

Application of *Penicillium sp* as Entomopathogenic Fungi to Control the Red Rust Beetle *Tribolium castaneum* (Hbst.) (Coleoptera:Tenebrionidae)

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Environmental concern issues controlled the chemical pesticides usage since residue might cause a health problem. An alternative safe way for pest control is the main objective of this investigation. The recent study was conducted to evaluate the effect of some Entomopathogenic Fungi (*Penicillium sp*) to control the rust red beetle (*Tribolium castaneum*). different concentrations of fungal extract (10, 30, 50%) were applied beside, 40% application of the whole fungal organ against insects. Results from this investigation showed that, the treatment with Entomopathogenic fungus *Penicillium sp*. affected significantly ($P < 0.05$) the number of living insects. Higher effect on the number of the living treated insects was observed by the third concentrations (50%) compared with other concentration (10% and 30%) in the first treatment. The treatment with the whole fungus organs showed high effect on the number of the live insects where after 7 days all the insects were dead. Generally the concentration of the extract, the days of exposure and the interaction between days and concentration had significant effect on the live number of insects. Furthermore, metabolomics study of extracts by the means of GC-MS must be studied for determination of the active compounds by means of chromatographic techniques.

Key words: Entomopathogenic fungi, *Penicillium sp*, Red Bettel Rust.

Radical decrease in Agricultural production resulting from numerous pests like fungi and insects is considered as global problem that sustain economic and environmental system. Chemical pesticides use for plant protection strategy is a well-known method applied in integrated pest management system of plants. Although, chemical application may lead to effective result in pest control but, different environmental hazards and mankind toxicity beside development of pesticide resistance species had led to look for an eco-friendly alternative strategy

in best management. This leads to increased development of compounds based on the models of naturally occurring toxins of biological origin, having various biological activities¹. biochemical pesticides might be a good alternative for best management without any harmful effect on the environment.² characterized the bio-pesticides as 1- Microbial pesticides that contain a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredients, 2- plant- Incorporated-Protectants (PIPs) which are pesticidal substances that plants produce from genetic material that has been added to the plant and 3- Biochemical pesticides which are naturally occurring substances that control pests by non-toxic mechanisms. In the present investigation microbial pesticides was applied in a form of Entomopathogenic fungi. ³ reported that fungal disease in insect are common and

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widespread, fungi infect the host by breaking the host cuticle, avoid insect immune system response by utilizing nutrient present in the haemocoel and producing toxin. The efficacy of seven strains of entomopathogenic fungi against *Ceratitis capitata* adults was assessed in vitro⁴. A recent study⁵ demonstrated the ability of entomopathogenic fungi *Metarhizium anisopliae*, *Cordyceps bassiana* and *Isaria fumosorosea* to control *Diaphorina citri* (Hemiptera: Psyllidae) in Persian lime under field conditions. Entomopathogenic fungus *Metarhizium anisopliae* against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes also has been stated by⁶ who indicated a positive effect and highly susceptibility to infection with this pathogenic fungus.⁷ documented a high pathogenicity level isolated entomopathogenic fungus *Heliothius armigera* and cotton aphids in vitro. Successful infection of entomopathogenic fungus depends primarily on the adherence and penetration ability of a fungus to the insect integuments by a variety of degradable extracellular enzymes that hydrolyze the epidermis of the insect such as lipases, proteases and chitinases⁸⁻⁹. Furthermore, entomopathogenic fungi can infect non-feeding stages such as eggs and pupae since fungi do not have to be ingested and can invade their hosts directly through the exoskeleton or cuticle¹⁰. The current investigation focused on Red flour beetle, *T. castaneum* (Col.; Tenebrionidae) which is diverse pest in flour mills and wherever dried foods processed or stored,¹¹ found different virulence ability among nine isolates of entomopathogenic fungi *B. bassiana* against adults of *Tribolium castaneum* (Herbst). *Lecanicillium muscarium*; *Beauveria bassiana* and *Calvatia carniiformis* were studied against rust red beetle (*Tribolium castaneum*) with different fungal concentration and showed different response in the ability to reduce the growth of insect¹².

Despite the information that *Penicillium sp.* is one of the entomopathogenic fungi that have the potential to be developed as biological control agent of pests there was few report about *Penicillium sp.* as Entomopathogenic fungi. Therefore, the aim of current investigation focused on *Penicillium sp.* and considered as Entomopathogenic fungi model against Red flour beetle, *T. castaneum*. Fungal enzymes, toxins,

spores and hyphae were applied and the live insects number were calculated after the treatment.

MATERIALS AND METHODS

The evaluation was conducted in the Biology section laboratory, Faculty of Science, Princess Nourah Bent Abdul-Rahman university, Riyadh, Saudi Arabia.

Entomopathogenic fungi

Penicillium sp. was as entomopathogenic fungi and deposited in the entomopathogenic fungi collection of PNU. The strain was successively sub-cultured on Potato Dextrose Agar (PDA) at 25°C, in complete darkness. Fungal strains maintenance was identified. In general, 14-day-old spores of studied strain were cultivated on PDA and used as inoculum in the growth media.

The bioassay

The experiment was performed on white flour where the biological control was tested using the fungus *Penicillium sp.* The treatments were as follows: control and different fungus concentrations (10%, 30% and 50%). For each concentration was represented in six replicates where the control was represented 2 times. The flour was distributed evenly in all cans.

METHODOLOGY

Fungi were grown on liquid Sabouraud dextrose, enriched with yeast extract and incubated for a period of 14 days at 25°C. After that the medium was filtrated using filter paper in sterilized volumetric flask (bio-control using fungal enzymes and toxins). Furthermore, the different concentrations (10, 30, 50%) were prepared from the original extract using distilled water. For each concentration treatment (10, 30 and 50%) 120 red rust beetle distributed in six cans and treated. Each can was tightly closed using sterilized tissue paper and incubated in electric incubator in 25- 27°C. data and observation was taken every 5 days for two weeks and the number of live beetle was recorded.

The second treatment

In a second experiment *Penicillium sp.* was cultivated in solid Sabouraud dextrose agar for 14 days at 25°C (bio-control using fungus enzymes, toxins, spores and hyphae). A sample

from the fungus was taken using sterile needle and placing in sterile water in special water sprayer system and well shaken to insure homogeneity. Thereafter the solution was used to spray on the red rust beetle in 2 flour cans each contain 20 beetles. Then all cans were tightly closed using sterilized tissue paper and incubated in electric incubator at 25- 27°C for one week. data and observation was taken on daily bases and the number of live beetle was recorded.

Statistical analysis

All results were computed and expressed as mean \pm standard deviation (SD) for six replicates. Statistical analysis was performed using JMP software (version 18.0) with analysis of variance (One-Way ANOVA).

RESULTS

Penicillium sp was cultured and identified as *Phialides* produced singly, in groups or from branched metulae, giving a brush-like appearance known as a penicillus (Figure 1). All

cells between the metulae and the stipes of the conidiophores are referred to as branches, fungal description was approved using Mycology on line (<http://www.mycology.adelaide.edu.au/>).

The ability of *Penicillium* sp as entomopathogenic fungi against Red flour beetle, *T. castaneum* was investigated in the present study. Results revealed that the application of different concentrations of fungal extract showed different responses of the Red flour beetle as a number of live insects. Table 1 below showed that the application of higher fungal concentrations (50%) on Red flour beetle resulted in low number of live insects compared with other concentration (10% and 30%). Furthermore, the longer the exposure days to the fungal extract, the lower the live insect number detected, in day 15 the number of the live insects was lower than the other days (Table 1). Significant effect of the treatment (different fungal concentration), exposure days and the interaction between the fungal concentrations and exposure days on the live insect number was well documented. Significant negative correlations

Table 1. The effect of different fungal extract concentrations treatment on the rate of the number of Red rust beetle (*Tribolium castaneu*).

Treatment(Con%)	Insects Numbers				Means
	Exposure duration (Days)				
	1	5	10	15	
Fungal Extract(10%)	20 \pm 0 a	18.6 \pm 0.4ab	16.3 \pm 0.5ab	14.5 \pm 0.4b	17.41667
Fungal Extract(30%)	18.6 \pm 0.4a	16.6 \pm 0.5ab	14.0 \pm 0.3ab	13 \pm 0b	15.66667
Fungal Extract(50%)	17.4 \pm 0.2a	15 \pm 0.3ab	11.3 \pm 0.5b	9 \pm 0c	13.16667

Treatment **

Exposure duration**

Treatment x date ***

** represent the significant effect in the $P < 0.05$. Different letter expressed significant variation in the insect live number during the experimental period for one concentration.

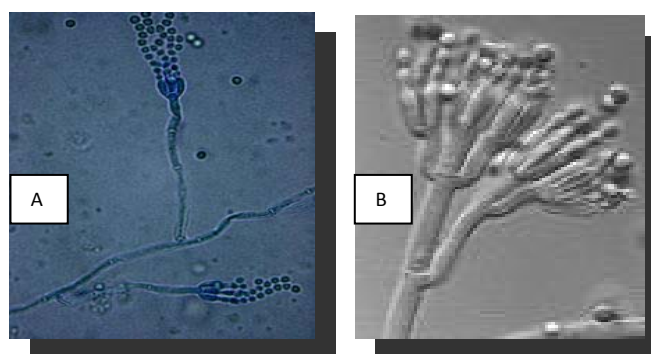


Fig. 1. Morphological structures and types of conidiophore branching in *Penicillium*. two-stage branched¹³.

between the number of the insects and the fungal extract concentration (-0.6^{**}) and between the number of the insects and exposure days (-0.8^{**}) were noticed.

Furthermore, the application of fungal extract with 40% concentration as a fungal, enzyme, toxin, hyphae and spores was investigated as second treatment against Red flour beetle, *T. castaneum*. Observed reductions in the insect live number after the treatment were observed. From a number of 20 insects in the 7th day of the treatment only one insect was alive. Moreover, significant effect was observed for the treatment, the exposure duration and the interaction between them. On the other hand, negative correlation (-0.99^{***}) was detected the number of the insect and the exposure days to the fungal extract. Effective results in suppression of insect growth treated with fungal extracts was observed when the insect treated with the whole fungus organs extract, since after day 7

there was no live insects. On the other hand, the number of the untreated insects was constant in both treatments.

DISCUSSION

The ever continuous use of conventional agrochemical insecticides may develop insecticide resistance organisms besides the chemical residue that may affect the human health and environment. Therefore an eco-friendly alternative is needed to reduce expected negative impacts for these materials. Using of living organisms as bio-pesticides which pose less threat to the environment and to human health might be a good alternative. Entomopathogenic fungi as bio-pesticides are important natural regulators of insect populations and have potential as myco-insecticide agents against diverse insect pests in agriculture¹. Special attention has been given to

Table 2. The effect of fungal extract (40%) treatment on the rate of the number of Red rust beetle (*Tribolium castaneu*) for one week.

Treatment	Insects Numbers							Means
	Exposure duration(Days)							
	1	2	3	4	5	6	7	
Fungal Extract(40%)	19.0 ± 0.3a	17.0 ± 0.4ab	14.0 ± 0.2b	9.0 ± 0.5cd	6.0 ± 0.2d	3.0 ± 0.1de	1 ± 0e	9.8 ± 5.5

Treatment **

Exposure duration**

Treatment x date ***

** represent significant effect in the $P < 0.05$. Different letter expressed significant variation in the insect live number during the experimental period.

entomopathogenic fungal research because they have the ability to penetrate through insect cuticle¹⁴. The present study used *Penicillium* sp as entomopathogenic fungi against red rust beetle (*Tribolium castaneum*). The ability of different fungal extract concentrations to kill the red rust beetle was approved positively, Same line of observation was documented by¹⁵ when he isolated Quinolactacide compounds from *Penicillium citrinum* which showed 88% mortality against green beach aphids. Furthermore, Positive reduction in the *Tribolium castaneum* living number when treated with different concentrations of pathogenic Fungi *Beauveria bassiana*, *Lecanicillium muscarium* and *Calvatia carniiformis* was observed¹². On the other hand,

positive correlation between the concentration and dead number and between the concentration and exposure days to the fungal extract might be due to increase number of fungal spores per space since¹⁶ mentioned that the death of the insect is due to the fact that fungi might infect the insect or the spores contacted the insect. When the spores contacted the insect cuticle it will grow and spread throughout the insect obtaining nutrients, leading to the death of the host by physiological starvation 3 – 7 days after infection⁹. Furthermore, whole fungus organ treatment showed good results since secretion of cuticle degrading enzymes such as chitinases, lipases, N-acetylglucosaminidases and esterases, is the mode by which Entomopathogenic fungi infect insects¹⁷.

Pathogenicity in the target host is well correlated by the production of the virulence protease Pr1 which plays a significant role in the success of entomopathogenic fungi in insect infection¹⁸⁻¹⁹. Furthermore, Entomopathogenic fungi may produce secondary intermediates and toxins, some of which have insecticidal activities after invasion of the fungus organ inside the insect which lead to rapid host death²⁰⁻²¹.

CONCLUSION

In conclusion of this study a significant role of *Penicillium* sp. biotic factors regulating Red rust beetle populations is well documented. Furthermore, the higher the concentration of the entomopathogenic fungal extract the higher effect was observed.

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