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Evaluation of some plant extracts and entomopathogenic fungi against the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychyidae) and some associated predators

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Abstract

Two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychyidae) is a polyphagous plant mite pest causing major economic losses. Avoiding the extensive application of chemical pesticides, which had many hazards to human health and the environment and to obtain satisfactory alternatives to pesticides to combat the, two plant extracts *Portulaca oleracea* L. (Family: Portulacaceae) and *Lepidium sativum* L. (Family: Brassicaceae) and two isolates of entomopathogenic fungi *Beauveria bassiana* (Bals) Vuill (Family: Cordycipitaceae) and *Paecilomyces lilacinus* (Thom) (Family: Ophiocordycipitaceae), were tested on in the laboratory; a side effects of highly virulent tested material was examined on some of the predators, *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* (McGregor), *Neoseiulus bicaudus* (Wainstein) (Acari: Phytoseiidae) and *Orius albidipennis* (Hemiptera: Anthocoridae). Mortality rates for *T. urtice* ranged between 21-91% for *P. oleracea* and 24-80% for *L. sativum*, while for the two fungi isolates, *Beauveria* and *Paecilomyces*, ranged between 38.2 - 74.5% and 31.3 - 49.1% on *T. urtice*, respectively. The side effects of the plant extract ranged between 24-35% on predatory mites and 3.85% on predatory insects. Whereas fungi effect was less than 30 % for all predators. Due to its effect on *T. urticae* and minimal effect on *O. albidipennis*, both proposed methods could be used to control the pest mite alone or incompatible with the predatory insect in joint programs. The detrimental effect of the tested material on predatory mite survival and progeny indicates the need for further studies to develop other strategies that combine these natural control agents.

Introduction

Spider mites are the most lethal mites belonging to the family Tetranychidae and are common pests of many crops. They can feed on over 180 different plant species, both in greenhouse environments and open fields (Chakraborty *et al.*, 2010). Among these mites, the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychyidae) is particularly noteworthy, it can undergo overlapping generations within a single crop cycle, leading to significant economic losses (Zhang, 2003 and Vacante, 2016).

The extensive application of chemical pesticides, particularly in horticultural crops, has had a dual impact. On one hand, it has resulted in the development of resistance among two-spotted spider mite populations, the other hand is a dangerous role for human health and environmental pollution (Van Leeuwen *et al.,* 2010). Recently, there has been a great interest in the use of alternative methods for controlling pest infestation in different crops. Biological and integrated control can be a trusted way to solve pesticide problems.

Some plant extracts have proven to be a viable option instead of synthetic acaricides due to their notable effectiveness against pests, their biodegradability and their minimal side effects on non-target organisms as well as on the environment (Isman, 2000; Cavalcanti *et al*., 2010 and Attia *et al*., 2011, 2012a, 2012b).

As pesticides, these extracts can impact pest behavior by repelling them or impeding their feeding activity. Furthermore, they can induce physiological changes in pests, including hindering molting and respiratory functions, reducing growth and fecundity, even causing disruption to their cuticles, also it can delay the evolution of resistance (Isman, 2000; Enan, 2001 and Gökçe *et al.,* 2011). Furthermore, using pesticidal plants requires frequent weekly application of plant extracts since the active components break down quickly and have low persistence (Casida, 1980).

Entomopathogenic fungi (EPF) may play a major role in the natural regulation of spider mite populations. They could be used in biological control programs, either as a stand-alone solution to replace synthetic acaricides or as a component of integrated mite management (Shi *et al.,* 2006 and Maniania *et al.,* 2008). Numerous isolates of *Beauveria bassiana* (Bals) Vuill can efficiently cause infection and mortality of both eggs (Shi *et al.,* 2008 and Zhang *et al.,* 2014) and active stages (Shi and Feng, 2009 and Wu *et al.,* 2016) of the two spotted spider mites.

Using predatory mites of the family Phytoseiidae has shown effective control methods in Integrated Pest Management (IPM) programs for controlling pest mites especially the two spotted spider mite *T. urticae* (McMurtry *et al.,* 2013). *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) is one of the most important predators of tetranychid mites and is widely found on various crops. It is considered one of the main predatory mites used in IPM in Egypt (El-Sharabasy, 2010). Moreover, the Bioecological and behavioral traits of *Neoseiulus* spp. make them promise for use in a variety of crops, climatic and environmental conditions, and different control strategies (Moraes *et al.,* 2004; Sousa Neto *et al.,* 2021 and Wang *et al.,* 2021). At least six species in the genus *Neoseiulus* have been mass-reared and commercialized (Lenteren, 2012; Knapp *et al.,* 2018; Lenteren *et al.,* 2018 and Sousa Neto *et al.,* 2021).

The most common *Orius* species in Mediterranean countries is *Orius albidipennis* (Reuter) (Hemiptera: Anthocoridae) (Al-Kherb, 2014), *O. albidipennis* is a biological control agent of greenhouse against many pests. *O. albidipennis* is among the predators of mites [(*T. urticae* and *Tetranychus telarius* (Linnaeus)], [(*Aphis gossypii* Glover and *A. maidis* (Hemiptera: Aphididae)], thrips [(*Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae)] and lepidopterous eggs and newly hatched larvae (Madadi *et al.,* 2009; Sobhy *et al.,* 2010 and El-Arnaouty *et al*., 2018). For this reason, understanding the interactions between the main natural enemies of *T. urticae* and using different biological control methods may help to improve and make their integrated use more compatible.

This work aims to evaluate the effect of two plant extracts and two Entomopathogenic fungi isolates on *T. urticae* and its side effects on some predators under laboratory conditions.

Materials and methods

1. *Tetranychus urticae* stock cultures:

T. urticae a delicate strain, was obtained from the laboratory of Acarology in Plant Protection Research Institute (PPRI). *T. urticae* colonies were raised on Acalypha *(Acalypha marginata*) leaves placed on moist cotton in arenas and kept in an incubator at 25±1 °C and 65 ± 5% RH. Acalypha leaves were replaced when necessary.

2. Rearing of predators:

Three predator mites were used in this study. *P. persimilis*, *Neoseiulus californicus* (McGregor) and *Neoseiulus bicaudus* (Wainstein) (Acari: Phytoseiidae). were obtained from Laboratory of Acarology in Plant Protection Research Institute (PPRI) and maintained on mix stages of the two spotted spider mite *T. urticae*. In addition to the Predator insect *O. albidipennis*. which was reared at a controlled climatic room at a temperature of 28±2°C, 75±10 % RH. and 16:8 hrs. L.D. photoperiod. The adults were placed in cylinder plastic container (12cm high x 6 cm diameter), covered with white muslin. Frozen eggs of *Sitotroga cerealella* (Oliv.), were used as a food source. Middle veins of lettuce were used as oviposition substrates. Veins of lettuce with *Orius* eggs were removed daily from the adult unit and placed in plastic boxes (20 x10 x 10 cm) till hatching. To prevent cannibalism some strips of paper were added to each box and water was supplied by adding moist cotton (Gaber *et al.,* 2011).

3. Plant extracts:

Seeds of plant; purslane *Portulaca oleracea* L. (Family: Portulacaceae) and Garden cress *Lepidium sativum* L. (Family: Brassicaceae), acetone has been chosen as the solvent for extracting the desired substance. Plant materials were obtained from Cairo Unveracity Faculty of Agriculture Ornamental Horticulture Department.

3.1. Extraction procedure:

A weight of 100 gm of each plant was ground in an electric grinder and put in 350 ml acetone as described by Su and Horvat (1981). After 2hrs the acetone extract was filtered and evaporated to dryness under vacuum using a rotary evaporator water bath at 60 ℃. The crude extract was then weighted and completed to 10 ml with acetone. The obtained extracts were used as crude extracts both crude plant extracts were kept in a refrigerator until use.

4. Fungal culture and preparation of conidial suspension:

Two isolates of entomopathogenic fungi were used in this study, *B. bassiana* (Balsamo) and *Paecilomyces lilacinus* (Thom), these isolates were procured from (PPRI).

4.1. Culturing of entomopathogenic fungi:

The two tested entomopathogenic fungi *B. bassiana* and *P. lilacinus* were cultured on autoclaved Sabouraud Dextrose Agar with yeast extract (SDAY) media and autoclaved for 20 minutes, then incubated at 25±1°C, 70±5% RH. in dark for 15 days (Hassan, 2008 and Hassan *et al.,* 2017).

5. Toxicity of tested plant extracts and entomopathogenic fungi against *Tetranychus urticae* females:

A petri-dish was prepared with a moist cotton bed, where separate leaf discs of *A. marginata* measuring 2 cm in diameter were carefully positioned upside-down. Each disc was then populated with a total of fifteen adult *T. urticae* individuals, and the direct spray technique was applied with a glass atomizer with 2ml suspension with different concentrations of the tested materials (at 30 cm height between atomizer and tested disks). Purslane extract was used at concentrations of (2, 3, 4 and 5 %) while Garden cress extract was used at

concentrations of (3, 4, 5 and 6 %). Each concentration was tested using four replicates, and the mortality percentages were measured at 1, 3, and 5 days after treatment. In addition to the entomopathogenic fungi, five concentrations of each isolate were prepared (10^6, $5x10^6$, 10^7, $5x10^7$, and10^8 spores/ml as well as the control for both experiments (2ml of distilled water with 0.01% Tween 80) were used on the same day after preparation and shaken before use (Abo-Shabana, 1980 and Hassan *et al.*, 2017). Four replicates were used for each concentration. Mortality was assessed daily for 7 days until no more mortality could be observed (Ayoub, 1984). All treated discs were kept at 25±2°C and 55%±5 RH. Mortality percentage, LC_{50}, LC_{90} and slope values were calculated.

6. Effect of *Portulaca oleracea* plant extract and *Beauveria bassiana* fungal spores on
mortality of the predators:

Predatory mites were transferred to a clean disc of Acalypha placed separately upside –down on moist cotton wool in a Petri-dish, with twelve discs as a replicate. The highly virulent tested material (i.e. Purslane plant extract and *B. bassiana*) on the adult stage of *T. urticae* was tested against the adult stage of three predators' mites *P. persimilis*, *N. californicus, N. bicaudus*, with LC_{50} concentrations by using direct spray technique, after spraying petri-dishes were kept in an incubator at 28 ± 1°C and 70% RH. Mortality percentages were calculated after 1, 3, 5 and 7 days until no more mortality could be observed of treatment and corrected by Abbott's formula (1925). While for the mortality rate of predator insect *O. albidipennis*, the previously selected materials were tested on the second instar larval. Treatment and control had five replications; every replicate had six larval instars. The percentage of mortality was recorded for 7 days.

7. Statistical analysis

The percentage of mortality was determined and corrected by Abbott's formula (1925). LC_{50}, LC_{90} and slope values were calculated according to Finney (1971), using "Ldp line" software by Bakr (2000). While for the predator's experiments, data were analyzed using two-way analysis of variance (ANOVA) and compared using LSD test by SAS statistical software. (SAS Institute, 2003)

Results and discussion

The present results showed the efficiency of two plant extracts (*P. Portulaca* and *L. sativum*) and two entomopathogenic fungi (*B. bassiana* and *P. Lilacinus*), against adult stages of *T. urticae* in laboratory experiments.

1.Toxicity of some plant extract against *Tetranychus urticae*:

Table (1) presents the findings, indicating that the acetone extract of *P. oleracea* demonstrated remarkable efficacy in combating adult female *T. urticae*. The extract resulted in a mortality rate ranging from 21% to 91.6% when applied at concentrations of 2-5% after 3 days. Conversely, the acetone extract of *L. sativum* exhibited a mortality rate of 24% to 80% when applied at concentrations of 3-6% after the same duration. The LC_{50} values obtained for *P. oleracea* and *L. sativum*, were 2.77 and 4.2 Figure (1), while LC_{90} were 4.8 and 7.2 and the slope values were 5.3 and 5.8, respectively. These findings align with the study conducted by Syed *et al.*, (2010) which highlighted the medicinal properties of *P. oleracea* and its effectiveness as a pesticide against various arthropod pests.

These results come parallel to Elkady *et al*. (2022) who reported that LC_{50} of *P. oleraceae* in all plant extracts with chloroform were 25158.97 ppm after 24h and 20507.56 ppm after 48 h, while with methanol extract the value of LC_{50} was 135284.70 ppm after 24h and 47158.62 ppm

after 48. Also, Rajabpour *et al*. (2018) used *P. oleraceae* against *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae) as acaricidal and repellent. The value of LC_{50} with *P. oleraceae* ethanolic extract was 0.00589 mg/kg and the repellency indices (IR) was 1.03. In addition to studies done by Choi *et al*. (2004) tested 53 essential oils, and found that caraway seed, citronella, lemon, eucalyptus, pennyroyal and peppermint were highly toxic to mite species, *T. urticae* and *P. persimilis.*

Table (1): Comparison of the toxicity of two plant extracts against *Tetranychus urticae* adult stage.

Tested material	Con.	Mortality %	LC_{50}	LC_{90}	Slope
Portulaca oleracea	2 3 4 5	21 67 76.9 91.6	2.77	4.8	5.3
Lepidium sativum	3 4 5 6	24 47 67 80	4.2	7.2	5.8

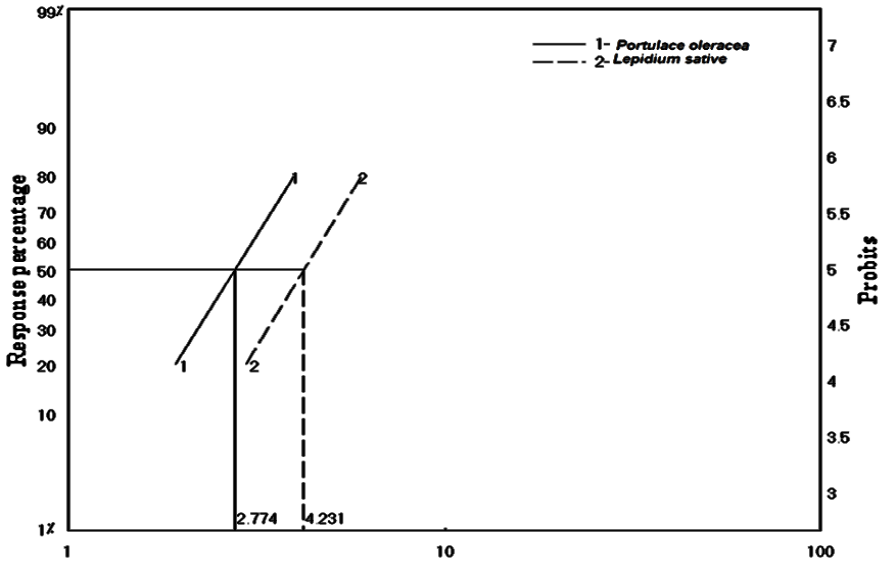

Figure (1): Percentage mortality regression lines of two plant extracts against *Tetranychus urticae* adult female.

The susceptibility of adult female *T. urticae* to entomopathogenic fungi *B. bassiana* and *P. lilacinus* was investigated in this study. Table (2) The percentage of mortality gradually increased along with spores' concentrations. The Lowest concentration of 10^6 spores/ml revealed 38.2 and 31.3 % While the highest concentration of 10^8 spores/ml revealed 74.5 and 49.1 % for both *B. bassiana* and *P. lilacinus* respectively, 7 days after treatment. The results obtained in the present study are comparable with those of other research that used fungal pathogens against mites. A study by Waked *et al*. (2015) recorded that isolates of *B. bassiana* and *P. fumosoroseus* were highly effective and virulent against females of *T. urticae* at four different concentrations. The *P. fumosoroseus* isolate had lower LC_{50} (1.8 x10^7 spores/ml) than *B. bassiana* (2.6 x

10^7 spores/ml). Furthermore, Hassan *et al.* (2017) tested two isolates of *Metarhizium anisopliae* (Metschnikoff) and four isolates of *B. bassiana* on both egg and adult stages of *T. urticae* the mortality ranged from (46 to 80%) and the most effective isolates were B4 with LC_{50} 6.57 x 10^6 spores/ml. Also, this is in line with the previous study of Islam *et al.* (2017) the results revealed that *B. bassiana* was highly effective in controlling *T. urticae* adults causing mortality 72 hrs. after fungal application. Notably, the acaricidal efficacy of *B. bassiana* exhibited a time-dependent pattern, whereby the toxicity increased as time progressed. Furthermore, it was observed that the deceased female *T. urticae* individuals infected with *B. bassiana* displayed mycosis symptoms, characterized by the presence of fungal conidia, whereas the control group of deceased mites did not exhibit any mycotic manifestations.

Also results in Table (2) and Figure (2), proved that *B. bassiana* was more effective against *T. urticae* adult females compared with *P. lilacinus*. The LC_{50} value was 2.03x $10^7$and 6.91x10^8 spores/ml, for *B. bassiana* and *P. lilacinus*, respectively. These findings are consistent with those observed by Ali *et al.* (2020) focused on testing two isolates of *B. bassiana* (B1, B2) and two isolates of *M. anisopliae* (M1, M2). Interestingly, the most effective isolate was identified as B2, with an LC_{50} value of 2.86×10^7 spores/ml.

Table (2): Comparison of the pathogenicity of two EPF against *Tetranychus urticae* adult stage.

Tested Isolates	Con.	Mortality %	LC_{50}	LC_{90}	Slope
Beaveria bassiana (Balsamo)	10^6 $5X10^6$ 10^7 $5X10^7$ 10^8	38.2 60.7 63.8 66.6 74.5	2.03 X 10^7	2.84 X 10^9	0.59
Paecilomyces lilacinus (Thom)	10^6 $5X10^6$ 10^7 $5X10^7$ 10^8	31.3 37.0 42.0 48.3 49.1	6.91 X 10^8	1.44 X 10^{11}	0.5524

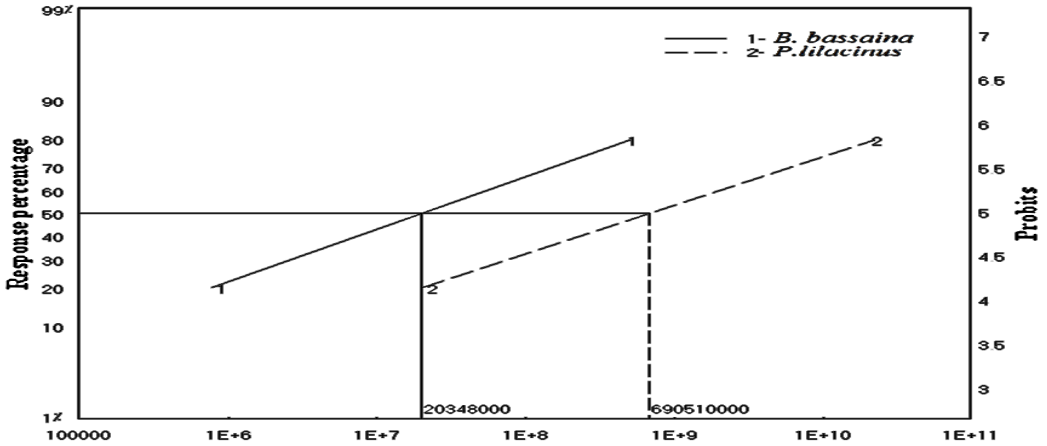

Figure (2): Percentage mortality regression lines of the (EPF) against *Tetranychus urticae* adult female's stage.

2. Effect of *Portulaca oleracea* plant extract and *Beauveria bassiana* fungal spores on mortality of the predators:

This part of the investigation was undertaken to evaluate the side effect of using the LC_{50} of the highly virulent materials of *P. oleracea* plant extract and (EPF) *B. bassiana* against predatory mites, *P. persimilis*, *N. californicus, N. bicaudus* and the predator insect *O. albidipennis*., the results in Table (3) show that there were no significant different when the three predator mites *P. persimilis*, *N. californicus* and *N. bicaudus* sprayed with *P. oleracea*, while there was a significant difference when applying the same plant extract on *O. albidipennis* . Also, there were no significant differences when using *B. bassiana* on all previous predators. When we use *P. oleracea* and *B. bassiana* the mean percentages of mortality, on *P. persimilis* were (24.45, 25.83 %) respectively, with LSD 15.13, (There were no significant differences), on *N. californicus* were (28.67, 25.8) respectively, with LSD 9.67, (There was no significant differences) on *N. bicaudus* were (35.36, 26.55) respectively, with LSD 7.99 (There was a significant differences) while on *O. albidipennis* were (3.85 and 29.49) respectively, with LSD 9.03 (There was a significant difference). The LSD between all the predators was 11.33 and 9.63 when applying *P. oleracea* and *B. bassiana*, respectively.

Notable observation revealed that the concentration LC_{50} of both *P. oleracea* and *B. bassiana* caused high mortality of the adult females *T. urticae*, was slightly harmful on *P. persimilis*, *N. californicus, N. bicaudus* and *O. albidipennis*.

Table (3): Mortality of some predators after treating with *Portulaca oleracea* and *Beauveria bassiana.*

Treatments	*Phytoseiulus persimilis*	*Neoseiulus californicus*	*Neoseiulus bicaudus*	*Orius albidipennis*	LSD
Portulaca oleracea	24.45 Aa*	28.67 Aa	35.36 A a	3.85 Bb	11.33
Beauveria bassiana	25.83 Aa	25.8 Aa	26.55 Ba	29.49 Aa	9.63
LSD	15.13	9.67	7.99	9.03	

*** Means with the same Capital letter within the same column and small letter within the same row are not significantly different at level 0.05.**

These active compounds possess a wide range of acaricidal properties, making them a comprehensive solution for mite control. However, it is important to note that these compounds have lethal effects on the predator species *P. persimilis*, *N. californicus, N. bicaudus* and *O. albidipennis* but *P. oleracea* doesn't affect *O. albidipennis* In a study conducted by Syahputra and Endarto (2013), the acaricidal activity of the aqueous extracts of *Mimusops elengi*; *Kalanchoe pinnata*; *Barringtonia asiatica*; *Brucea javanica* and *Jatropha curcas* against the citrus rust mite *Phyllocoptruta oleivora* (Ashmead) was evaluated and gave 100% Mortality four days after treatment and it was observed that none of the aqueous extracts caused mortality in the larvae of the predator *Harmonia axyridis* (Pallas). The findings of Momen *et al.* (2014) revealed that *N. californicus* is extremely less sensitive to Melissa oil and Melissacide (Mineral and vegetable oils) compared to *T. urticae* under laboratory conditions. When *N. californicus* was subjected to the (LC_{50} and LC_{90} values reported on *T. urticae*), female mortalities ranged between 8.5–13%, respectively.

Our result is in line with the findings of Michereff *et al.* (2022), who observed that two strains of *B. bassiana* caused *T. urticae* mortality rates exceeding 70 %. Also, three species of *Neoseiulus* mites were found to be vulnerable to both strains, however, *N. barkeri* was less susceptible to fungal infections compared to the other *Neoseiulus*

species, with mortality rates below 65 % and without reduction of oviposition. These results showed that the simultaneous use of *B. bassiana* and *Neoseiulus* had a detrimental effect on predatory mite survival and progeny. Additionally, more investigations have indicated that the incorporation of entomopathogenic fungal conidia into predatory mites can elevate the infection rates in pest populations, thereby enhancing their effectiveness as biocontrol agents (Lin *et al.,* 2019).

Moreover, Chandler *et al.* (2005) found that under field conditions, the release of *P. persimilis* may be an effective alternative to biocontrol of *T. urticae*, and it can be complemented with applications of the entomopathogenic fungus *B. bassiana.* Therefore, evaluating the compatibility of using predatory mites and entomopathogenic fungi to control *T. urticae* is a critical issue for the implementation of IPM programs in production areas. Also, Seiedy *et al.* (2015) investigated the direct spray of the entomopathogenic fungus *B. bassiana* (Three strains) against the predatory mite *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) in laboratory conditions. *A. swirskii* adults were highly susceptible to one of the three isolates on the seventh day. The study showed that *A. swirskii* was susceptible to *B. bassiana* when conidia were applied directly to the mites.

These results in contrast with those of Numa Vergel *et al.* (2011) *P. persimilis* was more susceptible than *N. californicus* to *B. bassiana*. The mortality rate was recorded on *P. persimilis* (43%) and on *N. californicus* (14%) after the application of the highest concentration of *B. bassiana*. However, the oviposition rate of *P. persimilis* increased when treatment with *B. bassiana*. This result is consistent with Duso *et al.* (2008) who reported that *B. bassiana* caused a mortality of 43% on *P. persimilis* and its fertility eventually decreased as the fungal conidial inoculum increased under laboratory conditions. The same entomopathogenic fungus generated reported mortalities above 10% in *N. californicus* under laboratory conditions (Castagnoli *et al.,* 2005).

Also, Pozzebon and Duso (2010) showed that exposure to *B. bassiana* had a significant effect on *P. persimilis* survival and fecundity. While Numa Vergel *et al.* (2011) showed that fecundity values for the two predators were decreased, after being treated with *B. bassiana* the *N. californicus* showed a lower fecundity rate (17 %) than *P. persimilis* (43 %). Moreover, for the *O. albidipennis*, a study conducted by Ludwig and Oetting (2001) reported that *Orius insidiosus* was least susceptible to direct infection of *Beauveria bassiana* strain JW-1 (4.9%). Also, Dunkel and Jaronski (2003) indicate that *B. bassiana* strain GHA, can be used safely without significant effect on predator *Xylocoris flavipes.* Zimmermann (2007) reported that *Orius sauteri* is not the host range of this strain of *B. bassiana*. In additions, Thungrabeab and Tongma (2007) stated that *B. bassinna* was not pathogenic to different natural enemies. Similar findings were also found by Gao *et al.* (2012), who examined the *B. bassiana* Bb-RSB on the predator *Orius sauteri*. The results indicated that Bb-RSB did not possess insecticidal properties mortality was very low (5%) and did not have any impact on the developmental rate of the predator *O. sauteri.*

In a more recent study by Pazyuk *et al.* (2022), the effect of entomopathogenic fungi belonging to the genus Lecanicillium on the fecundity of *Orius laevigatus* (Fieber) was investigated. The researchers concluded that the mycelium EPF strains did not have any negative effects on the fecundity of *O. laevigatus*. Similarly, Samy *et al.* (2021), conducted a study on the pathogenicity and side effects of indigenous *B. bassiana* on *Coccinella undecimpunctata* and *Hippodamia variegata*. The findings

revealed that there were no adverse effects on either predator. These findings align with the results obtained by Galland (2023), who reported that *B. bassiana* MUCL 1555 exhibited no lethal effects on *O. laevigatus,* which may be attributed to the fact that *O. laevigatus* have strongly sclerified body segments preventing the adhesion of spores to their cuticle (Sharma and Sharma, 2021).

This means that the plant extract *P. oleracea* and entomopathogenic fungus *B. bassiana* could be used with some precautions in an Integrated Pest Management (IPM) program for controlling *T. urticae.* (i.e. Releasing the predatory mites or insects after the degradation of the plant extracts or the incubation period of the fungi, without harm to them).

Further knowledge is needed to adjust the timing of various releases of both biological control agents to obtain maximum effectiveness in the greenhouse with minimum impact of the fungus on the predator.

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