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The Impact of Entomopathogenic fungi on the Mortality rate of *Rhyzopertha dominica* (F.) on wheat grains under in laboratory condition

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Abstract
Effectiveness of two entomopathogenic fungi, *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschn.) against *Rhyzopertha dominica* was checked under *in vitro* condition. During the study, a homogenous culture of lesser grain borer was developed and tested under controlled lab conditions. Three concentrations (1×10^8, 5×10^7 and 25×10^6 CFU) of each fungus were used by spraying 50 gram of wheat in the combined and separate form at 30±2ºC with 65±5% R.H. Pacer (R) (*Metarhizium anisopliae)* and RACER (TM) (*Beauveria bassiana*) commercially available product by Agri Life India were considered in this study. Mortality data were recorded at three exposure time intervals (5, 10 and 15 days). Research outcomes publicized that by increasing the concentration of fungi their efficacy was also increased. Moreover, in the combined application of (*Beauveria bassiana* + *Metarhizium anisopliae*) gave the highest mortality percentage of *Rhyzopertha dominica* F. as compared to a single application.

Keywords: wheat, mortality rate*, Beauveria bassiana, Metarhizium anisopliae, Rhizopertha dominica*

Introduction

Wheat (*Triticum aestivum* L.) is a common cereal crop cultivated worldwide for commercial purposes and used as a staple food in many countries (Taylor and Koo, 2012) [19]. Globally wheat emerge as a third-largest food crop and Pakistan is the 8th largest wheat producer in the world (Shuaib *et al*. 2007) [17]. Although all sources are being used to increase the production of crops to meet the world's food requirements but unfortunately every year almost, one-third of the harvested crops are damaged by the attacking pests (Dubey *et al*. 2008) [6]. These insect pests not only decrease the foodstuff quantitatively but also ruined the quality of food. Stored grain products attacked by the insect pest not only lose their weight but nutrients deficiency also occurs (Phillips and Throne, 2010) [14]. In Pakistan, wheat is stored in a bulk quantity for food supply purposes (Syed *et al*. 2001). However, this wheat crop when stored in 3 to 4 months are susceptible to many insect attacks. Attack of insect pests on stored grain products start from the field and continue even when they are stored in different structures and places. Stored grain insect pests play a vital role in deteriorating the food commodities which are placed for storage (Singh *et al*. 1990) [18]. More than twenty insect species have been reported as the most destructive insect pests of stored products. Coleoptera's order is important in this regard to having more than 70% of stored product insects (Vinuela *et al*. 1993) [21]. *Rhyzopertha dominica* is the most destructive pest of wheat, maize and other stored grains. This stored product pest is a strong flier and can move easily from one place to another. In this way more than one storage house is exposed to this notorious pest (Khan and Marwat, 2004) [8]. It can survive in dry conditions and also can damage the grains in the summer season by producing off-odor (Vassanacharoen *et al*. 2008) [20]. By attacking on the internal side of grains it continues to feed and consumed the whole-grain only the seed shell is left behind (Bashir, 2002) [2]. Usually its grub stage is known as internal feeder as most of the lifetime is spent inside the grains. Due to internal feeder, controlling this pest by using synthetic chemical insecticides is a difficult task. At the surface of grains female first lay eggs then first instar grubs appeared to make holes in the grains and continue to feed inside the grain. They remain in the grains until the emergence of adults with a life span of 3-6 months (Peter, 2012; Edde, 2012) [13, 7]. Different synthetic chemicals are used to control the stored grain insects in different forms, such as Chlorpyrifos-methyl, pirimiphos-methyl, Deltamethrin and Cyfluthrin. Although these insecticides have the potential to control stored insects but their application may cause health and environmental problems. Continuous use of chemical insecticides developed resistance to insects with time and responsible for the elimination of beneficial insects (Batta, 2005) [3]. So researchers and scientists are working to use some new strategies and substances which should be environmentally safe. Entomopathogenic fungi can give promising results against stored product insects by keeping the environment safe. These fungi are usually host-specific and most probability is there that they will not cause any harmful effects for non-targeting beneficial insects (Padin *et al*. 2002) [12]. Conidia is the basic unit of these entomopathogenic fungi which are produced asexually and through which they infect their host. When these conidia come in contact with the exoskeleton of insects and the environmental conditions are feasible, they germinate and penetrate the insect bodies (Richard *et al*. 2011) [16]. Commercial formulations of these entomopathogenic fungi are now available in markets that are being used against stored product insect pests like lesser grain (Quarles, 1995) [15]. For many years, the potential use of *M. anisopliae* and *Beauveria bassiana* can harmful to stored insect pests because of their desiccation stability. Keeping in all the view, in this study application of entomopathogenic fungi against *Rhyzopertha dominica* have an eco-friendly approach and non-hazardous to the environment.

Materials and Methods

Research work was carried out in Grain Research, Training and Storage Management Cell of Department of Entomology, University of Agriculture, Faisalabad, during the year, 2015.

Rearing the insect culture

The wheat which infested with the *Rhyzopertha dominica* was collected from Grain Market, farmer's storages and cereal storage rooms of Mailsi (Vehari). This infested wheat was sieved out to separate the adults of lesser grain borer (*Rhyzopertha dominica*). Controlled conditions (30±2ºC and 60±5% R.H) for two weeks were provided to maintain the homogenous adult population of lesser grain borer and further consumed in a lab bioassay.

Fungi and their concentrations

Two different bio-insecticides Racer® and Pacer® *(Beauveria basiana* and *Metarhizium anisopliae* respectively) were taken from Agri-Life® Hyderabad, Andhra Pradesh, India. Three concentrations (1×10^8, 5×10^7 and 25×10^6) were made in separate as well as in a combined form with (1:1) ratio of each fungus. The availability of these bio-pesticides are in powder form, therefore for preparing formulation distilled water was used.

Effectiveness of two entomopathogenic fungi in separate and combined form against *Rhyzopertha dominica* (F.)

Standardized population of equally aged newly emerged adults of *Rhyzopertha dominica* was released on treated wheat with Racer® 0.1 g (25×10^6 CFU), 0.5 g (5×10^7 CFU) and 1g (1×10^8 CFU) and Pacer® 0.1 g (25×10^6 CFU), 0.5 g (5×10^7 CFU) and 1g (1×10^8 CFU) and with their combination with 1:1 ratio. Three replications and one control of each treatment were used. Plastic jars were used in this research. Each treatment consists of 50-gram sterile wheat grain which were placed in plastic jars and 30 insects from the homogenous population were released in each treatment. Formulation of entomopathogenic fungi were sprayed on these jars and controlled was sprayed by distilled water. For this experiment artificial environment was maintained according to previously described controlled conditions. To find out the Mortality % Abbott formula was applied

$$\% = 1 - (\text{n in T after treatment})/(\text{n in c after treatment}) \times 100$$

(Abbott, 1925)[1]. Where: n = Insect population, T = treated, Co = control.

Analysis of Data

The data was analyzed by Complete Randomize Design (CRD) design using statistical software and means were separated using Tukey's HSD at P>0.05.

Results

Effectiveness of two entomopathogenic fungi in separate and combined form against *Rhyzopertha dominica* (F.)

05 day of exposure

After 05 d of exposure, the results were illustrated that synergistic effect of *B. bassiana and M. anisopliae* showed maximum mortality percentage at all applied concentrations (9.6%) at 1×10^8 CFU, (8.2 %) at 5×10^7 CFU and (6.4%) was observed at 25×10^6 followed by *B. bassiana* 5.8 percent at 1×10^8 CFU, 5.4 % at 5×10^7 and 3.1 % at 25×10^6 CFU. While the minimum mortality (1.02 %) was obtained at 25×10^6 CFU of *M. anisopliae* respectively Fig. 01. Results revealed that the percentage of mortality was increased with the increase in concentration both in combined as well as single fungal formulation.

10 day of exposure

Similar findigs regarding mortality percentage of *Rhyzopertha dominica* (F.) were obtained during 20 d of exposure indicated that mortality percentage at all applied concentrations (1×10^8, 5×10^7, 25×10^6 CFU) was found maximum by using the combined effect of *B. bassiana and M. anisopliae* (18.6%, 14.2 %, and 10.2%) followed by *B. bassiana* (13.2, 10.4, 8.6 percent). While the minimum mortality (4.02 %) was calculated at 25×10^6 CFU of *M. anisopliae* respectively Fig 02.

15 day of exposure

Statistical evidences at 30 days of exposure are demonstrated that mortality % (38.1, 34.5, 30.3) was achieved at 1×10^8, 5×10^7, 25×10^6 CFU of *B. bassiana + M. anisopliae* mixture followed by *B. bassiana* 29.4, 26.13, 22.4 % while the minimum mortality (23.3%, 17.4% and 13.2%) was measured in *M. anisopliae* respectively Fig 03. By increasing the concentrations in singly or combined form, the mortality percentage was also increased.

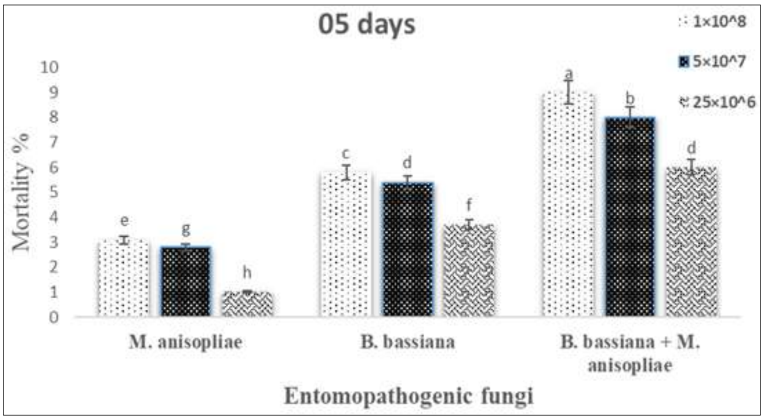

Fig 1: Comparison of mean values of the data regarding percent adult mortality of Rhyzopertha dominica (F.) at five day of exposure against single as well as combined effects of treatments and concentrations. Standard error with the sign of ± represents the mean value

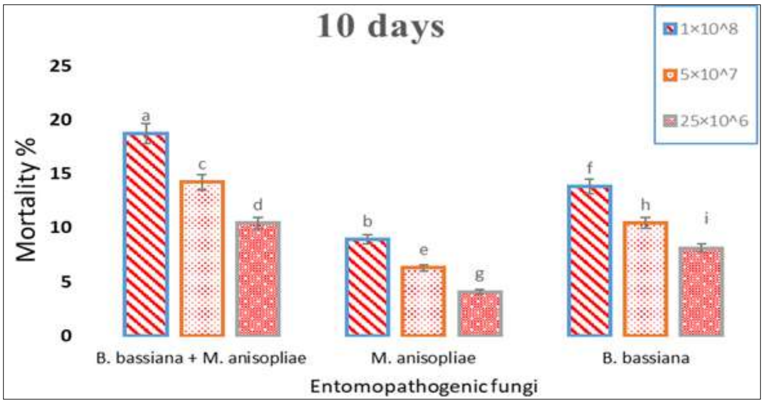

Fig 2: Comparison of mean values of the data regarding percent adult mortality of Rhyzopertha dominica (F.) at ten day of exposure against single as well as combined effects of treatments and concentrations.

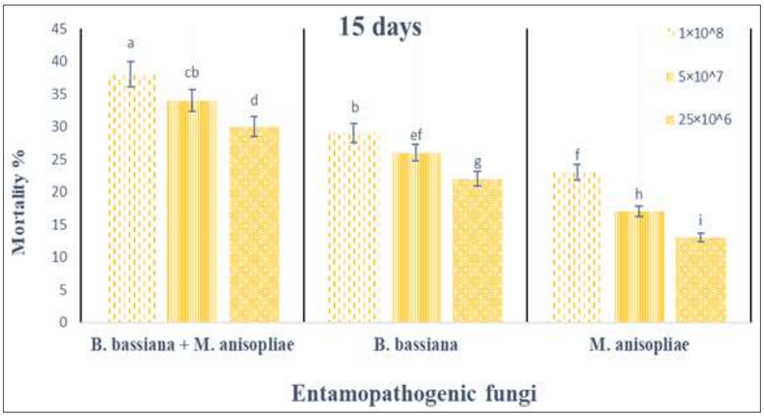

Fig 3: Comparison of mean values of the data regarding percent adult mortality of Rhyzopertha dominica (F.) at fifteen day of exposure against single as well as combined effects of treatments and concentrations.

Discussion

The use of entomopathogenic fungi against stored product insect pests is getting common day by day and commercially available as a biopesticides in local markets (Meikle *et al*. 2001) [11]. Zimmermann (2007) [22] elaborated that entomopathogenic fungi are responsible for the mortality of the insects in 6 different steps. In the first step adhesion of conidia occurred to the insect cuticle. In the second step these adhered conidia start germination on the insect body and in third these conidia penetration in the cuticle of their host. After this step they defeat the host defense system. In the fifth step the vegetative growth of these fungi starts inside the insect body. In the final step mortality of the population is occurred. According to our study the first outcome of this research regarding the mortality of adult *Rhyzopertha dominica* (F.) through entomopathogenic fungi illustrated that percentage mortality increases by increasing the concentration of these entomopathogenic fungi. The second outcome of the study is that the time interval also plays an important role in mortality percentage. Similarly, Bello *et al*. (2001) [4] experimented by using *Metarhizium anisopliae* against *S. oryzae* and examined the mortality of insect pests at different intervals of times. they experimentally proved the mortality data from 3 to 14 days and found 40% mortality occurred at the 14th day of exposure. Secondly, the synergistic effect of both entomopathogenic fungi *B. bassiana and M. anisopliae* caused the highest mortality with the comparison to the single application that was matched with work conducted by (Bugeme *et al*. 2008) [5] who checked the Combine effect of *Metarhizium anisopliae* and *Verticillium lecanii* against *Callosobruchus chinensis* and *Rhyzopertha dominica* showing 50.11% and 43.31% mortality respectively at 15 days' interval. But when these bio-pesticides were sprayed individually they did not give good results. Lord (2001) [10] also proved experimentally the synergistic interaction between several entomopathogenic fungi against *R. dominica* (F.). Single formulation of entomopathogenic fungi against insect pests is less affected as compared to their combined application (Kryukov *et al*. 2009) [9].

Conclusions

It is concluded that the combined application of *Beauveria bassiana* and *Metarhizium anisopliae* gives the best results under lab conditions to control the homogenous population of *Rhizopertha dominica* F.

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