



Efficacy of entomopathogenic fungi isolated from the nest of the samsun ant, *Pachycondyla sennaarensis* against larvae of the mosquitoes *Aedes caspius* and *Culex pipiens*

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With 2 figures and 1 table

Abstract: The use of entomopathogenic fungi is a promising alternative to chemical control against mosquitoes. In February 2013, two fungi were isolated from the Samsun ant, *Pachycondyla sennaarensis*, Mayr, (Hymenoptera: Formicidae) collected in Riyadh, KSA. One of these fungi, identified as *Fusarium solani* was highly pathogenic against larvae of the mosquito, *Aedes caspius* (Diptera: Culicidae). *F. solani* killed 100% of *Ae. caspius* within 5 days of exposure to the concentration of 5×10^7 cfu/ml. Also, results indicated that *F. solani* was more virulent to the mosquito larvae than *F. chlamydosporum* at the concentration of 8×10^7 cfu/ml. The histopathological examination indicated that spores of *F. solani* are invasive to the wall of *Ae. caspius* larvae. Both *F. solani* and *F. chlamydosporum* were not affected against *Ae. caspius* and *Culex pipiens*. These results suggested that *F. solani* could be an effective tool to manage *Ae. caspius* proliferation and hence diseases transmission.

Keywords: Entomopathogenic fungi, *Pachycondyla sennaarensis*, *Aedes caspius*, *Culex pipiens*, biocontrol

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Introduction

Vector borne diseases are a major source of illness and death worldwide. Mosquitoes are one of the most important vectors that alone transmit diseases to more than 700 million people per annum (Taubes 1997). Malaria alone kills 3 million each year, including 1 child every 30 sec (Shell 1997). *Ae. caspius* is widely distributed in different regions of Saudi Arabia such as Riyadh district (Al-Khrejji 2005), the eastern (Buttiker 1981) and south western regions (Abdullah & Merdan 1995). *Ae. caspius* is the most abundant mosquito in Al-Ahsaa (Ahmed et al. 2011). Omar (1996) reported that local *Culex pipiens* mosquitoes might act as a potential vector of introduced Bancroftian filariasis in Saudi Arabia.

In Saudi Arabia, the most common mosquito borne diseases include dengue (Khan et al. 2008), filarial (Hawking 1973), malaria (Abdoon 2004), and Rift valley fever (Balkhy & Memish 2003, Al-Hazmi et al. 2003, Madani et al. 2003). Control of such diseases is becoming alarmingly complex because of increasing resistance in mosquitoes to many synthetic insecticides. Therefore, there is an increasing interest in alternative control methods, including biocontrol using entomopathogenic fungi.

Many studies have been carried out to investigate the potential of entomopathogenic fungi for the control of adult malaria vector mosquitoes (Scholte et al. 2003, Blanford et al. 2005, Scholte et al. 2005, Mnyone et al. 2009, Farenhorst et al. 2009, Bilal et al. 2012, Seye et al. 2012). Bukhari et al. (2010) stated that, spores of two fungus species, *Metarhizium anisopliae* and *Beauveria bassiana* have potential to kill the mosquitoes, *Anopheles gambiae* and *Anopheles stephensi* in the larval stage and that mortality rate depends on fungus species itself, larval stage targeted, larval density and amount of nutrients available to the larvae.

Many Fusarium species are well-known pathogens of plants, insects, and humans. More than 13 Fusarium species are pathogenic to insects, and the group has a host range that includes Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera (Humber 1992, Scholte et al. 2004). Gupta et al. (1991) isolated the toxin beauvericin, a cyclodepsipeptide, from Fusarium. Those authors showed that beauvericin can kill 50% of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), larval test populations at a 663-ppm dose. Beauvericin, isolated from Fusarium, also is lethal to dipteran insects such as mosquitoes and blow flies (Tanada & Kaya 1993). According to Humber (1992), *Fusarium solani* (Mart.) Sacc. is infectious to pupae of the dipteran family Anthomyiidae.

In this study, we screened the soil sample from ant's nest of *P. sennaarensis* for entomopathogenic fungi for their relative pathogenicities to 2nd instar larvae of *Aed. caspius* in order to evaluate their potential as biological control agents.

Materials and Methods

Isolation and Identification of Fungi

Pachycondyla sennaarensis workers and soil samples were collected from ant's nest of *P. sennaarensis* in Dir'ya region, near Riyadh City, Saudi Arabia. *P. sennaarensis*

were preserved in the laboratory until death and then superficially sterilized by placing the specimens in 70% ethanol for a few seconds, washed in sterile distilled water and dried. The ants were distributed in 10 Petri dishes (3 ants/plate) containing 10 mg sterilized starch and mixed with the soils samples suspended in 15 ml of sterile distilled water. They were incubated at 25 ± 2 °C and 85 ± 5 % relative humidity (RH) for 14 days. A Petri dish containing the same media was used as control. The colonies of fungi on ants were observed each day and images were taken. These colonies were isolated in Petri dishes on Potato dextrose agar (PDA) and the subcultivations were performed to obtain the pure fungal colonies. The pure fungal isolates were identified by macro-and-micro-characteristics according to Leslie & Summerell (2006). The macro-characteristics including colony morphology on PDA and micro-characteristics including macroconidia, microconidia, chlamydospores and Sporodochia were studied. To confirm the identification, the isolates have been deposited into the cultural collection of Assiut University Mycological Center (AUMC), Arab Republic of Egypt.

Conidial suspensions

Fungal spore suspensions of two *Fusarium* isolates were prepared from 14 days old cultures on PDA. Twenty ml of sterile distilled water were added to each culture and were gently mixed, then the spores were harvest and purified by centrifugation at 3000 rpm to remove the hyphae. The available spores were estimated by standard plate count (SPC) according to Pitt et al. (1992). Seven decimal dilutions of fungal spore suspensions were performed on test tubes containing 9 ml of sterilized saline solution (0.89% of NaCl). Each decimal dilution was tested, and the mortality of *Ae. caspius* larvae was reported.

Rearing of mosquitoes

Ae. caspius larvae used in this study was maintained at Department of Zoology, College of Science, King Saud University. They were reared indoor at 27 ± 2 °C, 50 ± 5 % relative humidity, a 14:10 light: dark photo-period and they were fed daily with fish feed until become pupae. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary. They were moved into mosquito cage where the emergent adults were fed with a 10% glucose solution in a jar with cotton wick. The adult were given a blood meal from a mouse placed in resting cages overnight for blood feeding by females *Ae. caspius*. Glass Petri dish lined with filter paper with 100 ml tap water kept inside the cage for oviposition.

Larvicidal bioassay

Fungal spore suspensions were distributed in Petri dishes. A tap water and spore free suspension were used as two control groups (Spores free suspension was prepared by filtering the 10 ml of spore suspension through 0.2 µm filter to remove the spores). In each bioassay, 20 larvae of *Ae. caspius* of 2nd instar larvae were added to each Petri dishes (n = 3). Bioassay was conducted at 7 different concentrations (5×10^8 , 8×10^6 ,

4×10^5 , 6×10^4 , 7×10^3 , 3×10^2 , and 5×10^1). The plates were incubated at 27 ± 2 °C, 50 ± 5 % relative humidity, a 14:10 light: dark photo-period for 5 days and the percent mortality of mosquito larvae was recorded.

Direct Microscopic Test

The direct microscopic observation method was carried out according to Gianni et al. (2001) to observe histopathological effects. The control and treated *Ae. Caspius* larvae groups were mounted on slides containing drops of 40% KOH and studied under light microscope.

Statistical analysis

The experiment was designed as completed random design (CRD). The data were expressed as means \pm standard error. Statistical analyses of mortality rate were performed by analysis of T-test (one sample T-test) (SPSS 13.0 for window 2004).

Results

The developing colonies of fungi on *P. sennaarensis* were shown after 5 incubation days. The colonies were completely around the outer surface of *P. sennaarensis* after 14 days. The hyphae, macro-conidia, micro-conidia and conidiophores were observed by microscopic examination (Fig. 1). Two pure isolates were cultivated and the study of macro- and micro-characteristics revealed that these isolates were *F. solani* and *F. chlamydosporum*. The pure isolates were sent to Mycological center, Assiut University (AUMC), Assiut, Egypt. The report of the mycological center confirmed that the two isolates were *F. solani* (AUMC 9304) and *F. chlamydosporum* (AUMC 9305).

Preliminary Bioassay with conidia suspensions of each isolate revealed that *F. solani* was highly pathogenic to *Aedes caspius* larvae. Mortality rates was 100% at the concentration of 8×10^7 cfu/ml within 5 days while the mortality did not reach more than 25% when using spore suspension of *F. chlamydosporum*. For that reason, *F. solani* was used in subsequent experiments. Seven decimal dilutions were tested (8×10^6 , 4×10^5 , 6×10^4 , 7×10^3 , 3×10^2 and 5×10^1 cfu/ml) and the mean (%) mortality of control and treated *Ae. caspius* larvae groups with *F. solani* after five days of treatment were presented in Table 1. The mean mortality rate in the treated groups with the *F. solani* spore suspensions showed a significant increase ($p < 0.05$) compared to the control groups. The mortality rate reached 100% in *Fusarium solani* spore suspensions at the concentration of 5×10^7 spore/ml, after that, the mortality rate showed a significant decrease ($p < 0.05$) compared to the first suspension of *F. solani* spores.

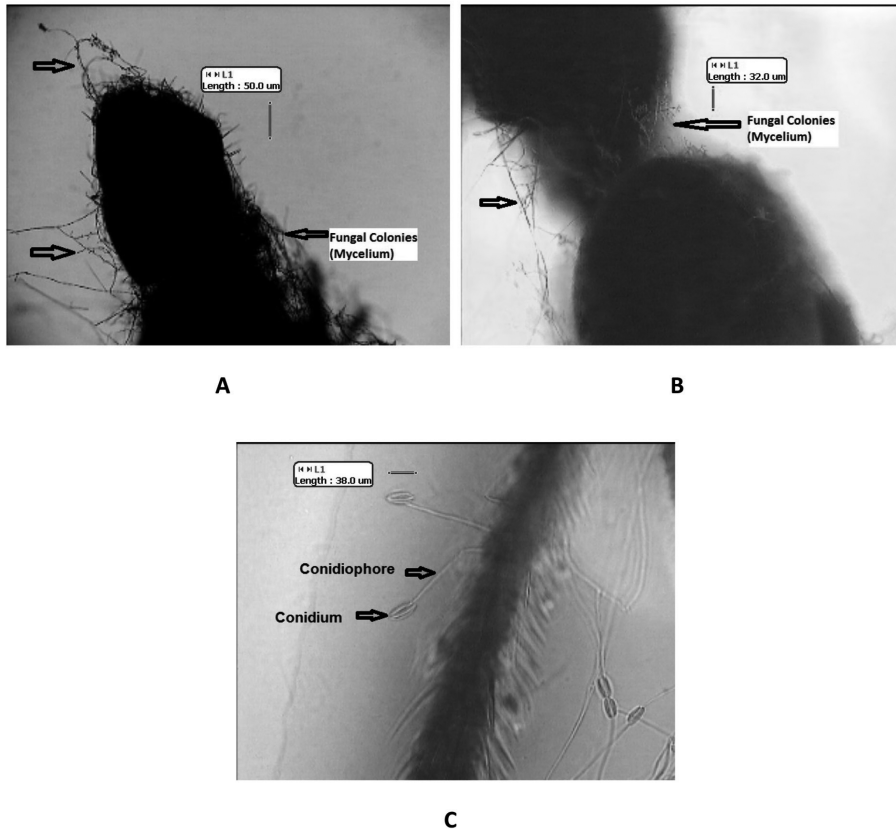


Fig. 1. Light microscope micrograph of the samsum ant shows developing fungal colonies, hyphae, conidiophore and conidia on outer surfaces. A: Abdomen, B: Petiole, C: Antenna.

In this stage, the effects of fungal spore concentrations on *Ae. caspius* population were studied and histopathologic effects on *Ae. caspius* were observed by direct microscopic method. The results of histopathologic test indicated that spores of *F. solani* infected primarily the wall of *Ae. caspius* larvae. Light microscopic micrograph for control groups and *Ae. caspius* larvae infected by *F. solani* spores showed that the spore were invasive to the wall (Fig. 2B, C). In the same figure, the wall of control groups appeared intact without fungal infection (Fig. 2A) compared to Fig. (2B) that showed fungal elements inside the wall of *Ae. caspius* larvae stained with KOH (blue color).

Results in Table 1 showed that neither *F. solani* nor *F. chlamydosporum* affected on *Cx. pipiens* larvae.

Table 1. Mean mortality rates (%) for *Ae. caspius* and *Cx. pipiens* larvae treated with fungal spore suspensions (average of three replicates).

Fungi	Species	Dose								Control
		5x10 ⁷	8x10 ⁶	4x10 ⁵	6x10 ⁴	7x10 ³	3x10 ²	5x10 ¹		
<i>Fusarium solani</i>	<i>Ae. caspius</i>	100 ± 0.0**	73.33 ± 15.27	43 ± 15.27	30 ± 17.32	33.33 ± 20.81	33.33 ± 28.86	33.33 ± 11.54	0.1 ± 0.0*	
	<i>Cx. pipiens</i>	11 ± 0.3	10.2 ± 2.6	7.12 ± 0.4	6.1 ± 0.3	2.81 ± 0.3	0.51 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	
<i>F. chlamydo-sporum</i>	<i>Ae. caspius</i>	18.4 ± 0.5	13.1 ± 1.9	12.2 ± 0.7	10.61 ± 0.7	5.1 ± 0.9	2.6 ± 0.5	0.7 ± 0.1	0.1 ± 0.0	
	<i>Cx. pipiens</i>	10.3 ± 0.8	7.14 ± 1.6	6.3 ± 0.2	3.8 ± 0.11	2.11 ± 0.3	0.81 ± 0.6	0.34 ± 0.2	0.0 ± 0.0	

Each value represent mean of three replicates and ± SE.

* A significant increase in mortality rate (%) compared to treated groups.

** A significant increase in mortality rate (%) compared to treated and control groups.

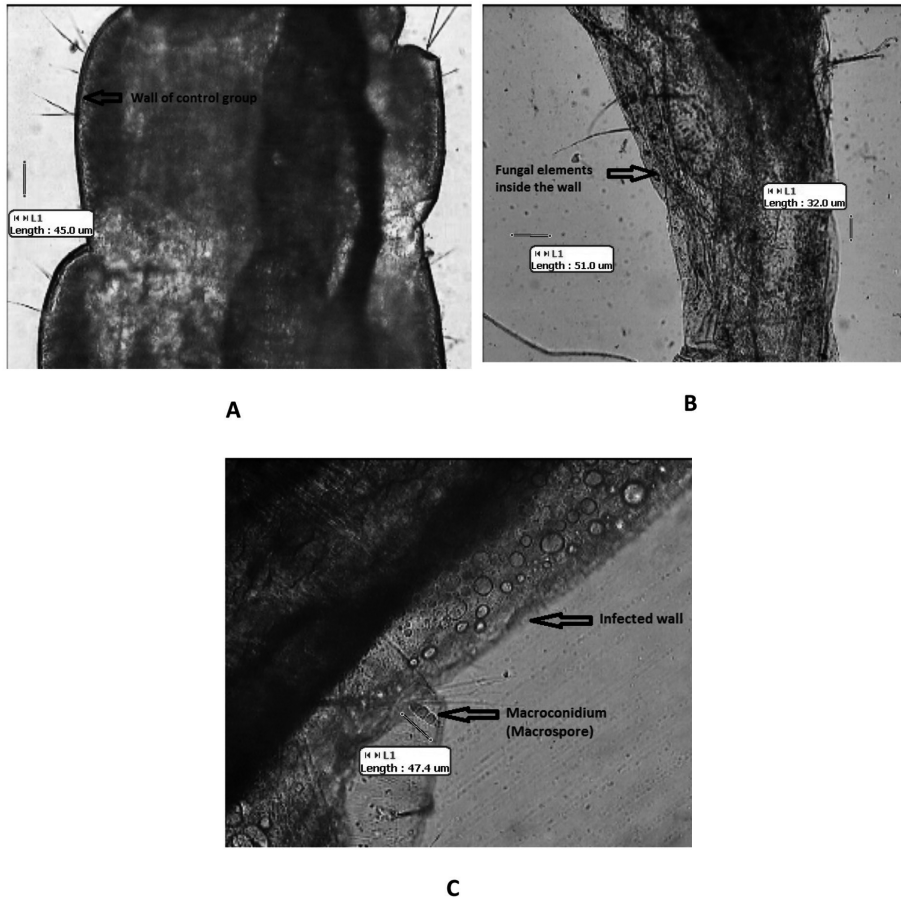


Fig. 2. Light microscope micrograph of *Ae. caspius* larvae control group (A) and infected groups by *F. solani* (B and C). Fungal elements appear as blue color inside the wall (B) and a macroconidium infects the wall (C).

Discussion

The present study was conducted to evaluate the soil sample fungi of *P. sennaarensis* nest as possible entomopathogenic fungi for the control of mosquito larvae. This is the first report to demonstrate the efficacy of *F. solani* and *F. chlamydosporum* as an entomopathogenic fungus on *Ae. caspius* larvae. However, *F. solani* was more virulent to the mosquito larvae than *F. chlamydosporum*. The immature stage of the vector is the most perfect stage for the biocontrol agent (Conti et al. 2010). Seye et al. (2013) suggested that the fungi *Metarhizium anisopliae* formulated with Suneem may provide a more sustainable management strategy for malaria vectors control at larval stages of *Anopheles gambiae*. *F. solani* showed a clear dose-dependent effect on mortality in *Ae. caspius* larvae. The reduction in *Ae. caspius* larvae survival will likely

reduce the number of adult mosquitoes and the blood meals taken, and as a result the possibility of the vector acquiring and transmitting a pathogen. The previous studies have revealed that infection of adult mosquitoes (*An. gambiae*, *Culex pipiens*, *Aedes aegypti* and *C. inornata*) with various fungi species caused significant reduction in their survival (Scholte et al. 2004). Reducing adult survival is therefore considered the most effective way to reduce disease transmission (Ansari et al. 2011). Also, Laerciana et al. (2013) stated that the microbial control of adult mosquitoes is thought to be more promising than that of larvae due to reduced half-life of the conidia in aqueous environments.

By and large, our results suggest that entomopathogenic *F. solani* is a potential fungus for targeting mosquitoes and the diseases transmission. Future research efforts should investigate the potential effects on non- target species, the risk to humans and the environment as well.

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