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ORIGINAL RESEARCH ARTICLE

Microbial biorationals as an effective alternative to pesticides for control of two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) on parthenocarpic cucumber grown under protected conditions

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Abstract

The use of chemical pesticides to control two spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae) in various crops has resulted in the development of various problems viz., residues in sprayed crop, pest resistance, pest resurgence and environmental pollution. For an effective alternate environmentally safe management tactic, the microbial biorationals constitute an important tool. In the present study, entomopathogenic fungi (EPF) and botanical pesticides were tested at different concentrations for the management of eggs and other active stages of *T. urticae* infesting parthenocarpic cucumber grown under poly-net house. Among these biorationals, spraying of Neem Baan at 10 and 15 ml/l resulted in significantly higher level of reduction in TSSM population (88.5 to 91.8% in egg and 86.6 to 90.9% in active stage of mites after 3rd spray, respectively) than with EPFs. Among the EPFs, *Lecanicillium lecanii* (Zimmerman) Viegas and *Beauveria bassiana* Balsamo (Vuillemin) [(at 10 & 15 ml/l (82.0 to 87.0% in egg and 79.2 to 84.1% in active stage of mites)] was found to be more effective than *Metarhizium anisopliae* (Metschnikoff) Sorokin [(at 10 & 15 ml/l (77.0 to 82.4% in egg and 76.4 to 80.4% in active stage of mites after third spray, respectively)] in reducing different life stages of *T. urticae* on cucumber. Significantly higher yields of cucumber were recorded from plot treated with Neem Baan at 10 and 15 ml/l (2350.7 to 2437.3 g/ plant) were used. As Neem Baan 1% increased growth and yield of cucumber by controlling *T. urticae*, effectively, it can act as a good alternative for the chemical pesticide which in most instances leads to residues, pest resistance and pest resurgence problems.

Keywords Botanical pesticide · Entomopathogenic fungi · Parthenocarpic cucumber · Protected condition · *Tetranychus urticae*

Introduction

Protected cultivation offers a new dimension for achieving higher productivity of high value vegetables within a limited area (Singh and Kaur 2020). It is a cultivation technology with human interventions that creates a favourable micro-climate around the plants for their sustained growth and development, offsetting the detrimental effects of the abiotic and biotic factors. It holds great potential in enhancing crop diversification, off-season, and year-round production of vegetables at remunerative prices (Sharma 2020). High value vegetable crops like tomato, cherry tomato, coloured capsicum, parthenocarpic cucumber, French beans (pole type), winter watermelon, muskmelon and strawberries can be grown successfully under protected cultivation (Singh et al. 2015). Parthenocarpic cucumber (*Cucumis sativus* L.) is one of the most widely grown vegetable crop in the world under protected environment generating high remunerative prices and profits during off-season (Senthilkumar et al. 2018; Ghongade and Sood 2019; Thakur and Sood 2022). The crop is grown for its tender fruits which have high water content and are consumed raw as salad (Sumathi et al. 2008; Ghongade et al. 2021). It is a rich source of vitamins B and C, carbohydrates, and minerals like calcium and phosphorus (Nagamani et al. 2019).

Parthenocarpic cucumber grown in protected environment is vulnerable to a diversity of insect pests. Amongst them, two spotted spider mite (TSSM), *Tetranychus urticae*

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Koch (Acari: Tetranychidae) is a key pest of greenhouse cucumber (Ghongade and Sangha 2020; Ghongade and Sood 2021). This is an economically important mite species that infests more than 900 different plant hosts worldwide and causes significant damage to at least 150 of them ((Martinez-villar et al. 2005; Aghamohammadi et al. 2016; Ghongade and Sood 2018). Under poly-net and net houses in India, the TSSM causes major losses causing significant decline in crop productivity (Ghongade and Sood 2021; Ghongade et al. 2022). TSSM is a highly polyphagous, cosmopolitan species and its damage includes appearance of characteristic yellowish-white chlorotic spots on leaves and webbing, affecting photosynthesis and adversely affecting the quality and quantitative yield of the crop (Park and Lee 2002; Aghamohammadi et al. 2016; Thakur and Sood 2022).

Presently, management tactic for TSSM control involves the use of chemical pesticides (Kumral et al. 2010). The TSSM congregates on the abaxial surface of the leaves that minimizes its contact with the insecticides thereby requiring frequent insecticidal applications for its control. The indiscriminate use of insecticides has led to the development of resistance in *T. urticae* to several classes of insecticides such as organophosphates, carbamates, synthetic pyrethroids, neonicotinoids and more recently ketoenols (IRAC 2022). Besides this, the intensive use of insecticides has detrimental effects on non-target organisms and food safety, thereby necessitating the reliance on alternative eco-friendly measures (Roy and Mukhopadhyay 2012; Titiksha 2019).

In recent years, biorational agents such as entomopathogenic fungi and botanical pesticides are widely used in integrated pest control (Bhullar et al. 2021; Thakur and Sood 2022). *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* (Zimmerman) (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae (Metsch.)* (Hypocreales: Clavicipitaceae), has been identified as pathogens of several mites' species (Faria and Wraight 2007; Nag et al. 2020) and is highly effective in reducing mites' populations. Numerous studies have been conducted to determine the possible use of EPFs in biological control of mites (Chandler et al. 2005; Bhullar et al. 2021). Plant extracts and botanical pesticides have showed great importance in agricultural fields due to their low cost and high toxicity against major pests such as thrips, aphids, jassids, whiteflies and mites in addition to being environmentally safe having no residual effects (Stumpf and Nauen 2001; Singh and Kaur 2020; Ghongade and Sangha 2023). Neem oils and extracts are considered as the best option for the insect pest management programs in vegetables because they are safe to beneficial organisms, are target pest specific and compatible with biological control agents (Tang et al. 2002; Ghongade and Sangha 2021). *Azadirachta indica* (Meliaceae) rich in azadirachtin has excellent insecticidal properties due to the presence of limonoids, which interfere with various physiological processes and chemical pathways thereby offering feeding deterrence and toxic effects to the insect-pests (Dougoud et al. 2019 and Bhullar et al. 2021).

Keeping in view the consumer health, environmental sustainability of biorationals, commercial value of parthenocarpic cucumber and the importance of TSSM as a key pest of cucumber, the present study was designed to evaluate the effectiveness of different biorationals for the management of two spotted spider mite, *T. urticae,* on parthenocarpic cucumber grown under protected conditions.

Materials and methods

Growing of crop

A parthenocarpic variety of cucumber '*F_1 hybrid Punjab Kheera-1*' (parentage: selection of a single plant from segregated material collected from the farmer's field) developed by Department of Vegetable Science, Punjab Agricultural University, Ludhiana was selected for cultivation under poly-net house conditions. The crop was grown at experiment farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana, Punjab, India in a naturally ventilated double door poly-net house having basic structure made of galvanized iron pipes covered with ultra violet (UV) stabilized plastic film (200-micron thickness) at the top and UV stabilized net of 40-mesh size on the sides. The poly-net house was 15 m × 7 m (105 m^2) with an arc-shaped roof. The maximum height of the poly-net house in the middle was 3 m and minimum height was 2 m at the sides. The poly-net house was constructed in the East–West direction to get the maximum benefit of the sunlight throughout the year and to minimize the adverse effects of wind.

During 2017 and 2018, the crop was transplanted in the last week of February and the first picking (harvest) was done in the month of April. Parthenocarpic cucumber seedlings (thirty days old, having 2–3 true leaves and 8–10 cm plant height) were transplanted on raised beds (90 cm width, 15 cm height) with spacing of 70 cm × 30 cm (row x plant) ($Temp_{Max}$ = 43.7 °C; $Temp_{Min}$ = 10.9 °C; RH_{Max} = 92.0%; RH_{Min} = 18%). Seedlings were maintained as per the Package of Practices for Vegetables Crops (Anonymous 2022). No insecticides/fungicides were used for the production of cucumber under naturally ventilated poly-net house. Cucumber plants were trained as single shoots using bamboos/wooden rods supported by nylon ropes. The extra shoots if any were pruned regularly to optimize the growth of plants. The irrigation was given at weekly intervals starting on the first day after transplanting (DAT). NPK was administered through fertigation.

Collection and rearing of two spotted spider mite

Potted French bean plants were used as host for rearing of *T. urticae*. These plants were grown in soil-less media comprising coco peat and vermicompost in the ratio of 1:1. Stock culture of TSSM was maintained at room temperature. Active stages of TSSM were initially collected from mite infested cucumber plants grown in nearby poly-net houses by use of a camel hair brush and released on potted French bean plants. The identity of the TSSM species was confirmed prior to starting of the cultures. Older and heavily infested plants were periodically replaced with fresh plants throughout the study period.

Preparation of biorationals

Biorational molecules belonging to different groups and exhibiting different mode of action were chosen for managing *T. urticae* under poly-net house conditions (Table 1).

Experiment protocol

The experiment was laid out in a randomized block design (RBD) with each concentration of biorational as one treatment and 3 replications/ treatment were kept. Apart from these biorational treatments like Neem Baan (1% W/W) (Azadirachtin 10,000 ppm), *Beauveria bassiana* 1.50% [1×10^9 CFU/ml], *Lecanicillium lecanii* 1.50% [1×10^9 CFU/ml], *Metarhizium anisopliae* 1.5% [1×10^8 CFU/ml] (2 dosages each at 10 ml and 15 ml/l), and one chemical, Dimethoate 30 EC (one dosage at 2 ml/l) and control check (no spray) was also maintained for comparison.

There were 10 plants per plot in each biorational treatment, separated by distance of 120 cm between 2 plots to avoid chemical interference owing to drift to adjacent treatments while spraying. Large numbers of TSSM adults were released with the help of camel hair brush at 35 days after transplanting (DAT) of cucumber in the poly-net house for early infestations. For foliar applications of the different biorationals, a battery-operated knapsack sprayer fitted with hollow cone nozzle (40 PSI pressure) was used. All the safety measures for the applicator like wearing gloves, goggles and masks at the time of application were followed. The biorationals were applied late in the afternoon hours.

Data collection

There were 3 foliar sprays for all treatments. The first treatment applications (sprays) were given 55 and 60 days after transplanting (DAT) in 2017 and 2018, respectively, and repeated at 11 days intervals. The data of *T. urticae* (eggs and active stages) in different treatments were recorded before the spray and 10 days after each spray. The leaves with eggs and active stages of mites were plucked and brought to the laboratory. Five leaves were plucked randomly per treatment and the number of different stages of TSSM was recorded from 3 spots/leaf under a stereo zoom binocular microscope. Percent reduction in the population of TSSM (eggs and active stages) over control was calculated, using Henderson and Tilton's formula (Henderson and Tiltons 1955). Before analysis, corrected mortality was calculated using the formula.

$$\text{Corrected Mortality \%} = \left[1 - \frac{\text{n in Co before treatment} * \text{n in T after treatment}}{\text{n in Co after treatment} * \text{n in T before treatment}}\right] * 100$$

where, n: mite population, T: treated, Co: control.

Marketable fruit yield

Mature cucumber fruits having attained marketable size (about 15 cm long) were harvested at regular (5 days) intervals and observations were recorded on fresh fruit weight per plant at each picking for each treatment. The fruit weight obtained at each picking was aggregated to obtain in cumulative fruit yield per plant during 2017 and 2018.

Table 1 Biorationals evaluated against *Tetranychus urticae* on poly-net house grown parthenocarpic cucumber

Treatment	Concentrations (% or ml/l)	Brand name	Formulator
Neem Baan (1% W/W) (Azadirachtin 10,000 ppm)	10 ml and 15 ml/l	Nimbecidine	T. Stanes and Company Limited
Beauveria bassiana 1.50% [1×10^9 CFU/ml]	10 ml and 15 ml/l	Bio-Power	T. Stanes and Company Limited
Lecanicillium lecanii 1.50% [1×10^9 CFU/ml]	10 ml and 15 ml/l	Bio-Catch	T. Stanes and Company Limited
Metarhizium anisopliae 1.5% [1×10^8 CFU/ml]	10 ml and 15 ml/l	Bio-Magic	T. Stanes and Company Limited
Dimethoate 30 EC	2 ml/l	Rogor	Gujarat Pesti-chem Industries
Control (No spray)	-	-	-

Statistical analysis

The mean population of the TSSM (eggs and active stages) and fruit weight ($\bar{x} \pm SE$) were subjected to analysis of variance (ANOVA). One-way ANOVA was conducted for all parameters, and mean values were compared by Tukey's-b test using the SPSS software (SPSS 2015), and treatment means were interpreted at a 5% level of significance.

Results

Efficacy of biorationals against eggs of *T. urticae*

In 2017, the pre-treatment population of the eggs of two spotted spider mite ranged from 70.9 to 74.7 eggs per five leaves and the variability was non-significant in different biorational treatments before the application of the treatments. All applied treatments demonstrated a statistically significant superiority over the untreated control in reducing the population of *T. urticae* eggs after the 1st, 2nd, and 3rd spray intervals. The highest percent reduction over control (PROC) after the 3rd spray was recorded in Dimethoate 30 EC at 2.0 ml/l (97.6%). However, it was statistically at par with Neem Baan at 15 and 10 ml/l (91.6% and 89.1% PROC), *L. lecanii* at 15 and 10 ml/l (84.6% and 80.9% PROC), *B. bassiana* at 15 and 10 ml/l (83.4% and 82.0% PROC) and *M. anisopliae* at 15 ml/l (79.3% PROC), respectively. Among all the biorational treatments *M. anisopilae* at 10 ml/l was found less effective in reducing eggs (77.0% reduction) of *T. urticae* (Table 2).

In 2018, the variation in the pre-treatment population on eggs of *T. urticae* in different treatments was statistically non-significant, indicating that the population was uniformly distributed in the poly-net house before the different treatments were imposed. The mean population of *T. urticae*, 1 day before imposing the treatments, was from 69.9 to 73.7 eggs per five leaves. The data recorded after the 3rd spray revealed that percent reduction of eggs ranged from 80.3 to 98.3% in different treatments (Table 3). All the biorational treatments were found significantly superior over control in reducing the percentage of eggs. Dimethoate at 2.0 ml/l (98.3% PROC) was found most effective in reducing the population of eggs, followed by Neem Baan at 15 and 10 ml/l (91.8 and 88.5% PROC), *L. lecanii* at 15 and 10 ml/l (87.0% and 84.4% PROC) and *B. bassiana* at 15 ml/l (86.4% PROC), which was statistically at par with each other. However, the treatments *B. bassiana* (at 10 ml/l (83.5% PROC)) and *M. anisopliae* (at 15 and 10 ml/l (82.4 and 80.3% PROC)) were found statistically at par amongst each other though significantly poorer than Dimethoate at 2.0 ml/l, Neem Baan at 15 and 10 ml/l, *L. lecanii* at 15 and 10 ml/l and *B. bassiana* at 15 ml/l, respectively (Table 3).

Efficacy of biorationals against active stages of *T. urticae*

In 2017, the mean population of *T. urticae* (active stages) on the cucumber leaves before application of the treatments ranged from 81.0 to 85.1 active stages per five leaves and the data on mean *T. urticae* incidence one day before spray did

Table 2 Evaluation of different biorationals against eggs of *Tetranychus urticae* on poly-net house grown parthenocarpic cucumber (2017)

Treatment	Concentrations (ml/l)	Per cent reduction in eggs of *T. urticae* per five leaves *(mean ± SE) after different sprays			
		1st Spray	2nd Spray	3rd Spray	Overall reduction (%)
Beauvaria bassiana (1 × 10^9 CFU/ml)	10	42.7 ± 1.8^{bcd}	59.7 ± 2.7^{b}	82.0 ± 3.9^{ab}	61.5 ± 11.4^{a}
	15	44.5 ± 2.4^{bcd}	61.0 ± 5.2^{b}	83.4 ± 4.6^{ab}	63.0 ± 11.3^{a}
Lecanicillium lecanii (1 × 10^9 CFU/ml)	10	43.5 ± 5.0^{bcd}	60.9 ± 2.4^{b}	80.9 ± 3.6^{ab}	61.8 ± 10.8^{a}
	15	45.8 ± 4.5^{bcd}	62.8 ± 3.1^{b}	84.6 ± 3.7^{ab}	64.4 ± 11.3^{a}
Metarhizium anisopilae (1 × 10^8 CFU/ml)	10	36.8 ± 2.0^{d}	58.3 ± 6.9^{b}	77.0 ± 6.9^{b}	57.4 ± 11.6^{a}
	15	38.0 ± 4.0^{cd}	59.4 ± 4.0^{b}	79.3 ± 4.4^{ab}	58.9 ± 11.9^{a}
Neem Baan (1% W/W)	10	54.2 ± 2.9^{abc}	69.3 ± 2.8^{b}	89.1 ± 2.5^{ab}	70.9 ± 10.1^{a}
	15	56.2 ± 2.8^{ab}	71.1 ± 0.5^{b}	91.6 ± 1.7^{ab}	73.0 ± 10.3^{a}
Dimethoate 30 EC	2.0	68.3 ± 4.1^{a}	87.4 ± 1.3^{a}	97.6 ± 1.1^{a}	84.4 ± 8.6^{a}
Control	-	15.8	24.3	24.9	21.7
F values		8.5	6.3	2.8	0.6
P		< 0.001	0.001	0.036	0.747

*Means ± standard error followed by the same letter within each column are not significantly different from other treatments according to Tukey's-b Test where p ≤ 0.05

Table 3 Evaluation of different biorationals against eggs of *Tetranychus urticae* on poly-net house grown parthenocarpic cucumber (2018)

Treatment	Concentrations (ml/l)	Per cent reduction in eggs of *T. urticae* per five leaves *(mean ± SE) after different sprays			
		1st Spray	2nd Spray	3rd Spray	Overall reduction (%)
Beauvaria bassiana (1 × 10⁹ CFU/ml)	10	42.5 ± 2.3[bcd]	60.4 ± 9.8[a]	83.5 ± 4.1[b]	62.1 ± 11.9[a]
	15	45.6 ± 1.6[bcd]	63.4 ± 6.9[a]	86.4 ± 2.6[ab]	65.1 ± 11.8[a]
Lecanicillium lecanii (1 × 10⁹ CFU/ml)	10	42.4 ± 2.2[bcd]	60.8 ± 8.7[a]	84.4 ± 2.8[ab]	62.5 ± 12.2[a]
	15	46.4 ± 1.7[bcd]	61.6 ± 1.5[a]	87.0 ± 1.2[ab]	65.0 ± 11.8[a]
Metarhizium anisopilae (1 × 10⁸ CFU/ml)	10	37.6 ± 0.5[d]	59.0 ± 9.0[a]	80.3 ± 4.4[b]	59.0 ± 12.3[a]
	15	40.8 ± 3.1 [cd]	60.9 ± 5.3[a]	82.4 ± 4.6[b]	61.4 ± 12.0[a]
Neem Baan (1% W/W)	10	53.2 ± 2.8[bc]	70.4 ± 3.9[a]	88.5 ± 2.9[ab]	70.7 ± 10.2[a]
	15	55.7 ± 3.8[b]	71.6 ± 4.5[a]	91.8 ± 0.6[ab]	73.0 ± 10.4[a]
Dimethoate 30 EC	2.0	68.8 ± 5.2[a]	87.9 ± 3.1[a]	98.3 ± 0.9[a]	85.0 ± 8.6[a]
Control	-	18.5	30.3	25.6	24.8
F values		11.3	2.1	3.3	0.5
P		< 0.001	0.097	0.018	0.834

*Means ± standard error followed by the same letter within each column are not significantly different from other treatments according to Tukey's-b Test where p ≤ 0.05

not differ significantly among treatments. The percent reduction over control (PROC), progressively increased after subsequent sprays during the season. After the 3rd spray, among the different treatments, Dimethoate at 2.0 ml/l gave maximum reduction in active stages of *T. urticae* (97.1%) and it was statistically at par with Neem Baan at 15 and 10 ml/l (89.0 and 86.5% reduction), *L. lecanii* at 15 ml/l (84.1% reduction), and *B. bassiana* at 15 ml/l (83.4% reduction), respectively. The lowest percent reduction was recorded in the treatment of *M. anisopliae* at 10 ml/l (76.4% reduction) and it was also statistically at par with *M. anisopliae* at 15 ml/l (79.4% reduction), *L. lecanii* at 10 ml/l (80.3% reduction) and *B. bassiana* at 10 ml/l (79.2% reduction), respectively (Table 4).

In 2018, the mean TSSM active stage population did not vary significantly (79.3 to 80.5 active stages per five leaves) at 1 day before spraying (DBS) during 2018 season, indicating uniform distribution of the pest. After 3rd spray, significantly most effective treatment was Dimethoate at 2.0 ml/l causing 98.2 percent reduction in active stages. It was statistically at par with Neem Baan at 15 ml/l (90.9% reduction) and 10 ml/l (88.4% reduction) but both of these treatments were also found to be statistically at par with rest of the treatments viz*.*, *B. bassiana* at 15 and 10 ml/l (84.3 and 80.2%), *L. lecanii* at 15 and 10 ml/l (83.8 and 79.7%) and *M. anisopliae* at 15 and 10 ml/l (80.3 and 77.8%), respectively (Table 5).

The pooled analysis of the 3 sprays for overall reduction of *T. urticae* egg and active stages revealed that among all the treatments, Dimethoate at 2.0 ml/l gave maximum reduction in egg (84.4% and 85.0% PROC in 2017 and 2018) and active stages (84.6% and 84.2% PROC in 2017 and 2018) of *T. urticae* and it was statistically at par with all other biorational treatments (Tables 2, 3, 4 and 5), respectively.

Effect on marketable fruit yield

In 2017, plants sprayed with Dimethoate at 2.0 ml/l resulted in the highest fruit yield (2453.3 g per plant), followed by Neem Baan (at 15 ml/l (2437.3 g per plant) which was at par with each other. Whereas, yield recorded in *L. lecanii* (at 15 ml/l (2358.7 g per plant) and *B. bassiana* (at 15 ml/l (2345.7 g per plant) was at par with each other though significantly lower than Dimethoate and Neem Baan. Least fruit yield was recorded in *M. anisopilae* at 10 ml/l (2283.4 g per plant). All treatments had significantly higher fruit yields when compared to control (no spray) (Table 6).

In 2018, the overall absolute fruit yields have slightly lower than 2017. Dimethoate (at 2.0 ml/l) and Neem Baan (at 15 ml/l) again recorded the maximum fruit yields of 2398.8 and 2397.9 g per plant, respectively. These yields were significantly superior to the rest of the treatments. Minimum fruit yield was recorded in the plants sprayed with *M. anisopilae* (at 10 and 15 ml/l (2222.9 and 2252.1 g per plant) followed by plant sprayed with *B. bassiana* (at 10 ml/l (2264.6 g per plant). All other treatments significantly increased the marketable fruit yields per plant after 60 days of transplanting as compared to untreated control (Table 6).

Table 4 Evaluation of different biorationals against active stages of *Tetranychus urticae* on poly-net house grown parthenocarpic cucumber (2017)

Treatment	Concentrations (ml/l)	Per cent reduction in active stages of *T. urticae* per five leaves *(mean ± SE) after different sprays			
		1st Spray	2nd Spray	3rd Spray	Overall reduction (%)
Beauvaria bassiana (1 × 10^9 CFU/ml)	10	42.6 ± 13.1^{b}	61.5 ± 2.1^{b}	79.2 ± 4.0^{b}	61.1 ± 10.6^{a}
	15	46.1 ± 5.2^{b}	63.7 ± 10.8^{ab}	83.4 ± 6.8^{ab}	64.4 ± 10.8^{a}
Lecanicillium lecanii (1 × 10^9 CFU/ml)	10	43.4 ± 3.8^{b}	60.9 ± 8.3^{b}	80.3 ± 4.1^{b}	61.5 ± 10.7^{a}
	15	45.1 ± 7.6^{b}	62.2 ± 7.4^{b}	84.1 ± 3.4^{ab}	63.8 ± 11.3^{a}
Metarhizium anisopilae (1 × 10^8 CFU/ml)	10	38.4 ± 8.7^{b}	57.7 ± 5.5^{b}	76.4 ± 5.5^{b}	57.5 ± 11.0^{a}
	15	40.3 ± 7.8^{b}	59.6 ± 7.5^{b}	79.4 ± 5.1^{b}	59.8 ± 11.3^{a}
Neem Baan (1% W/W)	10	48.3 ± 2.5^{ab}	65.6 ± 8.4^{ab}	86.6 ± 5.1^{ab}	66.8 ± 11.1^{a}
	15	51.7 ± 0.8^{ab}	68.2 ± 8.0^{ab}	89.0 ± 3.0^{ab}	69.6 ± 10.8^{a}
Dimethoate 30 EC	2.0	70.0 ± 6.3^{a}	86.6 ± 2.9^{a}	97.1 ± 1.6^{a}	84.6 ± 7.9^{a}
Control	-	12.6	19.3	18.9	16.9
F values		1.8	1.4	1.9	0.6
P		0.154	0.257	0.116	0.787

*Means ± standard error followed by the same letter within each column are not significantly different from other treatments according to Tukey's-b Test where $p \leq 0.05$

Discussion

The use of pesticides in combating *T. urticae* has several limitations such as, residue problems, resistance development in the mites and high cost of pesticide sprays. Various biorationals (neem products and EPFs) have been found to be effective in the control of TSSM in this study. Neem Baan, *L. lecanii, B. bassiana* and *M. anisopilae* can all be helpful in addressing the various issues related to chemical management of TSSM. Azadirachtin is the chief constituent of Neem Baan 1% (10,000 ppm) found highly effective in the management of *T. urticae.* Azadirachtin exhibits high level of miticidal activities such as oviposition and fecundity deterrence, growth inhibition and antifeedant action (Sharma et al. 2014; Bezzar-Bendjazia et al. 2017). Azadirachtin has the capacity to modify or inhibit the juvenile and moulting hormone titres by acting upon the synthesis and release of allatrotropins and prothoracic hormone from the *corpus*

Table 5 Evaluation of different biorationals against active stages of *Tetranychus urticae* on poly-net house grown parthenocarpic cucumber (2018)

Treatment	Concentrations (ml/l)	Per cent reduction in active stages of *T. urticae* per five leaves *(mean ± SE) after different sprays			
		1st Spray	2nd Spray	3rd Spray	Overall reduction (%)
Beauvaria bassiana (1 × 10^9 CFU/ml)	10	41.3 ± 4.6^{b}	60.8 ± 6.8^{b}	80.2 ± 4.5^{b}	60.8 ± 11.2^{a}
	15	44.6 ± 8.3^{b}	62.1 ± 7.7^{ab}	84.3 ± 5.7^{ab}	63.7 ± 11.5^{a}
Lecanicillium lecanii (1 × 10^9 CFU/ml)	10	41.7 ± 3.2^{b}	60.9 ± 7.9^{b}	79.7 ± 5.6^{b}	60.8 ± 11.0^{a}
	15	45.4 ± 4.1^{b}	64.5 ± 7.0^{ab}	83.8 ± 5.5^{ab}	64.6 ± 11.1^{a}
Metarhizium anisopilae (1 × 10^8 CFU/ml)	10	37.2 ± 7.5^{b}	56.5 ± 6.2^{b}	77.8 ± 3.7^{b}	57.2 ± 11.7^{a}
	15	40.8 ± 8.1^{b}	58.8 ± 12.0^{b}	80.3 ± 6.6^{b}	60.0 ± 11.4^{a}
Neem Baan (1% W/W)	10	50.2 ± 3.5^{ab}	69.6 ± 6.5^{ab}	88.4 ± 3.1^{ab}	69.4 ± 11.0^{a}
	15	52.3 ± 2.5^{ab}	71.2 ± 5.1^{ab}	90.9 ± 2.1^{ab}	71.5 ± 11.1^{a}
Dimethoate 30 EC	2.0	66.9 ± 2.8^{a}	87.5 ± 2.7^{a}	98.2 ± 1.1^{a}	84.2 ± 9.2^{a}
Control	-	13.4	23.4	21.6	19.5
F values		2.7	1.7	2.1	0.6
P		0.038	0.171	0.093	0.796

*Means ± standard error followed by the same letter within each column are not significantly different from other treatments according to Tukey's-b Test where $p \leq 0.05$

Table 6 Marketable yield in different biorational treatments in parthenocarpic cucumber

Treatment	Concentrations (ml/l)	Yield (g/plant ± SE)	
		2017	2018
Beauvaria bassiana (1×10^9 CFU/ml)	10	2318.8 ± 2.9^{d}	2264.6 ± 3.1^{ef}
	15	2345.7 ± 4.3^{c}	2306.9 ± 3.2^{d}
Lecanicillium lecanii (1×10^9 CFU/ml)	10	2322.9 ± 3.9^{d}	2272.6 ± 4.4^{e}
	15	2358.7 ± 5.5^{c}	2329.8 ± 5.5^{c}
Metarhizium anisopilae (1×10^8 CFU/ml	10	2283.4 ± 5.0^{e}	2222.9 ± 2.1^{g}
	15	2308.7 ± 3.8^{d}	2252.1 ± 3.4^{f}
Neem Baan (1% W/W)	10	2400.7 ± 7.1^{b}	2350.7 ± 5.1^{b}
	15	2437.3 ± 5.5^{a}	2397.9 ± 3.3^{a}
Dimethoate 30 EC	2.0	2453.3 ± 3.2^{a}	2398.8 ± 4.2^{a}
Control (Untreated)	-	1925.6 ± 5.7^{f}	1906.3 ± 3.6^{h}
F values		933.7	1302.6
P		<0.001	<0.001

Yield ± standard error followed by the same letter within each column are not significantly different from other treatments according to Tukey's-b Test where $p\leq0.05$

cardiacum (Bezzar-Bendjazia et al. 2017). Azadirachtin has been reported to acts as a growth regulator, thereby modifying metamorphosis process (Thakur and Sood 2017). Similar to present study Dimetry et al. (2012) also found Nimbecidine 0.03% (Azadirachtin 300 ppm) was effective in the reduction of *T. urticae* population compared to untreated control in cucumber under plastic house conditions followed by Bio-Catch (*L. lecanii* spores at 1×10^9) in alternation, with four successive sprays. Singh et al. (2018) observed that the application of Neem oil at 4 percent (4 ml/l) caused 58 percent reduction in active stages of mite population after seven days of first spray. This is in concurrence with our study, wherein, Neem Baan 1% (10,000 ppm) was recorded to be effective against *T. urticae* on cucumber in poly-net house conditions. In addition to controlling pest occurrence in cucumber, the plant growth stimulant effect of Neem Baan improved the growth and yield of the protected plants. Similarly, Thakur and Sood (2022) are also supportive to present investigations as they also observed the efficacy of Neem Baan on cucumber plants and significantly increased the marketable crop yield. Besides cucurbits, neem sprays resulted in increased yields of many other crops (Nag et al. 2020; Bhullar et al. 2021).

EPF foliar sprays resulted in 36 to 87% reduction of eggs, and active stages of *T. urticae* infesting cucumber under poly-net house conditions in this study. For effective management of TSSM, 3 sprays of *L. lecanii* and *B. bassiana* (at 15 ml/l) at 10-day intervals, starting at 35 days after transplanting of cucumber were appropriate. Several studies have exhibited the utility of *B. bassiana, M. anisopliae*, *L. lecanii, Metarhizium brunneum* (Metschnikoff), *Metarhizium flavoviride* Gams and Rozsypal, *Isaria cateniannulata* (ZQ Liang), and *Neozygites floridana* (Weiser and Muma) as entomopathogens for the control of mites in poly-net house as well as field crops. Similar to the present results, earlier Wekesa et al. (2005) recorded different biopesticidal formulations against spider mite, *Tetranychus evansi* Baker & Pritchard, on tomato plants to control adult stages of tobacco spider mite, *T. evansi*. *B. bassiana* isolate GPK (82.6%) and *M. anisopliae* isolates ICIPE78 (77.9%) were highly effective in reducing the adult mite population after 10 days post treatment. The higher efficacy here than our study might be dose different mite species. Further, the findings of Amjad et al. (2012) have reported the efficacy of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for the management of the TSSM on cotton under field conditions.

Sreenivas et al. (2005) demonstrated the high pathogenicity of *M. anisopliae*, *B. bassiana* and *V. lecanii* against red spider mite, *Tetranychus neocaledonicus* Andre (different from our species *T. urticae*) under glasshouse conditions and Chavan et al. (2008) observed that with increase in concentration of *V. lecanii* and days after treatment per cent mortality of red spider mite increased till fourteen days after spray in their study. However, in contrast to these studies, in the present work, per cent mortality of mite decrease after eleven days of spray and this might be attributed to lower concentration of colony forming units (1×10^9 CFU/ml) of *V. lecanii*. Similar results were reported by Geroh et al. (2014) wherein with increase in conidial concentration of *B. bassiana*, per cent mortality of *T. urticae* also increased in okra under field conditions. *B. bassiana* caused 15 to 70% mortality against *T. urticae* on maize after 7 days post treatment when applied at the concentration of 3.3×10^6 and 7.8×10^9 conidia/ml, respectively (Elhakim et al. 2020). Nag et al. (2020) reported the efficacy of *B. bassiana* and *V. lecanii* at 3 concentrations (10 ml/l, 15 ml/l and 20 ml/l) against egg and active stages of two spotted spider mite, *T. urticae* on green pepper under nethouse conditions and found that with an increase in concentration of *B. bassiana* and *V. lecanii* (at 20 ml/l), percent mortality of *T. urticae* increased continually until ten days after spray.

The efficacy of EPFs depends upon different factors such as the concentration (CFUs) in the formulations, the potency of the strain of the fungus used for formulation, type of formulation (whether oil based or extract) and above all the inherent tolerance/resistance in the mite species. In our study, *M. anisopliae* (1×10^8 CFUs) was the least effective as the CFUs were less compared to *L. lecanii* (1×10^9 CFUs) and *B. bassiana* (1×10^9 CFUs). The inherent potential efficacy of *M. anisopliae* strain might be less compared to other EPFs. Variable efficacy are reported in literature can also be attributed to type of formulations (kernel extracts 58% to oil > 75%). Regarding strains, *T. evansi* (Wekesa et al. 2005) or *T. neocaledonicus* (Sreenivas et al. 2005) could be more susceptible to biorationals compared to *T. urticae* in our study.

Significantly high yields were obtained by the use of Neem Baan (1%) (26.17% higher than control) that it was statistically at par with the chemical treatment. Even the least effective EPF, *M. anisopliae* gave 17.59% higher yield than the control. The additional intangible benefit of getting nutritionally better and residue free produce cannot be overlooked.

Conclusions

Neem Baan formulations proved better than all the fungal formulations of *B. bassiana*, *L. lecanii*, and *M. anisopliae* in reducing the populations of *T. urticae* (eggs, and active stages) in parthenocarpic cucumber grown in poly-net house. The effectiveness of Neem Baan 1% was equivalent to Dimethoate 30 EC hence provides an equally efficacious environmentally safe option for control. Three sprays of *L. lecanii* and *B. bassiana* (at 15 ml/l) at 10 days interval starting at 35 days of the transplanting can help in reduce the population load of mite early in the season. The integration of biorationals in IPM modules for management of *T. urticae* on parthenocarpic cucumber grown under protected environment is a promising preposition.

Data availability All data and materials are mentioned in the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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