Journal of Applied Entomologist, 2023; 3(1):15-19 ISSN NO: 2583-1917

Efficiency of entomopathogenic nematode and certain bio-agents as biological control against the tow-spotted spider mite, *Tetranychus urticae* Koch

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Received 26 Dec 2022; Accepted 2 Feb 2023; Published 7 Feb 2023

Abstract

The objective of this study was to assess the efficiency of biocontrol agents such as entomopathogenic nematodes (EPNs) *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* to Abamectin against the two-spotted spider mite *Tetranychus urticae* under laboratory settings. The mortality percentages of *H. bacteriophora* were 13.33 to 26.66% and 10 to 23.33% when using 2000 and 1000 juveniles, respectively. Moreover, mortality percentages from 6.6 to 26.66% for *S. capocapsae* were identical for both two levels. In contrast to Abamectin, Bioarc (*Bacillus megaterium*), Biozeid (*Trichodema album*), and Baroque agents were tested against *T. urticae* under greenhouse conditions. Bioarc had the highest mean reduction (96.28%) compared to Abamectin (99.16%), although Biozeid and Baroque were approximately equivalent in terms of mortality reduction (95.48% and 95.2%, respectively).

Keywords: *T. urticae, Heterorhabditis bacteriophora, Steinernema carpocapsae, Bacillus megaterium,* and *Trichodema album*

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most significant mites, inflicting severe yield losses and economic losses on a wide range of plant hosts. The excessive use of chemical acaricides controls spider mites below the economic threshold level, but at the expense of environmental contamination and inherent threats to plant, animal, and human life Wilson (1993) [32] and Wilson *et al*., (1991) [31]. Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are promising biocontrol agents against significant soil-dwelling pests Lacey and Georgis (2012) [23]. The symbiotic bacteria associated with EPNs are poisonous to a wide variety of insect pests Guo (1999) [17]. They are considered safe alternatives to chemical pesticides for humans, plants, and cattle Ehlers (2001) [14]. Entomapathogenic nematodes (EPNs) *Heterorhabditis bacteriophora and Steinernema carpocapsae* are employed to manage numerous insect pests Ebssa, (2005) [13]. They are appealing alternatives to chemical insecticides and are safe for use around humans, animals, and plants Ehlers (2001) [14]. Biological control agents include predatory mites, parasitoids, and bacterial pathogens contribute to the natural regulation of numerous plant pests (Al-Azzazy, 2002, 2005; Attia *et al*., 2013) [1, 2, 10]. As a category of significant natural enemies of spider mites, pathogenic bacteria demonstrate varied ways of action. These include parasitizing, competition for resources, generation of poisons, antibiotics, or enzymes, and induction of plant systemic resistance (Alsohim *et al*.,2014; Alsohim and Fouly, 2015) [7, 8]. The entomopathogenic fungi play a significant role in the regulation of phytophagous mite populations, decimating them on occasion Cander and Van der Geest *et al*., (2000) [11] and so reducing the use of acaricides. Egypt's climate is thought to be more favorable for fungal infections Sewify (1989) [30]. Several species of pathogenic fungi were used to combat mite pests: *Hirsutella thompsonii* Fisher against the citrus rust mite, *Phyllocoptruta oleivora* (Ashmed) (McCoy 1975; Latge *et al*., (1988) [27, 24] against *T. urticae* Hanna and Heikal (1995) [18] and *Aceria gurreronis* Kumar (2006) [22]; pathogenicity of two fungi, *T. harzianumand*; *C. herbarium* on *T. urticae* in the laboratory and semi-field conditions Afifi *et al*., (2007) [7]. Therefore, the purpose of this study was to assess the efficacy of specific entomopathogenic nematodes (EPNS) and two commercial products, Bioarc (*Bacillus megaterium)* and Biozeid *(Trichoderma album*), as biological control agents and Baroque Chitin production inhibitors versus Abamectin.

Materials and methods

Culture of *T. urticae*

P. valgaris seeds were planted in 20-centimeter-diameter containers while adhering to all agricultural practices. The plants were infested with spider mites, T. urticae, three weeks after planting and left for roughly 60 days until the mite population developed.

Bio-agents tested

A) Entomopathogenic nematodes were utilized, specifically *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. The inoculum of entomopathogenic nematodes utilized in this study was obtained from the Plant Protection Research Institute, Agricultural Research Center (ARC) Dokki in Giza, Egypt.

B) Bioarc (2.5g/L of distilled water): An indigenous commercial formulation of *Bacillus megaterium* (25×106 cfu/g) phosphorus solubilizing bacteria.

C) Biozeid (2.5g/L of distilled water): A commercially available preparation of the nematophagous fungus *Trichoderma album* (25×106 cfu/g).

The Ministry of Agriculture registered both bio-products obtained from the Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

D) Baroque: Artificial insect growth regulators (Etoxazol 10%W/V).

Chitin production inhibitors

E) Abamectin, (1.8% EC)

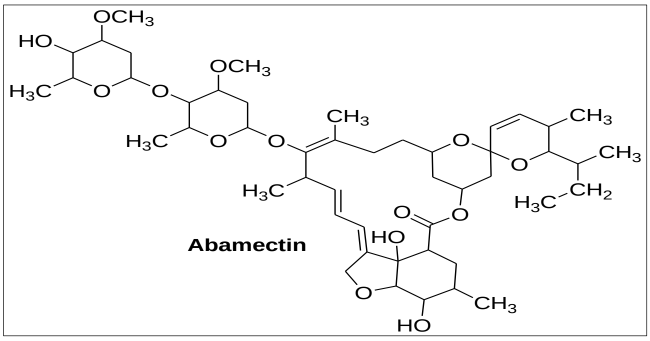

Fig 1

Laboratory experimental

In order to analyze the two pathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* against *T. urticae*, the treatments were evaluated in Petri dishes with 1000 and 2000 juveniles at the pair level. Selected *P. valgaris* leaves were plucked from the plant, transported to the laboratory, and cleaned with distilled water before being dried in the air for 30 minutes. Leaf discs (1.2 cm in diameter) were cut and placed on cotton wool moistened in Petri dishes with covers (9 cm in diameter) Daily additions of 10 mature females of *T. urticae* were made to Petri plates placed in transparent plastic boxes with lids and placed in an incubator at 25.2 ^{O}C and 65.5% RH. Water and mites alone were employed as a negative control. Abamectin was utilized as a standard acaricidal agent, at concentrations of 25 and 50 mg/mL for comparative evaluation. Mites' mortality was examined after one, three, five, and seven days of therapy and compared to the control

Experimental technique

Certain bio-agents Bioarc, Biozeid, and Synthetic insect growth regulators were the subject of experiments. In the experiment, Baroque was compared to abamectin as an acaricide on the reproduction of *T. urticae* on *Phaseolus valgaris* L. plants grown in a greenhouse.

25-day-old kidney bean seedlings were infected with *T. urticae*, left for 15 to 20 days, and then treated with agricultural techniques. Each treatment and control were replicated four times. In every trial, each pot had three plants. Each of the tested products were sprayed using a hand sprayer. Mites were enumerated and documented one, three, five, seven, and fourteen days following treatment for all treatments and the untreated control.

Statistical analysis

The analysis of variance (ANOVA) program Gomez and Gomez (1984) [16] was used to analyze the data, followed by the Multiple Range Test to compare means Duncan (1955) [12]. According to the equation of Henderson and Tilton, the reduction % was determined and corrected (1955) [19].

Henderson and Tilton formula

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

Results and discussion

This study was done to determine the impact of two entomopathogenic nematodes, *H. bacteriophora* and *S. carpocapsae*, on *T. urticae*. Studies (Ashwini *et al*., 2022) [6] that recovered EPN from various places, isolated and purified bacterial colonies, described the bacteria using morphological and molecular techniques, and evaluated the efficacy of various bacteria in controlling polyphagous *Tetranychus truncatus* Ehara mites. The average mortality of the two-spotted spider mites, *T. urticae*, was revealed by the results given in Table (1). Two concentrations of Abamectin produced the highest *T. urticae* mortality rates. Nonetheless, two pathogenic nematodes *H. bacteriophora* and *S. carpocapsae* level 2000 Juveniles after Abamectin as the mean mortality values were 9.33 and 9.66 after 7 days for concentrations 25 and 50 mg/mL, followed by *H. bacteriophora* and *S. carpocapsae* by level 2000 Juveniles 2.66 and 2.66 after 7 days respectively.

Table 1: Impact of two entomopathognic nematodes against the spotted spider mites

Treatments	Nematode level	1day	3day	5day	7day
Heterorhabditis bacteriophora	2000	$1.33^{b}\pm0.66$	$2^{b}\pm0.0$	$2^{bc}\pm0.0$	$2.66^{b}\pm0.66$
	1000	$1^{b}\pm0.57$	$1^{b}\pm0.57$	$2.33^{b}\pm0.66$	$2.33^{b}\pm0.66$
Steinernema carpocapsae	2000	$0.66^{b}\pm0.66$	$2^{b}\pm1.15$	$2.66^{b}\pm0.66$	$2.66^{b}\pm0.66$
	1000	$0.66^{b}\pm0.66$	$1.33^{b}\pm0.66$	$2.66^{b}\pm0.66$	$2.66^{b}\pm0.66$
Abamactin	(25mg/mL)	$6.33^{a}\pm0.66$	$6.66^{a}\pm0.88$	$7.66^{a}\pm1.2$	$9.33^{a}\pm0.66$
	(50mg/mL)	$6.66^{a}\pm0.66$	$7.33^{a}\pm0.88$	$8.0^{a}\pm1.0$	$9.66^{a}\pm0.33$
Control	——	0^{b}	0^{b}	0^{c}	0^{c}
LSD 5%	——	1.83	2.19	2.22	1.75
F-value	——	22.15	15.88	16.91	42.20
P-value	——	0.0000	0.0000	0.0000	0.0000

Each value presented the mean of four replicates. Values followed by the same letter (s) in a column are not significantly different according to analysis of variance (ANOVA) test at level 0.05.

T. urticae mortality rate utilizing two levels of entomopathogenic nematodes *H. bacteriophora* and *S. carpocapsae* was depicted in Table (2). Results indicated that the mortality percentages of *H. bacteriophora* ranged from 13.33 to 26.66 % and 10 to 23.33 % when used consistently with 2000 and 1000 juveniles. Nonetheless, the mortality rate ranged from 6.66 to 26.66 % when *S. carpocapsae* was administered at both dosages. The mortality of *T. truncates* was lower when treated with *P. aeruginosa* as opposed to *P. lumine-scens*, similar to the findings of Ashwini *et al*., (2022) [6].

Table 2: Mortality percentage of *T. truncates* after using two levels of entomopathogenic nematodes

Treatments	Nematode Level	1 day	3 day	5 day	7 day	General Mean Reduction %
H. bacteriophora	2000	13.33	20	20	26.66	20
	1000	10	10	23.33	23.33	16.66
S. carpocapsae	2000	6.66	20	26.66	26.66	20
	1000	6.66	13.33	26.66	26.66	18.32
Abamectin	(25mg/m)	63.33	66.67	76.67	93.33	60.75
	(50mg/m)	66.67	73.33	80	96.67	79.16

According to reports, the bacteria *Ochrobactrum triticiisolated* from the EPN *Oscheius chongmingensis* is responsible for 93.33 % of *Galleria mellonella* deaths Fu and Liu (2019) [15]. In this investigation, however, *Ochrobactrum* isolates exhibited no significant activity against *T. truncatus*. Total mortality was 16.66% for *H. bacteriophora* and 18.32% for *S. carpocapsae* per 1000 juveniles in a cup. In contrast, the total mortality of 2000 juveniles caused by two entomopathogenic nematodes was 20%.

Effect of two bio-agents (bioarc and biozeid), baroque inhibit metamorphosis and the chemical pesticide, agrinate on *T. urticae*.

Table (3) demonstrates the effectiveness of two bioagents, *B. megaterium* (Bioarc) and *T. album* (Biozeid), as well as Baroque and Abamectin (18% chemical pesticide), in reducing *T. urticae*.

Table 3: The effectiveness of a particular bioagent as a biological control agent against *T. urticae* under greenhouse conditions

Compound	Before Treatment	After Treatment				
		1 day	3 days	5 days	7 days	14 days
Biozeid	10.06^{c} ±0.492	1.2^{b} ±0.33	0.6^{c} ±0.16	0.4^{b}±0.19	1.2^{b} ±0.44	0.133^{b}±0.09
Bioarc	10.93^{c} ±1.02	1.0^{b}±0.29	2.6^{b} ±0.62	1.26^{b}±0.30	0.6^{b}±0.32	0.8^{b}±0.35
Baroque	10.26^{c} ±0.628	1.2^{b} ±0.44	1.7^{bc}±0.24	0.8^{b}±0.29	0.33^{b} ±0.15	0.2^{b}±0.10
Abamectin	21.86^{a}±1.12	1.06^{b} ±0.31	0.33^{c}±0.18	0.26^{b}±0.11	0.06^{c}±0.06	0.0b
Check	16.33^{b} ±1.06	39.73^{a}±1.7	41.9^{a}±1.33	43.8^{a}±1.49	30.4^{a}±2.37	28.8^{a}±1.53
LSD	2.549	2.324	1.90	1.97	3.079	1.99
F-value	32.46	438.73	725.97	755.70	150.26	326.20
P-value	0.0000	0.0007	0.0000	0.0000	0.0000	0.0000

Using a significance level of 0.05, Duncan's test reveals that values in a column that are followed by the same letter are not substantially different.

According to the data presented in table (3), the mean reduction in the population of *T. urticae* reduced from 21.86 to 0.33, 0.26, 0.06, and 0.00 mites 3, 5, 7, and 14 days after Abamectin therapy, respectively. After 14 days of Biozeid application, the mean number of *T. urticae* reduced from 10 to 0.133. Afifi *et al*. (2007) [4] reported that, under semi-field settings, the average reduction in mite population caused by *T. harzianum* and *C. herbarium* suspensions was 42.4% and 43.9% after 10 days of spraying, whereas it increased to 58.2% when the two fungi were combined. Both *T. Harzianum* and *C. herbarium* fungi had a favorable effect on the pathogenicity of the two-spotted spider mite, *T. urticae*, although it is recommended to apply a combination of both fungi for more effective control.

According to Lenteren (2000) [25], greenhouses provide optimal circumstances for the rapid development of pests and illnesses, necessitating repeated chemical treatments. There are over 100 types of beneficial organisms commercially available for the treatment of all significant mites and insect pests. The best-documented diseases of Acari are fungi, according to Chandler and Van der Geest (2006) [11]; close to 60 species of fungi have been found to infesting at least 70 species of acarines across nearly all orders. In addition, Kovach (1996) [21] and Lola-Luz (2003) [26] revealed that the fungus *T. harzianum* might treat certain plant diseases without harming mammals or other creatures. It prevented the development of the fungus *Botrytis cinerea*, which causes gray mould disease in strawberry fruits. Currently, several formulations of these biocontrol agents are being manufactured. After 14 days of treatment with Baroque and Bioarc, the mean number of *T. urticae* mites reduced from 10.26 to 0.2 and 10.93 to 0.8, respectively. Across all treatments, a substantial reduction in *T. urticae* was seen in comparison to the control. Al-Azzazy *et al*. (2020) [3] tested the effectiveness of three types of bacteria. *Acinetobacter* sp. (2.237×108 cfu /mL), Bacillus subtilis (2.470×108 cfu/ mL), and *Bacillus qassimus* (3.320×08cfu/mL) were evaluated against the two-spotted spider mite, *Tetranychus urticae*, under laboratory and greenhouse settings. Sprays of *Acinetobacter* sp. demonstrated maximum effectiveness against *T. urticae*. In the laboratory and greenhouse, mite mortality reached 87.15 and 77.29 %, respectively, three days after treatment. Kidist and Fikre (2020) [20] studied the effectiveness of these naturally occurring bacteria in controlling the red spider mite in rose flower farms. This experiment was conducted on the Menagesh, Gallica flower farm with the Limbo flower type during the flowering stage. Using a motorized backpack sprayer for two cycles at a rate of 7 mL/L for four consecutive months. Even 21 days after the second spray, the analysis of variance on mite count data following the administration of Bitoxybacillus (Bt) and Abamectin 1.8%EC revealed no significant difference (p>0.05). In contrast, the spider mite population density in Bt-treated plots was significantly lower at all sampling times compared to untreated controls with Abamectin. The pest population after three weeks of Bitoxybacillin treatments was 68.1 per stem compared to Abamectin 1.8%EC (125.1) and control (110.57), demonstrating that the registered miticide failed to suppress the rose mite population.

Santos-Matos *et al*., (2017) [29] showed that neither *Escherichia coli* nor *Bacillus megaterium* infection induced a response in *T. urticae* as determined by genome-wide transcriptome analysis.

In accordance with this, spider mites died within days even when injected with tiny quantities of non-pathogenic *Drosophila bacteria*. Additionally, bacterial populations within the infected spider mites expanded dramatically. According to data in Table (4), Bioarc had the highest mean reduction (96.28%) compared to Abamectin (99.18%), although Biozeid and Baroque had identical reductions in mortality (95.48 and 95.2%, respectively).

Table 4: Average percentage of mortality in spider mite *T. urticae* population treated with the two bio-agents Baroque inhibit metamorphosis and the chemical pesticide Abamectin

Treatment	1 day	3 days	5 days	7 days	14 days	Redaction%
Biozeid	95.1	97.7	98.5	93.6	92.5	95.48
Bioarc	95.19	93.44	97.09	96.78	98.92	96.28
Baroque	96.33	90.74	95.9	97.5	95.84	95.2
Abamectin	96.99	99.41	99.55	99.85	100	99.16

In addition, Affokpon *et al*., (2011) [5] and Oliveira *et al*., (2009) [28] discovered that the soil fungus *Trichoderma* spp. is a possible nematode bio-control agent on a variety of foods, vegetables, and crops. In addition, *Trichoderma* spp. and *B. megaterium* are frequent soil-beneficial bio-fertilizers that belong to plant growth-promoting rhizobacteria and have been utilized for root-knot nematode management.

Conclusion

The objective of the present study is to investigate the possibility of using entomopathogenic nematodes (EPNs) such as *H. bacteriophora and S. carpocapsae* to control two-spotted spider mite, *T. urticae*. It is concluded that, although *Trichoderma album* and *Bacillus megaterium* effectively controlled the spotted spider mites *Tetranychus urticae*, the highest mean of reduction was recorded using Bioarc (96.28%). Biozeid and Baroque were approximately equal in the reduction of mortality which was 95.48% and 95.2 % respectively.

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