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EVALUATION OF THE EFFICACY OF LOCAL AND COMMERCIAL ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST *ORYZAEPHILUS SURINAMENSIS* (COLEOPTERA: SILVANIDAE)

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Abstract

The saw toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), is a key pest of storage products worldwide. This study was conducted to evaluate two Iraqi isolates of entomopathogenic fungi (EPFs), including *Isaria fumosorosea* (Wize) (Clavicipitaceae) and *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) and two commercial formulations, Naturalis-L (*B. bassiana* strain ATCC 74040) and Met 52 EC (*Metarhizium anisopliae* strain F52) against *S. oryzaephilus*. The effect of fungal infection on adult fecundity was also evaluated. *Isaria fumosorosea* and *B. bassiana* caused the highest mortality levels against the adult stage of *O. surinamensis* at all three conidial concentrations, compared to Naturalis-L and Met 52 EC. Corrected mortality of *O. surinamensis* caused by both *B. bassiana* and *I. fumosorosea* after 7 days of exposure at 1×10^8 conidia ml^{-1} was 48.07 and 45 ± 1.1 %, respectively. First and third instar larvae were high mortality compared to fifth larval instars and beetle adults. All fungi caused nearly 60% reduction in total fecundity of the *O. surinamensis* female. The outcomes reveal that the two EPFs isolated from Iraq, *I. fumosorosea* and *B. bassiana* have possible application as a biocontrol agent against *O. surinamensis*. Therefore, further studies are needed to develop commercially EPFs of native isolated.

Key Words: *Beauveria bassiana*, *Oryzaephilus surinamensis Metarhizium anisopliae*, EPFs, Iraq.

Introduction

Oryzaephilus surinamensis L. (Coleoptera: Silvanidae) is an important and widespread stored insect pest of grain products worldwide. This insect can attack several grain types including wheat, barley, maize, oilseeds, dry fig and dates (Al- Qazzaz and Al-Musawi, 2012). Chemical insecticides are the main way of controlling and reducing the damage of *O. surinamensis* and other stored insect pests. However, extensive use of chemical insecticides caused several issues, such as resistance of insect for insecticide, chemical residues and ecological pollution with damaging side effects on people health (Sullivan, 2002). These insecticide problems have encouraged to search for alternative methods of control stored insect pests.

Several entomopathogenic fungi (EPFs) have been evaluated against stored grain insect pests including *B. bassiana*, *M. anisopliae*, *I. fumosorosea*, and *Lecanicillium* spp. (Mohammed *et al.,* 2019). These EPFs have been used commercially as biocontrol agents against a wide range of insect pests (Wakil and Ghazanfar 2010; Dent, 2000). Even though there are more than 50 microbial application products known worldwide, only 3 myconisecticides have been used against stored grain insects (Faria and Wraight, 2007).

There is not enough study about the efficiency of EPFs against *O. surinamensis* in Iraq, therefore, the study was aimed to assess two Iraqi entomopathogenic fungal isolates and two commercial formulations against different developmental stages of *O. surinamensis* then examine sublethal impacts of EPFs infection on the fecundity of *O. surinamensis* beetle females.

Materials and Methods

Rearing of *O. surinamensis*

Oryzaephilus surinamensis was collected initially from nuts from many stores in Karbala city and rearing in Entomology laboratory at Agriculture Faculty, the University of Kerbala in 2019. The insects were

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recognized by a microscope using an identification key (Hedges and Lacey,1996; Halstead, 1993). The *O. surinamensis* were reared on whole sterilized rice. Beetle cultures were kept in glass jars (1 litre) covered with a muslin cloth and tied with rubber bands and kept in laboratory at 27±2 °C and 65±2% R.H.

Preparation of entomopathogenic fungi

Beauveria bassiana and *I. fumosorosea,* were obtained from the Plant Protection Dept., University of Kufa, which were originally isolated from aphids. These fungal isolates were cultured on potato dextrose agar (PDA) at 25°C as described by (Jasim and Mohammed 2019). Commercial formulations Naturalis-L (Fargro Ltd, Littlehampton, West Sussex, UK) and Met 52 EC (Belchim Crop Protection Limited, Eaton Socon, UK). Aerial conidia of both *B. bassiana* and *I. fumosorosea* were prepared and collected as described by Mohammed *et al.,* (2019). Three different conidial concentrations of each fungal isolate and commercial formulation were prepared (1×10^4, 1×10^6,1×10^8 ml^{-1}).

Effect of different conidial concentrations of *B. bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC against *O. surinamensis* adults.

Five plate replicates, each with 10 adults was tested for either *B. bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC. Individuals of each replicate were treated with 2 ml of each conidial suspension (1×10^4, 1×10^6, 1×10^8 ml^{-1}) using a handheld sprayer. Controls were treated with sterile 0.001% aqueous Tween 80 only. After drying for 20 min at room temperature, the dishes each with 5 g of sterilized rice grain were sealed with Parafilm and incubated at 27±2°C and 65±2% RH. After 24 h, lids of Petri dishes were replaced with new lids have ^{1}-cm hole enclosed with nylon mesh and incubated into the growth chamber for 7 days. Dead beetles took out from the dishes and mortality was documented after 1, 3, 5 and 7 days after treatment. Dead beetles were surface sterilized by rinsing two times with 70% ethanol for 30 seconds then with purified distilled water and located on water agar (3 gram of agar/L of water) in (9 cm) Petri dishes for 5 days to check infection by EPFs (Mohammed and Hatcher 2016). A cadaver was regarded as having died from infection by these fungi if conidia were recovered from it.

Effect of *O. surinamensis* developmental stages on the efficacy of *B. bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC

In this experiment, a conidial concentration of 1×10^8 ml^{-1} of either *Beauveria bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC were nominated based on the level of *O. surinamensis* death in the multiple-dose bioassays. Different stages of *O. surinamensis* (first, third and fifth instar larvae and adults) were gained from the lab using the technique mentioned by Nadeem *et al.,* (2011). Each stage of beetle and fungal species combination were replicated five times. The protocol of experiment and evaluation were identical to the effect of different conidial concentrations bioassays described above.

Effect of EPFs infection on the fecundity of *O. surinamensis*

To validate the sublethal outcome of EPFs infection on the fecundity of *O. surinamensis* adult females, male-female (newly emerge) couples (each couple is a replicate) were put in each Petri dish (9-cm) containing Whatman No. 1 filter papers and Individuals of each replicate were treated with 2 ml of conidial suspension (1×10^8 ml^{-1}) of either *B. bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC using a handheld sprayer. Controls were treated with sterile 0.001% aqueous Tween 80 without conidia. Every treatment was replicated 5 times. After 24 hours, each male-female couple was moved to individual sterile Petri dish with 3 g of rice covered with a muslin cloth and rubber bands and kept at 27 ± 2°C and 65 ± 5%. The number of eggs produced from each female was counted every day until the female's death using an appropriate magnifying lens.

Statistical analysis

The mortality was corrected using Abbott formula (Abbott, W.S. 1925). Experiments were statistical analyses using GenStat (version 16). Data were transferred using arcsine square root transformation when it was needed to meet the assumption of normality. The effect of life stage of *O. surinamensis* on the efficacy of either *B. bassiana, I. fumosorosea,* Naturalis-L and Met 52 EC was analyzed using two-factor repeated measurement analysis ANOVA. The effect of conidial concentration of each fungal isolate on the mortality of *O. surinamensis* was analysed separately using two-factor repeated measurement analysis ANOVA. The outcome of EPFs infection on the fecundity of females was analysed using one-way ANOVA. Mean comparisons were performed using the LSD test at 5% level of significance ($P<.05$).

Results and Discussion

Effect of different conidial concentrations of *B. bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC against *O. surinamensis* adults

Adult mortality varied significantly among fungal

isolates and conidial concentration ($P < 0.01$). For example, the highest mortality level of local isolates *B. bassiana* and *I. fumosorosea* at 1×10^8 ml^{-1} were reached 48 and 45%, respectively, compared to 25 and 32% for commercial formulations Naturalis-L and Met 52 EC, respectively table 1. The effect of period time after EPFs application on the mortality level was significant ($P < 0.01$), in all treatments, the highest mortality rates were achieved 7 days post-treatments table 1. The results were supported with Abdel-Raheem *et at.,* 2015, who found that *M. anisopliae* and *B. bassiana* were affected against *O. surinamensis* other storage products insects. Also, Al-Zurfi (2019) found that *B. bassiana* and *M. anisopliae* were affected against *Tribolium. castaneum*.

Table 1: The effect of different conidial concentrations of either *B. bassiana*, *I. fumosorosea*, Naturalis-L or Met 52 EC on the corrected mortality (±SE) of *O. surinamensis* after 1, 3, 5 and 7 days of application.

Fungal isolate	Conidial concentration	Corrected mortality (±SE)			
		1 d	3 d	5 d	7 d
Beauveria bassiana	1×10^8	0.0	6.6 ± 0.1	28 ± 0.6	48 ± 1.4
	1×10^6	0.0	0.0	13.2 ± 0.9	28 ± 0.7
	1×10^4	0.0	0.0	6.6 ± 0.1	14 ± 0.2
Isaria fumosorosea	1×10^8	0.0	6.6 ± 0.1	35 ± 0.8	45± 1.1
	1×10^6	0.	0.0	18± 1.1	27 ± 0.9
	1×10^4	0.0	0.0	0.0	13.2 ± 0.2
Naturalis-L	1×10^8	0.0	3.2 ± 0.1	14 ± 0.2	25 ± 1.1
	1×10^6	0.0	0.0	0.0	13 ± 0.7
	1×10^4	0.0	0.0	0.0	6.6 ± 0.1
Met 52 EC	1×10^8	0.0	0.0	18 ± 1.2	32 ± 1.3
	1×10^6	0.0	0.0	0.0	14 ± 0.3
	1×10^4	0.0	0.0	0.0	6.6 ± 0.1
$L.S.D_{(0.05)}$ for Conidial concentration = 2.75; $L.S.D_{(0.05)}$ for days = 1.23 $L.S.D_{(0.05)}$ for interaction = 4.22					

Table 2: Corrected mortality (±SE) of three instar larvae and adults of *O. surinamensis* treated with 1×10^8 conidia ml^{-1} of either *B. bassiana* , *I. fumosorosea*, Naturalis-L or Met 52 EC after 1, 3, 5 and 7 days of application.

Fungal isolate	Developmental stage	Corrected mortality (±SE)			
		1 days	3 d days	5 d days	7 d days
Beauveria bassiana	1st instar	46 ± 1.1	90 ± 2.2	100 ± 0.0	100 ± 0.0
	3rd instar	42 ± 0.9	91 ± 2.9	100 ± 0.0	100 ± 0.0
	5th instar	12.2 ± 1.2	35 ± 2.5	49 ± 3.3	61 ± 1.6
	Adult	6.6 ± 0.3	21 ± 0.7	21 ± 2.1	48 ± 1.4
Isaria fumosorosea	1st instar	40 ± 3.3	78 ± 5.2	90 ± 2.8	100 ± 0.0
	3rd instar	42 ± 0.6	78 ± 1.4	83 ± 3.2	93 ± 3.4
	5th instar	10 ± 1.1	28 ± 0.9	35 ± 1.5	56 ± 0.8
	Adult	6.6 ± 0.2	21 ± 1.0	35 ± 2.2	35 ± 1.1
Naturalis-L	1st instar	40 ± 3.1	78 ± 4.0	100 ± 0.0	100 ± 0.0
	3rd instar	12 ± 0.3	33 ± 0.8	52 ± 2.5	78 ± 2.7
	5th instar	6.6 ± 0.5	27 ± 1.7	44 ± 3.6	49 ± 2.8
	Adult	13.2 ± 1.0	14 ± 0.8	21 ± 1.2	27 ± 1.7
Met 52 EC	1st instar	40 ± 1.6	85 ± 4.2	100 ± 0.0	100 ± 0.0
	3rd instar	20 ± 0.1	50 ± 1.1	63 ± 1.9	74.3 ± 3.2
	5th instar	6.6 ± 0.3	7 ± 0.4	28 ± 1.3	39 ± 1.1
	Adult	6.6 ± 0.1	11 ± 0.3	28 ± 0.9	22 ± 1.7
$L.S.D_{(0.05)}$ for developmental stage = 3.56; $L.S.D_{(0.05)}$ for days = 2.03 $L.S.D_{(0.05)}$ for interaction = 4.57					

The difference in efficacy of biopesticides fungi might at results of their diverse proteins that make up (approximately 70%) of the majority of beetles' body wall cuticle (Hepburn 1985, Charnley 2003). Hence, the degrading goings-on of proteases might show a role in the express penetration of beetle cuticle, which reflects the virulence of the EPFs (Bidochka and Khachatourians 1990). Zare *et al.,* (2014) stated a strong high association among the proteolytic activity and virulence of seventeen isolates of *B. bassiana* against Khapra Beetle.

Effect of *O. surinamensis* developmental stages on the efficacy of *B. bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC

The results of this experiment showed that the efficacy of *B. bassiana* , *I. fumosorosea*, Naturalis-L and Met 52 EC were varied among *O. surinamensis* developmental stages, with a 100% mortality rate for the first instar larvae treated with either *B. bassiana* , *I. fumosorosea*, Naturalis-L and Met 52 EC table 2. Also, mortality rates for third instar larvae ranged from 100 to 74.3%. There was important difference of EPFs *B. bassiana* and *I. fumosorosea* to each stage of beetle ($P <0.01$). The mortality in the control treatment ranged from 2–4%.

The findings were supported by Khashaveh *et al.,* (2011), who found that the increased death caused by *M. anisopliae* (DEMI001) isolated from Iran, touched 100% against Khapra Beetle larvae and 83.9% for beetle adults. The difference in developmental stage exposure of *O. surinamensis* to EPFs *B. bassiana*, *I. fumosorosea*, Naturalis-L and Met 52 EC might be described by biochemical variations through host cuticle growth (Kirkland *et al.,* 2004) or differential conidial gaining of *O. surinamensis* larvae (first instars), compared to beetle adults (Barahona *et al.,* 2018). Nevertheless, further investigations are needed to evaluate these factors.

Effect of EPFs infection on the fecundity of *O. surinamensis*

The fecundity of *O. surinamensis* was affected by the EPFs application. Beetles in the control treatment were produced 98.4± 1.5, which was higher than those to *B. bassiana* (46± 1.1eggs), *I. fumosorosea* (49.5± 3.2eggs), Naturalis-L (47.4± 2.4 eggs) and Met 52 EC (41± 1.7 eggs). However, there was no significant difference among fungal treatments and control treatment in the percentage of hatched eggs table 3.

Table 3: Effect of EPFs infection on the mean numbers of eggs produced per beetle adult and percentage of hatched eggs, compared to the control.

Treatment	Fecundity (No. of eggs per adult)	% of hatched eggs
Beauveria bassiana	46± 1.1	80.86± 2.7
Isaria fumosorosea	49.5± 3.2	78.78± 3.5
Naturalis-L	47.4± 2.4	82.7± 1.9
Met 52 EC	41± 1.7	78.04± 1.8
Control	98.4± 1.5	80.28± 3.8
$L.S.D_{(0.05)}$	3.53	4.79

The decrease in the average of egg production of infected *O. surinamensis* could be recognized to histological and cytological injuries to the ovaries (Sikura *et al.,* 1972). As well as, attack of host tissues and production of secondary metabolites by EPFs might affect egg production (Furlong *et al.,* 1997). Additionally, beetle infected by EPFs might result in a significant delay at the beginning of calling, mating and/or the damage of the EPFs to the testes of exposed males which could explain the lack of eggs deposited by the females. No research is presented on the sub lethal outcome of EPFs on *O. surinamensis* beetle females, but significant effects of EPFs on the fecundity of numerous beetles have been informed (Branson & Sutter 1985, Mulock & Chandler 2001, Ondiaka *et al.,* 2008). For instance, *B. bassiana* reduced the whole number of eggs laid per female fecundity of *L. decemlineata* by about 56% compared to untreated females (Fargues *et al.,* 1991).

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