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Controlling of *Stomphastis thraustica* (Meyrick) (Lepidoptera: Gracillariidae) by Using Entomopathogenic Fungi

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A B S T R A C T

Stomphastis thraustica is a very important pest of Jatropha plant, it causes great losses in the yield (seeds) of the plant which used for production of biodiesel. This work was conducted under laboratory conditions to evaluate the efficiency of two fungus isolates for controlling this pest. This study discover a new biological control method against this pest that benefit for environmental conservation. Our results indicated that the fungus *Beauveria bassiana* gave 92.2% mortality against both 5th larval instar and pupa at 1x10^{8} conidia / ml concentration while this percentage decreased to 74.4% with the same previous concentration of *Metarhizium anisopliae* against the same larval instar. The higher pathogenicity of *B. bassiana* than *M. anisopliae* against 5th larval stage is more obvious with LC50 and LC90 values where they recorded 2.5x10^{5} and 3.7x10^{7} conidia / ml for *B. bassiana* and 1.1x10^{7} and 8.9x10^{8} for *M. anisopliae*. Although the two fungus isolates are virulent against larvae and pupae of *S. thraustica*, the higher pathogenicity of *B. bassiana* gave it the priority for controlling this pest and the two fungus isolates considered promising alternatives to chemical insecticides and recommended in IPM programs.

Introduction

Jatropha curcas considered one of the most important wonder plants in recent years. This plant is cultivated in dry tropical conditions for producing biodiesel (Wani *et al.*, 2006; Kumar *et al.*, 2002). This plant is infested by many insect pest among them is *Stomphastis thraustica*; the leaf miner of *Jatropha* plant. This pest causes great losses in most parts of *Jatropha* plant especially the foliage which consequently negatively affects the yields of produced seeds, the main target for biodiesel production (Narayana *et al.*, 2006; Gao, *et al.*, 2010; Rao *et al.*, 2001; Rodrigues *et al.*, 2011). Many trials were conducted for using insecticides to control the insect pests of *Jatropha* plant (Cassimo *et al.*, 2011) but till now, there is no any trial to use the biological control agent for controlling this pest. This make up the first attempt to use two strains of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, for controlling this pest.

Materials and Methods

Fungus culture

Isolates of *Beauveria bassiana* and *Metarhizium anisopliae* were obtained from Assiutt University, Mycological center,

Faculty of Science. The isolates were cultured on potato dextrose Agar (PDA) medium containing 20 g glucose, 20 g starch, 20 g agar, and 1000ml of distilled water in test tubes. These tubes were autoclaved at 21 C^0 for 15-20 min and incubated at 27 C^0 ±1, 80 % ± 5% RH and photophase of 12 h for 15 days. The conidia were harvested by scraping the surface of the culture with inoculation needle. The mixture was stirred for 10 minute the hyphal debrise was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using Haemocytometer. Serial dilutions were prepared in distilled water containing 0.1 % tween- 80 and preserved at 5C^0 until used.

Pathogenic bioassay

Insect's culture

Jatropha leaves infested with *Stomphastis thraustica* were collected from the field in cloth bags and transferred to the laboratory for examination. The infested leaves introduced into covered cage measured 50x25x50 cm till adult emergence. After then, the mature adults (males and females) were gathered by aspirator and released into other cage provided with Jatropha pots to acts as an oviposition site. The culture was established and maintained under 22± 2C^0, 55 ±3% RH and 16:18:D (Ebadah *et al.*, 2017).

Mortality test under laboratory conditions

The concentration of the stock suspension was adjusted to $1x10^6$, $1x10^7$ and $1x10^8$ conidia /ml using Neubaour haemocytometer. To evaluate the pathogenicity of each of the fungal isolate, infested *Jatropha* leaves with different larval instars were dipped in the fungal suspension with improved concentration for 5 seconds (five replicate were applied) then the treated leaves were kept in petri dish (15cm) contain wet tissue for keeping the humidity. After 4 days of observation, all counted larvae were collected to determine how many larvae were dead without being infested with fungal isolates. A control treatment was sprayed with distilled water only.

Re-isolation

To know the reasons of larval death, they were collected and placed on selective agar discs to show if the morality due to fungal infestation or not. In addition to examine the die larva under stereomicroscopy and pick picture to record symptoms of death (Fig. 1).

Statistical analysis

The mean number of live larvae per leaf was tested for percent mortality. The data were subjected to analysis of variance (ANOVA) and the means were compared by LSD test at 0.05 levels, using SAS computer program (SAS, 2009). Pathogenicity analysis was done according to Gomez and Gomez (1984).

CM (%) = T(%)-C(%) /100-C(%)

Where;

CM (%)-corrected mortality
T-Mortality in treatment
C-Mortality in control

Results and Discussion

Table 1 shows the mean percent mortalities of both isolates of entomopathogenic fungi on the different stages of *S. thraustica* the data showed that both tested isolates had more or less degree of Pathogenicity against larvae and prepupae of *S. thraustica* in case of isolate *B. bassiana*, the least concentration ($1x10^6$conidial/ml) gave the lowest mortality percent in all larval instar started from 27.8%

in 2nd larval instar and ended with 65.6% in the 5th larval instar, while the highest tested concentration ($1x10^8$ conidial/ml) started with 64.4% mortality in 2nd larval instar and ended with 92.2%mortality in the 5th larval instar. Also, prepupal stage recorded 50.0% mortality at ($1x10^6$ conidial/ml) and this percent raised to 92.2% at ($1x10^8$ conidial/ml).

In case of isolate *M. anisopliae* the same previous trend of mortality was observed where the mortality percent has positive relationship the applied concentration in all tested staged of *S. thraustica* where the highest mortality percent (74.4%) was recorded with 5th larval instar at ($1x10^8$ conidial/ml).

Generally, the data in the table 1 shows asymmetric trend of mortality percent in relation to applied concentration of both tested isolated. From these data it is observed that *B. bassiana* is more virulence than *M. anisopliae* against larvae and pupae of *S. thraustica* at all tested concentrations especially *B. bassiana* recorded 94.4% and 92.2% mortality in 5th larval instar and pupal stage at ($1x10^8$ conidial/ml) this highest mortality percentage give the priority to isolate *B. bassiana* for controlling this pest.

The higher pathogenicity of *B. bassiana* against *S. thraustica* than *M. anisopliae* is more obvious through LC_{50} and LC_{90} values in table 2.

The effected of entomopathogenic fungi were evaluated to determine whether the tested concentrations have high efficacy against larvae and pupae of *S. thraustica* under laboratory conditions.

Both fungal isolates were found pathogenic to larvae and pupae of *S. thraustica* and there is no relationship between the age of the treated larval instar and both corresponding LC_{50} and LC_{90} where LC_{50} for 5th and 3rd larval instar were $2.5\ x10^5$ conidia /ml and $6x10^6$ conidia / ml of *B. bassiana*, respectively and LC_{90} values of the same isolate for the same previous larval instar were $3.7x10^7$ and $7.8\ x10^8$ conidia/ml, respectively. The same previous trend of LC_{50} and LC_{90} towards different larval instars was also observed with *M. anisopliae.*

Table.1 Mean percent mortality of *Beauveria bassiana* and *Metarhizium anisopliae* at different concentrations on the larvae and pupae of *Stomphastis thraustica* under laboratory condition

Treatment	Mean percent mortality after treatment					
	Conc. (Conidia /ml)	Larval stage				Prepupal stage
		2nd	3rd	4th	5th	
Beauveria bassiana	$1x10^6$	27.8	38.9	34.4	65.6	50.0
	$1x10^7$	57.8	69.9	64.4	82.2	74.4
	$1x10^8$	64.4	78.9	71.1	94.4	92.2
Metarhizium anisopliae	$1x10^6$	11.1	21.1	17.8	25.6	35.6
	$1x10^7$	29.9	38.8	34.4	49.9	46.7
	$1x10^8$	53.3	67.8	62.2	74.4	60.0
control	water	7.8	11.1	7.8	2.2	13.3

Table.2 Mean lethal concentrations (LC_{50}) and (LC_{90}) of *Beauveria bassiana* and *Metarhizium anisopliae* on the larvae and pupae of *Stomphastis thraustica* under laboratory condition

Treatment		95% confidence limit		95% confidence limit	
	Treated stage	LC_{50}	slope	LC_{90}	slope
Beauveria bassiana	2nd larval instar	$1.2x10^7$	0.8 ± 0.16	$1.3x10^9$	0.6 ± 0.1
	3rd larval instar	$6x10^6$	0.9±0.18	$7.3x10^8$	0.6±0.1
	4th larval instar	$4.3x10^6$	0.5±0.1	$5.5x10^8$	0.6±0.1
	5th larval instar	$2.4x10^5$	0.8±0.19	$3.7x10^7$	0.59±0.12
	Prepupal stage	$1.8x10^6$	0.6±0.11	$8.8x10^7$	0.8±0.11
Metarhizium anisopliae	2nd larval instar	$9.2x10^7$	0.5±0.1	$3.1x10^9$	0.8±0.1
	3rd larval instar	$5.1x10^7$	0.8±0.12	$2.8\ x10^9$	0.7±0.1
	4th larval instar	$3.8x10^7$	0.7±0.2	$1.6x10^9$	0.8±0.1
	5th larval instar	$1.1x10^7$	0.9±0.1	$8.9x10^8$	0.7±0.1
	Prepupal stage	$5.7x10^7$	0.8±0.12	$1.5x10^{11}$	0.4±0.12

Fig.1 Reisolation on agar media to distinguish between larval mortality due to fungus (A) or due to other reason (B)

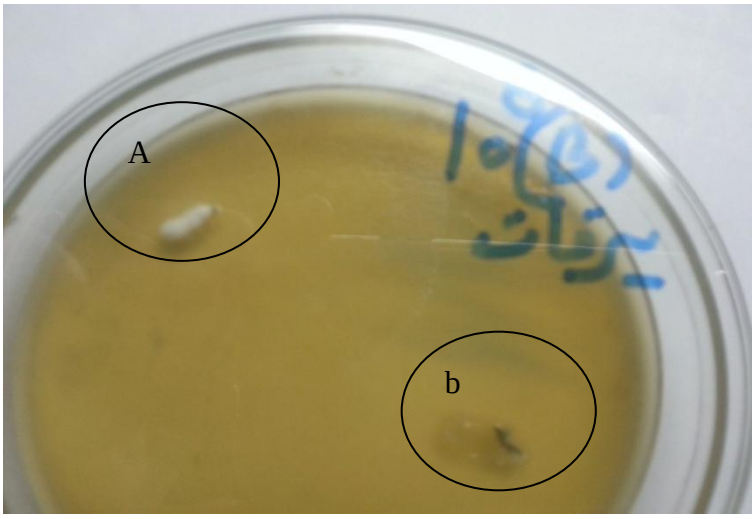

Fig.2 Larval mortality symptoms after 96 hr of fungal treatment. Arrow indicated to hyphal growth on the cuticle surface. A= 3rd larval instar, B= 5th larval instar

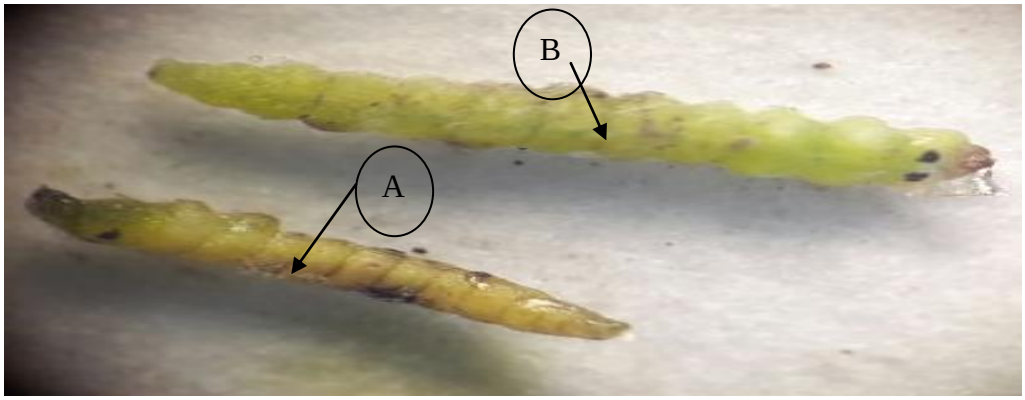

Fig.3 Pupal mortality symptoms after 96 hr of fungal treatment

The data in table 2 shows that there are negative relationships between LC_{50} and LC_{90} and the age of the treated instar, this is mean that the earlier instar is less sensitive to both fungal isolates than older ones. Also, it is noticed that prepupae of *S. thraustica* is more sensitive to both fungal isolate than larval stage at any concentration. The symptoms of the larval death due to fungal infection are clearly observed in figures 1, 2 and 3.

The amount of conidia used to attain concentration and thus, achieving an efficacious penetration of the fungus on the cuticle of the treated insect stage and causing host death. Similar finding by Garcia *et al.*, 2011 were obtained on the efficiency of *B. bassiana* and *M. anisopliae* on *Spodoptera fungiperda* and *Epilachna varivestis* larvae at six concentration (10^4 to 10^9); *B. bassiana* was more virulent for *E. varivestis* larvae with 93.3% mortality. *B. bassiana* strain presented the highest mortality on *S. fungiperda* larva. On the other hand, our results disagreed with Khalid *et al.*, 2012, evaluating the pathogenicity of both *B. bassiana* and *M. anisopliae* on *G. mellonella* larvae using concentration 10^2 to 10^6 conidial /ml.

Our results agree with that of Cabello *et al.*, 2009 where stated that; the higher mortality of *T. absoluta* larvae under laboratory condition indicated that *B. bassiana* cause moderate larval mortality.

Our results confirmed the finding of Shalaby *et al.*, (2013) on *T. absoluta* they stated that the earlier larval instar is more sensitive to *M. anisopliae* than the older larval instars. Dahliz (2014) have reported similar results with *M. anisopliae.* Our results confirmedly agreed with the study of Inani and Oldarge (2012)**;** they compared the pathogenicity of *B. bassiana* and *M. anisopliae* on *T. absoluta* larvae and indicated the higher efficiency of *B. bassiana* against *T. absoluta* larvae.

Our results also indicated the potential of *B. bassiana* isolate in controlling the larvae of *S. thraustica* and can be included in IPM programs. Neves and Alves (2002) noted,as more conidia penetrating, more toxins or enzymes are released, increasing the insect mortality, though, the fungus action speed depends, besides the concentration, of the host species involved (Sosa-Gomez and Moscardi, 1992)**.** According to Klespies and Zimmermann (1998), variation in virulence of entomopathogenic fungi is a result of differences in the enzymes and toxins production in conidia germination speed, mechanical activity in the cuticle penetration,

colonization capacity and cuticle chemical composition.

The use of entomopathogenic fungi different insect pest seems to promising insecticide alternative and its efficiency trusted by many authors. Our results indicated that the most effective percent mortality of tested fungal isolates was found in *B. bassiana* than *M. anisopliae* in all treated larval instars besides the pupa of *S. thraustica.* Both isolates could be very well utilized as safe alternatives to chemical insecticides for controlling *S. thraustica* especially our study make up the first attempt to use the entomopathogenic fungi against *S. thraustica.* It might be concluded that *B. bassiana* than *M. anisopliae* show different pathogenicity cause mortality of insect from early to last larval instar besides the pupal stage and the higher efficiency of *B. bassiana* against *S. thraustica* give it the priority for controlling this pest than *M. anisopliae.* Therefore, further study under field conditions should be occurred to evaluate the efficiency of these formulations for controlling this pest.

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