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Evaluation of the acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans against *Tetranychus truncatus* Ehara (Prostigata:Tetranychidae) on cucumber under protected cultivation

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ABSTRACT: The efficacy of two concentrations of *Acremonium zeylanicum* (Petch), two acaricide molecules *viz*., spiromesifen and diafenthiuron and two botanicals *viz*., neem oil and azadirachtin against *Tetranychus truncatus* (Ehara) on cucumber in polyhouse was evaluated. The results revealed that fourteen days after treatment, *A. zeylanicum* significantly reduced mite population to the tune of 55.03 to 58.98 per cent at 1×10^8 spores ml^{-1} and 72.71 to 74.51 per cent at 1×10^9 spores ml^{-1}. However its efficacy was not comparable with that of the acaricides and botanicals which recorded significantly higher percent reduction in mite population.

KEY WORDS: *Mite*, acaropathogen, acaricide, cucumber

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a popular vegetable crop grown in polyhouses in Kerala. Under polyhouse, cucumber is prone to attack by a number of insect and non-insect pests. Among the non-insect pests, spider mites of the family Tetranychidae, are considered as serious pests. A study on the diversity of mite pests associated with vegetable crops grown in polyhouses of Thrissur district, Kerala, identified *Tetranychus truncatus* Ehara (Prostigata:Tetranychidae) as the predominant species of spider mite (Lenin *et al*., 2015). Farmers depend solely on acaricide molecules for mite management in polyhouses, which can lead to development of resistance in mites to acaricides, in addition to probable health hazards. Hence, there is an increasing interest in natural pesticides which are derived from plants and microorganisms, since they are perceived to be safer than the synthetic chemicals. Recently, an acaropathogen, *Acremonium zeylanicum* was isolated from *Tetranychus urticae* Koch infesting brinjal grown under protected cultivation in Thrissur district by All India Network Project on Agricultural Acarology (AINPAA), where it was observed to cause significant mortality to the spider mite (Krishna *et al*., 2014). Bioefficacy studies conducted earlier in the laboratory against *T. truncatus* had shown that *A. zeylanicum* significantly reduced the mite count at concentrations of 1×10^8 and 1×10^9 spores ml^{-1},

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indicating that the local isolate of *A. zeylanicum* can be a potential candidate in biological control of spider mites on vegetable crops (Sherief *et al*., 2017). With this background, the present study was conducted to evaluate the efficacy of *A. zeylanicum* against *T. truncatus* on cucumber in polyhouse.

MATERIAL AND METHODS

An experiment was conducted in the polyhouse of All India Network Project on Agricultural Acarology (AINPAA), College of Horticulture, Kerala Agricultural University from March to May, 2017 to evaluate the efficacy of *A. zeylanicum* against *T. truncatus* on cucumber (variety Sania). The experiment was laid out in Completely Randomized Design with seven treatments and three replications. The treatments evaluated included *A. zeylanicum* at two different concentrations *viz*., 1 × 10^7 spores ml^{-1} and 1 × 10^8 spores ml^{-1}; two novel acaricides *viz*., spiromesifen (100g ai ha^{-1}) and diafenthiuron (400g ai ha^{-1}); two botanicals, neem oil (2%) and azadirachtin (0.005%) along with an untreated control.The crop was raised in the polyhouse as per the Package of Practices Recommendations of Kerala Agricultural University, 2016 at a spacing of 60×30 cm in plots of 1.6 m × 1.3 m size. Mites from the laboratory culture of AINPAA, maintained on mulberry leaves were released on three leaves of twenty five days old cucumber plants at the rate of 20 active mites/leaf by stapling mite infested mulberry leaf bits of 5 cm^2size on top, middle and bottom leaves of cucumber plant.

Treatments were imposed three weeks after the release of mites. Spray solution was prepared by thorough mixing of measured quantity of different treatments and required amount of water to form a uniform suspension. The treatments were applied using a hand sprayer. A control treatment was maintained with water spray. Observations were recorded on the count of eggs and active stages of *T. truncatus in situ* from three windows of 1 cm^2 each from three leaves per plant representing the top, middle and bottom canopy using a hand lens of 10 X magnification.The population counts were recorded one day before spraying and 1, 3, 7, 10 and 14 days after spraying. To confirm the results, the experiment was repeated on the same crop. However, the population of *T. truncatus* was found to be negligible in all treatments except control, hence a second release of the mite was made two weeks after the release, and the same treatments were imposed. Observations were recorded in a similar manner. The data were statistically analysed using analysis of variance technique (ANOVA) considering population counts prior to the first application.

RESULTS

Efficacy of treatments - First experiment:

The mean mite counts before the application of treatments ranged from 18.05 to 20.80 per cm^2 leaf area (Table 1). The data indicated that *A. zeylanicum* significantly reduced the mite count over untreated control at both the concentrations evaluated. At $1×10^7$ spores ml^{-1} in the acaropathogen treated leaves the mite count was 15.30, 10.67, 8.85, 11.25 and 9.09 per cm^2 leaf area after 1, 3, 7, 10 and 14 days of treatment, respectively as compared to the pre-treatment mite count of 19.68 per cm^2 leaf area. *A. zeylanicum* at the concentration of $1×10^8$ spores ml^{-1} was more effective on *T. truncatus* than the concentration $1×10^7$ spores ml^{-1} in which, the population was 10.81, 8.70, 5.08, 7.35 and 7.14 per cm^2 leaf area after 1, 3, 7, 10 and 14 days of spray, respectively as compared to pre-treatment mite population of 18.62 per cm^2 leaf area. However, the acaricides and botanicals caused significantly higher reduction in mite count compared to *A. zeylanicum*. On Spiromesifen treated plants the mite count was 4.97, 1.38, 0.92, 0.68 and 0.46per cm^2 leaf area after 1, 3, 7, 10 and 14 days of spray, respectively. On Diafenthiuron treated plants 5.08, 1.97, 1.07, 0.85 and 0.57 mites per cm^2 leaf area were recorded after 1, 3, 7, 10 and 14 days of spray, respectively as compared to the pre-treatment mite count of 18.29 per cm^2 leaf area, and was on par with spiromesifen. Plants treated with neem oil had mite population of 8.29, 3.46, 2.05, 2.38 and 3.33 per cm^2 leaf area while those treated with azadirachtin had 8.78, 4.15, 2.95, 3.62 and 4.03 per

Table 1. Effect of *Acremonium zeylanicum* in comparison to acaricides and botanicals on *Tetranychus truncatus* infesting cucumber in polyhouse- experiment 1

Sl. No.	Treatments	Pre-treatment count	Mean no. of mite/cm² leaf area			Per cent reduction after 7 days	Mean no. of mite/cm² leaf area		Per cent reduction i after 14 days
			1 DAS	3 DAS	7 DAS		10 DAS	14 DAS	
1	*Acremonium zeylanicum* 1×10^7 spores ml^{-1}	19.68	15.30 ^{b} (15.31)	10.67^b (10.69)	8.85 ^{b} (8.80)	55.03	11.25^b (11.26)	9.09 ^{b} (9.13)	53.81
2	*Acremonium zeylanicum* 1×10^8 spores ml^{-1}	18.62	10.81^c (10.80)	8.70 ^{c} (8.69)	5.08 ^{c} (5.10)	72.71	7.35 ^{c} (7.34)	7.14 ^{c} (7.12)	61.65
3	Spiromesifen 100g ai ha^{-1}	18.50	4.97 ^{e} (4.95)	1.38 ^{f} (1.36)	0.92 ^{e} (0.94)	95.02	0.68 ^{f} (0.66)	0.46 ^{e} (0.43)	97.51
4	Diafenthiuron 400g ai ha^{-1}	18.29	5.08 ^{e} (5.06)	1.97 ^{f} (1.94)	1.07 ^{e} (1.10)	94.14	0.85 ^{ef} (0.84)	0.57 ^{e} (0.52)	96.88
5	Neem oil 2 %	18.05	8.29 ^{d} (8.27)	3.46 ^{e} (3.42)	2.05 ^{de} (2.09)	88.64	2.38 ^{de} (2.36)	3.33 ^{d} (3.27)	81.55
6	Azadirachtin 0.005 %	18.68	8.78 ^{d} (8.77)	4.15 ^{d} (4.14)	2.95 ^{d} (2.96)	84.20	3.62 ^{d} (3.61)	4.03 ^{d} (4.01)	78.42
7	Control	20.80	22.39 ^{a} (22.42)	29.33^a (29.39)	34.77^a (34.67)	-	30.03 ^{a} (30.06)	30.03^a (30.14)	-

DAS = Days after spraying. Means followed by same letters do not differ significantly by DMRT (p = 0.05), Values in the parentheses are adjusted treatment means.

cm^2 leaf area, respectively after 1, 3, 7, 10 and 14 days and were on par with each other. Plants in control plots had mite population of 20.80, 22.39, 34.77, 30.03 and 30.03per cm^2 leaf area after 1, 3, 7, 10 and 14 days of spray, respectively.

Seven days after treatment, *A. zeylanicum* reduced mite population by 72.71 and 55.03 per cent at concentrations of 1×10^8 spores $ml^{-1}$and 1×10^7 spores ml^{-1} respectively. However, spiromesifen caused the highest per cent reduction in the mite population (95.02%) closely followed by diafenthiuron (94.14%), neem oil (88.64 %) and azadirachtin (84.20%). Fourteen days after treatment, spiromesifen, difenthiuron, neem oil and azadirachtin reduced the mite numbers by 97.51,96.88, 81.55 and 78.42 per cent respectively, while on the acaropathogen, *A. zeylanicum* treated plants 61.65 and 53.81 per cent reduction in mite numbers at concentration of 1×10^8 spores ml^{-1} and 1×10^7 spores ml^{-1} respectively was observed.

Efficacy of treatments - Second experiment:

The mean mite count, before imposing second spray in different plots ranged from 17.16 to 19.29 per cm^2 leaf area (Table 2). The results showed a similar trend in the second experiment also, *A. zeylanicum* significantly reduced mite count on cucumber over control. On 1×10^7 spores ml^{-1} treated plants 13.41, 10.46, 7.71, 9.63 and 8.37 mites per cm^2 leaf area were recorded after 1, 3, 7, 10 and 14 days of treatment, respectively as against the pre-treatment mite population of 18.80 per cm^2 leaf area. On 1×10^8spores ml^{-1} treated plants the mite population was 10.10, 7.82, 4.73, 5.42 and 6.34 mites per cm^2 leaf area, after 1, 3, 7, 10 and 14 days of treatment, respectively as compared to pre-treatment mite population of 18.56 per cm^2 leaf area. The acaricides spiromesifen and diafenthiuron caused significantly higher reduction in mite population followed by the botanicals neem oil and azadirachtin. On Spiromesifen treated plants 3.73,

Table 2. Effect of *Acremonium zeylanicum* in comparison to acaricides and botanicals on *Tetranychus truncatus* infesting cucumber in polyhouse- experiment 2

Sl. No.	Treatments	Pre-treatment count	Mean no. of mite/cm² leaf area			Per cent reduction after 7 days	Mean no. of mites/cm² leaf area		Per cent reduction after 14 days
			1 DAS	3 DAS	7 DAS		10 DAS	14 DAS	
1	*Acremonium zeylanicum* 1×10^7 spores ml^{-1}	18.80	13.41[b] (13.69)	10.46[b] (10.72)	7.71 [b] (7.79)	58.98	9.63 [b] (9.53)	8.37 [b] (8.41)	55.47
2	*Acremonium zeylanicum* 1×10^8 spores ml^{-1}	18.56	10.10 [c] (10.26)	7.82 [c] (7.98)	4.73 [c] (4.77)	74.51	5.42 [c] (5.37)	6.34 [c] (6.36)	65.84
3	Spiromesifen 100g ai ha^{-1}	17.45	3.73 [e] (3.34)	1.94 [e] (1.57)	1.36 [e] (1.25)	92.20	0.85 [f] (0.97)	0.54 [f] (0.47)	96.90
4	Diafenthiuron 400g ai ha^{-1}	17.16	4.65 [e] (4.11)	2.12 [e] (1.61)	1.5 [e] (1.35)	91.25	0.93 [f] (1.104)	0.60 [f] (0.50)	96.50
5	Neem oil 2 %	18.28	7.87 [d] (7.89)	3.17 [de] (3.19)	2.21 [de] (2.21)	87.91	2.70 [e] (2.692)	3.24 [e] (3.24)	82.27
6	Azadirachtin 0.005 %	18.05	8.62 [d] (8.53)	4.27 [d] (4.18)	3.27 [d] (3.24)	81.88	3.73 [d] (3.76)	4.33 [d] (4.31)	76.01
7	Control	19.29	20.66 [a] (21.19)	27.6 [a] (28.10)	30.84 [a] (30.98)		27.33 [a] (27.16)	31.19[a] (31.28)	

DAS = Days after spraying. Means followed by same letters do not differ significantly (p = 0.05) Values in the parentheses are adjusted treatment means.

1.94, 1.36, 0.85 and 0.54 mites per cm^2 leaf area were recorded after 1, 3, 7, 10 and 14 days of spray, respectively as compared to pre-treatment mite population of 17.45 per cm^2 leaf area. The population of mites on cucumber plants, sprayed with diafenthiuron, was 4.65, 2.12, 1.50, 0.93 and 0.60 per cm^2 leaf area after 1, 3, 7, 10 and 14 days of spray respectively as compared to pre-treatment population of 17.16 per cm^2 leaf area. Mite population on the botanicals, neem oil and azadirachtin treated plants after 1, 3, 7, 10 and 14 days of spray was 7.87, 3.17, 2.21, 2.70 and 3.24 per cm^2 leaf area and 8.62, 4.27, 3.27, 3.73 and 4.33 per cm^2 leaf area, respectively. In control plots, the mite population increased of 19.29 per cm^2 leaf area to 31.19 during this period.

Seven days after treatment, spiromesifen significantly caused reduction in mite population (92.20%), closely followed by diafenthiuron (91.25%). The next best treatment was neem oil with a mean reduction in mite count of 87.91 per cent followed by azadirachtin (81.88%). The acaropathogen *A. zeylanicum* at 1×10^8 spores ml^{-1} reduced mite population by 74.51 per cent and at 1×10^7spores ml^{-1} by 58.98 per cent. Fourteen days after treatment, application of spiromesifen, diafenthiuron, neem oil and azadirachtin resulted in 96.90, 96.50, 82.27 and 76.01 per cent reduction in the mite population, respectively. The acaropathogen, *A. zeylanicum* at 1×10^8 spores ml^{-1} reduced the mite population by 65.84 per cent followed by 55.47 per cent at 1×10^7 spores ml^{-1}.

DISCUSSION

In the polyhouse, *A. zeylanicum* significantly reduced mite population on cucumber seven days after treatment at both the concentrations of 1×10^8 spores ml^{-1} and 1×10^7 spores ml^{-1}. The study clearly indicated the potential of *A. zeylanicum* in bringing down the population of the spider mite, *T. truncatus*. In the polyhouse, at the higher concentration of 1×10^8 spores ml^{-1} the acaropathogen could reduce the mite population by 72.71 and 74.51 per cent on seventh day in first and second experiments

respectively. At a lower dosage of 1×10^7spores ml^{-1} it brought about a reduction of 55.03 and 58.98 per cent in the mite population by seven days in the first and second experiment, respectively. Pathogenicity studies conducted earlier with several acaropathogens have indicated the potential to reduce mite numbers of the local isolates of pathogenic fungi. For instance, an entomopathogen, *Cladosporium cladosporioides* isolated from *T. urticae* on okra at Coimbatore caused 96.5 per cent mortality of mites when tested in the laboratory (Jeyarani *et al*., 2011). Similarly, local strain of *Hirsutella thompsoni*, when evaluated against *Oligonychus coffeae* in tea caused mortality of 65 per cent in laboratory (Amarasena *et al*., 2011).

A. zeylanicum isolated from sugarcane woolly aphid from northern Karnataka was evaluated for pathogenicity against important sucking pests of different crops in the laboratory at Dharwad. The fungus proved to be highly pathogenic to cabbage aphid (*Brevicoryne brassicae* Linn.), sorghum aphid (*Melanaphis sacchari* Zehnt.) and sugarcane wooly aphid (*Ceratovacuna lanigera* Zehnt.). However, it was relatively less pathogenic to chilli mite (*Polyphagotarsonemus latus* Banks) and brinjal spider mite (*Tetranychus neocaledonicus* Andre) (Divan and Mallapur, 2011). But in the present study, the fungus *A. zeylanicum* was found to be highly pathogenic to the spider mite *T. truncatus*.This might be because the fungal isolate evaluated in the present study was isolated from a mycosed spider mite and hence is highly adapted to the host and locality. Pena *et al*. (1996) found that fungal isolates originating from *Polyphagotarsonemus latus* Banks (Tarsonemidae) were more pathogenic to *P. latus* species than those isolated from other hosts.

In the present study, though the mite population significantly declined by seventh day, after application of *A. zeylanicum* in the polyhouse, there was an increase in population from seventh day to fourteenth day. In an earlier study with the acaropathogen, laboratory bioassay has indicated comparatively poor ovicidal action of *A. zeylanicum* against *T. truncatus* (Sherief *et al*., 2017). As a result, it could be that a considerable proportion of eggs in the population would have hatched during this period, leading to increase in population by fourteenth day in the polyhouse. The study showed that the efficacy of *A. zeylanicum* was not comparable with that of novel acaricides and botanicals. The new acaricide molecules, spiromesifen and diafenthiuron were effective and superior to the fungus in reducing the population of *T. truncatus*. Efficacy of these acaricides in reducing mite population was observed from the first day after spray application itself. In the present study spiromesifen caused 97.51 and 96.70 per cent reduction in mite population after 14 days, in first and second experiment, respectively. Baloch *et al*. (2016) also reported that spiromesifen resulted in significant reduction of 96.27 per cent in the population of *T. urticae* 15 days after treatment, on okra. Study on the efficacy of spiromesifen against *T. urticae* on ridge gourd showed that the molecule caused more than 90 per cent mortality (Reddy and Latha, 2013). Under field conditions, spiromesifen could result in complete suppression of population of *T. urticae* in ten days (Sato *et al*., 2011).

Diafenthiuron also caused significant reduction of 96.88 and 96.50 per cent in mite population, respectively, at fourteen days after treatment, in first and second experiments which was on par with spiromesifen. High efficacy of diafenthiuron in suppressing population of spider mite in different crops was earlier reported by several workers (Patil, 2005; Bhaskaran *et al*., 2007, Aswin *et al*., 2015). Among the botanicals evaluated, neem oil (81.55% and 82.27%) and azadirachtin (78.42% and 76.01%) recorded considerable reduction in mite population in the first and second experiment. This was in accordance with the observation of Krishna and Bhaskar (2016) who reported that two per neem oil cent caused 81.15 per cent reduction in population of *T. urticae* on okra. Kumar (2007) observed that neem oil two per cent was effective in managing mite population on rose cultivated under polyhouse condition. Acaricidal property of azadirachtin was reported by Bernandi *et al*. (2012) who recorded 94 to 100 per cent reduction in the population of *T. urticae* on strawberry. The results clearly indicate that *A. zeylanicum* has potential in suppressing mite

population and it can be suggested as an ideal candidate for incorporation in integrated mite management programme in crops, however its safety to humans and beneficial organisms need to be established.

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