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New Methods for the Control of Lesser Grain Borer, Rhyzopertha Dominica

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Abstract-The aim of this work was evaluation of the efficacy of entomopathogenic fungi and new natural superoxide water (Envirolyte-Egypt) to control the lesser grain borer, Rhyzopertha dominica (Coleoptera: Bostrichidae). For this purpose: (1) isolated Trichoderma album was evaluated [Individual solutions of 0 (untreated control, water only), 10, 102, 103, 104 and 107 spores .ml-1 of the strain of fungus /100 g corn grains and poultry ration were used]. (2) Fogging solutions of 0 (untreated control, water only), 1:100 and 1:250 of Envirolyte-Egypt /100 g corn grains and poultry ration were used. Trichoderma album caused 20 % mortality within 7 days post spraying at the lowest concentration and 100% mortality at the highest concentration of the spores at 7 days post spraying. Trichoderma album proved to be fatal against the lesser grain borer, Rhyzopertha dominica. Envirolyte-Egypt caused 40% mortality within 7 days post spraying at the lowest concentration and 80% mortality at the highest concentration. Envirolyte-Egypt has potential effect on Rhyzopertha dominica.

Index—**Biological control; Entomopathogenic fungi; Envirolyte-Egypt; Rhyzopertha Dominica.**

I. INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (*Coleoptera: Bostrichidae*) is a primary pest of stored grains in many regions of the world. The lesser grain borer is an internal feeder of various stored raw grains. This species is well adapted to dry conditions [1] and is generally regarded as a strong flier; hence it can easily disperse from one storage facility to another and create new infestations [2], [3]. Females lay eggs on the surface of grain kernels, and upon hatching, the larva enters the kernel ([4], [5] and remains inside until maturity [6] . The developing larva feeds inside the kernel, causing weight loss and damage to the germ and endosperm. The mature adult emerges from the kernel by boring a large exit hole, producing what is commonly referred to as an insect-damaged kernel (IDK) in wheat. Significant physical damage and weight loss result from internal and external feeding by larvae and mature adults, respectively. Therefore, the best method to manage this pest is to control adults before they colonize and reproduce in the grain, or control neonates before they enter the kernels. Applying a grain protectant may offer long-term protection against adults and neonates of *R. dominica* in stored grains. The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. *Trichoderma* strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism [7]. In this paper, we apply recent development in biological control of the lesser grain borer, *Rhyzopertha dominica*. We used non pathogenic entomopathogenic fungi (*Trichoderma album*) as well as new natural superoxide water (Envirolyte-Egypt) to control the lesser grain borer in corn grains and poultry ration.

II. MATERIALS AND METHODS

Isolation of entomopathogenic fungi

Selective media (Sabourud dextrose agar) rich with antibiotics and growth substances are used for the isolation of *Trichoderma* from soils, using soil dilution plating. Submerged fermentation used for production of blastosproes and conidia. Blast spores are produced relatively in a large quantities using liquid medium (corn steep). Haemocytometer was used to count numbers of propagules.

Envirolyte-Egypt

Envirolyte-Egypt (nontoxic, contains various mixed oxidants predominantly hypochlorous acid and sodium hypochlorite [($HClO$, ClO_2, $HClO_3$, $HClO_4$, H_2O_2, O_2, ClO^-, ClO_2^-, ClO_3^-, O^-, HO_2^-, OH^- - working substances), pH 2.5-3.5, ORP>1150mV, C_{active} ~500mg/l

Corn grains and poultry ration

Harvested yellow corn grains and broiler-starter poultry ration were used. The composition of the basal diets is presented in the following Table:

†Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D_3 , 9790 IU; vitamin E, 121 IU; B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

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Table 1. Composition of basal diets (%)

Ingredients	Starter (0 – 21 d)
Yellow corn	50.86
Corn gluten meal	5.00
Soybean meal	35.0
Soy oil	5.80
Dicalcium phosphate	2.50
Lime stone	0.13
Common salt	0.33
DL-Methionine	0.05
L-lysine	0.03
Broiler premix†	0.30
Nutrient Profile:	
ME (kcal/kg)	3184.21
Crude protein%	22.82
C / P ratio	139.5
Crude fat%	7.3
Crude fibre%	4.3
Total ash%	6.1
Calcium%	0.9
Non-phytate phosphorus%	0.46

Grain and ration treatment

Entomopathogenic fungi The experiment was replicated five times at 1-week intervals. For each replicate, five individual lots of 100 g of corn grains and poultry broiler starter ration were weighed out. Individual solutions of 0 (untreated control, water only), 10, 10^2, 10^3, 10^4 and 10^7 spores .ml^{-1} of the strain of *fungus* /100 gm grain or ration were prepared in sterile distilled water. Each of the individual lots was sprayed using a Badger 100 artists' airbrush to mist 0.4mL of solution from a particular concentration directly onto the material. For each concentration, the treated material were put in 0.95-L glass jars and hand-tumbled for 30 seconds to ensure uniform treatment with *fungus*. About twenty unsexed adults of *R. dominica*, obtained from colonies maintained on poultry ration in constant darkness inside an incubator set at 27 °C, 60 % relative humidity, were placed inside the vial for each treatment combination. All vials were placed in plastic humidity boxes containing a saturated sodium chloride (NaCl) solution to maintain r.h. at 75%, which is equivalent to about14.0–14.5% grain moisture content. After the vials were placed in the boxes, each box was placed in a temperature incubator at 32 °C. This temperature was optimum for progeny production of *R. dominica* on wheat ([8], [9], so 32 °C was selected for this test as well. Temperature and humidity inside the test boxes were monitored. After 1 week of exposure, *R. dominica* adults were sieved from vials in each treatment combination to assess mortality.

Envirolyte-Egypt

About twenty unsexed adults of *R. dominica*, obtained from colonies maintained on poultry ration were sprayed using a Badger 100 artists' airbrush to mist 0.4 mL of solution from a particular concentration of 1:250 and 1:100) and then placed inside the vial for each treatment. After 1, 2, 4 and 7 days of exposure, *R. dominica* adults were sieved from vials in each treatment combination to assess mortality.

Histopathological Examination

Histopathological examination of insects was carried out after 7 days of exposure by fixing in buffered 10 % formaldehyde PH 7.0 (phosphate puffer) for 48 hr, fixed in embedded paraffin and stained with hematoxylin and eosin.

Statistical analysis

The obtained data were subjected to analysis according to [10]. Differences between means were done at the 5% probability level, using Duncan's new multiple range test.

III. RESULTS AND DISCUSSION

Trichoderma album caused 20 % mortality of the adult *R. dominica* at the lowest concentration of the spores and 100% mortality at the highest concentration after 7 days of treatment. Mortalities of adult *R. dominica* after one-week exposure were differed significantly (P>0.05) among fungal concentrations. Data for mortalities were described by Table 3 and Fig. (1A). Recent development in pest control research have proves the urgent need for developing biological control methods with the use of microbial pathogens ([11], [12]. *Trichoderma album* proved to be fatal against the poultry red mite *D. gallinae*, [13]. We think that, the spores of the fungi germinate on the host cuticle, penetrate them and spread through the body (Photo. 1A). In addition, lytic enzymes secreted by the fungus may be played a role in the process of damage 14] . After the fungus has been killed the insect, it can grow out of the insect cadaver and produce more spores and increasing the chance for others to be killed [15], [13]. Fungi of the genus *Trichoderma* have been used for the production of lytic enzymes and to control a wide range of plant pathogenic fungi and weed management [14]. *Trichoderma virens* (*Gliocladium virens*), which releases the herbicidal molecule viridiol which applied in Composted Chicken Manure (CCM) and provided a significant weed control. "Reference [16] shows that, the *Trichoderma virens* UKM1 secrets a significant amount of exochitinase which degraded the chitinous materials of the shrimp shells. In the present paper we found that, *the spores of T. album* were attached and penetrate the cuticle of adult *R. dominica*, resulted in severe damage of cuticle and endodermoid of the treated insect, as shown in Photo. 2B and C. The fungus can grow out of the insect cadaver and produce more spores (Photo. 3). As a contact insecticide, entomopathogenic fungi invade their host through the cuticle, covered by an outermost lipid layer mainly composed of highly stable, very long chain structures [17].

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Table 2: Effect of spore-concentration of *T. album* on the mortality of adult *R. dominica* after 7 days of exposure.

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Concentration	Log 10					Control
	1	2	3	4	7	
Mortality %	20^b	35^b	40^b	70^b	100^b	0^a
Histopathological altrations	+	++	++	+++	+++	-

The same letter in the same raw is not significantly different at P<0.05. +: Mild (Lesions in cuticle layer);++: Moderat (Lesions in cuticle layer and the endodermoid) ;+++: Severe (Lesions in cuticle, endodermoid and other organs) -: No histopathological alterations.

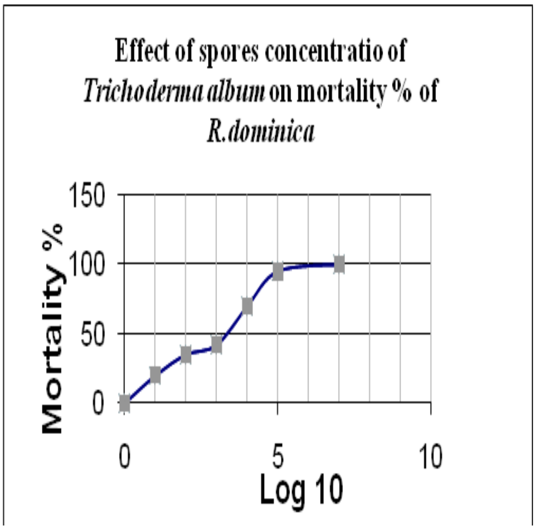

Fig. 1A: Effect of spore-concentration of *T. album* on the mortality of adult *R. dominica* after 7 days of exposure.

IV. CONCLUSION

Recent development in pest control research have proves the urgent need for developing biological control methods. *Trichoderma album* proved to be fatal against *R. dominica*. In the present paper we found that, *the spores of T. album* were attached and penetrate the cuticle of adult *R. dominica*, resulted in severe damage of cuticle and endodermoid of the treated insect and the fungus can grow out of the insect cadaver and produce more spores. Envirolyte-Egypt caused 40% mortality within 7 days post spraying low concentration and 80% mortality of high concentration. *Supper oxide water* has potential effect on *Rhyzopertha dominica*.

V. ACKNOWLEDGMENT

We thank Faculty of Veterinary Medicine, Cairo University (Department of Veterinary Hygiene, Department of Parasitology and Department of Pathology) and Ministry of Agriculture for providing the technical and financial support for this study.

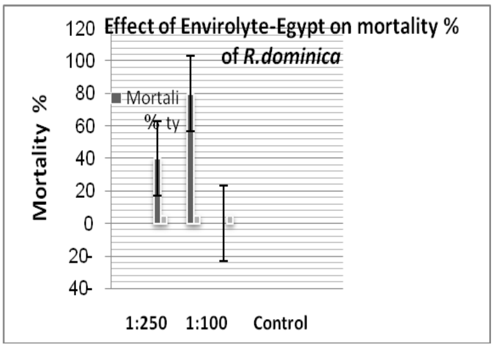

Fig. 1B: Effect of *Envirolyte-Egypt* on the mortality of adult *R. dominica* after 7 days of exposure.

Envirolyte-Egypt caused 40% mortality of adult *R. dominica* within 7 days post spraying at the lowest concentration (1:250) and 80% mortality at the highest concentration (1:100), as shown in Table 2 and Fig. 1A. Observations with a simple compound microscope showed that hair-like structures on the *Rhyzopertha dominica* (trichomes) is considered to be one of the most difficult insects to control because adults are slow moving, are larger than some of the common external feeders in stored grains, and are not as pubescent as the more susceptible species [20]

Table 3: Effect of *Envirolyte-Egypt* on the mortality of adult *R. dominica* after 1, 2, 4 and 7 days of exposure.

Concentration	1:100					1:250					Control
Period of exposure (Days)	1	2	3	5	7	1	2	3	5	7	7
Mortality %	10	12	40^b	60^b	80^b	0	8	18	32^b	40^b	0^a
Histopathological altrations	+	++	++	+++	+++	-	-	+	++	++	-

The same letter in the same raw is not significantly different at P<0.05. +:Mild (Lesions in cuticle layer);++:Moderat (Lesions in cuticle layer and the endodermoid) ;+++:Severe (Lesions in cuticle, endodermoid and other organs) -:No histopathological alterations.

The entomopathogenic fungus is known to infect over 200 insect pest species, including termites [18] . It is currently being used as a biological tool to control a number of pests such as termites, mites, etc. and its use in the control of malaria-transmitting mosquitoes is under investigation. *Beauveria bassiana* and *Metarhizium anisopliae* were produced mortalities against *Sitophilus oryzae*, *S. zeamais* (*Col.*, *Curculionidae*), and *Rhyzopertha dominica* [19].

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APPENDIX

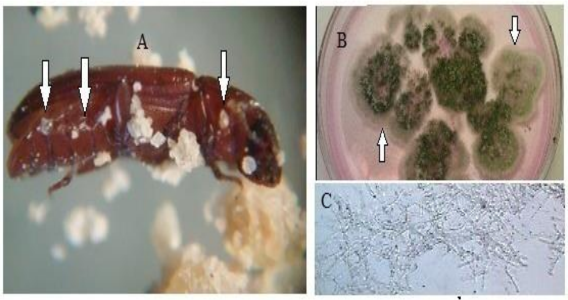

Photo. 1: Entomopathogenic fungi infect their hosts through the cuticle, penetrate them and spread through the body; A: T.album penetrate the cuticle of adult R. dominica after the one-week exposure and grew on the insect cadaver (The arrows) (X40); B: Colonies of T.album; C: Hyphae of T. album (X 100).

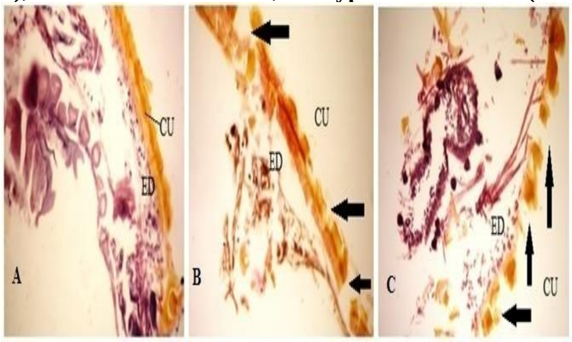

Photo. 2: Longitudinal section of the insect wall, A; Normal cuticle (CU) and endodermoid component (ED),B; Damage of cuticle and endodermoid (Arrows) due to the effect of Envirolyte-Egypt, C; Severe damage of cuticle and endodermoid (Arrows) due to the effect of T.album.(X100).

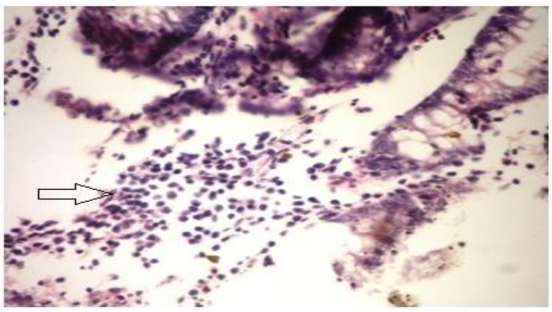

Photo. 3: Spores of T.album attaching the insect wall *(Rhyzopertha dominica)* and induced damage of cuticle and endodermoid (Arrow) (X400)

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