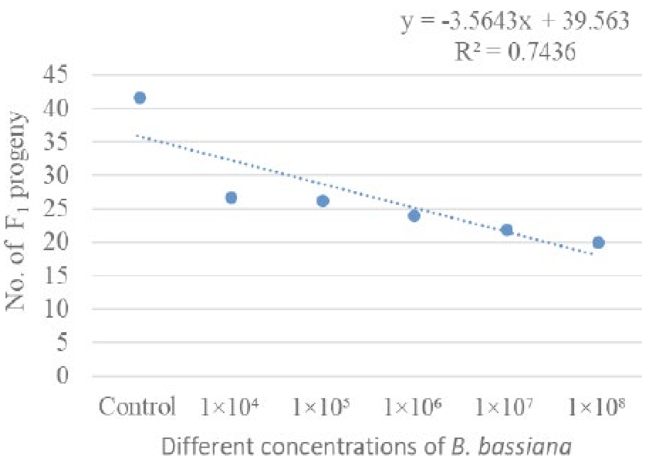

Figure 5: *Modeling trend for emergence of F_1 adults of pulse beetle in response to different concentrations of B. bassiana.*

Percent inhibition rate of F_1 adults

Maximum percent inhibition rate (52.45) in pulse beetle was noted with highest concentration of *B. bassiana* (1 × 10^8) which was significantly different with all other treatments. Almost similar percent inhibition i.e. 35.52 and 35.48 was recorded with *B. bassiana* concentration of 1 × 10^4 and 1 × 10^5 respectively. Minimum percent inhibition rate was seen in control.

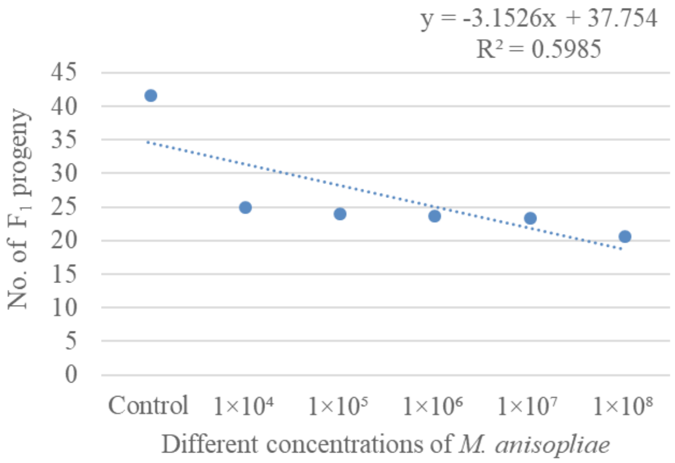

Figure 6: *Modeling trend for emergence of F_1 adults of pulse beetle in response to different concentrations of M. anisopliae.*

Whereas percent inhibition rate of 41.32 and 42.57 was recorded with concentration of 1 × 10^5 and 1 × 10^6 of *M. anisopliae* that was significantly different from each other as well as from all other treatments. Lowest percent inhibition rate of *C. chinensis* was however recorded in control. Further, maximum percent inhibition rate (50.23) was observed with highest concentration of *M. anisopliae* (1 × 10^8) that was also significantly different from all other treatments (Figure 7).

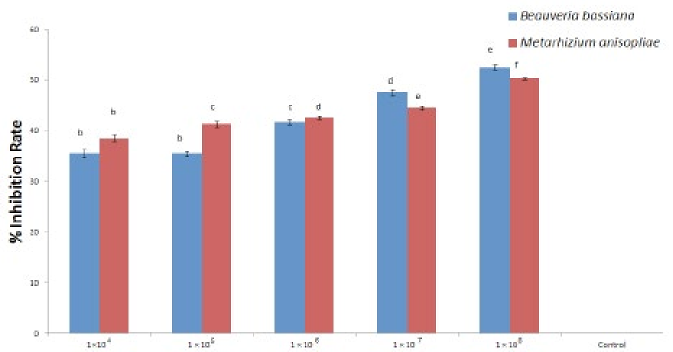

Figure 7: *Percent inhibition rate (Mean ± SE) of pulse beetle in stored chickpea treated with different concentrations of B. bassiana and M. anisopliae.*

Linear regression model was used to check effects of dissimilar dilutions of *B. bassiana* regarding % inhibition rate. Positive effects of fungi dilutions regarding inhibition of F_1 emerged were observed as indicated by modeled equation (Y=8.6994x + 4.992). The intercept (a) value retained 4.99 while slope (b) was 8.69. So, as fungal dilutions increased, inhibition rates were also enhanced at the rate of 8.69. Coefficient of determination (R^2) was 0.76 that revealed that dilutions (independent variable) had 76% effect on the dependent variable (inhibition's rate). The value of R^2 furthermore verified correctness of model to anticipate effects of fungal treatments for inhibition rate of F_1 (Figure 8).

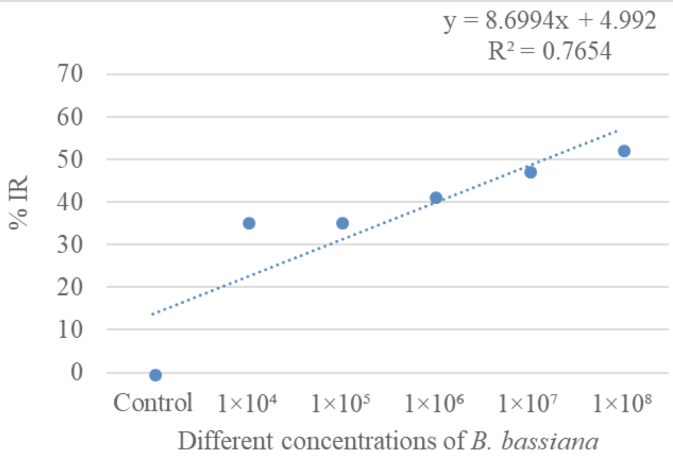

Figure 8: *Modeling trend of percent inhibition rate (Mean ± SE) of pulse beetle in stored chickpea treated with different concentrations of B. bassiana.*

Linear regression model was also applied to find out the effect of dissimilar concentrations of *M. anisopliae* on the percent inhibition rate. Positive effect of fungal concentration on the inhibition of F_1 emerged was observed as shown by the modeled equation (Y=7.7214x + 9.16). The intercept (a) value remained 9.16 while slope (b) was 7.72. So, as the fungal concentration was increased, inhibition rate was also increased at the rate of 7.72. Coefficient of determination (R^2) was 0.63 which shows concentrations (independent variable) have 63% effect on the inhibition rate (dependent variable). The value R^2 further confirmed accuracy of model to predict effect of fungal treatments on inhibition of newly emerged (Figure 9).

Number of days to 100 percent mortality of F_1 adults Minimum number of days (7.3) to 100% mortality was observed with highest concentration of *B. bassiana* which was significantly different to all other treatments. Yet, maximum days (18.3) to 100% mortality were seen in control. There was no difference observed in number of days (9) and (8.3) to 100% mortality between concentration of 1×10^6 and 1×10^7 respectively. The reason may be minimal difference of concentration between them. More number of days to 100% mortality was measured in highest concentration of *B. bassiana* i.e. 1×10^8.

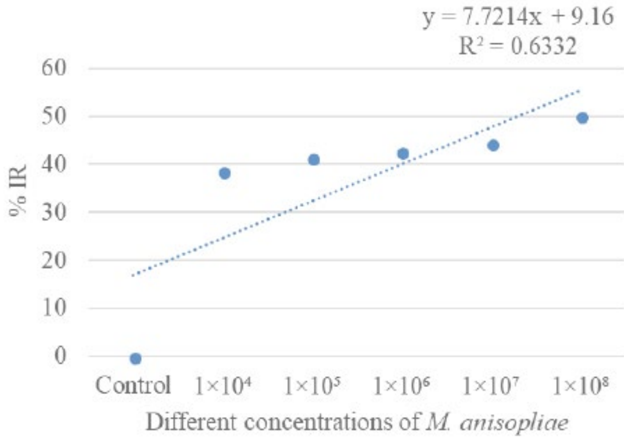

Figure 9: *Modeling trend of percent inhibition rate (Mean ± SE) of pulse beetle in stored chickpea treated with different concentrations of M. anisopliae.*

There was no considerable variation observed in concentration of 1×10^7 and 1×10^6 that took 8.6 and 07 days to 100% mortality which was significantly different with all other treatments. Whereas, more number of days to 100% mortality was seen in lowest concentration of *M. anisopliae* (1×10^4) which was significantly different to all other treatments (Figure 10).

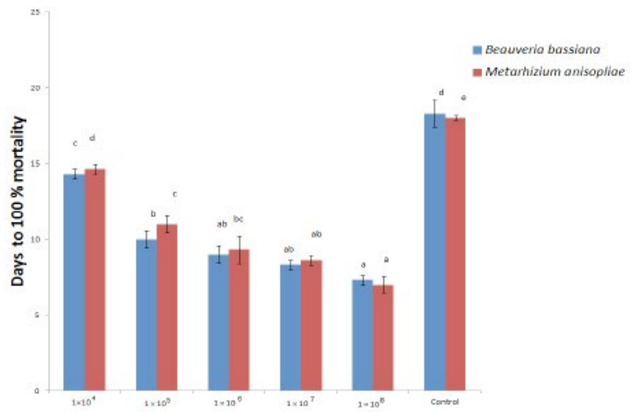

Figure 10: *Modeling trend of days to 100% mortality of F_1 adults (Mean ± SE) of pulse beetle in stored chickpeas treated with different concentrations of B. bassiana and M. anisopliae.*

To determine effectiveness of diverse dilutions regarding death rate of new appeared adults, linear regression models were applied. When dilutions of fungi were used as obvious from the regression equation (Y= -2.1143x + 18.6), negative impacts on percent mortality of F_1 adults were noted. The intercept (a) value remained 18.6 but slope (b) was -2.11.

Consequently, as dilution of *B. bassiana* enhanced, numbers of days to 100% mortality of F_1 were also reduced @ -2.11. Coefficient of determination (R^2) was 0.87 that discovered that dilutions of fungi have 87% effects on the dependent variable. The R^2 furthermore verified correctness of models to anticipate effects of fungi treatments on the days required to 100% mortality (Figure 11). Similarly, to observe the effect of diverse concentrations on the mortality rate of newly emerged adults, linear regression model was used. When the fungal concentration was applied as evident from the regression equation (Y= -2.1343x + 18.887), the negative impact on the percent mortality of F_1 adults were recorded. The intercept (a) value remained 18.88 but slope (b) was -2.13. Hence as the concentration of *M. anisopliae* increased, days to 100% mortality of F_1 was reduced @ -2.13. Coefficient of determination (R^2) was 0.93 which revealed that the fungal concentrations had 93% effect on the dependent variable. The R^2 further confirmed the accuracy of model to predict effect of fungal treatments on the days required to 100% mortality (Figure 12).

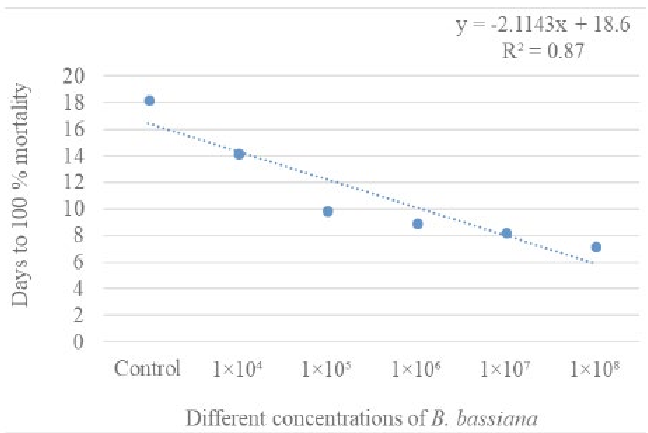

Figure 11: *Modeling trend of days to 100% mortality of F_1 adults (Mean ± SE) of pulse beetle in stored chickpeas treated with different concentrations of B. bassiana.*

Likewise, more than 50% weight loss was observed in concentration of *M. anisopliae* 1×10^6 as compared to 35.03% observed in 1×10^7 concentration. The lowest percent weight loss (13.7%) was observed with highest concentration of *M. anisopliae* (1×10^8), while maximum weight loss was recorded (87.1%) in control. Percent weight loss in chickpea caused by *C. chinensis* in response to diverse dilutions of fungi was experimented by linear regression model (Figure 14). Unfavorable impacts of dilutions of fungi on the feeding of pulse beetle were noted as showed by the modeled equation (Y= -7.5503x + 93.396). The intercept (a) and slope (b) value remained 93.39 and -7.55, separately. So, as dilution of *B. bassiana* enhanced weight loss caused by pulse beetle lowered @ -7.55.

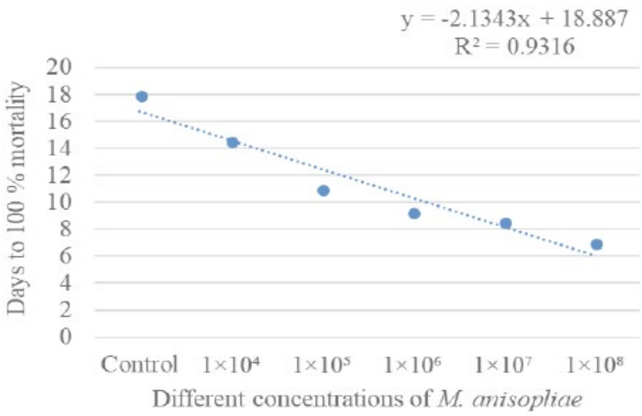

Figure 12: *Modeling trend of days to 100% mortality of F_1 adults (Mean ± SE) of pulse beetle in stored chickpeas treated with different concentrations of M. anisopliae.*

Percent weight loss of chickpea grains

Highest percent weight loss was measured (85.33%) in control (Figure 13). Second to it, the percent weight loss (81.16%) was lowered in lowest concentration (1×10^4) of *B. bassiana*. No significant percent weight loss was recorded in concentration of *B. bassiana* (1×10^5) and 1×10^6 respectively. Lowest percent weight loss was shown in highest concentration of *B. bassiana* i.e. (1×10^8).

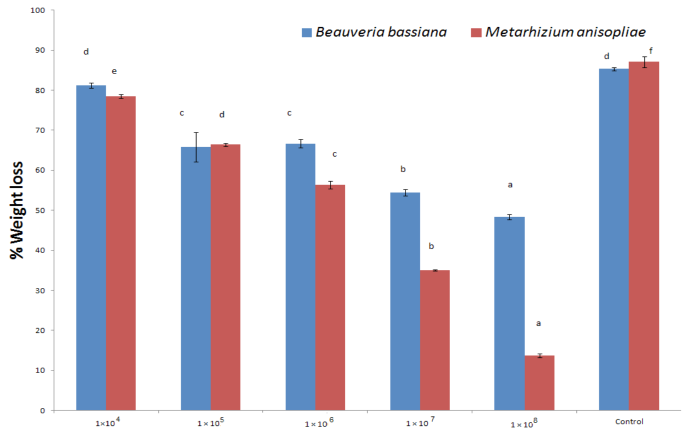

Figure 13: *Percent weight loss (Mean ± SE) caused by pulse beetle in stored chickpeas treated with different concentrations of B. bassiana and M. anisopliae.*

Determination co-efficient (R^2) was 0.95 which indicated that dilutions of fungi have 95% effects on the percent weight loss. The R^2 furthermore verified correctness of model.

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Similarly, percent weight loss caused by *C. chinensis* in response to diverse fungal concentrations was experimented by linear regression model (Figure 15). Unfavorable impact of fungal concentrations on the feeding of pulse beetle was observed as presented by the modeled equation (Y= -14.498x + 113.4). The intercept (a) and slope (b) value remained 113.4 and -14.49, separately. So, as the concentration of *M. anisopliae* increased weight loss caused by pulse beetle decreased with the rate of -14.49. Determination coefficient (R^2) was 0.96 which showed that the fungal concentrations have 96% effects on the percent weight loss. The R^2 further confirmed the accuracy of model.

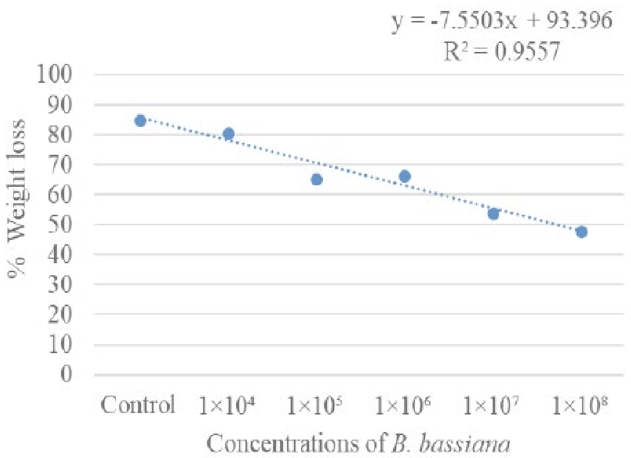

Figure 14: *Modeling trend of percent weight loss (Mean ± SE) caused by pulse beetle in stored chickpeas treated with different concentrations of B. bassiana.*

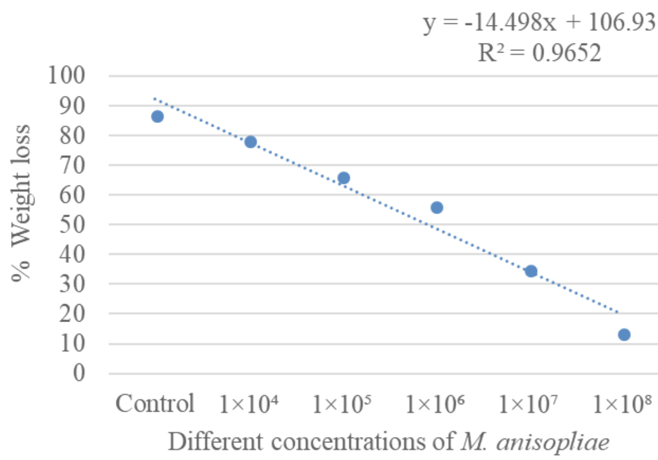

Figure 15: *Modeling trend of percent weight loss (Mean ± SE) caused by pulse beetle in stored chickpeas treated with different concentrations of M. anisopliae.*

Percent damage of chickpea grains

More than 50% damage (56.33) was recorded by *C. chinensis* in *B. bassiana* with concentration of 1×10^6 as compared to treatments at the rate of 1×10^7 and 1×10^8. Maximum percent damage (94%) was observed in control. Minimum percent damage (29%) by *C. chinensis* was enumerated in highest concentration of *B. bassiana* which was significantly different with all other treatments. Maximum damage (95.55%) was seen by *C. chinensis* in control. Minimum percent damage (28.5%) was calculated with maximum concentration of *M. anisopliae* which was significantly different with all other treatments. More damage (73.66%) was observed in lowest concentration of *M. anisopliae* (1×10^4) than all other treatments (Figure 16).

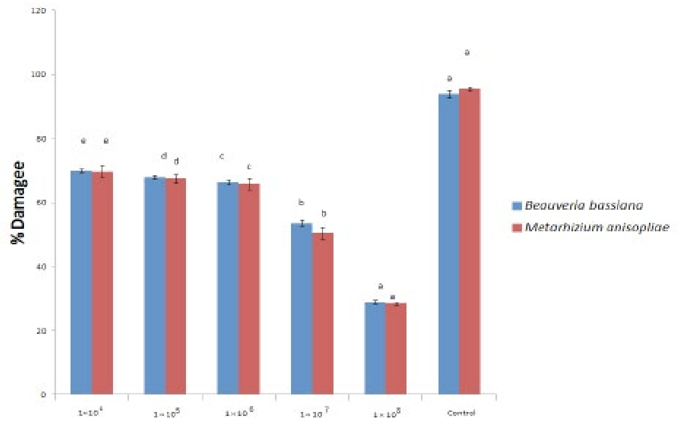

Figure 16: *Percent damage (Mean ± SE) caused by pulse beetle in stored chickpeas treated with different concentrations of B. bassiana and M. anisopliae.*

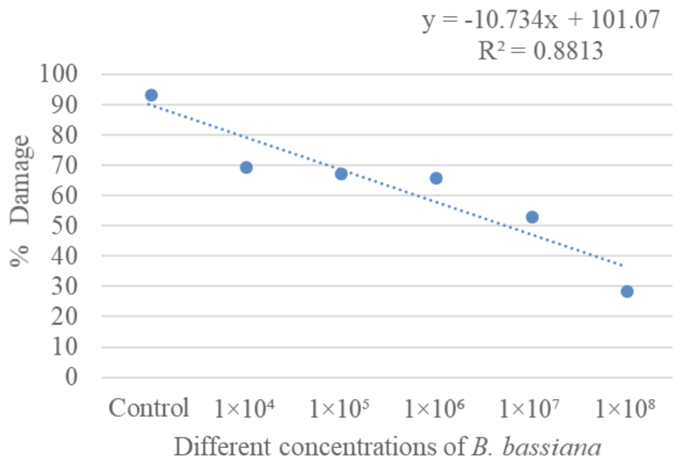

Figure 17: *Modeling trend of percent damage (Mean ± SE) caused by pulse beetle in stored chickpeas treated with different concentrations of B. bassiana.*

Percent damage caused by *C. chinensis* in response to diverse dilutions of fungi were experimented by linear regression model (Figure 17). Unfavorable impacts of dilutions of fungi on the feeding of pulse beetle was noted as showed by the modeled equation (Y= -10.734x + 101.07). The intercept (a) and slope (b) value remained 101.07 and -10.73, separately. So, as dilution of *B. bassiana* enhanced, percent damage caused by pulse beetle lowered with the rate of -10.73. Determination co-efficient (R^2) was 0.88 which indicated that dilutions of fungi have 88% effects on the percent damage. The R^2 furthermore verified correctness of model.

Similarly, percent damage caused by *C. chinensis* in response to diverse fungal concentrations was experimented by linear regression model (Figure 18). Unfavorable impact of fungal concentrations on the feeding of pulse beetle was observed as presented by the modeled equation (Y= -11.268x +102.37). The intercept (a) and slope (b) value remained 102.37 and -11.26, separately. So, as the concentration of *M. anisopliae* increased, percent damage caused by pulse beetle decreased with the rate of -11.26. Determination coefficient (R^2) was 0.89 which showed that the fungal concentrations have 89% effects on the percent damage. The R^2 further confirmed the accuracy of model.

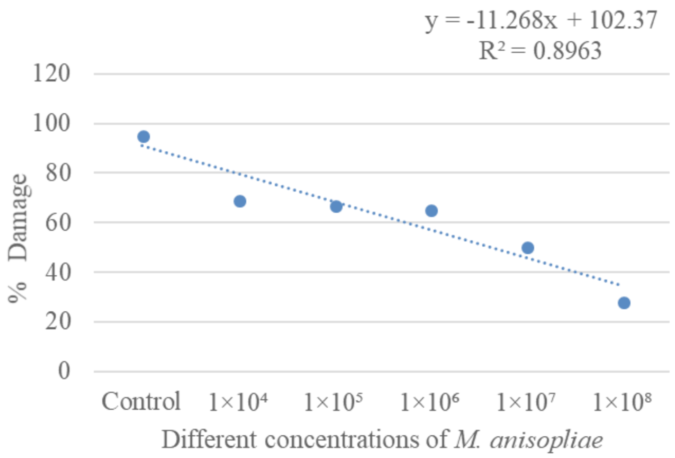

Figure 18: *Modeling trend of percent damage (Mean ± SE) caused by pulse beetle in stored chickpeas treated with different concentrations of M. anisopliae.*

Some scientists also used these entomopathogenic fungi for management of insect pests. The findings of studies are in conformity with Abdel-Raheem (2015) who evaluated efficacy of entomopathogenic fungi, *Beauveria* and *Metarhizium spp.* against some stored insect pests. In another similar study undertaken by Shaheen et al. (2016), they used entomopathogenic fungus, *B. bassiana* as natural management agent to control pulse beetle *C. chinensis* in chickpea grains at different temperatures. In this study mortality of pulse beetle was found directly proportional to concentrations of *B. bassiana*.

In a similar study, by Radha (2012) similar results were found when she assessed the efficiency of two Entomopathogenic fungi *M. anisopliae* and *B. bassiana* against *C. maculatus*. Mahdneshin et al. (2011), evaluated pathogenic viability of five isolates of *B. bassiana* and *M. anisopliae* against *C. maculates* by concentration assay technique at 27±1°C and 60±5 percent relative humidity under controlled conditions. The IRAN (441C) of *B. bassiana* proved the most detrimental against test insect because it had lesser LC_{50} and LT_{50} and it yielded maximum deaths (76 percent) in application having 1×10^8 conidiaml^{-1}. The *Beauveria bassiana* found more virulent than *M. anisopliae* against test insect. In another study undertaken by Khashaveh (2013), it was observed that adults of test insects were vulnerable to whole isolates of *M. anisopliae*. When studied complete (03) isolates, death percentage of the two species enhanced with rise in conidial formulation and considerable variation was recorded between dilutions. The factors of probit test illustrated non-overlap of 95 percent of LC_{50} and LC_{95} and noteworthy variation was recorded in three isolates examined in opposition to test insects.

Author's Contribution

Mohsin Iqbal: Conducted research and wrote manuscript.
Farid Asif Shaheen: Conceived and supervised research and wrote manuscript.
Farah Naz: Supervised pathological aspects of research.
Muhammad Usman Raja: Proof checking and supervised pathological aspects of research.
Muhammad Fiaz: Analyzed data and helped in manuscript writing.
Muhammad Nadeem: Data collection and collection of experimental materials.

References

Aslam, M. 2004. Pest status of stored chickpea beetle, *Callosobruchus chinensis* linnaeus on chickpea. J. Ento. 1(1): 28-33. https://doi.org/10.3923/je.2004.28.33

Abdel-Raheem, M.A., I.A. Ismail, R.A. Rahman, N.A. Farag and I.A. Rhman. 2015. Entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) As biological control agents on some stored product insects. J. Entomol. Zool. Stud. 3(6): 316-320.

Atif, H.M., N. Javed, S.A. Khan and S. Ahmed. 2012. Virulence of *Xenorhabdus* and *Photorhabdus* bacteria and their toxins against juvenile's immobilization of *Meloidogyne incognita*. Pak. J. Phytopathol. 24(2): 170-174.

Bakkali, F., S. Averbeck, D. Averbeck, M. Idaomar. 2008. Biological effects of essential oils. Rev. Food Chem. Tox. Vol. 46 (2): 446-475. https://doi.org/10.1016/j.fct.2007.09.106

Consultative group on international agricultural research. 2017. (https://www.cgiar.org)

Correa, J.P., A. SáenzApontel, Rodríguez, M.X. Bocanegra. 2016. In vitro interaction of *M. anisopliae* Ma 9236 and *B. bassiana* Bb9205 with *Heterorhabditis bacteriophora* HNI0100 for the control of *Plutella xylostella*. Springer Plus. 5: 2068.

Food and agriculture organization. 2016. (www.fao.org)

Halstead, D.G.H. 1963. External sex differences in stored-products Coleoptera. Bull. Entomol. Res. 54(1): 119-134. https://doi.org/10.1017/S0007485300048665

Hidalgo, E. 1998. The effect of different formulations of Beauveria bassiana on Sitophilus zeamais in stored maize. Journal of Stored Products Research, 34(2): 171-179.

Jung, S. and Y. Kim. 2006. Synergistic effect of entomopathogenic bacteria (*Xenorhabdus* sp. and *Photorhabdus temperata* ssp. temperata) on the pathogenicity of *Bacillus thuringensis* ssp. aizawai against *Spodopteraexigua* (Lepidoptera: Noctuidae). Environ. Ento. 35(6): 1584-1589. https://doi.org/10.1093/ee/35.6.1584

Khashaveh, A. 2013. Laboratory bioassay of Iranian isolates of entomopathogenic fungus, M. anisopliae (Ascomycota: Hypocreales) against two species of storage pest. Agriculturae Conspectus Scientificus, 78(1): 35-40.

Kryukov, V.Y., V.P. Khodyrev, O.N. Yaroslavtseva, A.S. Kamenova, B.A. Duisembekov and V. Glupov. 2009. Synergistic action of entomopathogenic hyphomycetes and the bacteria *Bacillus thuringiensis* ssp. morrisoni in the Infection of colorado potato beetle. Appl. Biochem. Microbiol. Vol. 45, (5): pp. 511–516. https://doi.org/10.1134/S000368380905010X

Mahdneshin, Z., S. Vojoudi, Y. Ghosta, M.H. Safaralizadae and M. Saber. 2011. Laboratory evaluation of the entomopathogenic fungi, Iranian isolates of *B. bassiana* (Balsamo) Vuillemin and *M. anisopliae* (Metsch) Sorokin against the control of the cowpea weevil, Callosobruchus maculatus F. (Coleoptera: Bruchidae). https://doi.org/10.5897/AJMR11.1223 Afr. J. Microbiol. Res. 5(29): 5215-5220.

Mburu, D.M., N.K. Maniania, A. Hassanali and M.W. Ndung'u. 2011. Comparison of volatile blends and gene sequences of two isolates of *M. anisopliae* of different virulence and repellency towards the termite *Macrotermes michaelseni*. J. Exp. Biol. 214 (6): 956-962. https://doi.org/10.1242/jeb.050419

Nabaei, N.A., S.M. Mehrvar and M. Bagheri. 2012. Efficacy of entomopathogenic fungi in combination with diatomaceous earth against *Callosobruchus maculatus* (Coleoptera: Bruchidae). Acta Entomol. Sinica. 55 (11):1282-1288.

Park, Y., J.K. Jung and Y. Kim. 2016. A mixture of *B. thuringiensis* sub sp *israelensis* with *X. nematophila*-cultured broth enhances toxicity against mosquitoes *Aedes albopictus* and *Culexpipien s*pallens (Diptera: Culicidae). J. Econ. Entomol. 109 (3): 1086-1093. https://doi.org/10.1093/jee/tow063

Park, Y. 2015. Entomopathogenic bacterium, *X. nematophila* and *P. luminescens*, enhances *B. thuringiensis* Cry4Ba toxicity against yellow fever mosquito, *Aedes aegypti* (Diptera: Culicidae). J. Asia-Pac. Entomol. 18(3): 459-463. https://doi.org/10.1016/j.aspen.2015.05.002

Radha, R. 2012. Insecticidal efficacy of entomopathogenic fungi in stored cowpea against cowpea bruchid, *Callosobruchus maculates* (F) (Coleoptera:Bruchidae). Asian Acad. Res. J. Multidicip. (1): 3.

Rajendran, S. and V. Srinanjini. 2008. Plant products as fumigants for stored-product insect control. J. Stored Prod. Res. 44: 126-135. https://doi.org/10.1016/j.jspr.2008.04.001

Shaheen, F.A., M.W. Akram, M.A. Rashid, M. Nadeem, M. Saeed, M. Husain and M. Khalid. 2016. Biological control of pulse beetle *Callosobruchus chinensis* L. (Bruchidae: Coleoptera) in stored chickpea grains using entomopathogenic fungus *Beauveria bassiana* Balsamo. J. Entom. Zool. 2016; 4(4): 1076-1083.

Shaheen, F.A., M.W. Akram, M.A. Rashid, M. Nadeem, M. Saeed, M. Husain and M. Khalid.

2006. Resistance of chickpea (*Cicer arietinum* l.) cultivars against pulse beetle. Pak. J. Bot. 38(4): 1237-1244.

Sony, S. and Y. Kim. 2010. Differential pathogenicity of two entomopathogenic bacteria, *Photorhabdus temperata* subsp. temperata and *Xenorhabdus nematophila* against the red flour beetle, *Tribolium castaneum*. J. Asia-Pac. Ento. (13): 209-213.

Sony and K. Yonggyun. 2010. Differential pathogenicity of two entomopathogenic bacteria, *Photorhabdus temperata* subsp. Temperata and *Xenorhabdus nematophila* against the red flour beetle, *Tribolium castaneum*. J. Asia-Pac. Ento.13: 209-213.

SeongchaeJ and K. Yonggyun. 2006. Synergistic effect of entomopathogenic bacteria (*Xenorhabdus* sp. and *Photorhabdus temperata* ssp. temperata) on the pathogenicity of *Bacillus thuringiensis*ssp. aizawai against *Spodopteraexigua* (Lepidoptera: Noctuidae). Environment. Entomol. 35(6):1584-1589. https://doi.org/10.1093/ee/35.6.1584

Tuan, A.N., J.K. Jeong, G.K. Seon and K. Keun. 2009. Production of blastospore of entomopathogenic *B. bassiana* in a submerged batch culture. Mycobiol. 37(3): 218-224. https://doi.org/10.4489/MYCO.2009.37.3.218

Zia, A., M. Aslam, F. Naz and M. Illyas. 2011. Bio-efficacy of some plant extracts against chickpea beetle, *Callosobruchus chinensis* Linnaeus (Coleoptera: Bruchidae) attacking chickpea. Pakistan J. Zool. 43(4).