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Biocontrol Potential of Different Entomopathogenic Fungi and Neem Oil for Control of Sucking Insects and Two-Spotted Spider Mites in Vegetables

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Greenhouse vegetable crops are significantly affected by infestations from three major groups of harmful sucking insects: aphids (*Aphis gossypii*), whiteflies (*Bemisia tabaci*), and two-spotted spider mites (*Tetranychus urticae*). Aim: The study aimed to assess the effectiveness of entomopathogenic fungi, including Metarhizium anisopliae, *Beauveria bassiana*, and *Paecilomyces fumosoroseus*, both individually and in combination with neem oil, against these pests. When treatments were applied six days prior to testing, all entomopathogens at a concentration of 100,000 spores per milliliter resulted in 84% mortality of *T. urticae*, A. gossypii, and *B. tabaci*. Higher doses of neem oil, when combined with entomopathogenic fungi, proved effective in controlling insect pests. The combination of entomopathogenic fungi at a concentration of 1 x 10^5 spores/mL and 1% neem oil in a 1:1 ratio exhibited significantly greater pest elimination success compared to the individual treatments. Specifically, the combination of *M. anisopliae* and neem oil (1:1) achieved 100% mortality of *T. urticae* and A. gossypii, while *P. fumosoroseus* with neem oil (1:1) was effective against *B. tabaci* six days post-treatment. This research demonstrated that combining entomopathogenic fungi with neem oil in a 1:1 ratio proved more effective than applying them individually. The study also highlighted the compatibility of these treatments, with all experimental samples resulting in mortality rates exceeding 100%.

Keywords: Biocontrol, Entomopathogenic Fungi, Neem Oil, Sucking Insects, Two-Spotted Spider Mite.

Introduction

In recent times, modifications in farming techniques, the progression of climate change, and the increased utilisation of input-intensive, high-yield crop varieties have significantly altered pest dynamics in the Mekong Delta region of Vietnam. These shifts have notably impacted vegetable ecosystems and orchards. Changes in both environmental and ecological parameters have contributed to the emergence of several insect pests and mites, which previously posed minimal threats to vegetable production in the region, as pressing concerns across various localities in the country (Deguine et al., 2023; Van Mele et al., 20010). Among these, sucking insect pests and two-spotted spider mites, specifically *Aphis gossypii*, *Bemisia tabaci*, and *Tetranychus urticae*, have gained recognition as critical constraints to vegetable cultivation not only in Vietnam but also globally (Van Mele, Cuc, & Van Huis, 2010; Van Mele et al., 20010). These pests are known for their polyphagous nature and feed on a wide spectrum of host plants such as cabbage, tomatoes, eggplants, and ornamental species like roses and lilies. Their geographical distribution spans extensively worldwide, with higher population densities typically recorded in temperate climates, which also exhibit greater species diversity than tropical zones (Żyła, Homan, & Wegierek, 2017).

In response to escalating pest infestations, farmers have resorted to frequent and heavy application of insecticides. This approach has led to several adverse outcomes, including the resurgence of targeted pests, the development of insecticide resistance, and the outbreak of secondary pests. Additionally, the residual presence of chemical pesticides in agricultural produce, water sources, and beverages has given rise to severe health and environmental repercussions. These include contamination of groundwater, human health hazards, and the mortality of non-target organisms such as pollinators and beneficial insects (Ambethgar, 2009; Deguine et al., 2023; Halder, Divekar, & Rani, 2021; Van der Sluijs et al., 2013). In this context, biopesticides offer a sustainable alternative by minimising dependency on synthetic chemicals (Deguine et al., 2023). Entomopathogenic fungi (EPFs) have demonstrated considerable efficacy in reducing pest populations across a diverse range of agricultural systems. Species such as *Metarhizium anisopliae*, *Beauveria bassiana*, and

Paecilomyces fumosoroseus are well-documented for their pest control potential. Studies focusing on the biological control of insect pests using EPFs have reported high levels of effectiveness, targeting up to 150 insect species. The most widely utilised biocontrol strains originate from four primary Hypocrealean fungal species: *M. anisopliae*, *B. bassiana*, *Isaria fumosorosea*, and *B. brongniartii*. Initial commercial trials have likely enhanced these success rates. However, a major limitation of EPFs lies in their relatively slow mode of action, as the time required to achieve insect mortality surpasses that of conventional chemical agents. This drawback can be mitigated by integrating EPFs with faster-acting compounds in pest management systems (Bitsadze et al., 2013).

The fungal pathogen *M. anisopliae* has shown effectiveness against more than 200 insect pest species. Research by González-Mas et al. (2019) identified significant control outcomes for A. gossypii, two-spotted spider mites (TSSM), and other related mite species. Further evidence from studies such as Cakmak et al. (2009) confirms that, following cuticular penetration, EPF conidia rapidly colonise the insect haemocoel, subsequently proliferating and sporulating within the host as well as on its external surface (Chandler, Davidson, & Jacobson, 2005). The success of EPFs in controlling TSSM and other sucking insects is influenced by the fungal strain, the concentration of the inoculum, and prevailing environmental conditions. *Azadirachta indica* A. Juss (commonly known as neem) has gained widespread application in pest control due to its efficacy against multiple insect species, accessibility, and cost-effective production. Efforts have been made to employ biocontrol agents and botanicals in equal proportions to assess their compatibility and maximise pest control outcomes (Gatarayiha, Laing, & Miller, 2010). The co-application of fungal biopesticides and neem oil has emerged as a promising strategy (Ullah & Lim, 2015). This integrated approach enhances both economic viability and environmental sustainability compared to chemical insecticides (Bugeme et al., 2014). Moreover, there are no recorded cases of resistance development to these biological control methods, and they are considered environmentally safe.

Accordingly, the present study aimed to investigate the effectiveness of selected entomopathogenic fungi, neem oil, and their low-concentration combinations in suppressing *A. gossypii*, *B. tabaci*, and *T. urticae*, as part of a biologically based pest management strategy.

Materials and Methods

Insect Collection and Rearing of A. gossypii, B. tabaci, and T. urticae

The present study involved the collection of sucking insect species from eggplant and tomato fields that were free from pest infestation and insecticide exposure in Can Tho City, Vietnam. The identified pests included aphids (*Aphis gossypii*), whiteflies (*Bemisia tabaci* species), and two-spotted spider mites (*Tetranychus urticae*). A research plot maintained by the University was utilised for this purpose. Seedlings were grown under regulated conditions in plastic pots with a diameter of 15 cm, using a sandy loam and organic matter mixture at a 2:1 ratio. The growth environment was maintained at a temperature of 25 ± 1°C with a relative humidity of 65 ± 10%. The pots were enclosed to facilitate the natural reproduction of aphids, whiteflies, and spider mites along with their respective nymphs, ensuring the absence of insecticidal interference. For the bioassay trials, second and third instar nymphs derived from the offspring of the aforementioned insect species were employed.

Preparation of Concentration of Entomopathogenic Fungi and Neem Oil

The fungal isolates *Beauveria bassiana* CTUBb28, *Metarhizium anisopliae* CTUMa01, and *Paecilomyces fumosoroseus* CTUPae025 were cultured on SDAY3 medium (Sabouraud Dextrose Agar Yeast), which comprised 40 g of dextrose, 10 g of peptone, 10 g of yeast extract, 20 g of agar, 0.5 g of magnesium sulphate ($MgSO_4$), 1.0 g of monopotassium phosphate (KH_2PO_4), and 1,000 mL of distilled water. The cultures were incubated at a constant temperature of 25°C for a duration of 14 days. Upon completion of the sporulation phase, conidia were collected using an inoculation loop, washed with sterile distilled water, and filtered through a double layer of gauze to remove any residual hyphal structures. To attain the desired concentrations for experimental application, conidial suspensions were prepared in a 0.01% Tween 80 solution. In accordance with the germination assessment procedure outlined by Ansari & Butt (2011), conidial viability was confirmed to exceed 90% across all evaluations. Neem oil emulsions were formulated by blending the oil with an emulsifying agent based on Triton-X-100.

Bioassay

Bioassays for aphids and whiteflies were carried out, while assessments involving TSSM were performed using Petri dish setups. In these experiments, leaves from eggplant plants were arranged with the abaxial (underside) surface facing upwards, resting on a bed of moistened cotton wool. The petioles of the leaves were inserted into the cotton layer to preserve turgidity and sustain adequate moisture levels throughout the testing period.

Effect of Different Doses of Neem Oil Against Aphids, Whiteflies, and TSSM

Five different concentrations of neem oil (1%, 2%, 3%, 4%, and 5%) were prepared, with each concentration being replicated three times. A total of 90 nymphs were assigned to each treatment group and placed in separate Petri dishes, each 9 cm in diameter. The mortality rates of aphids, whiteflies, and TSSM were observed individually at 24-hour intervals over a six-day period.

Bioassay of Entomopathogenic Against Aphids, Whiteflies, and TSSM

The fungal strains *Beauveria bassiana*, Metarhizium anisopliae, and *Paecilomyces fumosoroseus* were prepared at four different concentrations: 1×10^4, 1×10^5, 1×10^6, and 1×10^7 conidia/mL. Each concentration was tested in triplicate. For each treatment, 75 nymphs were placed into individual Petri dishes, each with a diameter of 9 cm. The mortality of aphids, whiteflies, and two-spotted spider mites was assessed separately at 24-hour intervals over a six-day period.

Bioassay of Neem Oil and Mix with Entomopathogenic Against Aphids, Whiteflies, and TSSM

A total of thirty insect samples were placed individually into separate Petri dishes, each with a diameter of 9 cm. Three independent trials were conducted for each experimental condition. The seven experimental groups were as follows: A1: neem oil alone; A2: *M. anisopliae*; A3: *B. bassiana*; A4: P. fumosoroseus; A5: *M. anisopliae* combined with neem oil (1:1); A6: *B. bassiana* combined with neem oil (1:1); A7: *P. fumosoroseus* combined with neem oil (1:1); and a control group treated with water. The application method involved the use of Potter's tower, applying different concentrations of neem oil mixed with entomopathogenic fungi and a 1:1 ratio of neem oil for 10 seconds at a rate of 4,000 g/cm². After spraying, the pots containing the treated insects were dried using a fan. The bioassays were performed under controlled laboratory conditions, with temperatures maintained at 25 ± 1°C and a relative humidity of 70 ± 5%.

Data Analysis

Daily observations were conducted to record the number of deaths among aphids, whiteflies, and TSSM. Abbott's formula was employed to adjust for control group mortality. The data were subsequently analysed using probit analysis to calculate the median lethal concentration (LC50) and median lethal time (LT50), with statistical computations carried out using the Statistical Package for the Social Sciences. Additionally, the cumulative corrected mortality percentages for each fungal treatment were subjected to analysis of variance (ANOVA), and treatment means were compared using Duncan's Multiple Range Test (DMRT).

$$C\ (\%) = \frac{A-B}{A} \times 100$$

Where:

C = Corrected mortality;

A = Average number of pests (aphid, whitefly, and TSSM) in the control group;

B = Average number of pests (aphid, whitefly, and TSSM) in the treatment group.

Results and Discussion

Effect of Range Dose of Neem Oil Against Two Kinds of Sucking Insects and TSSM

The maximum biological efficacy under laboratory conditions was recorded daily following treatment. The results indicated that, after six days, the treatment with 5% neem oil exhibited the highest efficacy against both sucking insects and two-spotted spider mites Figure. 1. The toxicological effects of varying doses of neem oil and their interactions with the nymphs of *T. urticae*, *A. gossypii*, and *B. tabaci* are illustrated in Figure. 1. For *T. urticae*, the highest nymph mortality rate (74.3%) was recorded at a 5% dose, though this was not statistically significant when compared to the 4% neem oil dose (62.7%). In contrast, the lowest mortality (45.7%) occurred with a 1% neem oil dose Figure. 1. Nymphs of *A. gossypii* displayed high susceptibility to neem oil at all concentrations, with mortality rates of 93.3%, 96.7%, and 100% observed at 3%, 4%, and 5%, respectively. Furthermore, the mortality rate of *B. tabaci* was found to be 90.3% at the 5% dose, which was statistically distinct from the other doses.

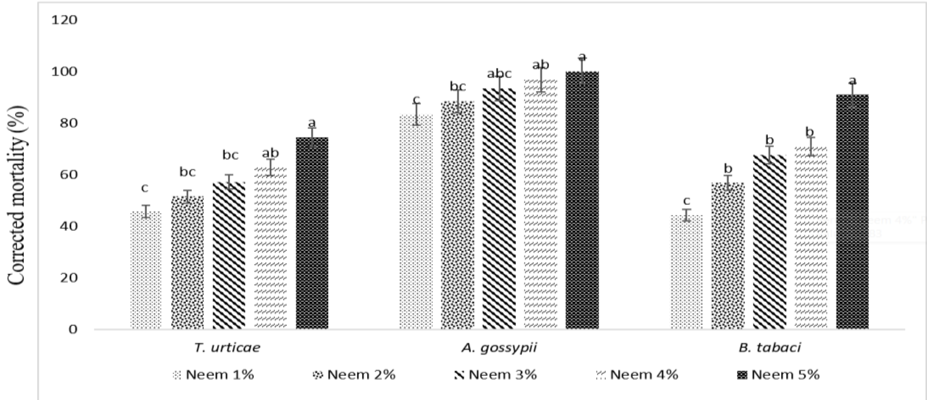

Figure 1: Corrected Mortality in *T. urticae*, *A. gossypii*, and *B. tabaci* after 6 Days of Application of Neem Oil at Different Doses under Laboratory Conditions.

Percent Mortality of Sucking Insects and TSSM to Entomopathogenic Fungi

** T. urticae*

Table 1 illustrates that the mortality percentage of nymphs was 60.0%, 67.6%, and 68.6% after the fifth day for *M. anisopliae*, *B. bassiana*, and P. fumosoroseus, respectively, at a concentration of 1 × 10^7 spores/mL. By the sixth day of infection, the mortality rates for *M. anisopliae*, *B. bassiana*, and *P. fumosoroseus* ranged from 66.7% to 84.8%, 56.2% to 85.7%, and 60.0% to 85.7%, respectively. The results indicated that all entomopathogenic fungi at the 1 × 10^7 spores/mL concentration were associated with a mortality rate exceeding 84% on the sixth day post-treatment.

* A. gossypii

Table 1 shows that the highest nymph mortality was recorded with *P. fumosoroseus* at a concentration of 1 × 10^7 spores/mL, resulting in 78.3% mortality, followed by *M. anisopliae* at 68.3%, and *B. bassiana* at 66.7% at the same concentration. By the sixth day of treatment, fungal applications at 1 × 10^7 spores/mL reached peak mortality rates, ranging from 85.0% to 91.7% under controlled laboratory conditions.

* B. tabaci

According to Table 1, *M. anisopliae* at a concentration of 1 × 10^7 spores/mL resulted in 50.0% nymph mortality by the fifth day post-infection. At the same concentration, *B. bassiana* caused 53.3% mortality, while *P. fumosoroseus* achieved 71.7%. By the sixth day, mortality rates had increased, with *M. anisopliae* reaching 88.3%, and *P. fumosoroseus* ranging from 91.7% to 93.3% at concentrations of 1 × 10^6 and 1 × 10^7 spores/mL, respectively.

Table 1: Percentage Mortality of Nymphs of Sucking Pests and Two-Spotted Spider Mites (TSSM) at Various Time Intervals Following Exposure to Entomopathogenic Fungi (EPF) under Controlled Conditions of 25 ± 1°C and 70 ± 5% Relative Humidity.

Treatments	Doses (Spores/mL)	Corrected Mortality (%)								
		T. urticae			*A. gossypii*			*B. tabaci*		
		4th DAT	5th DAT	6th DAT	4th DAT	5th DAT	6th DAT	4th DAT	5th DAT	6th DAT
M. anisopliae	D1	27.6	48.6	66.7	35.0	51.7	76.7	13.3	28.3	70.0
	D2	35.2	50.5	78.1	40.0	55.0	85.0	16.7	41.7	78.3
	D3	39.1	52.4	82.9	46.7	61.7	85.0	23.3	51.7	91.7
	D4	44.8	60.0	84.8	50.0	68.3	88.3	26.7	61.7	93.3
B. bassiana	D1	29.5	38.1	56.2	25.0	48.3	68.3	11.7	18.3	70.0
	D2	32.4	49.5	68.6	28.3	53.3	78.3	13.3	23.3	73.3
	D3	41.9	61.0	76.2	31.7	60.0	81.7	15.0	41.7	86.7
	D4	49.5	67.6	85.7	33.3	66.7	85.0	16.7	53.3	81.7
P. fumosoroseus	D1	28.6	43.8	60.0	36.7	58.3	81.7	13.3	36.7	80.0
	D2	32.4	44.8	61.9	41.7	66.7	83.3	28.3	53.3	81.7
	D3	38.1	48.6	68.6	50.0	71.7	90.0	33.3	58.3	83.3
	D4	52.4	68.6	85.7	53.3	78.3	91.7	35.0	71.7	88.3

D1: 1 × 10^4 Spores/mL; D2: 1 × 10^5 Spores/mL; D3: 1 × 10^6 Spores/mL; D4: 1 × 10^7 Spores/mL; DAT: Days after Treatment.

The Median Lethal Concentration (LC50)

The LC_{50} values and toxicity levels of the three species of entomopathogenic fungi were compared against sucking insects and TSSM, as shown in Table 2. The results revealed that the lowest LC50 values were 0.87 × 10^5 spores/mL and 0.10 × 10^7 spores/mL for *M. anisopliae* against *T. urticae* and *B. tabaci*, respectively. Meanwhile, the lowest LC_{50} value for *P. fumosoroseus* was 0.58 × 10^3 spores/mL against *A. gossypii*.

Table 2: Dose Mortality of EPF Against Sucking Insects and TSSM 25 ± 1°C and 70 ± 5% R.H.

EPFs	Dose Mortality of Entomopathogenic Fungi					
	T. urticae		*A. gossypii*		*B. tabaci*	
	LC50 (Spores/mL)	95% Fiducial Limits (Spores/mL)	LC50 (Spores/mL)	95% Fiducial Limits (Spores/mL)	LC50 (Spores/mL)	95% Fiducial Limits (Spores/mL)
M. anisopliae	0.87 x 10^5	0.21 x 10^3 - 0.93 x 10^6	0.81 x 10^4	0.39 x 10^1 - 0.12 x 10^6	0.10 x 10^7	0.79 x 10^6 - 0.54 x 10^9
B. assiana	0.13 x 10^6	0.28 x 10^5- 0.38x10^6	0.17 x 10^5	0.11 x 10^2 - 0.20 x 10^6	0.15 x 10^8	0.43 x 10^7 - 0.72 x 10^8
P. fumosoroseus	0.19 x 10^6	0.72 x 10^4- 0.12x10^7	0.58 x 10^3	0.27 x 10^2 - 0.12 x 10^5	0.60 x 10^7	0.15 x 10^7 - 0.28 x 10^8

The Median Lethal Time (LT50)

As shown in Table 3, the LT_{50} values decreased as the spore concentration increased. At the highest tested concentration of 1 × 10^7 spores/mL, the LT_{50} for *M. anisopliae*, *B. bassiana*, and *P. fumosoroseus* was recorded as 3.55, 3.75, and 3.37 days, respectively, for *T. urticae*. For *A. gossypii*, the corresponding LT_{50} values were 2.70, 2.72, and 2.42 days, while for *B. tabaci*, the LT_{50} values were 4.04, 4.88, and 4.05 days, respectively.

Table 3: Time Mortality of EPF Against Sucking Insects and TSSM 25 ± 1°C and 70 ± 5% R.H.

EPFs	Median Lethal Time (Days) of Entomopathogenic Fungi											
	T. Urticae				*A. gossypii*				*B. Tabaci*			
	D1	D2	D3	D4	D1	D2	D3	D4	D1	D2	D3	D4
M. anisopliae	4.44	4.04	3.80	3.55	3.32	3.10	2.88	2.70	5.64	5.04	4.30	4.04
B. bassiana	4.80	4.40	3.90	3.75	3.32	3.07	2.85	2.72	5.92	5.80	5.05	4.88
P. fumosoroseus	4.71	4.52	4.15	3.37	3.17	2.85	2.58	2.42	5.41	4.92	4.65	4.05

D1: 1 x 10^4 spores/mL; D2: 1 x 10^5 spores/mL; D3: 1 x 10^6 spores/mL; D4: 1 x 10^7 spores/mL

The Combination of Entomopathogens with Neem Oil Against Sucking Insects and TSSM

A. gossypii and B. tabaci

The examination of the effects of various entomopathogens, both individually and in combination with neem oil, revealed significant differences among the three types of EPFs, whether used alone or together. *M. anisopliae* demonstrated substantial potential as an entomopathogen against two sucking insects, aphids and whiteflies. The highest mortality rate observed was 81.1% at a concentration of 10^5 spores/mL and 75.0% six days after treatment (DAT) on *A. gossypii* and *B. tabaci*, respectively. *B. bassiana* showed corresponding mortality rates of 74.4% and 80.0%, respectively Table 4. In contrast, *P. fumosoroseus* exhibited the most efficacy against the nymphs of *A. gossypii* (66.7%, 81.1%, and 87.8% at 4, 5, and 6 DAT, respectively) and *B. tabaci* (51.24%, 72.88%, and 85.38% at 4, 5, and 6 DAT, respectively). Neem oil (1%), however, showed lower efficacy as a botanical treatment for the emerging pests, with mortality rates at 4, 5, and 6 DAT of 42.2%, 55.6%, and 72.0% for *A. gossypii*, and 20.0%, 31.67%, and 51.67% for *B. tabaci*. When these entomopathogenic fungi were applied in combination with neem oil at a 1:1 ratio, mortality rates significantly increased in all cases compared to their individual effects. This suggests compatibility among these essential biopesticides. The treatments combining *M. anisopliae* and neem oil, as well as *P. fumosoroseus* and neem oil at a 1:1 ratio, achieved maximum effective control of 100% against *A. gossypii* Table 4. The mixture of *B. bassiana* with neem oil resulted in 95.6% pest control. A similar trend was observed in *B. tabaci*, with complete (100%) control of thrips nymphs attained through the combined application of *P. fumosoroseus* and neem oil, *M. anisopliae* with neem oil, and *B. bassiana* with neem oil.

T. urticae

The present study highlighted the effectiveness of EPFs and neem oil in controlling TSSM. *M. anisopliae* exhibited an average effectiveness of 78.1% when used alone, but when combined with neem oil at a 1:1 ratio, it resulted in a maximum mortality rate of 100%. This was followed by a 98.1% efficacy for the combination of *P. fumosoroseus* and neem oil at the same ratio. The mixture of *B. bassiana* and neem oil demonstrated an efficacy of 86.7%.

Table 4: Impact of Various Entomopathogens Individually and in Combination with Neem Oil on *T. urticae, A. gossypii*, and *B. tabaci* at 25 ± 1°C and 70 ± 5% R.H.

Treatments	The Mortality (%)								
	T. urticae			*A. gossypii*			*B. tabaci*		
	4th DAT	5th DAT	6th DAT	4th DAT	5th DAT	6th DAT	4th DAT	5th DAT	6th DAT
A1	41.9e	48.6e	53.3e	42.2e	55.6f	72.0f	20.00d	31.67d	51.67c
A2	65.7c	76.2c	78.1c	61.1cd	73.3de	81.1d	42.5c	61.67c	75.00b
A3	51.4de	60.0de	65.7d	55.6d	67.8ef	74.4e	40.00c	65.00c	80.00b
A4	58.1cd	68.6cd	74.3cd	66.7c	81.1cd	87.8c	51.24d	72.88b	85.38b
A5	92.4a	95.2a	100a	90.0a	95.6ab	100a	49.17c	63.33c	78.33b
A6	77.1b	81.0bc	86.7b	84.4b	90.0bc	95.6b	59.61cd	77.62b	88.97b
A7	87.6a	92.4a	98.1a	93.3a	97.8a	100a	87.49b	95.47a	100.00a

Note: Identical letters within the same column indicate no significant differences ($P \leq 0.05$). In this case, A1 refers to neem oil applied at a 1% concentration; A2 corresponds to *M. anisopliae* at 1×10^5 spores/mL; A3 represents *B. bassiana* at 1×10^5 spores/mL; and A4 indicates *P. fumosoroseus* at 1×10^5 spores/mL. Treatments A5, A6, and A7 represent combinations of *M. anisopliae*, *B. bassiana*, and *P. fumosoroseus* with neem oil, respectively, each mixed in a 1:1 ratio. The term DAT denotes the number of days after treatment application.

Discussion

The potential of entomopathogenic fungi, including *Metarhizium anisopliae*, *Beauveria bassiana*, *Paecilomyces fumosoroseus*, and neem oil, as biological control agents against *Tetranychus urticae*, *Aphis gossypii*, and *Bemisia tabaci* appears promising for future applications. Higher concentrations are likely to enhance efficacy in controlling these insects. The study also highlighted that increased doses of neem oil or fungal conidia concentrations can significantly improve the effectiveness against the targeted pests. When exposed to the same concentration of conidia, *Aphis gossypii* and *Tetranychus urticae* showed greater susceptibility to *Metarhizium anisopliae* CTUMa01 and *Paecilomyces fumosoroseus* CTUPae025 compared to *Beauveria bassiana* CTUBb28. Abdel-Raheem, Saad, & Abdel-Rahman (2021) examined the mortality rates of Myzus persicae, *Aphis gossypii*, and Aphis citricola at spore concentrations between 10^6 and 10^7 spores/mL over four days of testing. Saranya et al. (2010) found that *Verticillium lecanii* and *Hirsutella thompsonii* achieved 100% mortality at 10^8 spores/mL, while *Beauveria bassiana* and *Metarhizium anisopliae* showed comparatively lower mortality rates. Smitha (2007) observed that as the fungal solution was diluted, mortality rates decreased. Additionally, the seventh-day infection of *Anagasta craccivora* resulted in 91% and 93% mortality rates, a trend also observed in this study. *Beauveria bassiana* spore concentration at 1.2×10^4 spores/mL and *Metarhizium anisopliae* at 2.45×10^6 spores/mL were examined by Chandler et al. (2005). Dogan et al. (2017) reported that *Metarhizium brunneum* (strains ARSEF 4556 and V275), *Lecanicillium lecanii* (strain UPH-0241), and *Beauveria bassiana* (strain UPH-1103) successfully reduced the population of two-spotted spider mites by 57.3% to 90.3% when exposed to a concentration of 1×10^7 conidia/mL under controlled conditions in Petri dishes.

The results of this study confirmed the significant pathogenic effects of the entomopathogenic fungi, as evidenced by the low LC_{50} values observed. However, a decrease in fungal spore concentration resulted in lower mortality rates. Previous studies have also reported low LC_{50} values for entomopathogenic fungi, such as 1.2×10^4 spores/mL for Brevicoryne brassicae and 2.7×10^4 spores/mL for *Aphis gossypii*. Among the tested fungal isolates, *Verticillium lecanii* and *Hirsutella thompsonii* exhibited higher virulence, achieving an LC_{50} of 2.5×10^4 spores. Similar findings were reported by researchers who recorded an LT_{50} of 3.17 days for *Beauveria bassiana*. Additionally, Saranya et al. (2010) found that *V. lecanii* induced mortality in Aphis fabae populations on bean crops within approximately 3.31 days.

Combining three types of entomopathogenic fungi with neem oil resulted in the highest mortality rates against *T. urticae*, A. gossypii, and *B. tabaci*. Azadirachtin in neem oil inhibits molting and reduces feeding, facilitating fungal mycelium penetration into the insect's chitin layer. The effectiveness of this combination is influenced by the chemical composition of neem oil, including phytoalexins, terpenoids, and triterpenoids, which affect the compatibility of EPFs. Ribeiro et al. (2012) noted that these changes can impact insect-pathogenic fungi, while Mohan et al. (2007) highlighted the role of genetic variability in fungal isolates' compatibility with plant protection products. A study demonstrated that a combination of *Verticillium lecanii*, *Beauveria bassiana*, and Azadirachtin reduced A. gossypii populations by 30% to 70%. Oil-based formulations of *B. bassiana* and *Metarhizium anisopliae* achieved a 96.67% mortality rate in leafhoppers, and *V. lecanii* caused mortality rates of 96.77% in aphids and 97% in thrips by day 10. Additionally, experiments showed no significant inhibition of *B. bassiana* growth when cultured with 5% neem seed extract.

Farmers increasingly use EPF as a sustainable pest management approach in agriculture, providing an effective alternative to chemical pesticides. As natural organisms, EPF offer excellent insect pest control, contributing to food security and supporting sustainable agricultural practices. IPM benefits greatly from EPF, offering a natural solution to concerns about the adverse effects of synthetic pesticides on human health, biodiversity, and ecosystem balance. EPF are effective biological control agents as they naturally infect and eliminate pests. Liu, Smagghe, & Liu (2023) conducted a study that highlighted the importance of EPF-host molecular interactions, focusing on glycans as crucial components for fungal adhesion and infection. Understanding the biochemical processes involved is essential for developing targeted biocontrol methods that yield effective results. By studying the biological complexity of these interactions, researchers can create more efficient EPF-based biopesticides. The knowledge gained from this study supports the development of precision agriculture and enhances the understanding of pathogen-host dynamics in pest management.

Trinh, Ha, & Qiu (2020) evaluated various EPF strains in laboratory conditions to assess their potential for controlling Megoura japonica bean aphids. They found fungal isolates that effectively eliminated aphid populations, highlighting the importance of native fungal strains for pest control. This research demonstrated the efficiency of EPF in developing pest management systems for aphid outbreaks in legume plants and other crops. Sani et al. (2020) investigated biological control for Bemisia tabaci, aligning with Trinh et al.'s findings. Their study showed that EPF is a valuable pest management tool, working well in various agricultural systems and in combination with other biocontrol agents like parasites and predators. They also explored different EPF formulation strategies and application methods, highlighting their role in eco-friendly pest management.

Aravinthraju et al. (2024) built on previous studies by examining how endophytic EPF enhances plant resistance to pests. The research revealed that EPF colonises plant tissues, triggering defensive mechanisms against stress. This approach can be used in pest and disease management, combining endophytic plants with fast-fluorescing bacteria. Mantzoukas et al. (2022) investigated EPF's pest control abilities across agricultural systems. Their research confirmed that EPF functions through two mechanisms: pathogen infection and ecological changes, affecting soil microbial communities, plant growth, and pest behaviour. Further interdisciplinary research and funding are needed to fully integrate EPF as a sustainable pest management solution. Halder et al. (2021) studied the use of EPF combined with botanical extracts for managing sucking pests on okra in outdoor conditions. They found that organic farming and integrated pest management are most effective when their components are well-matched, prioritising ecosystem health. Their data supports the development of integrated biological and botanical pest control products for sustainable crop protection.

Aquino-Bolaños et al. (2023) demonstrated that EPF in oil-based suspensions could effectively control the agave weevil, *Scyphophorus acupunctatus*. Laboratory tests showed that oil preparations helped fungi survive and attach to insect exoskeletons, increasing the likelihood of fungal infection. These findings are significant for agave-dependent regions, highlighting the potential of EPF as a sustainable, environmentally friendly pest control method. Du, Xia, & Jin (2022) used biotechnological methods to enhance EPF strains by modifying key transcription factor genes, including MaSom1. This led to increased virulence, conidial production, and environmental tolerance. Their research shows how genetic engineering can improve EPF as an effective biopesticide, while emphasising the need for proper regulation and risk assessment for genetically modified organisms in agriculture. Sharma, Sharma, & Yadav (2023) reviewed the role of EPF in sustainable agriculture, highlighting their benefits in pest management, pesticide reduction, soil quality improvement, and biodiversity protection. They suggest that EPF fungi should be incorporated into national and international agricultural strategies to address current ecological challenges. Their findings provide valuable resources for policymakers, researchers, and practitioners aiming to promote environmentally friendly agricultural systems.

Ebani & Mancianti (2021) expanded their investigations into the veterinary domain, demonstrating the effectiveness of EPF in managing livestock parasites, such as flies and mites, when combined with beneficial bacteria. This combined approach underscores the versatility of EPF, positioning them as key elements in integrated health systems for both agricultural and animal husbandry practices, thereby providing novel opportunities for the development of sustainable biocontrol methods. In a separate study, Kutalmış et al. (2023) examined the phylogenetic relationships among various EPF species to evaluate their effectiveness in controlling the pest *Tropinota hirta* (syn. *Epicometis hirta*) and the fungal pathogen *Venturia inaequalis*, which causes apple scab. Their findings highlighted the importance of understanding evolutionary connections when selecting the most suitable biocontrol agents. This research illustrates how phylogenetic studies can aid in making informed decisions about pest management, leading to the creation of more efficient and ecologically responsible biocontrol strategies. The body of scientific literature surrounding EPF continues to expand, reinforcing their potential as eco-friendly alternatives to chemical pesticides. The critical role of EPF in shaping the future of global agriculture is becoming increasingly apparent, offering targeted solutions to pest management issues. Ongoing advancements in research on EPF's biology, ecological interactions, and application techniques will be instrumental in realising their full potential within sustainable agricultural frameworks worldwide.

Conclusion

In conclusion, this study highlighted the effectiveness of neem oil and EPF in controlling TSSM, aphids, and whiteflies, particularly when applied at high concentrations. When used in combination at lower concentrations, these agents demonstrated notable potential in managing the populations of these pests. However, further investigation into optimal application rates and field spraying timings is essential to maximise the synergistic effects of these treatments for future practical applications.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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