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ORIGINAL ARTICLE

Effect of certain entomopathogenic fungi and nematode on the desert locust *Schistocerca gregaria* (Forskal)

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Abstract The efficiency of entomopathogenic fungi, *Beauveria bassiana* and *Entomophthora* sp. as well as the nematode, *Steinernema carpocapsae*, against 3rd, 5th instar nymphs and adults of the desert locust, *Schistocerca gregaria* (Forskal) had been studied under laboratory conditions. Fungi at the concentrations 2.3×10^5, 7×10^7, 3.9×10^9 and 4×10^5, 6.7×10^7, 2.2×10^9 spores/ml and nematode at 5000, 2500, 1250 IJS/ml, respectively were applied on the desert locust by two different treatment methods; spray and soil application. The obtained results revealed that all the locust nymphs and adults were susceptible to fungi and nematode with high mortality rate records. The nematode killed approximately 100% of the tested locust individuals within 3–5 days postinfection and was found more effective than fungi in less time. On the other hand entomopathogenic fungi and nematode were found to be more effective when applied on the soil surface rather than spray treatment. This was common at any concentration used. Based on mortality percentages, all tested fungi and nematode had high potentials for biocontrol agents against *S. gregaria*.

Introduction

The desert locust, *Schistocerca gregaria* Forskal (Orthoptera: Acrididae) is among the major insect pests due to its catastrophic damage to crops in large parts of Africa and Asia. The major control strategy adopted against the desert locust is based on the use of insecticides. The continuous use of chemical pesticides against pests has led dramatically to a resistance to the pesticides action and causes rapid increase of insect tolerance against any type of neurotoxic insecticide Elbanna et al. (2012). In addition, the intensive use of these chemicals gave rise to problems such as residual toxicity (pollution) and harmful effects on beneficial insects, human beings and their domestic animals. Such problems have become a reason for searching for safe pesticides, Gabarty et al. (2013). Recently, the biological control, specifically, use of entomopathogenic microorganisms through their various species, easy dissemination, specificity of action and persistence in the environment is a very promising alternative to ensure effective pest control.

The microorganisms used in microbial control belong to several taxa namely; bacteria, viruses, fungi, nematodes and protozoa Halouane et al. (2013) .Among the microorganisms

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used, more than 700 species of fungi are entomopathogenic agents against arthropods insects Khan et al. (2012). The largest number of pathogens is in the class Zygomycetes, but most employees come from Deuteromycetes, such as *Beauveria, Metarhizium, Verticillium, Entomophthora* and *Entomophaga* Halouane et al. (2013). Like other entomopathogenic fungi, *Beauveria bassiana* possess the potential to produce infections conidia, which penetrate the insect's cuticle, indicating that, *B. bassiana* induces on appropriate mechanism to overcome the insect's cellular defense system Abood et al. (2010).

Entomopathogenic nematodes (EPN) belonging to families Steinernematidae and Heterorhabditidae are soil inhabiting insect pathogens that possess potential as biological control agents due to their broad host range, host seeking abilities, high virulence, safe for vertebrates and plants and can be easily mass-produced and applied using conventional equipment Shamseldean et al. (2013). Both *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* species are the main insect pathogenic nematodes used as biological control agents against economic insect pests with a wide host range. It was believed that the nematode kills the host because the nematode evades recognition as non-self, allowing times to release its symbiotic bacterium. In turn, the bacteria tolerate the insects cellular and noncellular activities and inhibit the immune responses (Youssef, 2008). When the nematode enters a target insect, the symbiotic bacteria are released into the hemocoel. After inducing host immune suppression, the bacteria multiply in the hemocoel and cause fatal septicemia (Jung and Kim, 2007).

Infective juvenile stage survives in the soil, finds and penetrates insect pests, reproduces in dead insect hosts, causes mortality of insects in the soil and causes insect populations to crash conspicuously. After the infective juveniles enter their host insects, they release the toxic, bacteria into the insect hemocoel Park and Kim (2000).

The objective of this study was to evaluate the efficacy of the entomopathogenic fungi *B. bassiana*, *Entomophthora* sp. and the nematode *S. carpocapsae* on different stages of the desert locust *S. gregaria* under laboratory conditions by direct spray and soil treatments.

Materials and methods

Insect culture

Astock culture of the desert locust, *S. gregaria* (Orthoptera: Acrididae) was obtained from the culture maintained for several generations in the gregarious phase, mainly nymphs and adults at the Locust and Grasshopper Research Department, Agriculture Research Center (ARC), Giza, Egypt. The immature and mature insects of the locust were reared under the laboratory conditions of 27 ± 2 °C, 70 ± 5% RH and 12:12 daily photoperiod, according to the method described by Hunter-Jones (1966). The locust food is maize leaves and stems.

Fungal culture

Culture of entomopathogenic fungi, *B. bassiana* was obtained from Plant Protection Research Institute, ARC, Giza, Egypt. *B. bassiana* was cultured at 25 ± 1 °C on Potatoes Dextrose Agar (PDA). After 15 days, conidia were washed by distilled water. Fungal cells were counted using a hemocytometer and were diluted in a sterile saline solution to the concentrations of 2.3×10^5, 7×10^7 and 3.9×10^9 spores/ml. The required different concentrations of spores were prepared after several preliminary tests.

Fungal isolation

Isolation was carried out by adapted dilution plate method (Johnson et al., 1959). Dead nymphal instars of *S. gregaria* were removed from the culture and the surface was sterilized by 5% sodium hypochlorite for 2 min, then 70% ethanol solution for one minute, and rinsed in plenty of sterile distilled water. Each nymph was placed in a sterile Petri dish (9 cm in diameter) containing 1 ml of 0.85% saline solution, cut into small pieces using sterile cutter, and distributed in sterile Petri dishes. Twenty milliliters of PDA medium was poured in each Petri dish and then the plates were incubated at 28 °C for 10 days Gamal et al. (2012). The developed colonies were picked, transferred to PDA plates and purified by using single spore technique. All fungal isolates were cultured on PDA medium and incubated at 30 °C for 7 days. Stock culture from the isolate was stored on agar slants at 5 °C until further use. The fungal isolates were identified by the Plant Pathology Institute, ARC. This fungal inocula *Entomophthora* sp. was diluted in sterile saline solution to the concentrations of 4.1×10^5, 6.7×10^7 and 2.2×10^9, spores/ml, and were prepared for further tests.

Nematode culture

Nematode individuals, *S. carpocapsae* were supplied by Nematology, Pest Plant Protection Dept., National Research Center, Giza, Egypt. The nematodes were extracted and used for propagation as mentioned by El-Kifl (1980). Water suspension of infective juveniles stage **(IJS)** was washed and prepared at a concentration of 5000 IJS/ml in sterile distilled water and maintained at 4 °C till use (El-Kifl and Sammour, 1989). Three different concentrations of nematode were used. (5000, 2500, 1250 IJS/ml).

Treatments

Spray treatment

Serial dilutions were prepared in 100 ml distilled water for both tested fungi; i.e., 2.3×10^5, 7×10^7 and 3.9×10^9 spores/ml for *B.bassiana*, 4.1×10^5, 6.7×10^7, and 2.2×10^9 spores/ml for *Entomophthora* sp and 5000, 2500, 1250 IJS/ml for nematode *S.carpocapsae*. Fifteen individuals from the 3rd and 5th instar nymphs and adults of *S. gregaria* were divided into three groups (5 individuals each) in plastic box (10×17 cm^2) lined with a filter paper were sprayed with ten milliliters from the conidial suspension of each fungus and the suspension of nematode on each nymphal group, using hand atomizer. Pieces of fresh maize stem and leaves were introduced. A group of nymphs and adult sprayed with distilled water as untreated check (control group). The infected and non-infected nymphs and adults were daily cleaned, fed and estimated the mortality rate. The boxes were kept at 27 ± 2 °C and 70 ± 5% RH. Accumulative mortality percentages of the host insect were calculated and recorded using Abbott formulation (Abbott, 1925).