Effect of entomopathogenic fungi *Verticillium lecanii* on lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in the stored-wheat

Research Project

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CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the **BSc**. degree in **Agricultural Science** – **Plant Protection** with my approval as a supervisor.

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DEDICATION

I dedicate this review article to:

The sake of Allah, my creator and my master, my great teacher and messenger: Mohammed (my Allah bless and grant him.), who taught us the purpose of life.

I dedicate all my efforts and struggles of the educational life to my dear parents; without them I'm meaningless.

Also, I devote the work of this internship report to respectable and honorable teachers

Yousif Alkhan Abdulhakeem 

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Abstract

Every year stored product pests cause significant losses to agricultural products. Their control depends heavily on the use of fumigants and other insecticides, which have many negative consequences for humans and the environment. Nowadays, the use of fungal entomopathogens is one of the most promising alternatives to reduce the use of chemicals in storage facilities in that we tested entomopathogenic fungi *Verticelium lecanii* against adults of the lesser grain borer *Rhyzopertha dominica* (Coleoptera: bostichidae). Insects were sprayed with a three-dose rate (0.2, 0.4 and 0.6) of *v. lecanii* at 1.87×108 spore/ml and dead insects counted after 3, 7, 14, 21, 28 and 35 days under laboratory conditions. Mortality in adult insects reaching 6, 46, 56, 66, 73 and 83% after 35 days at 0.6g the insect mortality increased with the highest concentrations. Mortality recorded the lowest after 3 days in all doses. This study confirmed that *v. lecanii* could be used as Biocontrol agents against *R. dominica*.

1- Introduction

The cereals are annual common grass members of the grass family (a monocot family Poaceae, also known as Gramineae), which usually have long, thin stalks, such as wheat, rice, maize, sorghum, millet, barley and rye, whose starchy grains are used as food (Sarwar, M. H. *et al* 2013). wheat is naturally a good source of proteins (8-12%), vitamins such as Vitamin E, minerals such as Iron, Zinc, and dietary fibers,so, Wheat flour is used as the major ingredient in most of the breads (Anjum *et al*., 2006).

Stored product insects are one of the top pest concerns in food production and storage across the globe, stored product insects not only eat these products, they breed in them and can spread quickly, contaminating a huge amount of product which is then unfit for human consumption (British pest control association, (2019). Pests of stored grain such as Lesser Grain Borer (*Rhyzopertha dominica* F) is one of the pests which are classified as primary pests that can be invested on various types of seeds such as wheat, corn, rice and other cereal crops (Fajarwati *et al*., 2019).

Rhyzopertha dominica is the most economically important primary pest of stored grain in the United States and is a strong flier that can tolerate high temperatures and dry grain (moisture content less than 12%). Heavy infestation with lesser grain borers can be identified by a sweetish, musty odor in the storage. This odor is a result of the male-produced aggregation pheromone that has been demonstrated to be an effective lure for use in traps (David K. *et al*, 2004).

Chemical insecticides have been widely employed for the control of storage grain pests. This has caused such problems as insecticide resistance along with contamination of foodstuffs with chemical residues.

Entomopathogenic fungi have low environmental impact and are generally considered environmentally safe agents with low mamma- lian toxicity (Rumbos and Athanassiou 2017). Entomopathogenic fungs do not decrease the quality of the grains and thus enhances the marketability of stored products (Steenberg, 2005). More than 700 species of fungi belonging to 90 genera among *Beauveria bassiana, Metarhizium aniso-pliae, Verticillium lecanii, Purpureocillium lilacinum*, and *Isaria fumosorosea* are the widely used ones as bio- logical control agent against many agricultural pests Entomopathogenic fungs do not decrease the quality of the grains and thus enhances the marketability of stored products (Steenberg, 2005). Especially, the species *B. bassiana* and *M. anisopliae* have a wide host range and have been tested against most of the major stored-grain pests (Batta 2018; Rumbos and Athanassiou 2017). The aim of this study is to using alternative methods (safe method) to chemical control and determine the economic losses caused by *Rhyzopertha dominica*.

2- Literature review

2-1: Importance of wheat

Cereals play an important role in world agriculture. They contribute significantly to the global food pool in achieving food and nutritional security. Cereal grains provide a major source of energy, protein, and dietary fiber in human nutrition (Vasal, 2002). The major cereals consumed worldwide are wheat, rice, maize, barley, oats, rye, millet, sorghum. Apart from being an important part of diet, these cereals are also rich in various health promoting components (Slavin, 2003).

Wheat is a various species of the genus *Triticum* and it is a grass with so many important uses that it is cultivated worldwide and is one of the most important cereal crops of the world and is a staple food for about one-third of the world's population (Hussain *et al*., 2002; Awoke *et al.,* 2017).). It is not only an important crop today; it has also influenced human history. Wheat was a key factor enabling the emergence of city-based societies at the start of civilization because it was one of the first crops that could be easily cultivated on a large scale, and had the additional advantage of yielding a harvest that provides long-term storage of food (Elasraag, 2015).

Wheat is nutritious food worldwide and provides proteins, minerals, B-group vitamins and dietary fiber more in quantity than other cereal crops and help in preparation of different types of foods. The wheat grain consists of three distinct parts: bran (13-17%), germ (2-3%) and endosperm (80-85%) and contains all essential nutrients. In general, 70% carbohydrates, 12% water, 2% fat, 12% protein, 1.8% minerals, and 2.2% crude fiber are found in wheat grain kernel, it is

also enriched with phosphorus, magnesium, manganese, zinc, selenium, iron, potassium and copper (Liu *et al*., 2012).

2-2: lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

2-2: 1: Description

R. dominica is a holometabolous insect, or it undergoes complete metamorphosis. The lifecycle includes four stages: egg, larva, pupa, and adult. Adults are 2.3 – 3.0 mm long, dark red 4 brown/black in color with a cylindrical body. The elytra have regular rows of coarse punctures covered with curved setae. When viewed from above, the head is not visible and has its antennae end in a distinctive 3-segmented club-shape. (Astuti et al. 2013).

Fig 1: stages of *R. Dominica*

https://grainscanada.gc.ca/en/grain-quality/manage/identify-an-insect/primary-insect-pests/lesser-grain-borer.html

The newly hatched grub is quite active and white in color, with a tiny brown head and live inside grain. Pupae is dirty white in color and irregular in shape (Sharma & Choudhary, 2008). The eggs are ovoid in shape, 0.6 mm in length, 0.2 mm in diameter, white when first laid, and turn rose to brown before hatchi (Edde, 2006).

2-2: 2: Economic importance

The lesser grain borer, Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae), is a major pest of stored grains, especially wheat, maize, rice, and sorghum in warmer regions of the world (Haines 1991).

Zacher (1932) gives information on the first food products to which the grain beetle *Rhizoperta dominica* F. has been found. He reports that the species was found in the Tut Ank Amon tomb in wheat dating from 1345 BC. *R. dominica* is a primary insect pest of stored grain with adults feeding on whole or cracked grain with larvae developing inside kernels. Adults lay eggs loosely near intact kernels, according to (Sharma & Choudhary, 2008) Both grubs and adults cause damage but the adult is more harmful than the grub. They feed within the interiors of the grains and the kernels are reduced to mere shell. The young grub eats out the starchy content by boring the grain as it is straight but in the later stage the grub is curved and hence is unable to penetrate the grain and thus feeds on the waste flour left by the adults.

Fig 2: wheat infestation

https://extension.entm.purdue.edu/publications/E-238/E-238.html

2-3: Entomopathogenic fungi *Verticillium lecanii*

Verticillium is a genus of fungi in the division Ascomycota. Within the genus, diverse groups are formed comprising saprophytes and parasites of higher plants, insects, nematodes, mollusk eggs and other fungi thus it can be seen that the genus is a wide-ranging group of taxa characterised by simple but ill-defined characters. The genus may be broadly divided into three ecologically based groups 1) mycopathogens 2) entomopathogens (Zare and Gams, 2001) and 3) plant pathogens and related saprophytes (Barbara and Clewes, 2003).

It is tolerance for a range of environmental conditions and makes its registration easier than for a foreign organism. High level of field efficacy, low cost, easily mass produced and applied, compatible with other tactics, non-toxic to mammals and minimal potential for development of pest resistance. The mode of action is based on the direct contact between spores and insects (Batta, y. A. & Kavalliieratos, N. G. 2017).

The using of EPFs for the control of the insect pests in stored-grain products is one of the most promising alternative control methods (*Moore et al.* 2000. Especially, the species *B. bassiana and M. anisopliae* have a wide host range and have been tested against most of the major stored-grain pests (Batta 2018; Rumbos and Athanassion 2017).

Many of these naturally occurring pathogens have been formulated and commercialized as insecticides. Among entomopathogens, fungi have garnered the most interest for research and use as biologically based insecticides (Cerruti, 2021).

2-4: Effect of Entomopathogenic fungi to stored product insects

Successful control of stored grain pests with entomopathogenic fungi has been reported by a number of researchers. The first study on application of entomopathogenic fungi as formulated materials for control of storage pests was carried out by Hluchi and Samsinakova (1989). They used Boverosil®, a formulation as wettable powder (containing 5.92×109 conidia g-1 powder) from *B. bassiana* for control of S. granarius adults. They noted that Boverosil® can cause mortality in this insect, since 90% mortality was recorded at 5.92×108 conidia ml-1.

(Mantzoukas, *et al* 2020) were used three entomopathogenic fungal species, *Beauveria bassiana , Metarhizium anisopliae* and *Isaria fumosorosea* on larvae of the three very common and destructive stored product pests of the larvae: khapra beetle (*Trogoderma granarium* Everts), the confused flour beetle (*Tribolium confusum* Jacquelin du Val) and the Mediterranean flour moth (*Ephestia kuehniella* Zeller) in rice seed and wheat grain were sprayed with 1 mL of conidial suspension (108 conidia/mL) from each isolate after 10 days: On wheat seeds, the mean mortality of larvae *ranged between 16.67% (M. anisopliae) and 53.33% (I. fumosorosea) for T. granarium*, between 23.33% (*M. anisopliae*) and 43.3% (*I. fumosorosea*) for *E. kuehniella* and between 26.67% *(B. bassiana)* and 63% (*I. fumosorosea*) for *T. confusum*, and significant differences were detected among fungal isolates. *I. fumosorosea* was the most virulent strain to the *T. confusum* larvae, in comparison with the larvae of the other species. On rice seeds: the mean mortality of larvae was calculated between 33.33% *(I. fumosorosea)* and 46.67% (*B. bassiana)* for T. granarium, between 26.67% *(B. bassiana)* and 46.67% *(I. fumosorosea)* for *E. kuehniella* and between 20% (*B. bassiana*) and 33.33% (*M.*

anisopliae) for *T. confusum*, and significant differences were detected among fungal isolates. *I. fumosorosea* was the most virulent strain to *E. kuehniella* larvae while *B. bassiana* was the most virulent for *T. granarium*, in comparison with the larvae of the other species.

In 2009 Mahdneshin *et al* indicated that the cumulative mortality of *R. dominica* after 7 days varied from 14.78 with the lowest concentrations of M. anisopliae (1.5x104 conidia·mL-1) to 89.35% (1.1x1010 conidia·mL-1) with the highest concentration of B. bassiana by using the immersion technique.

The mortality of three entomopathogens fungi, *Metarihizium anisopliae*, *Isaria furnosoroseus* and *Beauveria bassiana* were evaluated for their virulence against the grubs of Khapra beetle, *Trogoderma granarium* (Everts) under laboratory conditions. The maximum mortality (98.33%) of *T. granarium* was observed in the application of *M. anisopliae* in a single concentration of 1 × 108 and differed significantly from other Entomopathogenic Fungi (EPF). Significantly minimum mortality (81.67%) of the pest was recorded in the application of *I. fumosoroseus*. The application of *B. bassiana* had an intermediate effect showing 90.83 percent mortality of the pest and differed statistically from the effect of other EPF (Iqbal, *et al*, 2021).

3- Material & Methods

3-1: Insect rearing

The experiment was conducted in incubator at the entomology lab (12) of plant protection department, Salahaddin University- Erbil.

Insect rearing Adults of *R. dominica* collected from infested stored wheat grain were reared on healthy wheat grains, the mouth of each glass jar was covered with muslin cloth and tied with the help of rubber bands. The glass jars were kept in an incubator at a constant temperature of 25 ± 5 0C and 65 ± 5% relative humidity.

Fig 3: Insect rearing

3-2: Entomopathogenic fungi *verticillium lecanii*

Commercially produce formulated conidia of *Verticillium lecanii* (Zimmerman), containing 1.87×10^{8} spore/ml conidia per gram of powder was used and kept at 4°C in refrigerator.

Each run of the experiment consisted of an untreated control and two different treatments powder of *verticillium lecanii* with wheat. Petri dishes containing treated and control insects were sealed with elastic bands. Mortality was recorded after 3, 7, 14, 21, 28 and 35 days.

3-3: Powder

Verticillium lecanii was applied as powder at three concentration (0.2, 0.4 and 0.6 g) in (1.87×10^8 spore/ ml). Petri dishes contain filter paper with (5gram wheat and 10 adults).

Fig 4: *Verticillium lecanii* concentration (Powder)

10 adults of R. dominica were transformed into petri dishes containing steril filter paper (9 cm diameter) then Verticillium lecanii was applied (1.87×10^8 spore/ ml) at three concentration were sprayed (0.2, 0.4 and 0.6 ml/g) powder aftersealed with acloth mesh to prevent them from escaping.

The Control group without *V. lecanii* and distils water.

Results and Discussion

The effect of entomopathogenic fungi *verticillium lecanii* on stored wheat at the dose rates of (0.2, 0.4 and 0.6 g) in (1.87×10^8 spore/ ml) of wheat and the bioassay was conducted at 25°C with 60% relative humidity.

Based on the data given in Tab. 1, our analyses show that the data regarding the mortality was recorded after 3 to 35 days exposure intervals. All the treatments gave the significant mortality of *R. dominica* and *V. lecanii* of 0.6 g was found to be the most effective after a 35 days exposure interval.

Table. 1: Mortality percentages of *R. dominica* indicated days treated with *Verticillium lecanii* at different concentrations:

Powder *V. lecanii*	Mortality percentages of *R. dominica* indicated days					
	3	7	14	21	28	35
0.2	6	10	23	30	36	41
0.4	6	23	30	31	36	48
0.6	6	46	56	66	73	83
Control	0	0	0	1	1	2

These results show that the effectiveness of entomopathogenic fungi depends on both the fungal concentration and the time elapsed after the treatment. EPFs can be effective when applied directly on the insect or when the insect contacts the applied surface (Castrillo *et al*. 2013).

Mortality after treatment varied from 6% at low concentration (0.2) to 83% at the highest concentration (0.6 g). Percent mortality of *R. domonica* was significantly

increased after 3 days exposure interval and exceeded 50% in all concentration, the highest mortality was reached to more than 80% at (0.6g) 35 days after treatment. Mortality reached more than 50% after 14 days with the highest concentration. These results are in agreement with those of Mahdneshin *et al*. (2009) who reported that isolates of *B. bassiana* and *M. anisopliae* were more effective against adult *R. dominica* with higher conidial concentrations and LC50 values of $9.6x10^5$ to $1.9x10^7$ conidia·mL

Abdel-Raheem et al. (2015) showed that *M. anisopliae* isolates were more effective against insect pests, where the LC50 values were $1.2x10^5$ and $2.7x10^5$ conidia·g-1, respectively, and the mortality percentages were 79.3% conidia g-1 and 50.3% conidia g-1 respectively. In 2019 (AK) was showed the efficacy of the five different entomopathogenes fungi, *Isaria fumosorosea, Simplicillium lamellicola, Beauvaria bassiana, Metarhizium anisopliae,* and, *Verticillium lecanii*, against adults of storage-grain pests *S. granarius* and S. oryzae at two different temperatures (20–25 °C) under laboratory conditions was evaluated. Among the EFPs, *I. fumosorosea* (92.69%) and *M. anisopliae* (90.35%) recorded the highest effects on *Sitophilus granarius* at 20 °C at the end of day 7, followed by *B. bassiana* (72.91%), *S. lamellicola* (62.02%), and *Verticillium lecanii* (33.91%). The same isolates were tested at 25 °C, where the highest effect, recorded at this temperature, was by *M. anisopliae* (90.48%), followed by *I. fumosorosea* (84.21%), *S. lamellicola* (59.26%), *B. bassiana* (56.14%), and *Verticillium lecanii* (22.81%).

In contrast, (DRAGANOVA, S. & MARKOVA, E. 2006) showed that the comparison between of *v. lecanii, B. bassiana* and *M. anisopliae* larvae of E. kuhniella and the influence of *B. bassiana* caused the highest lethal effect to larvae - 87.78% ± 13.18, following by isolates 399Bb and 382Bb of *B. bassiana* 60.00%

± 8.14 and 58.89% ± 8.75, respectively. Isolates 15Ma and 31Ma of M. anisopliae and the isolate 32Vl of *V. lecanii* showed less lethal effect in comparison with the other tested isolates. It has been established that only one isolate (383Bb *of B. bassiana*) was with efficacy over 70% to larvae of E. kuhniella.

Conclusion

Our study once again confirmed that the efficacy of a given fungal species is always strain-specific and virulence may vary depending on the host. Using of entomopathogenic fungi *Verticillium lecanii* has potential for effective and economically feasible control of stored product insects because chemical control has many side effects, increasing environmental toxicity and resistance to pest. In addition, chemicals cause damage to beneficial organisms, causing a loss of natural balance.

Verticillium lecanii was more efficient against adults of *Rhyzopertha dominica*, based on the result of our study it is one of the best methods we can use as biological control agents to control of this pest and storage pests

Recommendation

The use of Entomopathogenic fungi as safe methods and biocontrol agents to control stored product insect instead of chemical insecticides. Although further research needs to be carried out, our preliminary results showed that some *V. lecanii* could be studied and used more deeply to develop the best effective methods to be used against R. dominica in storage.

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