

Potency of Entomopathogenic Fungi, *Trichoderma album* Preuss in Controlling, *Rhizopertha dominica* F. (Coleoptera: Bostrichidae) under Laboratory Conditions.

Ghada S. Mohamed¹ and Eman Taha²

¹Plant Protection Department, Faculty of Agriculture, South Valley University, Qena, Egypt.

²Food Science and Technology Department, Faculty of Agriculture, South Valley University, Qena, Egypt.

E-mail: d.ghada1@yahoo.com Email: dr_eman76@yahoo.com.



ABSTRACT

A laboratory strain of *Trichoderma album* Preuss was assessed against the first larval instar and adult stage of *Rhizopertha dominica* F. under laboratory conditions by dipping bioassay. Four dose rates of *T. album* (7×10^4 , 7×10^5 , 7×10^6 and 7×10^7 conidia /ml) were applied. The count for mortality was made after 1, 3, 5 and 7 days. The treated wheat with *T. album* well as untreated wheat sample were stored for 6 months after infestation with *R. dominica* and the quality of the stored wheat was determined. The results showed that the first larval instar was more highly susceptible than the adult stage. The highest mortality percentages were 94% and 74% for *T. album* against *R. dominica* first larval instar and adult stages, respectively, after 7 days exposure at 7×10^7 conidia /ml. the LC_{50} value was 5.61×10^5 and 3.32×10^6 conidia /ml while the LT_{50} recorded at 4.02 and 6.43 days at 7×10^7 conidia /ml on *R. dominica* first larval instar and adult stages, respectively. Wheat properties were enhanced during storage for treated wheat comparing to untreated wheat which it reduced weight loss by 26% also gluten content and color quality of wheat were improved after treatment with *T. album*. These results indicate that the entomopathogenic fungus *T. album* has the potential to be a biocontrol agent for controlling *R. dominica* and a suitable candidate further for research and development.

Keywords: *Rhizopertha dominica*, *Trichoderma album*, biological control, wheat quality

INTRODUCTION

The most important component of the human diet throughout the world are cereal grains. Cereal grains production is seasonal and its consumption is continuous. Therefore, most of the product should be store safely. If the storage conditions are not suitable the grains could be damaged (Delcour and Hosenev, 2010).

The most damage of cereal grains during storage is caused by insects. Insect pest infestation has been reported to reduce the quality of cereals in terms of protein, gluten, starch, amino acids ,etc. also it may cause around 50% loss of the total production of cereals in some countries (Fornal *et al.*, 2007) owing to insect feeding on grains. As well as mixing of insect fragments that making it unfit for consumption. One of the most harmful pests of stored cereals grain especially wheat is *R.dominica*, that commonly called as lesser grain borer. *R.dominica* presents throughout warmer regions of the world. The effects of *R.dominica*, on some properties of grain and flour during storage have been reported (Keskin and Ozkaya, 2013). Valuable physical damage, weight loss and Lack of vitality result from internal and external feeding by *R.dominica* larvae and adults, respectively. The mature adult emerges from the kernel by dull a sizable exit hole, producing great damage for wheat kernel (Ozkaya *et al.*, 2009).

Chemical control of stored-grains pests is very hazardous because the stored products are used for human feed. Also the environment is damaged by the chemical insecticides. (Gandhi *et al.*, 2011). On the other hand the effectiveness of some chemical controls is often reduced because stored product insect pests population is often located in protected sites, such as inside the grain, where pesticide penetration is difficult (Arnaud *et al.* 2005).

Therefore, in recent years, many researchers have been looking for safe alternative methods for protection of stored grain in warehouses. A number of

entomopathogenic fungi have been reported in various studies effectively as biological control agents of stored product insect pests (Michalaki *et al.* 2006, Wakil and Ghazanfar 2010). *Trichoderma* spp. has been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers. Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defense system are typical biocontrol actions of these fungi (Khaleil, 2016). At recent days members of the genus *Trichoderma* can be effectively used against different insect pests. It has been recorded to stored product insects pest (Hussein *et al.* 2013). Recycling of the disease in the neighboring area occurred by infected insects, Moreover these infected insect greatest opportunity in the recurrence of the disease as the effectiveness of mycopesticide (Wood and Thomas 1996). Therefore more research in this field is increasing essential. The aim of this study to evaluate the biocontrol potential of *T. album* against *R.dominica* under laboratory condition and during 6 months of wheat storage.

MATERIALS AND METHODS

1. Insect rearing:

Insect culture *Rhizopertha dominica* was collected from the public wheat storages and reared on whole wheat grains. Insect cultures were maintained in plastic jars (1 liter) covered with muslin cloth. All *R. dominica* insects were reared under condition of $27 \pm 1^\circ\text{C}$ and $75 \pm 2\%$ R.H. in the laboratory of Plant Protection Department, Faculty of Agriculture, South Valley University, Qena, Egypt.

2. Source of fungi:

1- Fungus, *Trichoderma album*

The species of the fungus, which was used in this work *T. album* Preuss, was obtained from Assiut University Mycological Center (AUMC). The

entomopathogenic fungus *T. album* was kept in the refrigerator until using them for conidiospores production.

2- Fungal conidial preparation:

The fungus was grown on a media of Potato Dextrose Agar (PDA), which consisted of 250g potatoes, 25g dextrose, 20g agar and 1000ml distilled water. The medium was autoclaved at 120°C for 20 minutes. Then, the medium was poured in Petri dishes (9cm diameter×1.5cm) under sterilized conditions. The fungus was inoculated under these sterilized conditions and then incubated at 25- 30°C in the dark for 15 days. Conidiospores were obtained by swilling with sterile distilled water containing 0.05% Triton x-100. Conidiospores were then washed twice in sterile distilled water by centrifugation at 5000 revolutions / minute for 5 minutes. A haemocytometer was used to determine the concentration of conidiospores in the primiered suspension. Sequent alleviation was then made to get the required concentration of the conidiospores.

3. Bioassay:

Trichoderma album bioassay:

Four concentrations of conidial suspension (7x10⁴, 7x10⁵, 7x10⁶ and 7x10⁷ conidia /ml) from the entomopathogenic fungi, *T. album* were prepared using the hemocytometer. All bioassay tests were carried out in the laboratory under controlled temperature of 25±1 °C. Ten (first larval instar and adult stage) of *R. dominica* insect were treated by dipping them for 10 seconds in the conidial suspension in 50 ml-conical flask at room temperature. After dipping in the suspension, the lesser grain borer (first larval instar and adult stage) were transmitting into a Petri dish (9x1.5 cm) with wet filter paper. Then, they kept for 7 days under conditions of 25±1 °C and 80-90% RH. A treatment with no fungus was included as a control. Each treatment was replicated three times with 10 insects per replicate. Dead insects were counted after 1, 3, 5 and 7 days. Data were analyzed by probit analysis using SPSS program to calculate LT50 and LC50, values.

4. Conidiospores production:

The fungal culture which prepared above at (2.2) was used to inoculate white rice (1 kg) .

First of all (1 kg) of rice was boiled into enough water for 10-15 sec, the resulted rice rinsed from water and equally divided on four autoclaveable plastic bags (250g.each) .The plastic bags were sterilized at 120°C for 20 Min. The fungal cultures which grown earlier on Petri dishes were transferred to the rice (2 Petri-dishes/plastic bag) under sterilized conditions. The bags then closed and kept under 25-30°C for 15 days. After that the rice was dried on strengthening paper and then grinded. Then 50 grams of ground rice inoculated with Conidiospores of, *T. album* fungi was added to (1 kg) of wheat. Each gram of rice powder loaded with fungus contained (7x10⁷ conidia /ml) which considered the highest concentration that gave the highest mortality rate in previous bioassay experiment, then the treated wheat with *T. album* in addition to control wheat were infested with an insect, *R. dominica* an early stage of the infection and stored in plastic jars (1 liter) covered with muslin cloth at 25±1 °C and 80-90% RH for six months.

5. Physicochemical properties of stored wheat

Healthy kernels of wheat variety without infestation was used as a control. Infested wheat, wheat treated with *Trichoderma album*s (treated wheat) after six months of storage as described above in addition to control sample were milled in a laboratory mill. The resulting flour samples were used for physiochemical analysis.

The kernels damaged by the insect in the wheat samples were detected visually, and weighed (w/w). The test weight was determined by using the approved method of the American Association of Cereal Chemists 55-10 and the results were reported in kg/hL.

The Thousand Kernel Weight was calculated from the weight of a representative 1000 grains from a cleaned grain sample and expressed in grams

Moisture content of the kernels was determined by the approved AACC method 44-1 (2000). Protein content was assayed using the Kjeldahl method and expressed using the conversion factor $N \times 5.7$ (*Official Methods of analysis* 1984) Ash content was determined using the approved AACC method 08-01(2000). Gluten content assayed according to (Anonymous, 1982)

Color analysis

The color of a sample is indicated by Hunter L^* , a^* and b^* method using lovibond glasses calibrated in accordance with (Giese, 2000).

RESULTS AND DISCUSSION

Several studies documented the high potential of entomopathogenic fungi for the control of insect pests in stored products as promising alternatives to fumigants. Current evolution in insect pest management research has definite the insistent requirement for improving biological control technique with the application of entomopathogenic agents (Wakil and Ghazanfar 2010).

Pathogenicity of *T. album* to the of *R. dominica*:

The susceptible stage of most insects to *T. album* was at larvae. Usually, eggs and pupae cannot be easily infected. To adults, the infection was different with insects species. Some insects were infected at most stages, and some only at one or two stages. Zero percent of mortality was noted in the control. All isolates of the tested entomopathogenic fungi, *T. album* were capable of infecting the first larval instar and adult stage of *R. dominica*. The Percentage mortality of the first larval instar and adult stage of ware dependent and increased with increasing concentration. Annual percentage rate mortality of lesser grain borer the first larval instar and adult stage of varied from (32- 94 to 18- 74 %) respectively as shown in (Fig. 1). The study goes in the same line of the findings of Hussein *et al.*, 2013whoobserved up to 100% mortality of the adult stage of lesser grain borer, *R. dominica* by various strains of *T. album*.

A good relationship between the conidiospore concentrations of *T. album* and percentage of mortality was observed. The lowest entomopathogenic fungi concentration (7x10⁴ conidia /ml) gave less control (32 - 18%) of the lesser grain borer first larvae and adult stage. Thus, the low virulence of entomopathogenic

fungi could be related to the lower conidia concentrations applied; by comparison, the highest concentration (7×10^7 conidia /ml) for the fungi caused high mortalities (94 -74 %). Hussein *et al.* 2013 determined a correlation between the conidial concentrations of *T. album* and mortality percentages of the lesser grain borer *R. dominica*. nevertheless low concentrations of the fungus *T. album* achieved high performance in the mortality rate of *R. dominica*.

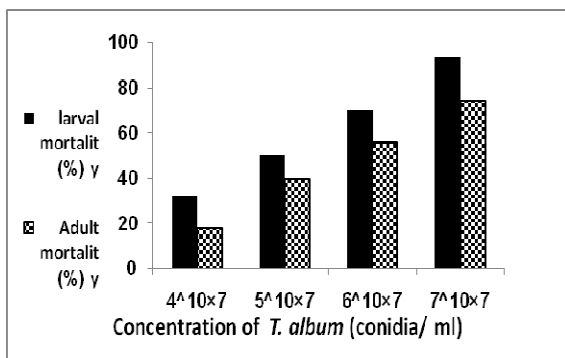


Fig. 1. Effect of increasing doses of *Trichoderma album* on the mortality of the first larval instar and adult stage of *Rhyzopertha dominica*.

The obtained results presented in Table (1) showed that the LT₅₀ values and slopes that were calculated from the mortality data of *T. album* on the first larval instar of *R.dominica*. LT₅₀ were decreased

with increasing of concentration. The difference between the times required to kill 50% of larval instar treated with the highest concentration (7×10^7 conidia /ml) of *T. album* and the lowest one (7×10^4 conidia /ml) was 9.18 days indicating a relatively powerful effect of *T. album* on the first larval instar of *R.dominica*.

The results in the same table showed the effect of *T. album* on the adult stage, results show the same trend of larval. The difference between LT₅₀ value of the highest and the lowest concentration was 7.25 days. Data also indicated that the first larval instar is a susceptible stage to the effect of *T. album* than the adult stage on different concentrations which used in this study. This relative response of first larval instar toward the fungi may be due to the heterogeneity of the cuticle formation in the different stages of this insect. In another hand, the sclerotization and tanning of the first larval instar cuticle may be playing a role in preventing the fungi to penetrate and delay the effect on adult stage of this insect. Batta (2008) also suggested that the cuticle of *R. dominic* playing an important role in the penetration of *B. bassiana*. The same author explained that the insect *R. dominic* achieved the highest percentage of mortality after 7 days of treatment with fungus *B. bassiana*. Quesada-Moraga, *et al.* (2006) explained that the effectiveness of the entomopathogenic fungi began clearly after 48 hrs. after inoculation and the hyphae penetrated the integument inside the trachea and the epithelial and epidermal cells.

Table 1. LT₅₀ Values of entomopathogenic fungi, *T. album* on the first larvae and adult of the lesser grain borer, *R. dominica*.

LT ₅₀ of the first larvae of the lesser grain borer, <i>R. dominica</i>				
Concentration (conidia/ml)	LT ₅₀ (days)	Confidence limits		Slope ± SE
		Lower	upper	
7×10^4	13.20	8.71	46.65	2.12 ± 0.59
7×10^5	8.16	6.27	13.68	2.17 ± 0.45
7×10^6	5.41	4.51	6.91	2.50 ± 0.42
7×10^7	4.02	3.64	4.98	4.49 ± 0.43
LT ₅₀ of adult of the lesser grain borer, <i>R. dominica</i>				
Concentration (conidia/ml)	LT ₅₀ (days)	Confidence limits		Slope ± SE
		Lower	upper	
7×10^4	13.68	9.06	48.76	3.17 ± 1.17
7×10^5	10.18	7.54	21.71	2.50 ± 0.62
7×10^6	7.53	5.37	8.95	2.55 ± 0.48
7×10^7	6.43	4.22	5.81	3.47 ± 0.94

Data presented in Table (2) and Figure (1) revealed that the mortality rates of *T. album* on the first larval instar and adult stage of *R. dominica*. The LC₅₀ values of *T. album* were 5.61×10^5 conidia /ml for the first larval stage, whereas it was 3.32×10^6 conidia/ml

for the adult stage respectively. Based on the result from the bioassay test, data also indicated that the tested of the entomopathogenic fungi *T. album* showed a high toxicity when it was applied against the first larval instar than the adult stage of *R. dominic*.

Table 2. LC₅₀ values of the entomopathogenic fungi, *T. album* on the first larval instar and adult stage of the lesser grain borer, *R. dominica*

Fungi isolation	Stages of exposure	LC ₅₀ conidia/ml	Confidence limits		Slope ± S.E
			Lower	upper	
<i>T. album</i>	Larvae	5.61×10^5	2.29×10^5	1.15×10^6	0.62 ± 0.09
	Adult	3.32×10^6	1.40×10^6	8.53×10^6	0.50 ± 0.09

Anonymous (2007) mentioned that the LC₅₀ and LC₉₀ values of *T. album* against *R. dominica* adult were

4.10×10^6 and 6.11×10^7 conidia /ml, *T. album* whereas (Abdel-Raheem, *et al.*2015) recorded that the LC₅₀

value was 1.20×10^5 conidia /ml after 11 days treatment by *B. bassiana*.

The obtained results clarified that the *T. album* motivated highly mortality against *R. dominica* and this accept with (Verma *et al.* 2007) who reported that, *Trichoderma spp.* have been openly used as entomopathogenic fungal agents against various pests. Also, *T. album* caused 100% mortality after 5 days of infection occurs against poultry red mites (Kaoud, 2010).

Dependence on the results of this investigation, it was cleared that the entomopathogenic fungus production tested may supply applicable alternatives to synthetic insecticides used in the control of the lesser grain borer, *R. dominica*. As well as in synchronism with good agricultural operations may be decrease the use of chemical pesticides and extend an element within an IPM system.

Wheat properties during storage

Analysis concerning the effect of insect-pest infestation on quality parameters of wheat are an important factor in assessing the nutritive value of grains. The effects of storage and insect infestation on the physical and chemical properties of the wheat samples were presented in Table (3) the results showed that over the 6-month storage period varied and progressive changes occurred in infested wheat samples with the increasing insect population. Test weight and kernel weights of the infested wheat samples decreased with the increasing insect populations. The thousand seed weight record 43.70 g for control while reached to 32.20 g for infested wheat by decrease percentage 26.31 %. It means that the infestation of wheat grains by insects could cause loss of wheat production estimated

at one quarter of total production of wheat grains. The test weight of control samples was 81.73kg/hL that decreased to 64.93kg/hL for infested wheat but the treated wheat kept their test weight at 84.66 kg/hL with little increase than control samples. Insects spend a great part of their life inside of wheat kernel as a results consume an amount of endosperm and decreased the kernel weight (Liscombe, 1962)a decrease in the ratio of endosperm to bran is a natural results for the development of insects inside the kernel as larvae, and consuming the endosperm and germ layers, (Nawrot *et al.*, 2006) all of this could be the reason for the decrease test weights. A clear effect for the wheat treated with *T. Album* fungi used in this study for stopping the serious damage of wheat kernels then keeping the weight at good percents comparing to untreated one

While protein content is important character for wheat quality, gluten considered the functional component of protein and determines many dough characteristics of wheat flour. In Table(3) the protein content for control samples was 13.4%while it increased to 14.00% and 16.74% for treated and infested wheat whereas gluten content was (16.93, 7.37) % for control and (13.31, 4.50) %for infested and (14.33, 6.10) % for treated wheat for both wet and dry gluten, respectively. The results indicated that protein content increased while gluten content decreased. The increases in the protein might be the result of the insect body parts and metabolic waste of insects. The decrease in gluten content in the infested samples was reported by (Keskin and Ozkaya., 2015)who showed that the reduction in gluten content due to the fact that gluten was brittle and disintegrated easily. A portion of it was lost during washing, and therefore, the gluten yield was lowered.

Table 3. Physiochemical properties of stored wheat samples

sample	Moisture %	Ash %	Protein %	Dry gluten g/100g	Wet Gluten g/100g	Test Weight kg/hL	Thousand seeds Weight g
Control	6.73	1.21	13.40	7.37	16.93	81.73	43.70
Infested wheat	6.70	2.13	16.74	4.50	13.31	64.93	32.20
treated wheat	6.16	1.13	14.00	6.10	14.33	84.66	46.00
Color measurement							
sample	<i>L*</i>		<i>a*</i>		<i>b*</i>		
Control	75.90		5.40		15.83		
Infested wheat	67.48		4.42		18.88		
treated wheat	77.74		4.15		18.60		

Ash content in flour is an indication of the bran content in the flour because ash is primarily concentrated in the bran by other meaning ash content indicates the amount of bran contamination in flour. Ash content of samples was 1.13%, 1.21% and 2.13 % for treated, infested and control wheat, respectively. A clear increase of ash content for infested wheat comparing with control or treated wheat was noted, the results indicated that the metabolic activity of insects caused destroy of the wheat grain thus the increasing of the bran content. On the contrary the treatment by using *T. album* fungi kept the ash content compares the control samples it means it had a good effect against insects actions in destroying the wheat grains.

Concerning Moisture content varied between 6.13-6.73 % for all samples. With regard to color analysis, the flour color affects the crumb and finished product color. Ash in flour can affect color, giving a darker color to finished product. Hunter *L** (lightness), *a** (red to green) and *b** (blue to yellow) values of wheat flour are shown in the same table. However a clearness for both the control and treated samples with *L** value (77.74 and 75.90) , respectively the infested wheat had the darkest color with *L** value 67.48, at the same time the values of yellowness color *b** value ranged from 15.83 to 18.88, the infested wheat *b** value express a little tendency of color towards yellow comparing to treated wheat while the control samples had the lowest *b**

value 15.83. The results in this study confirmed that both treated and infested samples had more yellow pigment than control. Wheat flours have a creamy color as a result of the presence of carotenoid pigments in the endosperm on the principle the level of these pigments is under genetic control. Therefore the color of the flour will vary depends on the level of these pigments that could be vary according to the insect activity during storage. A shortage of pigments carotenoids has occurred during the term of the flour storage, for 2 to 3 months at ambient temperature, and for several months on the degree of 39°C have been reported by (Mahmoud and Abdel-Halim, 1994). In the same table it is clear that the a^* value for control is a little higher comparing to other samples. L^* values of all samples indicated that insect metabolic system did not relate to the pink color of samples.

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فعالية الفطر الممرض للحشرات، *Trichoderma album* Preuss في السيطرة على حشرة ثاقبة الحبوب الصغرى، *Rhizopertha dominica* F تحت ظروف المعملية.

غادة صلاح محمد^١ و إيمان محمد ممدوح عبدالظاهر^٢

^١ قسم وقاية النبات - كلية الزراعة - جامعة جنوب الوادي

^٢ قسم الصناعات الغذائية والألبان - كلية الزراعة - جامعة جنوب الوادي

تم تقييم القدرة المرضية للسلالة المعملية لفطر *Trichoderma album* Preuss على العمر اليرقي الأول و الحشرة الكاملة لثاقبة الحبوب الصغرى عن طريق تجربة التقييم الحيوى بواسطة الغمر تحت الظروف المعملية. تم تعريض العمر اليرقي الأول و الحشرات الكاملة لثاقبة الحبوب الصغرى لأربعة تركيزات مختلفة من من فطر *T. album* (١٠×٧، ١٠×٧، ١٠×٧، ١٠×٧ جرثومة / مللى لتر) على حده ، ثم تسجيل معدلات الموت فى الحشرات بعد ١، ٣، ٥، ٧ يوم على التوالي. تم أيضا تخزين القمح المعامل بفطر *T. album* والقمح الغير معامل بالفطر بعد الإصابة بحشرة *R. dominica* لمدة ستة أشهر فى المعمل ، قد تم تقدير جودة الحبوب المخزونة لمدة ستة أشهر عن طريق بعض التقديرات الكيميائية والفيزيائية والتي شملت كل من الفحص الظاهرى للحبوب المصابة ومن ثم تقدير وزن الحبوب بالإضافة الى تقدير وزن الألف حبة وكذلك تقدير التركيب الكيماوى العام من حيث نسبة الرطوبة والبروتين والرماد والكربوهيدرات وكذلك تقدير نسبة الجلوتين بالإضافة الى قياس لون عينات الدقيق في كل من الحبوب المصابة والمعاملة بالفطر مقارنة بالكنترول أظهرت النتائج أن العمر اليرقي الأول للحشرة كان الأكثر حساسية للإستجابة لفطر *T. album* عن الحشرة الكاملة. كما أظهرت النتائج أن أعلى نسبة موت كانت (٩٤ % ، ٧٤ %) للعمر اليرقي الأول والحشرة الكاملة على التوالي بعد سبعة أيام من المعاملة عند تركيز ١٠×٧ جرثومة / مللى لتر. أظهرت النتائج أيضا أن قيمة التركيز اللازم لقتل ٥٠ % من الحشرات المعاملة هي (١٠×٥، ٦١، ١٠×٣، ٣٢، ١٠×٣، ٣٢ جرثومة / مللى لتر) بينما كانت قيمة الوقت اللازم لقتل ٥٠ % من الحشرات المعاملة هي (٤، ٠٢، ٤، ٤٣، ٦، ٤٣ يوم) عند تركيز ١٠×٧ جرثومة / مللى لتر للعمر اليرقي الأول و الحشرات الكاملة لثاقبة الحبوب الصغرى على التوالي. أوضحت نتائج التحليل الكيماوى للحبوب ان خصائص حبوب القمح المخزونة والتي تم معاملتها بفطر *T. album* قد تحسنت أثناء التخزين مقارنة بالعينات الكنترول أو الغير معاملة حيث انه تم خفض نسبة الفقد في وزن الحبوب المخزونة بفعل الحشرة بنسبة وصلت الى ٢٥ % مقارنة بالحبوب الغير معاملة وكذلك قد تحسنت نسبة الجلوتين في الحبوب المعاملة بالفطر وخصائص لون الدقيق قد أعطت نتائج أفضل من الحبوب الغير معاملة. على ضوء ما سبق أظهرت النتائج فاعلية فطر *T. album* الممرض للحشرات و إستخداماته الواعدة فى مجال مكافحة البيولوجية لحشرة ثاقبة الحبوب الصغرى.