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Research Article

Pathogenicity of Entomopathogenic *Metarhizium anisopliae* Against *Rhyzopertha dominica* (Bostrichidae: Coleoptera)

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Abstract – *Rhyzopertha dominica* is regarded as the most devastating insect pest among stored wheat grains. Indiscriminate use of pesticides and fumigants to control lesser grain borer has resulted in insecticide resistance, environmental hazards, residual toxicity and pest resurgence so biological control measures have attained high significance i.e. entomopathogenic fungi like *Beauveria bassiana and Metarhizium anisopliae* were used as biological-control against this pest. Research work on pathogenicity of entomopathogenic fungus, *M. anisopliae* was executed against lesser grain borer at its different concentrations at PMAS Arid Agriculture University Rawalpindi. Results revealed that the higher concentrations were more effective than the lower concentrations of entomopathogenic fungus. Against lesser grain borer the fungal efficacy was directly proportional to the time exposure. Conducted research depicted that for *M. anisopliae*, concentration of 1×10^8 spores/ml caused the maximum 93.33% mortality after 72 hours' interval. The consequences of the research provided an effective and safe biological control measures and in future will lead to an operative IPM management for this monetary pest.

Keywords – Rhyzopertha Dominica; Entomopathogenic Fungi; Biological Control, Mortality, Metarhizium Anisopliae.

I. INTRODUCTION

Triticum aestivum L. is at 3rd position in food crops cultivated all over the world and Pakistan is at 8th number in wheat production (Shuaib et al., 2007). It is cultivated 37% of total area in Pakistan and total 76% food production (Jilani, 1981).Total wheat stored 65-75% in Pakistan as for food and also stored seed (Choudhry & Anwar, 1988). More than three months stored wheat grain provide an opportunity to insect pest to grow and insects are the main reason for wheat destruction in biotic factors in Pakistan (Latif et al., 1991).Range 10.5– 13.5% is the moisture content in North America (Hagstrum, 1987; Reed & Pan, 2000). *Rhyzopertha dominica* (LGB)can cause extensive quantitative losses by reducing total weight of stored wheat grains, losses in total physical weight. It is estimated that 10 – 25 % of total food crop is lost each year due to this pest the worldwide. Storage crop loss not only caused by pest direct

damage but also nutrition loss, inhibition of seed germination and low marketing value, netting and insect bodies also occur. (Aziz, 2011).

Grain protectants have been proven to be ineffective in managing this pest due to resistance issues (Edde, 2012). Adults and larvae of the (LGB) *Rhyzopertha dominica* are able to damage the whole grains. (Mayhew & Phillips, 1994). Some changes in Temperature and other is humidity occurs in the large way of stored grain product but sometimes entomopathogenic fungi's growth increases grain storage temperature. In hot season temperature, range is 27–34 °C for maximum insect activity (Hagstrum, 1987). The greater end of that variety's top end displays growing of the many *B. bassiana* separates and it is use for the commercial study purposes GHA (Fargues et al., 1997). Temperature range has the main role in *B. bassiana* potential for pest management in grain the primary target pests.

Pesticides are used in controlling the pest is the one way to prevent some loss but it causes problems like insect resistant, chemical accumulation, expensive food and environmental (Sullivan, 2002). Some reports tell us that the DE combine with *B. bassiana* has increased insecticidal outcome than it was broken when the fungal measures were used singly (Kavallieratos et al., 2006).

Entomopathogenic fungi cause the infection in its host insects with the support of conidia/spores, which are produced asexually. These conidia/spores grow and then enter in the exoskeleton that host in appropriate environmental condition. Insect cuticle that offers secure structure and the defense to body of insect and it made from chitin and other mechanisms like is protein (Richard *et al*., 2011). Firstly the fungal spore/conidia attach on the external of its appropriate host and then with the penetration of the germ tube inside the cuticle of pest. It germinates inside cuticle of host. (Bateman *et al*., 1996). In host of the cuticle penetration is accomplished with the help of mechanical and other is enzymatic ruin, which documents of the germ tube to propagate in the body cavity (haemocoel). In the body, production of EPF endotoxins cause the death of the insect. Later, fungi can develop out from the cadaver and with the development of spores/conidia external the cadaver complete lifecycle of pest. By way of the fungal spores disperse in air and land on another host, its infection cycle starts again (Inglis *et al*., 2001).

Entomopathogenic fungi are mostly used in stored grain pests in many researches (Wakil & Ghazanfar, 2010). Food quality does not effected by both of them and market value remain same (Steenberg, 2006).The use of the bio-pesticide based on EPFs *B. bassiana* and *M. Anisopliae* against LGB can be a novel and highly effective measure to protect wheat stored grains. The major outcomes of this research were to determine death rate of LGB used by these EPFs. Microbial formulations may be the outcome and recommended to control LGB and that will also be eco-friendly. The objective of the proposed research is the determination of pathogenicity of insect pathogenic fungi *Metarhizium anisopliae* against the *Rhzopertha dominica* in stored wheat grains.

II. MATERIALS AND METHOD

Maintenance of *Rhyzopertha dominica*culture

Infested samples of stored wheat grains were collected from different godowns/storages in different areas of Punjab. *R. dominica* culture was sustained in the 'Stored Product Entomology Laboratory' of the University in an incubator at 70 ± 5 % RH and 30 ± 2 °C temperature. Adults male were differentiated by the presence of distinct transvers, punctuate grove on the fifth abdominal sternal of the male while that was absent in female (Ajaykumara, 2018)

Culture maintenance and inoculum preparation of *Metarhizium anisopliae*

Culture of entomopathogenic fungus, *M. anisopliae* was maintained in the 'Fungal Plant Pathology Laboratory' of the University. Initially the culture was grown in Potato Dextrose Agar (PDA) at 25°C 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 were grown on PDA medium. Later on spores/conidia were counted by Haemocytometer at 24 hours interval (Tuan *et al*., 2009). Different concentrations of spores/conidia were established in distilled water in addition with Tween 80 (0.02%). For analysis, diverse concentrations of *M. anisopliae* were equipped. At that point for mass culturing of fungi Petri

plates were kept in the incubator given at 75 ± 5% R.H and other is 28± 1°C temperature. At the end, culture was oven dried and conidia were harvested from culture with the help of rubber scalpel.

Insect bioassays using different concentrations of *Metarhizium anisopliae*

Experiment No. 1

In each plastic jar, 50g of grains were put and enclosed with tightened muslin cloth and kept at 30°C temperature in incubator. Ten pairs of *R. dominica* were released into each jar. Different concentrations of 1×10^6, 1×10^7 and 1×10^8 (conidia/spores/ml) of fungi were prepared for the experiment.

The insecticidal aptness of different concentrations of fungi against *R. dominica* was studied according to the following parameters.

Eggs number

Average eggs numbers per grain laid by *R. dominica* were counted to determine the influence of different treatments on its egg laying capacity (fecundity). From each jar grains were haphazardly nominated and number of eggs was counted. Finally, to check the eggs number on grains in every jar, average was calculated.

F_1 adults emerged numbers

Number of F_1 (freshly developed) matures in every jar were counted to check inhibition of *Rhyzopertha dominica* development by changed concentrations of both entomopathogenic fungi.

Percent inhibition rate (% IR):

Following formula was applied to check percentage decline in development of F1 adults or inhibition ratio:

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

Wherethat is,

Cn = It is number of newly developed adults (matures) in an unprocessed plastic jar (control)

Tn =It is number of freshly developed adults (mature) in treated plastic jar

Days to 100% mortality of F_1 emerged R. dominica:

Days to 100% mortality of F_1 *R. dominica* was similarly calculated to check the result of managements on newly developed generation. Among F_1 generation up to 100% mortality on weekly basis data was documented.

Percent weight loss (%):

Weight loss percentage was counted through following formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight – weight of sound \& damaged grains}}{\text{Initial weight}} \times 100$$

Among F1generation up to 100% mortality on weekly basis data was counted

Experiment No.2

Mortality of*Rhyzopertha dominica*

Mortality of *R. dominica* was determined in treated and untreated of stored grains in each petri-plate of 0.7cm diameter (38.5 cm2), which had a filter paper (Whatman No. 1). Different concentrations of fungi were applied to grains. Each treatment were 3 replications. The death rate of *R. dominica* due to diverse concentrations of both fungi was be observed after 24, 48 and 72 hours. Six beetles of *R. dominica* were released. Petri-plates were placed in an incubator at 30ºC with 70 ± 5% RH.

Statistical analysis

The statistics recorded were exposed to statistical analysis that was using appropriate statistical packages like SPSS 22 , Microcal origin for Windows and Endnote etc.

III. RESULTS

Experiment 1

Mean number of eggs per grain laid by *Rhyzopertha dominica* in wheat grains treat with different concentrations of *M. anisopliae*

Statistically all the treatments were different from 39 others but concentration of 1×10^7 and other is 1×108 spores/ml. The highest reduction in fecundity of LGB was observed when grains were treat with fungal of these concentrations of 1×10^7 and other is 1×10^8 spores/ml with 5.66 and 5.33 numbers of eggs per grain, respectively. The maximum numbers of eggs (6 eggs)were recorded in the concentration (1×10^6 spores/ml). All treatments had proven better in reducing fecundity rather than the control.

Table 1: Mean number of eggs (Mean ± SEM) per grain laid through *R. dominica* in wheat grains treat with different concentrations of *M. anisopliae*

Sr. No.	Concentrations (spores/ml)	Number of eggs (Mean ± SEM)
1	1×10^6	6 ± 0.57a
2	1×10^7	5.66 ± 0.33a
3	1×10^8	5.33 ± 0.33a
4	Control	17 ± 0.57b

Mean number of F_1 adults emergedof pest is R. dominica in wheat grains treat with different concentrations of *M. anisopliae*

Different concentration of *M. anisopliae* was increased the emergence rate of F_1 adults decreased as compared to the control and all the concentrations that were the significantly different from other. The maximum numbers (22 F_1 adults) recorded when we applied 1×10^6 spores/ml of concentration in the wheat grains . On the other hand, the least number of newly appeared adults 18 reported enter in the jar that was treated with concentration of 1×10^8 spores/ml applied.

Table 2: Mean number of F_1 adults emerged (Mean ± SEM) of *R. dominica* in wheat grains treat with different concentrations of *M. anisopliae*

Sr. No.	Concentrations (spores/ml)	Number of F_1 adult emerged (Mean ± SEM)
1	1×10^6	22 ± 0.57 b
2	1×10^7	19 ± 0.57 a
3	1×10^8	18.66 ± 0.33 a
4	Control	47 ± 0.57 c

Percent inhibition rate (Mean ± SEM) of *R. dominica* in wheat grains treated with different concentrations of *M. anisopliae*

Direct relationship among percent inhibition rate and the fungal concentrations. All the treatments were statistically dissimilar from each other. In this fungal concentration of 1×10^8 spores/ml reported with in the maximum inhibition rate (73%) followed by concentration of 1×10^7 and other is 1×10^6 spores/ml by being less than 73% IR. Concentration of 1×10^7 and 1×10^6 is 64% and 58%. Results depicted that all the treatments had proven effective against LGB apart from control

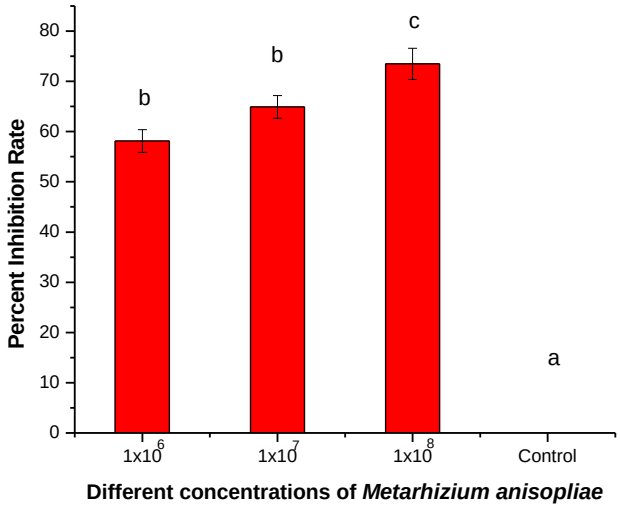

Figure 1: Percent inhibition rate (Mean ± SEM) of *R. dominica* in wheat grains treated with different concentrations of *M. anisopliae*

Days to 100% percentage mortality of F_1 emerged (Mean ± SEM) of *R. dominica* in wheat grains treat with different concentrations of this fungi *M. anisopliae*.

For the 100% mortality of newly emerged pulse beetle the least 9.33 days was required when jars were treated with 1×10^8 spores/ml of entomopathogenic fungus concentration. The maximum 13 days were reported for 100% mortality of F_1 adults upon application of concentration 1×10^6 spores/ml. All the treatments performed well other than the control.

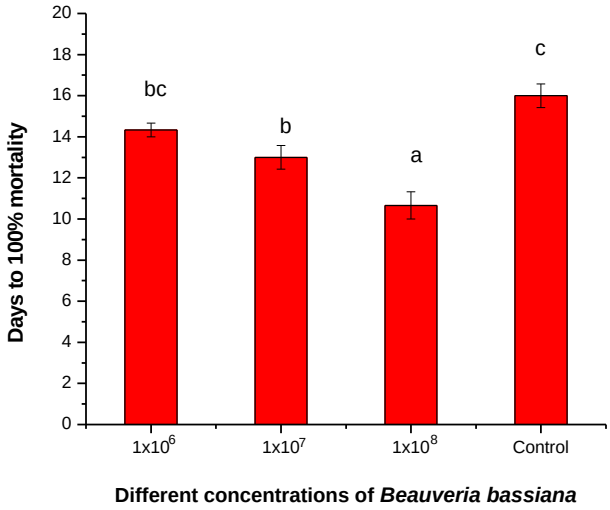

Figure 2: Days to 100% percentage mortality of F_1 emerged (Mean ± SEM) of *R. dominica* in wheat grains treat with different concentrations of *M. anisopliae*

Percent weight loss (Mean ± SEM) caused by *R. dominica* in wheat grains treated with different concentrations of *M. anisopliae*

All the fungal concentrations were statistically differ from other and resulted in effective virulence against LGB except the control as 92% weight loss was reported. Among fungal concentrations, the highest percent weight loss (25%) was caused by LGB when the concentration (1×10^6 spores/ml) was applied to grains. Whereas concentration of 1×10^8 conidia/ml was most effective with less than 18% weight loss to stored wheat grains.

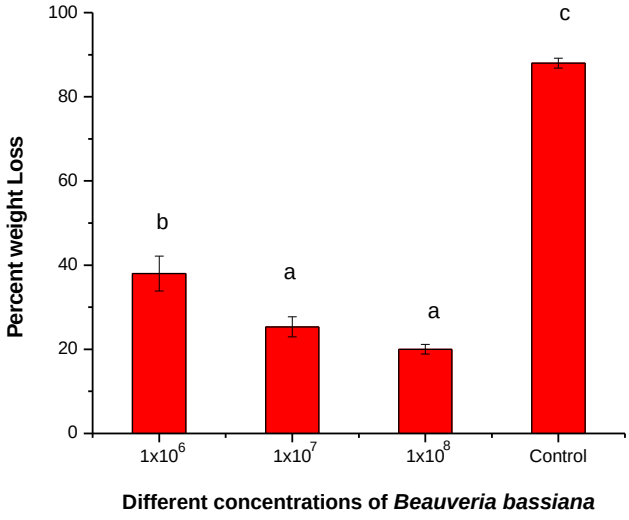

Figure 3 : Percent weight loss (Mean ± SEM) caused by *R. dominica* in wheat grains treat with different concentrations of *M. anisopliae*

Experiment No. 2

Mortality of *R. dominica* after hours 24, 48 and other is 72 hours

Results revealed that fungal concentrations of 3 times directly proportional to the percent death rate and the time intermission. The extreme 60% percent mortality of LGB was obtained after 24 hours with the concentration (1×10^8 spores/ml) of *M. anisopliae*. Statistically all treatments had proven better

results and dissimilar results in causing mortality after 24 hours rather than the control. 10% and 36.66% mortality was noted when treated with concentration of 1×10^6 and 1×10^7 spores/ml, respectively.

After 48 hours of experiment, near about 75 percent mortality caused by the fungal concentration 1×10^8 spores/ml that was significantly different from the pathogenicity of the concentrations (1×10^6 and 1×10^8 spores/ml) with 22% and 50% mortality of *R. dominica* adults, individually.

A significant mortality of 93.33% was observed after 72 hours when wheat grains were treated with the fungal concentration 1×10^8 spores/ml while the concentration (1×10^7 spores/ml) caused 60% mortality after same time interval. The least 40% mortality was reported when 1×10^6 concentration of EPF was used, which was considerably more than the mortality in control.

The concentration 1×10^8 after 24 hours, 1×10^7 after 48 hours and 1×10^7 spores/ml after 72 showed significantly similar results with being more or less than 60% mortality. Meanwhile same consequences were revealed by 1×10^6 spores/ ml of fungal concentration after 24 and 48 hours of interval.

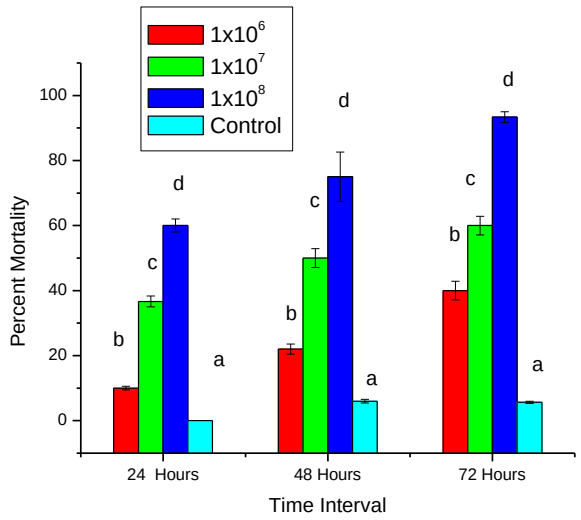

Figure 4: Mortality (%) of *R. dominica* after 24, 48 and 72 hours after application of entomopathogenic fungus *M. anisopliae*

IV. DISCUSSION

Results of this research were similar to Anitha *et al.* (2015) who observed that the mortality rate was directly proportional to the fungal concentration as well as exposure. Riasat *et al.* (2011) conducted research and revealed that the maximum mortality of stored product insect pests like beetles was resulted with the highest dose and exposure time interval and similar behavior was observed in our research experiment. Conducted experiment revealed that entomopathogenic fungi can cause remarkable mortality along with the reduction in the fecundity rate at the higher fungal concentrations whereas, similar effects was reported by Vanmathi *et al.* (2011) using entomopathogenic fungus *B. bassiana* against *R.dominica.*

Pathogenicity comparison among *B. bassiana* and other is *M. anisopliae* reported by the Abdel-Raheem *et al.* (2015) determined that more virulence was obtainable by this fungi *M. anisopliae* toward the all stored grains insect pests (lesser grain borer, saw-toothed grain beetle and other is rice weevil). In our study, efficacy of entomopathogenic fungus *M. anisopliae* was greater than *B. bassiana*. Death rate of adults was rise with the change in the fungal different concentration while oviposition rate, emergence of F_1 adults and damage rate decreases with the increase in the fungal concentration. However, both *M. anisopliae* and *B. bassiana* fungi had proven better in controlling stored product insect pest.

We find in our study that *M. anisopliae* was more efficient against *R. dominica* and *O. surinamensis* than the fungus *B.bassiana* and proved that *M. anisopliae* had higher virulence than *B. bassiana*. *M. anisopliae* and *B. bassiana* were less efficient against S. *oryzae*. The entomopathogenic fungi *B. bassiana* and *M. anisopliae* have potential for effective and economically feasible control of stored product insects according (Salem *et al* ,2015)

V. CONCLUSION

Results revealed that the higher concentrations were more effective than the lower concentrations of EPF. Fungal efficacy was directly proportional to the time exposure. EPF fungus *M. anisopliae* was virulent against *R. dominica* in stored wheat grains.

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