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Side Effect of Indigenous Entomopathogenic Fungi on the Predatory Mite, *Cydnoseius negevi* (Swirski and Amitai) (Acari: Phytoseiidae)

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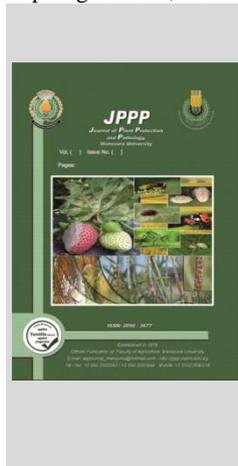
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ABSTRACT

The present study deals with the side effect of the four entomopathogenic fungi EPF, *Metarhizium anisoplia* (Metchnikoff), *Beauveria bassiana* (Balsamo), *Paceliomyces fumosoroseus* (Vassiljevsky) and *Cladosporium cladosporioides* (Fresen), which used mainly against insect pests and spider mites, on the phytoseiid predatory mite, *Cydnoseius negevi* (Swirski and Amitai) in laboratory. Spore suspensions of LC₅₀ (1x10⁶=10⁶ conidia/ml) of the tested fungi, which achieved a significant reduction% in populations of the two-spotted spider mite, *Tetranychus urticae* Koch, were sprayed on different stages of *C. negevi* individuals. In general, a slight negative toxic effect of EPF observed where no adverse effect was noticed on eggs while a negative effect was increasingly observed on larvae, protonymphs and deutonymphs. Mortality percentage of treated adult females increased by increasing time after treatment, where the highest mortality achieved 8 days after exposure. *Paceliomyces fumosoroseus* had the highest rate of reduction in egg production and followed by *B. bassiana* then *M. anisoplia*, while *C. cladosporioides* came last. Same ranking was observed regarding the life table parameters, where LC₅₀ of *P. fumosoroseus* caused the lowest R₀, T_m and e^{max} values and prolonged the mean generation time T and doubling time D₁ (days). Accordingly, in IPM program against insect and mite pests, we should use EPF as biocontrol agents, that are safe to biocontrol agents.

Keywords: Entomopathogenic fungi EPF, LC₅₀, *Cydnoseius negevi*, life tables and biological control.



INTRODUCTION

Agriculture crops globally infested by insect, mite pests as well as microorganism such as fungi, bacteria and viruses causing lot of diseases and considerable damages. The two-spotted spider mite, *Tetranychus urticae* Koch (Acari:Tetranychidae), is a widespread and common mite pest of many plant species in greenhouses and open fields worldwide (Zaher, 1986 and Fouly *et al.*, 2011). Spider mites frequently reach their economic injury level on over 200 host plant species in all continents (Zhang *et al.*, 2008). Application of chemical pesticides is still the main control method, so far, and perfect solution to get rid of these pests and to reduce mite populations fast under the economic threshold level. Although, the chemical pesticides can easily kill insect and mite pests, but they cause lot of problems for human, natural enemies, soil, water and raise the pollution to different environmental issues (Van Leeuwen *et al.*, 2007).

Entomopathogenic fungi (EPF) found to be the first microorganisms used for biological control of pests. Many species of fungi belonging to 90 genera are pathogenic to insect and mite pests (Sanjaya *et al.*, 2016). Fungal species have a wide host range, with individual isolates being more specific, such as *Metarhizium anisoplia* (Metchnikoff) and *Beauveria bassiana* (Balsamo) (Sanjaya, *et al.*, 2016). Generalist fungi observed on mites and could considered as potential biocontrol agents against major mite pests of agricultural crops such as spider mite, *T. urticae* (Chandler *et al.*, 1997). The spider mite *T. urticae* considered as one of the most important phytophagous mites (Krantz, 1978 and Zaher, 1986). It feeds on plant juice of economic crops like vegetables (eggplant, beans, tomato and strawberry), fruit

trees, ornamental plants and weeds causing a considerable damage, crop losses and may also transfers microorganisms such as virus and bacteria (Van Leeuwen *et al.*, 2007).

Using predators as bio-control agents for controlling pests in biological control is very effective especially Phytoseiid mites (Fouly, *et al.*, 1994; Fouly, 1997; Fouly, *et al.*, 2011 and 2014). As they are playing an important role as biocontrol agents in all over the world (Helle and Sabelis 1985; Gerson *et al.* 2003; McMurtry *et al.*, 2013 and Fouly *et al.*, 2014). Many species of phytoseiid mites are successful biocontrol agents for controlling phytophagous mites especially spider mites and other insect species (McMurtry *et al.*, 2013).

Cydnoseius negevi (Swirski & Amitai) (Acari: Phytoseiidae) is one of the important predators and is widespread in the Middle East (Abou-Awad *et al.*, 1998; Palevsky *et al.* 2009; and Hountondji *et al.* 2010). Also, the variety of food range for *C. negevi*, as it has a lot of sources such as eriophyid and spider mites like *T. urticae*, thrips, and different kind of pollen, that gave it a high survival potentiality (Momen 1997; El-Banhawy *et al.* 1999; Momen *et al.* 2009; Negm *et al.* 2014 and Hussein *et al.* 2016).

Few researches have conducted to study sub lethal effects of fungi on life table parameters of mite pests and their natural mite enemies (Wekesa *et al.* 2006; Shi and Feng, 2009). Therefore, the present protocol aimed to determine the side effect of the LC₅₀ of the tested EPF, *Metarhizium anisoplia* (Metchnikoff), *Beauveria bassiana* (Balsamo), *Paceliomyces fumosoroseus* (Vassiljevsky) and *Cladosporium cladosporioides* (Fresen), which mainly used against spider mites infesting eggplant in Mansoura, Egypt,

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on different biological aspects and life table parameters of the predatory mites *C. negevi* under laboratory conditions.

MATERIALS AND METHODS

Entomopathogenic Fungi Cultures

The entomopathogenic fungi EPF, *C. cladosporoides*, *M. anisopliae* and *P. fumosoroseus* were isolated from infected mites by picking off mycelial growth, then transferred to the surface of Potato Dextrose Agar medium (PDA) amended with rose bengal (0.003%) and streptomycin sulfate (0.01%) in Petri dishes and incubated at 26±2 °C, 70%±5 R.H for 4-7 days in dark. The fungal growth was individually transferred into PDA medium. Purification of fungus was carried out using hyphal tip technique (Goh, 1999). Furthermore, the purified fungal isolates were identified in Mycological Centre (AUMC), Assuit University, Egypt, 2019. The isolate of *B. bassiana* was directly purchased from AUMC.

Preparation of spore Suspensions

Fungi were grown on PDA media for 15 days at 26±2°C, 70%±5 R.H for producing spores. After that, spores were collected from plates by gently rubbing the agar surface after adding 0.5ml of Tween-80 solution (0.02% v/v) to the sporulated cultures in each plate. The spore suspension was filtered through cheese cloth to remove mycelial fragments, which combined and centrifuged at 10 ml for 5 min. Serial dilutions were done, where the number of spores was recorded in ml by using Haemocytometer slide by the following the equation of Chohan *et al.* (2015).

No. spore ml⁻¹ =

$$\left[\frac{\text{No. of spore in the 4 large squares} \times \text{dilution factor}}{64} \right] \left[4 \times 10^6 \right]$$

Cydnoseius negevi culture

Mite individuals were collected from eggplant leaves growing in the Acarology greenhouse, Fac. Agric., Mansoura University (2019). Mites were identified by the aid of a stereoscopic Microscope (Optika) according to the terminology of Evans (1963), Lindquist and Evans (1965), Evans and Till (1966 and 1979), Bregotova (1977) and Zaher (1986).

A pure culture of the predatory mite maintained on the developmental stages of two-spotted spider mite *T. urticae* as a prey and left to lay eggs on leaf discs of *Acalypha marginares* plants and kept on a moist cotton pad in a Petri dish (15 cm in diameter). Suitable moisture was added daily to keep the leaf discs fresh for longer time and for collecting the deposited eggs easily under laboratory conditions.

Bioassay tests

The newly deposited eggs were collected daily for 10 consecutive days and singly transferred into small discs of *Acalypha marginares* leaves (2 cm in diameter). Discs were limited with tangle foot of a mixture of Canada balsam, citronella oil and castor oil to prevent mites from escaping. Some droplets of water were added daily to maintain suitable moisture in cotton pad. Two hundred eggs were divided into five groups of 40eggs/each and maintained singly on hibiscus leaves on cotton pads in 12 cm Petri-dishes. All Petri-dishes were placed in a closed box and kept under controlled conditions of 26±2°C, 70±5% R.H and a photoperiod of 16:8 LD. The first four groups of eggs of *C. negevi* were sprayed with 2 ml of LC₅₀ (1x 10⁶ conidia/ml), which was previously obtained from a preliminarily experiment against the two-spotted spider mite, *T. urticae* of the prepared spore suspensions of the tested fungi. The fifth group was treated

with only distilled water and treated as a check. Each treatment was checked daily until all eggs hatched. Egg hatchability and duration of incubation period were determined. After that, as soon as egg hatched, the same technique was followed with all immature stages (larvae, 1st nymphs and 2nd nymphs) where duration (in days) of developmental period of each stage and life cycle were measured. In all cases, untreated mobile stages of spider mite *T. urticae* were introduced as a prey.

Determination of life table analysis

Five groups of newly emerged adult females (30 female/each) were subjected to the same concentration (LC₅₀) of the tested fungi suspensions and provided with untreated mobile stages of *T. urticae* for their longevity. Moreover, a group of untreated newly mated females was used as a check and all were kept at 26±2°C, and 70±5% R.H. Just after female emergence, one male from the culture was introduced to each replicate to insure mating. Number of deposited eggs for each female was counted until the last female died, sex ratio (♀/♂+♀), survivorship and longevity were determined and used for life table analysis according to Andrewartha and Birch (1954) and Laing (1968) and the basic computer program of Abou-Setta *et al.* (1986) where L = number of female alive, x = actual female age (in days), Mx = age specific fecundity rate (mean number of daughters born in an interval to a mother of age x), Lx = rate of survival at day x (the fraction of females surviving from age 0 until at least age x), Ro = the net reproductive rate (∑Lx Mx) (the total females born in two successive generations or the rate of multiplication in one generation), T = the mean generation time (∑Lx Mx . x)/∑ Lx Mx, rm=the intrinsic rate of natural increase (females/female/day), erm (λ)=the finite rate of increase (number of times the population multiplies in a unit of time (Birch, 1948) and Dt=the time required for a mite to double its population.

Statistical analysis

The average mortality percentages were corrected using Abbott's formula (Abbott, 1925) then data was statistically analyzed using analysis of variance (one-way classification, ANOVA (CoHort Software, 2004) using Duncan multiple range-test at 5% probability level to compare means significance (Duncan, 1955).

RESULTS AND DISCUSSION

Table (1) showed that within an average of 10 mite individuals of *C. negevi*, deutonymph was more susceptible than larva and protonymph treated with LC₅₀ of *B. bassiana*. Immature stages of *C. negevi*, which were treated with *M. anisopliae*, *C. cladosporoides* and *P. fumosoroseus* didn't show any significant differences. In comparison with untreated mite individuals, *B. bassiana*, *M. anisopliae*, *C. cladosporoides* and *P. fumosoroseus* caused an average of 1.58, 9.88, 14.55 and 19.09% mortality for larvae. Protonymph individuals were reduced by an average of 6.92, 13.74, 17.00 and 23.04%, while deutonymphs were killed by an average of 17.79, 10.71, 14.27 and 21.70% when they were subjected to LC₅₀ of the same previous EPF, respectively (Table 2). That means the suspension of *P. fumosoroseus* was more toxic to immature stages of *C. negevi* than other fungi where *C. cladosporoides* came second and followed by *M. anisopliae* and *B. bassiana*. The previous data clarified that there was a light effect of the tested EPF on the immature stages of *C. negevi* and that may be

because of the immature stages molt fast and change their cuticle, so the spores could not germinate and penetrate the cuticle and remain on the old cuticle. Similar conclusion was mentioned by Seyed-Talebi *et al.* (2012).

Table 1. No. of alive immature stages of the predatory mite *Cydnoseius negevi* treated with LC₅₀ (1x10⁶) of four entomopathogenic fungi under lab conditions (26±2°C and 70%±5 RH).

Fungi	larva	1st nymph	2nd nymph
<i>Beauveria bassiana</i>	9.33 ab±0.33	9.00 ab±0.00	7.67 bc±0.33
<i>Metarhizium anisopliae</i>	8.66 bc±0.33	8.33 bc±0.88	8.33 b±0.33
<i>Cladosporium cladosporoides</i>	8.00 c±0.58	8.00 bc±0.58	8.00 bc±0.00
<i>Pacilomyces fumosoroseus</i>	7.67 cd±0.33	7.33 c±0.33	7.33 c±0.33
Control	9.48 a±0.00	9.67 a±0.00	9.33 a±0.00
F	4.00	4.66	13.17
P	0.0015 **	0.0005 ***	0.0000 ***
L.S.D (P≤0.05)	1.32	1.20	0.76

Means ± SE followed by different letters in each column have significant differences (P≤0.05) by Tukey's test

Table 2. Mortality % of immature stages of *Cydnoseius negevi* treated with LC₅₀ (1x10⁶) of four entomopathogenic fungi after different time intervals under lab conditions (26±2°C and 70%±5RH).

Treatment	Mortality %		
	larva	1 st nymph	2 nd nymph
<i>Beauveria bassiana</i>	1.58	6.92	17.79
<i>Metarhizium anisopliae</i>	9.88	13.74	10.71
<i>Cladosporium cladosporoides</i>	14.55	17.00	14.27
<i>Pacilomyces fumosoroseus</i>	19.09	23.04	21.70

Mortality% of immature stages corrected according to Abbott formula (Abbott, 1925)

The present results also agree with those obtained by Ludwig and Oetting (2002); Wu, *et al.*, (2016) and Dogan, *et al.* (2017) who proved that immature stages of the predatory phytoseiid mite, *Ipheseius degenerans* (Berlese) were hardly influenced by the fungus *M. anisopliae* and followed by *Verticillium lecanii* and *B. bassiana*. Other researchers also supported the present observations, where Bugeme *et al.* (2014) indicated that, because development time is short, the spores cannot take enough time to germinate and be able to penetrate the mite cuticle where the molting operation may prevent these spores from sticking on the mite body. Moreover, Dogan *et al.* (2017) added that the spores failed to penetrate the old cuticle. Concerning eggs, which were sprayed with LC₅₀ of the four tested fungi, the present results showed that there is a low egg mortality was achieved where 90.02, 92.66, 93.33 and 94.33% of total number of treated eggs of *C. negevi* hatched after an incubation period of approximately one day as shown in Table (3). Although, some previous researchers found that mite eggs to be highly susceptible to EPF infection, other scientists found eggs to be more tolerant. For example, Irigaray *et al.* (2003) stated that

exposing spider mite eggs to high doses of *B. bassiana* resulted in 70% mortality. This suggests that some EPF strains exhibit greater ovicidal activity than others.

The low egg mortality reported here is probably due to the fungi strain having low ovicidal activity. Indeed, Shi and Feng (2009) screened 10 isolates of *B. bassiana*, *M. anisopliae* and *P. fumosoroseus*, where they found only 4 isolates to be highly effective in killing mite eggs but they can do only at high concentrations. They also mentioned that lower concentrations of these isolates caused significantly lower egg mortality. Erler *et al.* (2013) compared the strain *M. anisopliae* F52 with a strain of the fungus *B. bassiana* and found the latter to be more effective against eggs of spider mite *T. cinnabarinus*. Accordingly, it can be concluded that egg mortality to be dose dependent (Irigaray *et al.*, 2003; Shi and Feng, 2009; Erler *et al.*, 2013 and Bugeme *et al.*, 2014). Furthermore, freshly laid eggs of *C. negevi* may also be unaffected presumably due to their short exposure time to conidia but the possibility of a kind of resistance should be also taken in consideration.

Present data showed that, except larvae treated with LC₅₀ of *B. bassiana*, it was found that the duration period of larval stage had no significant differences with untreated one, while *B. bassiana* generally shortened the duration period of other immature stages as compared with those in untreated control. In general, data showed that immature stages, which were treated with LC₅₀ of *P. fumosoroseus*, gave the longest life cycle (8.00 days) compared with 7.00 days in control group (Table 3). Nevertheless, Seyed-Talebi *et al.* (2014) showed that the development periods for all immature stages of *T. urticae* not affected by fungal infection of *B. bassiana*. In contrary, Liu *et al.* (2019) found that LC₁₀ and LC₃₀ of the strain *B. bassiana* ICMP 8701 didn't show any significant difference between the duration periods of immature stages of the phytoseiid mite, *Amblydromalus limonicus* Garman & McGregor. They found that larvae, 1st nymph and 2nd nymph took an average of 1.36, 1.60 and 1.33 days for LC₁₀, while they lasted for 1.36, 1.62 and 1.54 days for LC₃₀ as compared with 1.53, 1.60 and 1.55 days for untreated individuals, respectively. Furthermore, various mechanisms have been reviewed to explain why the different developmental stages differ in their susceptibility to EPF with much emphasis being given to the physical-chemical properties of the arthropod cuticle and ecdysis (Butt *et al.*, 2016). Therefore, it can be concluded some observations found that EPF had no effect on the duration of developmental stages (Liu *et al.*, 2019) while other researchers found the opposite (Seiedy *et al.*, 2012; Lacey *et al.*, 2015 and Dogane *et al.*, 2017). In other word, EPF may have a positive or negative effect on predatory mites and that may be due to the susceptibility of mite species, strain of tested fungus and method of treatment.

Table 3. Duration (days) of incubation period, immature stages and life cycle of the predatory mite, *Cydnoseius negevi* treated with LC₅₀ (1x10⁶) of four entomopathogenic fungi under lab conditions (26 ±2°C and 70%±5 RH).

Treatment	Hatching %	Incubation period	Larva	1 st nymph	2 nd nymph	Life cycle
<i>Beauveria bassiana</i>	93.33	1.00a±0.00	0.83b±0.17	1.83b±0.17	3.01a±0.00	6.77b±0.23
<i>Metarhizium anisopliae</i>	94.33	1.00a±0.00	1.16ab±0.17	2.00a±0.00	3.04a±0.00	7.17b±0.17
<i>Cladosporium cladosporoides</i>	92.66	1.00a±0.00	1.00a±0.00	2.17a±0.17	3.02a±0.00	7.17b±0.17
<i>Pacilomyces fumosoroseus</i>	90.02	1.16a±0.17	1.17a±0.17	2.33a±0.33	3.18a±0.17	8.00a±0.29
Control	100.00	1.00 a±0.00	1.00a±0.00	2.00a±0.00	3.16a±0.17	7.00b±0.00
F	--	0.94	1.2	1.78	0.79	4.22
P	--	0.50	0.32	0.11	0.66	0.001 **
L.S.D. (P≤0.05).	--	0.23	0.24	0.38	0.26	0.62

Means ± SE followed by different letters in each column have significant differences (P≤0.05) by Tukey's test

Data in Table (4) gave the indication that *B. bassiana* was the most effective fungus on female mites especially after six days post treatment. Mortality of mite females, which were sprayed with *B. bassiana*, *M. anisopliae*, *C. cladosporoides* and *P. fumosoroseus* averaged 15.24, 2.62, 0.00 and 1.00% after 6 days post spray, respectively. These values increased two days later to 14.3, 7.60, 7.28 and 14.34%, respectively. In other words, entomopathogenic fungi need for at least six days to show their act on adult mite females. Some researchers have suggested that the immature stages are less susceptible than matures to EPF because of their small size and limited movement, which reduce their chance to be contacted with conidia (Bugeme et al., 2014). Moreover, Sholla et al. (2020) reported that the mortality% of the phytoseiid mite *Euseius scutalis* (A.-H.) females, which fed on *T. urticae* previously subjected to *B. bassiana* (LC₅₀), averaged 23% and 39% compared to only 5% for untreated mites. Similarly, *A. swirskii* adult females were highly susceptible to isolate *B. bassiana* F (total mortality 49.62% on the seventh day) but least susceptible to isolate *DEBI008* after 7 days (Seiedy et al., 2015). The same authors found that *A. swirskii* was susceptible to *B. bassiana* when conidia applied directly to mite bodies. Some researchers discussed the impact of EPF on phytophagous and predatory mites and found that fungal virulence is not just dependent upon the fungal strain, but also the concentration, formulation and frequency of application. Wekasa et al. (2005) and Shi and Feng (2009) found that more than 80% mortality of adult females of the two spider mite species, *T. evansi* and *T. cinnabarinus* was obtained with virulent strains of *B. bassiana*. Similar results were obtained with oil formulations of *M. anisopliae* and *B. bassiana*, which were applied against *T. cinnabarinus* on okra (Naik and Shekharappa, 2009). Bugeme et al. (2014) showed that the formulated *M. anisopliae* strain (ICIPE78), which used at 1×10^8 conidia/ml, was as effective as Abamectin insecticide in reducing *T. urticae* densities on protected and field crops.

It is feasible that *M. brunneum*4556 and V275 could have given even greater control of *T. urticae* if used at the higher dose and/or applied several times at set intervals. Moreover, Wu et al., (2016) mentioned that adult females of *T. urticae* were more susceptible to the fungus *M. brunneum* V275 than the predatory mites either *P. persimilis* or *N. californicus* when they were exposed to the same spore concentration. No obvious oviposition or behavioral changes observed in infected predatory mites or spider mite at least shortly before death. They added that the susceptibility of *P. persimilis* and *N. californicus* to EPF appears to be dependent upon the strain and dose. In general, it can be concluded that the current study showed that female predatory mites of *C. negevi* will freely lay eggs right up till they died by EPF. Furthermore, none of their progeny (eggs, juveniles, adults) become infected. Vergel, et al. (2011) found that the two fungal species *B. bassiana* and *P. fumosoroseus* at 1.25×10^7 conidia/ml caused 43% and 14% female mortality of *P. persimilis* and 31% and 16% of *N. californicus*, respectively. In contrast, Wu et al. (2014) found that a thrips pathogenic strain of *B. bassiana* wasn't harmful to neither adults nor larval stages of the predatory mite, *Neoseiulus barkeri* Hughes.

Table 4. No. of live females of *Cydnoseius negevi* treated with LC₅₀ (1x10⁶) of four entomopathogenic fungi after different time intervals under lab conditions (26±2°C and 70%±5 RH).

Treatment	Time after treatment (days)		
	4	6	8
<i>Beauvaria bassiana</i>	4.33c±0.33	3.67d±0.33	4.00c±0.00
<i>Metarhizium anisopliae</i>	5.00a±0.00	4.66b±0.33	4.67a±0.33
<i>Cladosporium cladosporoides</i>	5.00a±0.00	5.00a±0.00	4.33b±0.33
<i>Pacilomyces fumosoroseus</i>	5.00a±0.00	4.32c±0.33	4.00c±0.00
Control	4.67b±0.33	4.36c±0.33	4.68a±0.33
F	29.56	50.53	45.49
P	0.000 ***	0.000 ***	0.000 ***
L.S.D. (P≤0.05)	0.27	0.24	0.26

Each mean in each treatment represents 3 replicates in each of which 10 adult females.

Table 5. Mortality% of *Cydnoseius negevi* females treated with LC₅₀ (1x10⁶) of four entomopathogenic fungi under laboratory condition (26±2°C and 70%±5 RH).

Treatment	Mortality% after different time (days)		
	4	6	8
<i>Beauvaria bassiana</i>	7.28	15.24	14.34
<i>Metarhizium anisopliae</i>	0.00	2.62	7.60
<i>Cladosporium cladosporoides</i>	0.00	0.00	7.28
<i>Pacilomyces fumosoroseus</i>	0.00	1.00	14.34

Mortality% of immature stages corrected according to Abbott formula (Abbott, 1925).

Table (6) showed that fecundity, as represented by average number of deposited eggs of each female of *C. negevi*, gradually declined by the time passed after treatment. There were significant differences between the average number of deposited eggs between untreated females and those treated with LC₅₀ of the four EPF during the whole period of study. *P. fumosoroseus* was the most harmful fungal extract in reducing mite fecundity, and followed by *B. bassiana*, *M. anisopliae*, while *C. cladosporoides* came last. On the 4th day after treatment, *P. fumosoroseus* reduced fecundity of *C. negevi* by 37%, followed by 27% for *B. bassiana*, while *M. anisopliae* gave 21% reduction compared with untreated mites, respectively. Similarly, the reduction% of egg lying increased steadily during the whole period of study. Similar results were obtained by Jacobson et al. (2001) who stated that *Amblyseius cucumeris* Oudemans was unaffected by spray of the fungi-bioagent *B. bassiana* (*Naturalis-L*). Contradicted results obtained by Seyed-Talebi et al. (2014) who reviewed that the female longevity, oviposition period and fecundity of spider mite species, *T. urticae* were significantly lower by the subjection of *B. bassiana*. Wekasa et al. (2006) demonstrated that the behavior of phytoseiid mites can be affected by the presence of an excessive number of conidia of the entomopathogenic fungus *Neozygites floridana* (Weiser and Muma) Remaudiere and Keller on *Phytoseiulus longipes* Evans.

The predator individual detected and removed most capilliconidia attached to its body by self-grooming (using the 1st pair of legs to clean the body) can be a possible reason for the absence of a significant difference in the viability of predatory mites following these treatments in the present study. Donka et al. (2008) demonstrated that *Lecanicillium muscarium* could have effect on the survival of *P. persimilis* through the indirect application of conidia. The results may differ according to spore density (concentration), grade of

contact and conditions. Spores could adhere to the body of the predatory mite; yet, after 24 hours, up to 85% of the spores had fallen off its body. As in other phytoseiid predators such

as *P. persimilis* and *A. swirskii*, the tested *C. negevi* females could avoid their prey if it infected with entomopathogenic fungi (Meyling and Pell, 2006 and Seiedy, 2014 and 2015).

Table 6. Average number of daily deposited eggs by *Cydnoseius negevi* females treated with LC₅₀ (1x10⁶) of four entomopathogenic fungi after different time intervals of its oviposition period under lab conditions (26±2°C and 70%±5 RH).

Treatment	Time after treatment (days)			
	4	6	8	10
<i>Beauveria bassiana</i>	0.73c±3.33	1.05bc±3.33	1.26c±3.33	1.27c±3.33
<i>Metarhizium anisopliae</i>	0.66b±3.33	1.12b±0.00	1.19c± 3.33	2.28b±3.33
<i>Cladosporium cladosporides</i>	0.93b±5.77	1.25b±5.77	1.46b±5.77	2.46b±5.77
<i>Pacilomyces fumosoroseus</i>	0.53d±5.77	0.85c±5.77	1.07c±5.77	1.07d±5.77
Control	1.02a±0.00	2.04a±0.00	3.04a±0.00	4.06a±0.00
F	7952.65	24349.23	63231.59	172804.37
P	0.000***	0.000***	0.000***	0.000***
L.S.D. (P≤0.05)	0.08	0.22	0.16	0.18

Means ± SE followed by different letters in each column have significant differences (P ≤0.05) by Tukey's test.

Above mentioned results concluded that LC₅₀ of the tested fungi had a negative effect on fecundity of the predatory mite *C. negevi*. Although, spraying the entomopathogenic fungi onto mite females caused a considerable reduction in mite fecundity, most surviving females still had the ability to laying eggs. These findings agree with those obtained by Sanjaya *et al.* (2016) and Dogan, *et al.* (2017) who noted that some predatory mite species have a kind of immunity which prevent conidia or hyphae from penetration mite bodies. Recently, many researchers proved that most entomopathogenic fungi can produce secondary metabolites, which may have an antioxidant role that accumulated lethal lipids in pest body, (Saad *et al.*, 2019). In other word, these metabolites may have insecticidal activities such as *C. cladosporides*, *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* extracts. In general, Dogan *et al.* (2017) showed that different mites are susceptible to various species of EPF with the adults being the most susceptible followed by the juveniles (larvae and nymphs), while the eggs being the least susceptible. Susceptibility of the spider and predatory mites appeared to be dependent upon the fungal species/strain, dose, and assay conditions. On the other hand, Butt *et al.* (2016) reported that EPF strains differ in their host specificity with fatty acids in the epicuticular waxes which often influencing spore adhesion, germination and/or penetration through mite cuticle. They also showed that mite females would freely lay eggs right up till the time they died by the fungus *M. brunneum* V275 and none of their progeny (eggs, juveniles, and adults) become infected.

Therefore, it was felt necessary to know more about the side effect of these bio-agents, which can be recommended against mite pests, on life table parameters of the forthcoming offspring of *C. negevi* inhabiting the same habitat of its prey species.

Effect of entomopathogenic fungi on life table parameters of *Cydnoseius negevi*

Data in Table 7 showed that egg hatchability was reduced from 96% in untreated mites to 86, 92, 90 and 92% when they were subjected to LC₅₀ of *B. bassiana*, *M. anisopliae*, *C. cladosporides* and *P. fumosoroseus*, respectively.

Concerning the forthcoming sex ratio, the present results indicated that there were no significant differences between sex ratio in treated and untreated mites, where No. of female proportion (Total No. ♀/♀+♂) averaged 0.57, 0.60, 0.58 and 0.56 when mites treated with the previous fungi compared to control mites (0.61), respectively. Although,

there was no big difference between these ratios but they have certainly affected the other reproductive parameters. Generally, life table parameters were calculated according to the following formula:

$$\text{Max } \sum L_x m_x / \exp. r_{mx} = 1$$

Where “mx” age-specific fecundity which is the number of daughters produced per female during the interval “x”. The value “Lx” age-specific survival, which is the fraction of live females at age “x”. The value “r_m” is the natural logarithm of the intrinsic rate of natural increase and it indicates to the number of times of population multiplication in a time unit. The net reproductive rate “R₀” is the mean number for female multi-placation in one generation. “T” is the mean length of generation period, which expressed in days (Birch, 1948) (Fig. 1 a-e).

Concerning the mean generation time, the present work proved that LC₅₀ of the tested EPF slightly prolonged T time (days) where it was 22.51 days and then increased to 24.47, 23.82, 23.06 and 25.47 days for mites treated with *B. bassiana*, *M. anisopliae*, *C. cladosporides* and *P. fumosoroseus*, respectively (Table 7 and Fig. 1a-e). Accordingly, doubling time D_t (the time required to double a generation) was also affect by spraying with EPF, where it prolonged from 10.29 days in control group to 13.30, 12.93, 12.47 and 16.02 days when mites treated with the previous mentioned fungi, respectively. Similarly, Liu *et al.* (2019) found that T time (days) was 12.50, 10.82 and 11.42 days while in control it was 14.36 days. Therefore, they stated that mean generation time of *A. limonicus* was shortened by increasing fungal concentration.

The net reproductive rate R₀, data proved that it was 13.60, 13.52 and 13.72 and then sharply decreased to 9.43 female/female/generation when mites were treated with *B. bassiana*, *M. anisopliae*, *C. cladosporides* and *P. fumosoroseus*, respectively as compared with untreated ones (22.08) (Table 7). Therefore, all EPF negatively affected the net reproductive rate of *C. negevi* but *P. fumosoroseus* was the worst one where the previous fungi decreased R₀ values by 38.40, 38.76, 37.86 and 57.29%, respectively (Fig. 2). Similar results obtained by Seyed-Talebi *et al.* (2014) who found that *B. bassiana* reduced reproductive potential of spider mite *T. urticae* where 1x10⁸ of conidia suspension significantly reduced R₀, r_m and e^{rm} values from 25.68, 0.24 and 1.270 to 14.30, 0.21 and 1.24 in untreated and treated *T. urticae* on bean plants.

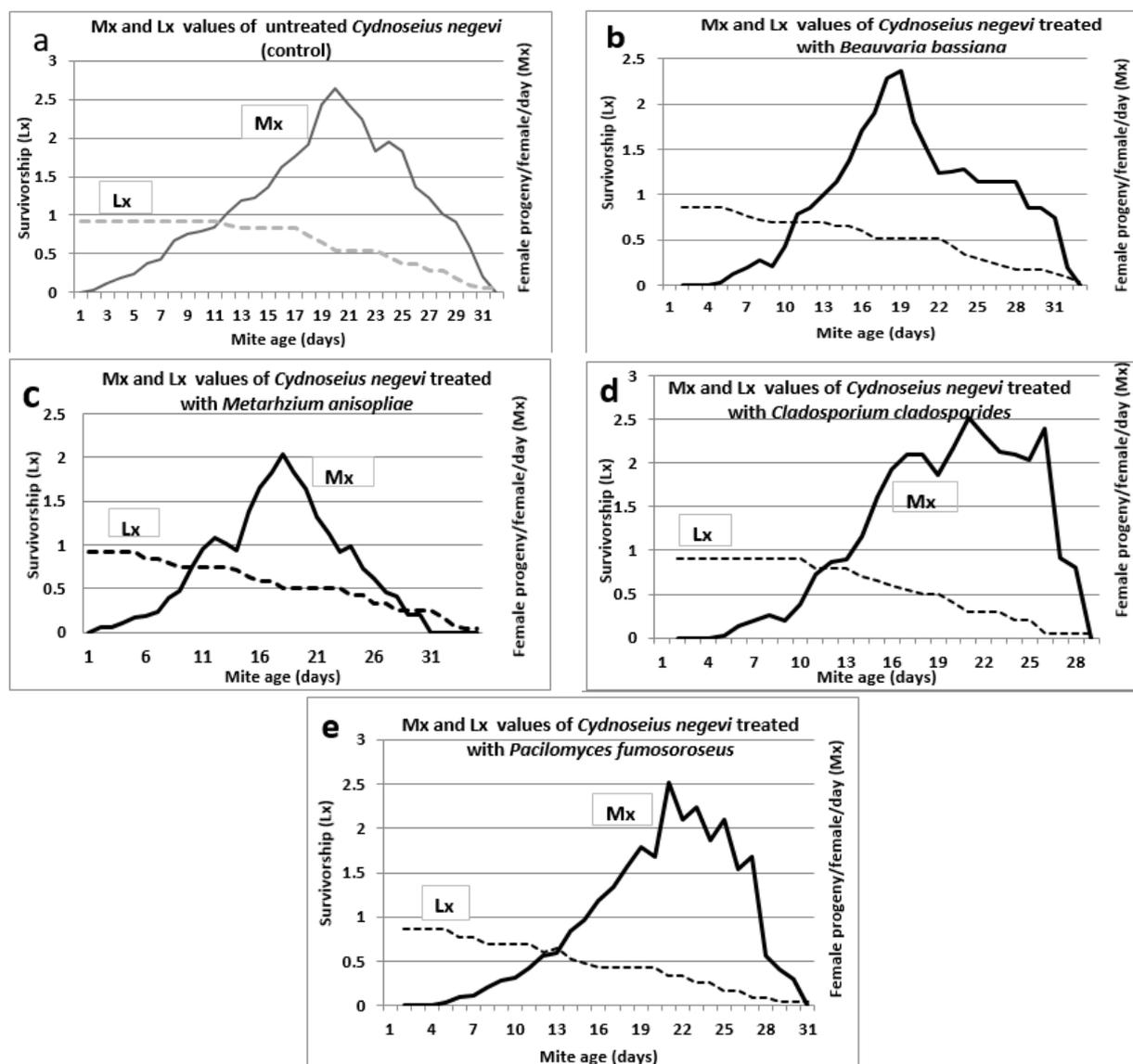


Figure 1. (a-e). Age specific survival (Lx) and age specific fecundity and (Mx) of untreated and treated *Cydnoseius negevi* with LC₅₀ (1x10⁶) of four entomopathogenic fungi under laboratory conditions (26°C and 70% RH).

Table 7. Life table parameters of both untreated (control) and treated *Cydnoseius negevi* with LC₅₀ (1x10⁶) of four entomopathogeni fungi under laboratory conditions (26°C and 70% RH).

Life table parameters	Entomopathogenic fungi				Untreated mites (control)
	<i>Beauvaria bassiana</i>	<i>Metarhizium anisopliae</i>	<i>Cladosporium cladosporides</i>	<i>Pacilomyces fumosoroseus</i>	
No. mite individuals	24	23	26	24	26
Hatchability %	86	92	90	92	96
Female proportion (No. ♀ / ♀ + ♂)	0.57	0.60	0.58	0.56	0.61
Mean generation time (T)	24.47	23.82	23.06	25.47	22.51
Net reproductive rate (R ₀)	13.60	13.52	13.72	9.43	22.08
Intrinsic rate of increase (r _m)	0.106	0.109	0.113	0.088	0.137
Finite rate of increase e ^{rm} (λ)	1.112	1.115	1.120	1.092	1.147
Doubling time (D _t)	13.30	12.93	12.47	16.02	10.29
Growth reproduction (GRR)	28.98	13.52	27.31	31.82	22.082

Also, it was observed that *P. fumosoroseus* was the most harmful fungus because it highly reduced the intrinsic rate of increase r_m by 35.76% comparing with untreated mites and followed by *B. bassiana*, *M. anisopliae* and *C. cladosporides* by reduction% of 22.62, 20.17 and 17.52%, respectively (Table 7). In other word, r_m value was 0.137 and declined to 0.088, 22.62, 20.43 and 0.113 female offspring/female (female-1day⁻¹) for untreated and treated mites with the same previous EPF, respectively (Table 7 and Fig. 2).

The same trend for the side effect of LC₅₀ of the tested fungi was observed on the intrinsic rate of increase $e^{rm}(\lambda)$ of *C. negevi*. Therefore, the current study showed that treatment of *B. bassiana*, *M. anisopliae*, *C. cladosporides* and *P. fumosoroseus* caused an average of 1.112, 1.115, 1.120 and 1.092 as compared with untreated mites by 1.147 eggs/female/day (day⁻¹). That means finite rate of increase was reduced by 3.05, 2.79, 2.35 and 4.79%, respectively (Fig. 2). According to the previous results, it was noticed that EPF

variably affected different biological aspects of the expected generation of the predatory mite. It may be due to the fungi species and their specifications as well as concentration. These observations have been discussed by Liu *et al.* (2019) who stated that sub lethal concentrations of *B. bassiana* could insignificantly influence the reproduction parameters in F1 of *Amblydromalus limonicus*, but there were no significant differences in the life table parameters. They found that r_m value was 0.135 and 0.128, while R_o was 5.40 and 4.00 while e^{tm} averaged 1.144 and 1.136 compared to 0.129, 6.42 in untreated group when mite individuals were treated with LC_{10} and LC_{30} of *B. bassiana*, respectively. That means there were no significant differences between treated and untreated mites and that means EPF had no effect the reproductive parameters of the predatory mite *A. limonicus*.

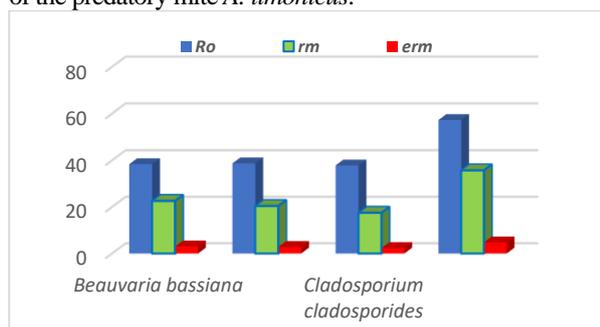


Figure 2. Reduction% in certain life table parameters of *Cydnoseius negevi* treated with four entomopathogenic fungi and kept at 26°C and 70% RH

CONCLUSION

The present results clearly indicated that it was important to determine the side effect of chemical or nonchemical pesticides, which could recommended against spider mites. Therefore, we had the attention to know more about the integrated strategy of combining the use and release of phytoseiid mites and entomopathogenic fungi to promote biological control as a part of IPM of plant pests. Furthermore, to the best of our knowledge, this is the first trial to study the side effect of entomopathogenic fungi on the predatory mite, *C. negevi*. As with certain biological control techniques, using a combination between predatory mite and myco-pesticides require a careful management to suit special crop and plant production. Finally, further studies are needed to test the compatibility of EPF and bio-control mite-agents including the most suitable and safer concentration which can reduce mite pest population and not harm the predatory mites. Selecting strains of EPF that kill pests but not beneficial predators and parasitoids will be vitally important in the development of robust, efficacious, cost effective pest control programs.

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التأثير الجانبي لمسببات الأمراض الفطرية الحشرية على النواحي البيولوجية للمفترس الأكاروسي سينوسيس نيجيفي نوران سعد محمد سعد*، عبد التواب عبد القادر يوسف و أحمد حسن فولي قسم الحيوان الزراعي- كلية الزراعة- جامعة المنصورة- جمهورية مصر العربية

إهتمت الدراسة بدراسة التأثير الجانبي لأربعة مستخلصات فطرية ميتاريزيوم انيسوبوليا و بيوفاريا باسيانا و باسيلوميسيس فيوموسوريوس و كلادوسبوريم كلادوسبورويدس والتي تستخدم ضد الآفات الأكاروسية والحشرية وذلك على الأكاروس المفترس سينوسيس نيجيفي تحت ظروف المعمل. تم عمل تركيزات عديدة لإستنتاج التأثير السام النصفى LC₅₀ على العنكبوت الأحمر نو البقعين ترانينكس أورتيكا وكان 1x10⁶ كونيديا/مل من المستخلصات الفطرية الأربعة. أوضحت الدراسة أن هناك تأثير سلبي بسيط على النواحي الإحيائية للمفترس الأكاروسي كما لم يكن هناك تأثير ملحوظ على طور البيض ولكن التأثير كان على الأطوار غير الكاملة والكاملة حيث يرتفع التأثير السلبي تدريجيا بزيادة الفترة الزمنية بعد تعرض الأطوار الكاملة للأناث وخاصة بعد ثمانية أيام بعد المعاملة. كان الفطر باسيلوميسيس أعلى الفطريات تأثيرا حيث تسبب في أعلى نسبة خفض لوضع البيض من الإناث المعاملة بالفطريات وتلاه في ذلك الفطريات بيوفاريا ثم الميتاريزيوم بينما احتل الفطر كلادوسبوريم المركز الأخير. هذا الترتيب من حيث التأثير الضار قد لوحظ على جداول حياة المفترس الأكاروسي حيث تسبب التركيز السام النصفى للفطر باسيلوميسيس في أعلى نسبة خفض في معدل الخصوبة الصافي R₀ ومعدل التزايد النوعي r_m ومعدل التضاعف الطبيعي في اليوم mth. كما أدت المعاملة بهذا الفطر إلى أطول فترة جيل T والدة الزمنية اللازمة لتضاعف الجيل Dt. ومن نتائج هذه الدراسة يتضح لنا أنه من الضروري عند استخدام المستخلصات الفطرية كواحدة من عناصر مكافحة المتكاملة للآفات الزراعية أن ندقق في نوعية تلك الكائنات حيث يفضل الأكثر أماناً على الأعداء الحيوية الطبيعية.