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Evaluation of the Entomopathogenic Fungus *Beauveria bassiana* (Balsamo) as a Biocontrol Agent against the Soft scale Insect, *Saissetia coffeae* (Walker) (Homoptera: Coccidae)

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ABSTRACT

Entomopathogenic fungi are considered as bio control agents against the insects, especially the piercing and sucking insects. Hereby a pioneer study in Egypt to evaluate the virulence of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) on the hemispherical soft scale insect, *Saissetia coffeae* (Walker) (Hamoptera: Coccoidea) infesting palmlike *Cycas revoluta* Thunb. in Egypt was carried out in laboratory. The pathogenicity of the fungus *B. bassiana* to nymphal stage was more than the adult females. The LC_{50} values were $1.57x10^3$ and $9.14x10^6$ spores /ml for nymph and adult females, respectively. The LT_{50} were 8.63 days for nymphs and 10.37 days for adult females. Field trial was conducted to evaluate the pathogenicity of *B. bassiana* on *S. coffeae* different stages infesting *C. revoluta* and *B. bassiana* reduced the population of different stages of *S. coffeae* where the reduction % on both nymphs and adult females after 30 days from treatment were 74.10 and 69.70 %, respectively.

Key Words: Entomopathogenic fungi, *Beauveria bassiana, Saissetia coffeae*, Biological control.

INTRODUCTION

Japanese sagopalm *Cycas revoluta* Thunb. is consider one of the wide spread plants in tropical and sub-tropical regions and a precious palm like . It grows slowly and ever green all over the year (Badr 1995). However, it is infested with many insect species, especially the sap sucking insects. The hemispherical soft scale insect *Saissetia coffeae* (Walker) (Homoptera: Coccidae) infests many ornamental plants including cycas (Hamon and Williams, 1984). Its main hosts are coffee, tea, citrus, guava, mango, olive, barbados, cherry and many other cultivated and wild plants (Hill 1983, Albuquerque *et al.*, 2002. and Abd-Rabou, 2005). Its infestation causes a serious damage to the leaves such as; yellowish and dryness, beside the honey dew secretion which helps the growth of the black sooty mould fungi covering the upper surface of the leaves preventing the photosynthesis and respiration leading to malformation of the green leaves (Hanafi, 1976) which consider the fortune of any ornamental plant. Also, hemispherical scales feed on plant juices causing a loss of vigor, spots on the foliage due to toxins in the insect saliva, deformation of infested plant parts, loss of leaves, retarded plant growth, and even death of the plant (Dekle,1965). According to Evans and Prior (1990), Petch in 1921 mentioned that entomopathogenic fungi were first noted on armored scale insects from willow and ash trees in France in 1848. Peña *et al.* (1987) noted occurrence of *Verticillum lecanii* associated with the scale insects *Philephedra tuberculosa* Nakahara and Gill in Florida, USA. Moreover, Evans & Prior (1990) summarized the pathogens recorded on diaspidid scale insects and mentioned the appearance of *V. lecanii* on *Aspidiotus nerii*, *Aspidiotus* sp., *Pseudoulacospis pentagona*, *Chionaspis salicis, Lepidosaphes ulmi*, *lepidosaphes* sp., *Mytilaxpis* sp. and *Unaspis citri.* Despite their being ubiquity principle pathogen to sucking insects, however, little is mentioned on entomopathogenic fungi as biological control agent of scale insects.

The present investigation was planned to evaluate the efficacy of the fungus *Beauveria bassiana* (Balsamo) as a biocontrol agent against *Saissetia coffeae.*

MATERIALS AND METHODS

Insect culture

C. revoluta palmlike leaflets heavily infested with *S. coffeae* were collected from El-Zohreya Botanical Garden, Cairo, Egypt. The leaflets were washed with distilled water to get rid of dusts and secreted honey dew from *S. coffeae* individuals and allowed to dry on tissue paper. Then, they were divided into pieces, each of 4-5 cm, to fit the diameter of Petri dishes.

Fungal culture and bioassay procedures

The fungal culture used in bioassay was *B. bassiana* isolate (isolated from unknown aphid species in Fayoum Governorate, Egypt by Nahla Ezz, Scale Insects and Mealybugs Department, PPRI). Propagation of

fungal isolate was carried out using Sabouraud dextrose agar (SDA) (10 g mycopeptone, 40 g dextrose, 15 g agar and 1000 ml distilled water) and incubated at 25° C at dark for 14 days. Fungal spores were harvested by scraping the culture surface with sterilized slide and suspended in sterilized 0.05 % Tween 80. Four conidial concentrations ($1x10^5$, $1x10^6$, $1x10^7$ and $1x\ 10^8$ conidia / ml.) were adjusted by estimation using a haemocytometer (Hirscmann 0.1 mm x 0.0025 mm^2).

Each concentration and control is represented with 5 replicates and each replicate consisted of 5 pieces of leaflets containing 50 individuals. The treatments were conducted by dipping the *S. coffeae* different stages located on the leaflets pieces into the fungal suspension concentrations for about 5 seconds and placed into 9 cm diameter Petri dishes. Check control was treated with only 0.05% Tween 80. All treatments were incubated at 25±1.5°C and > 90% RH and monitored daily by the aid of a binocular for determination of LC_{50} and LT_{50}. The dead individuals of *S. coffeae* were observed for fungal symptoms. Data were analyzed for determination of LC_{50} and LT_{50} using Ldp line analysis software.

Field experiment

1. Fungal production

B. bassiana isolate tested in a bioassay experiment was used in field trial. The isolate was allowed to grow for 2 weeks at 25°C on barely substrate medium (50 g Barely, 35 ml distilled water and 2 ml sunflower seed oil) (Aregger, 1992). Conidia were harvested by filtration in distilled water with 0.05% Tween 80 and homogenization. The final spore concentration of 5.8×10^9 spores /ml was formulated with sunflower oil.

2. Experimental design

A trial was carried out in middle of July at El-Zohreya Botanical Garden, Cairo, Egypt. Five replicates, each of 5 leaflets heavily infested *C. revoluta* palmlike leaves with *S. coffeae* different stages. Pre treatment counts were inspected. The palmlike received the normal agricultural practices. The fungal suspension was applied to *S. coffeae* by spraying using one liter hand sprayer atomizer. The check control sprayed with 0.05% Tween 80. Samples were taken every 5 days throughout one month and transferred immediately to be examined in the laboratory using a binocular. Reduction % in *S. coffeae* different stages estimated by using Stafford and Summers equation (1963).

$$\text{Reduction \%} = \frac{\text{Pre treatment count} - \text{Post treatment count}}{\text{Pre treatment count}} \text{X100}$$

Dead cadavers of *S. coffeae* in both treatments and check control were kept in < 90% R.H. and 25° C to observe the fungal growth.

RESULTS AND DISCUSSION

Susceptibility of *Saissetia coffeae* different stages to the entomopathogenic fungi *Beauveria bassiana*

Different stages of *S. coffeae* were susceptible to the entomopathogen *B. bassiana* (Fig 1.). Concentration and time mortality - response and estimated parameters LC_{50}, LT_{50} and slope (Table 1 and Figs 2 & 3) indicated pathogenicity of the entomopathogenic fungus, *B. bassiana* against *S. coffeae*. Susceptibility of nymphal stage was higher than adult female stages. The LC_{50} values were $1.57x10^3$ and $9.14x10^6$ spores /ml for nymph and adult females stages, respectively (Table 1). The LT_{50} were detected on concentration $1x10^8$ spores/ml recorded 8.63 and 10.37 days, for nymphs and adult female's stages, respectively (Table 1).

Field application of *Beauveria bassiana* against *Saissetia coffeae* infesting *Cycas revoluta* palmlike

Field application of the entomopathogenic fungi *B. bassiana* against *S. coffeae* infesting *C. revoluta* palmlike showed obvious effect in reduction percent of *S. coffeae* population (Table 2 and Fig. 4). The reduction percent began with low rates after 5 days from spraying in both nymphal and adult stages (16.67 and 7.58%, respectively). It increased afterward to record 74.10 % after 30 days from spraying for nymphal stage and 69.70% in adult stage.

Table (1): LC_{50} in spores /ml and LT_{50} in days for *Saissetia coffeae* nymphal and adult stages treated with the entomopathogenic fungus, *Beauveria bassiana* under laboratory conditions.

Insect stage	LC_{50}b (Spores/ml.)	Slope ± SE	LT_{50} (Days)	Slope ± SE
Nymphs	$1.57x10^3$	0.204±0.066	8.623	2.5±0.193
Adult	$9.14x10^6$	0.507±0.067	10.373	3.185±0.322

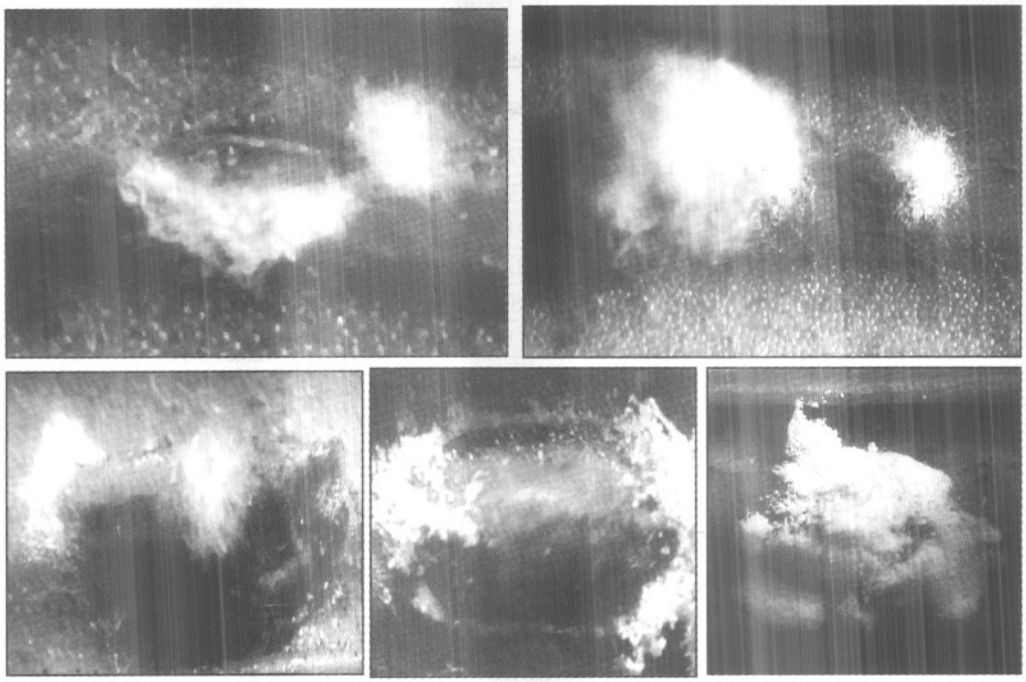

Fig. (1): Growth of entomopathogenic fungus, *Beauveria. bassiana* on nymphs and adults of the hemispherical soft scale, *Saissetia coffeae*.

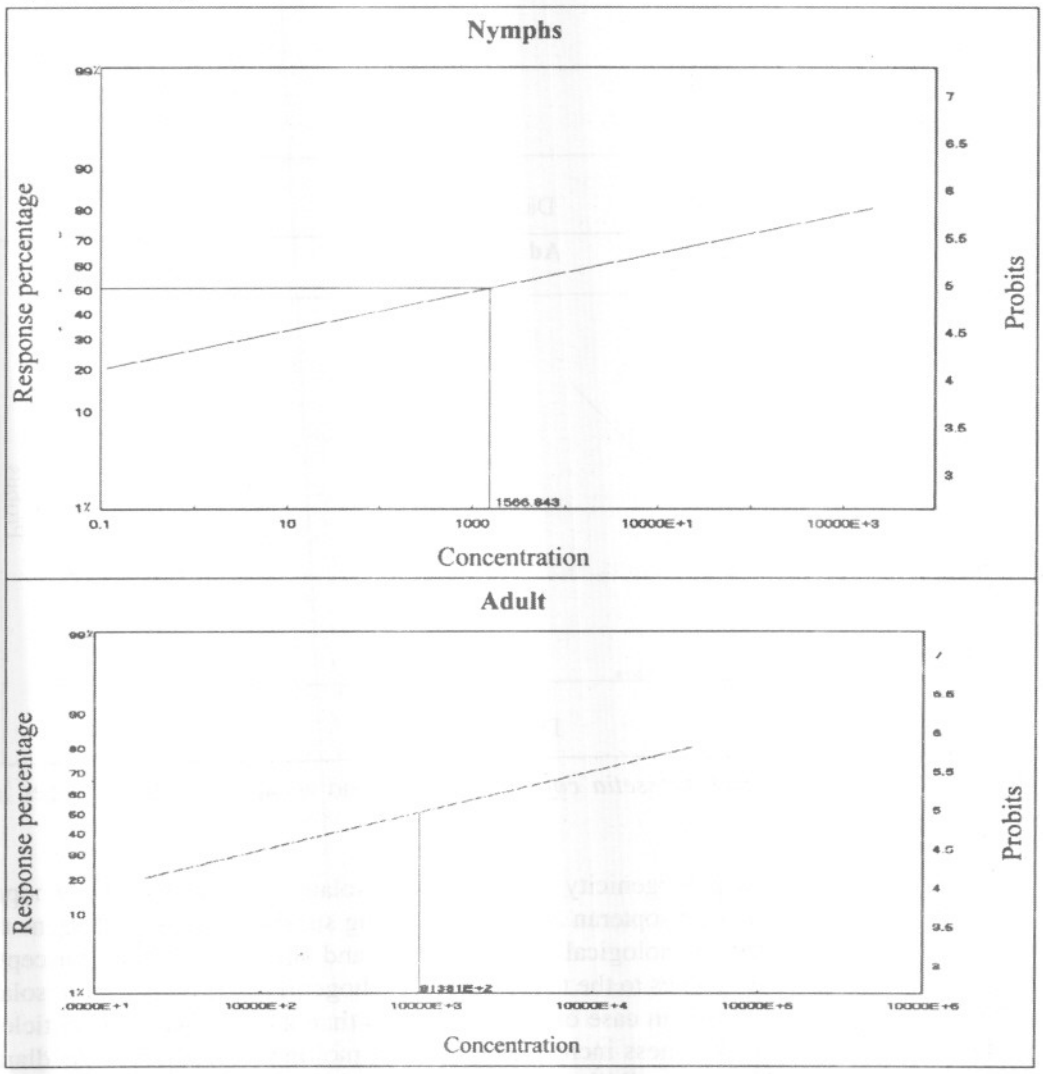

Fig. (2): Concentrations mortality–response of *Saissetia coffeae* nymphal and adult stages to fungi under laboratory conditions.

Table (2): Efficiency of *Saissetia coffeae* population by application the entomopathogenic fungus, *Beauveria bassiana* under field conditions.

Days after treatment	Reduction %	
	Nymphal stage	Adult stage
5	16.67	7.58
10	48.33	24.24
15	68.89	39.4
20	68.06	54.55
25	65.28	62.12
30	74.10	69.70

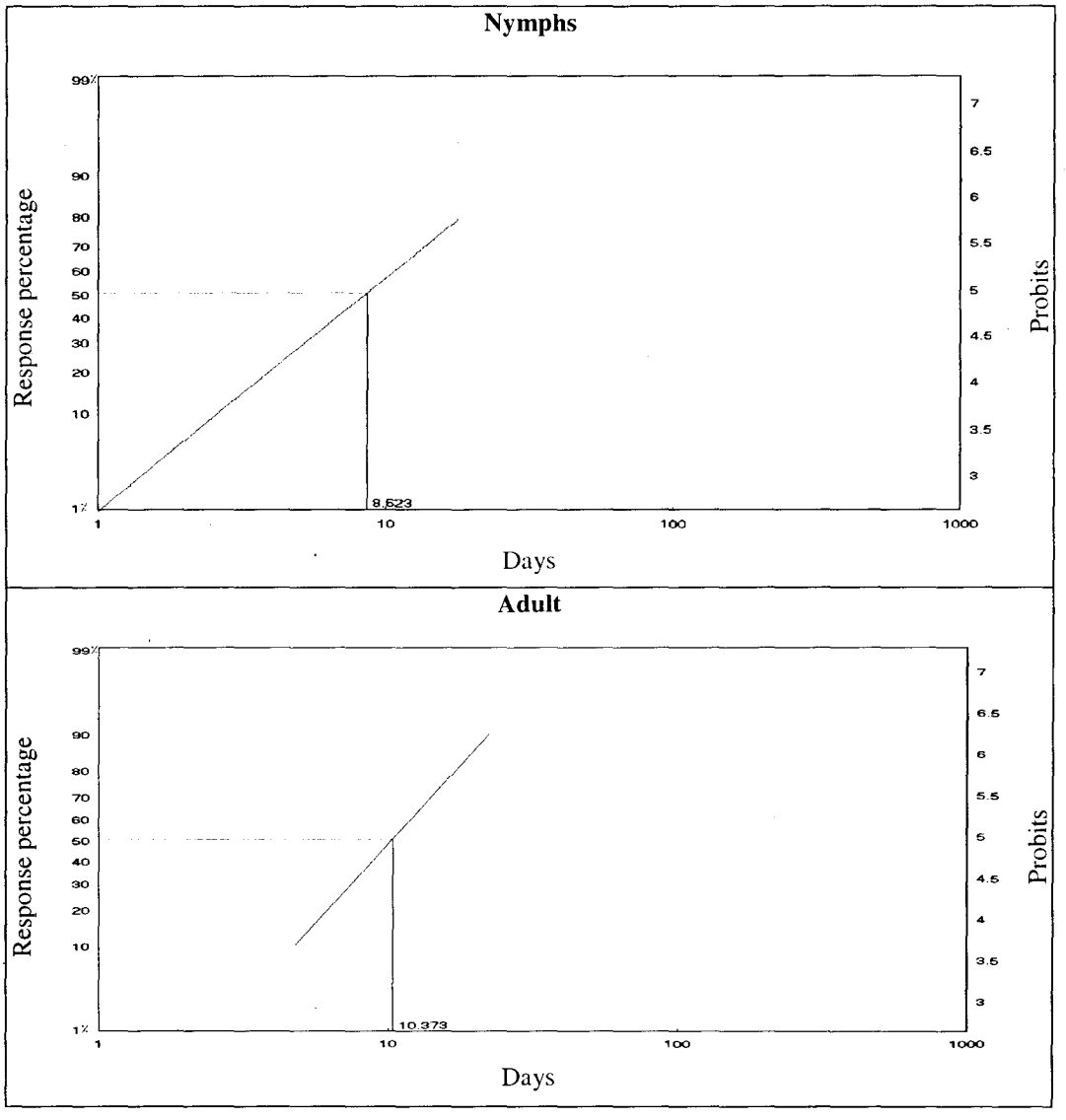

Fig. (3): Time mortality – response of *Saissetia coffeae* nymphal and adult stages fungi under laboratory conditions.

Laboratory bioassay revealed the pathogenicity of the fungus isolate to *S. coffeae*. Entomopathogenic fungi are the principle pathogen to the homopteran *S. coffeae*. Among sucking insects, pathogens that infect through the gut wall cannot be used in biological control (Hajek and St. Leger, 1994). Susceptibility of nymphal stage was higher than adult females to the tested entomopathogenic fungi *B. bassiana* isolate, which reflected by the low LC_{50} and LT_{50} values in case of treated nymphs than adults. The insect cuticle acts as a barrier for fungal penetration and its thickness increases with every molting (Boucias and Pendland, 1991). This can explain the differences in the susceptibility of the two stages to the entomopathogenic fungi.

Results showed obvious effect in reduction percent of *S. coffeae* population. However, the reduction percent began with low rates after 5 days from spraying and increased afterward to reach high percentages in 30 days in both nymphal and adult stages. This is in agreement with Srivastava and Fasih (1988) who recorded reduction in the mango mealybug *Drosicha mangiferae* green population from 33.3 to 100% in 10 days after spray application with *B. bassiana* suspension. Valand and Vyas (1991) found that the entomopathogen fungus *Aspergillus niger* reduced the number of coccids on leaves of the pointed gourd (*Trichosanthes dioica*).

Despite that the first of entomopathogenic fungi was noted on armored scale insects in France in 1848 (Evans & Prior, 1990; according to Petch 1921) the information about using this entomopathogens against scale insects over the world is very limited. Howard *et al.* (2001) mentioned that *B. bassiana* has a wide host range. Current study cleared that the entomopathogenic fungus *B. bassiana* is a promising biocontrol agent against the soft scale *S. coffeae*. It needs more focus and more field trials to assure its effectiveness and capability of application.

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الملخص العربى

تقييم الفطر الممرض للحشرات *Beauveria bassiana* (Balsamo) كعامل مكافحة حيوية ضد الحشرة القشرية نصف الكروية *Saissetia coffeae* (Walker)

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تمثل الفطريات الممرضة للحشرات عنصر ضبط بيولوجى مهم للحشرات الثاقبة الماصة. وتعد الدراسة الحالية الأولى من نوعها فى مصر حيث تقوم بتقييم التأثير الممرض للفطر *Beauveria bassiana* (Balsamo) ضد الحشرة القشرية الرخوة النصف كروية *Saissetia coffeae*(Walker) التى تتبع فوق فصيلة Coccoidea و تصيب نبات الزينة *Cycas revoluta* Thunb. . تم قياس القدرة المرضية للفطر *B. bassiana* ضد الأطوار المختلفة لحشرة *S. coffeae* فى المختبر و كان من الواضح التأثير الممرض للفطر على كل من الحوريات و الإناث البالغة. وأظهرت حوريات الحشرة حساسية أعلى من الإناث البالغة حيث كان التركيزالنصفى المميت (LC_{50}) للحوريات ١,٥٧ x ١٠^{٣} جرثومة/ملل. بينما كان فى حالة الإناث البالغة ٩,١٤ x١٠^{٦} جرثومة/ملل. وكان الوقت النصفى المميت (LT_{50}) لأعلى تركيز تم إختباره (١x١٠^{٨} جرثومه /ملل) ٨,٦٣ يوم للحوريات بينما الإناث البالغة سجلت ١٠,٣٧ يوم. أجرى التقييم الحقلى للفطر *B. bassiana*علي حشرة *S. coffeae* على أشباه نخيل السيكس *C. revoluta*. وكان من الواضح أن الفطر الممرض ذو تأثير على تعداد الأطوار المختلفة للحشرة قيد الإختبار، حيث بلغ الإنخفاض فى تعداد كل من الحوريات والطور الكامل للحشرة بعد ٣٠ يوم من المعاملة ٧٤,١٠ و ٦٩,٧٠ % على التوالى.