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Ovicidal and adulticidal effect of acaropathogenic fungi, neem oil and new acaricide molecules on *Tetranycus urticae* Koch

Aswathi R Krishna* and Haseena Bhaskar

Department of Agricultural Entomology, College of Horticulture,
Kerala Agricultural University, Vellanikkara, Thrissur 680656, Kerala, India.
E mail: achu.krishna12@gmail.com; bhaskarhaseena@yahoo.co.in

ABSTRACT: The relative toxicity of two acaropathogenic fungi (*Hirsutella thompsonii* and *Beauveria bassiana*), neem oil and three new acaricide molecules *viz*., fenazaquin 10 EC, spiromesifen 240 SC and diafenthiuron 50 WP to two-spotted spider mites (egg and adults) were evaluated against a standard check and untreated control under laboratory conditions. 24 hours after treatment, fenazaquin 10 EC excelled in ovicidal activity with a mean egg mortality of 40.81 per cent. The next best treatment was spiromesifen 240 SC which recorded 15.17 per cent egg mortality. Both fenazaquin 10 EC and diafenthiuron 50WP exhibited 100 per cent adult mortality within 24 hours of treatment application. After 72 hours, all the treatments except *B. bassiana* caused significantly high egg mortality. Neem oil (41.00 per cent) and *H. thompsonii* (31.98 per cent) emerged as the next best candidates with respect to adult mortality, while spiromesifen 240 SC recorded the lowest adult mortality of 3.40 per cent.

KEYWORDS: Bioassay; *Tetranychus urticae*; fenazaquin 10 EC; spiromesifen 240 SC; diafenthiuron 50 WP; *Hirsutella thompsonii*; *Beauveria bassiana*; neem oil

Two spotted spider mite (TSSM), *Tetranychus urticae* Koch is a highly polyphagous pest of numerous vegetable crops. Okra is one of the major vegetables cultivated in India throughout the year. One of the limiting factors in the cultivation of okra is the incidence of mite pest, *T. urticae,* especially during summer season which causes severe damage and yield loss up to 17.5 per cent (Ghosh *et al.,* 1996). The intense use of synthetic chemicals against this pest has

* Author for correspondence

resulted in the development of resistance to a wide range of chemicals. This has necessitated the development of newer chemicals with novel modes of action. There is also an increasing interest for natural pesticides which are derived from plants and micro organisms, since they are perceived to be safer than the synthetic chemicals (Yaner *et al*., 2011). The objective of the study was to compare the ovicidal and adulticidal effects of two acaropathogenic fungi(*Hirsutella thompsonii* and *Beauveria bassiana)*, neem oil and new acaricide molecules along with a standard check (fenazaquin 10 EC, spiromesifen 240 SC, diafenthiuron 50 WP and dicofol 18.5 EC) and untreated control on *T. urticae*, which may be considered as a study for developing suitable IPM for *T. urticae*.

Bioassay studies on *T. urticae* were conducted in the Acarology laboratory, Department of Agricultural Entomology, College of Horticulture, Vellanikkara at a temperature of 30 ± 3^0C and 58 ± 1.4 % relative humidity.

The stock culture of *T. urticae* was established in polyhouse on potted plants of okra. To obtain fixed age eggs/females of *T. urticae,* mites from the stock culture were also reared on okra leaves with their upper surface down on wet cotton bed in *Petri* plates in the laboratory at 30 ± 2^0C and 58 ± 1.4 % relative humidity. The culture was observed daily and the leaves were changed periodically.

Topical application method was employed to study the ovicidal effect of two acaropathogenic fungi, neem oil and three new acaricide molecules along with a standard check and untreated control on *T. urticae* (Table 1).

To obtain *T. urticae* eggs of uniform age, gravid females were taken from the mite culture with the help of a moistened zero size camel hair brush and kept individually on okra leaf discs (2 cm^2) placed underside up in p*etri* plates with wet cotton pad. The females, 24 hours after oviposition were subsequently removed for performing the bioassay on eggs. Leaf discs containing *T. urticae* eggs of uniform age were sprayed with the treatments to be tested using a hand atomizer (2 ml/disc) untreated control, sprayed with water. Dicofol 18.5 EC was used as the standard check. The treated leaf discs with eggs were air dried at room temperature and placed in Petri plates. All treatments were replicated three times. Observations on mortality of eggs were recorded at 24, 48 and 72 hours intervals with a stereo binocular microscope. The per cent mortality values were then corrected for control mortality using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality (\%)} = \frac{\{\text{Test mortality (\%)} - \text{Control mortality (\%)}\}}{100 - \text{Control mortality (\%)}} \times 100$$

In adulticidal bioassay leaf dip bioassay method was employed to study the effect of different treatments listed in Table 1. Leaf discs of 2 cm^2 diameter, were dipped in aqueous solution of prepared concentration of the acaricides for ten seconds and then air dried for completely evaporating the water droplets. Ten gravid females of uniform age taken from the stock

Table 1. Ovicidal and Adulticidal effects of different treatments on *T. urticae* in okra under laboratory conditions

Sl. No	Treatments& dosage used	Corrected Mortality (%) over untreated control water spray					
		Ovicidal effect			Adulticidal effect		
		24H	48H	72H	24H	48H	72H
1	Fenazaquin 10 EC – dosage 25 ì L /10 ml	40.81 [a]	51.79 [a]	94.21 [a]	100 [a]	100 [a]	100 [a]
2	Spiromesifen 240 SC – dosage 8 ì L/ 10 ml	15.17 [b]	34.49 [bc]	90.21[a]	0	0	3.40 [b]
3	Diafenthiuron 50 WP @ 400g ai/ha – dosage 16mg/10 ml	4.18 [d]	39.79 [b]	94.88[a]	100 [a]	100 [a]	100 [a]
4	Standard Check (Dicofol 18.5 EC @250g ai/ha) –dosage 25 ì L/10 ml	11.16 [bc]	32.12[bc]	70.54[a]	100 [a]	100 [a]	100 [a]
5	Neem oil 2% - dosage 200 ì L /10 ml	1.51 [d]	21.66 [c]	67.55[a]	37.67 [b]	37.67 [b]	41.00 [a]
6	*Beauveria bassiana* @ 10^7 spores/ml - dosage 100 mg/10 ml	0.04 [d]	3.59 [d]	7.29 [b]	0	0	6.73 [b]
7	*Hirsutella thompsonii* (@ 10^7 spores/ml) –dosage 100 mg/10 ml	2.11 [d]	37.45 [bc]	86.88 [a]	0	0	31.98 [a]

Means followed by same letters do not differ significantly by DMRT (p = 0.05)

culture, were released on to the treated leaf disc kept on wet cotton pad in Petri plate. Such three plates were maintained for each treatment. Leaf discs dipped in only water served as control, while those dipped in Dicofol 18.5 EC served as standard check. Observations on mortality of adult mites were recorded at 24, 48 and 72 hours interval using a stereo binocular microscope and per cent mortality was calculated. The values were then corrected for control mortality using Abbott's formula (Abbott, 1925).

Data corrected for mortality in the control using Abbott's formula were after square root transformation subjected to one- way analysis of variance ($P < 0.05$). Means were compared by Duncan's Multiple Range Test (DMRT) to determine significant differences at $P < 0.05$.

Ovicidal bioassay: It is evident from the table that the ovicidal activity of all the treatments increased from 24h to 72h. 24 hours after treatment, fenazaquin 10 EC excelled in ovicidal

activity with a mean egg mortality of 40.81 per cent. The next best treatment was spiromesifen 240 SC which showed 15.17 per cent egg mortality. It was statistically on par with dicofol (11.16 %). Per cent mortality in diafenthiuron 50 WP (4.18), neem oil 2% (1.51), *B. bassiana* (0.04) and *H. thompsonii* (2.11) indicated that they were on par with each other though inferior to dicofol. 48 hours after treatment, fenazaquin continued to be the better treatment causing 51.79 per cent egg mortality. It was followed by diafenthiuron, *H. thompsonii* and spiromesifen with a mean mortality of 39.79, 37.45 and 34.49 respectively. These treatments were also on par with the standard check. After 72 hours, all the treatments except *B. bassiana* caused significantly high egg mortality ranging from 70.54 to 94.88 per cent. *B. bassiana* proved ineffective on eggs of *T. urticae* .

Adulticidal bioassay

The new acaricide molecules, fenazaquin 10 EC and diafenthiuron 50WP caused complete adult mortality within 24 hours of treatment application. Both the chemicals were superior to other treatments and were on par with the standard check, dicifol 18.5 EC, which also caused cent per cent adult mortality within 24 hours time. The next best treatment was neem oil 2% with a mean mortality of 37.67 per cent. However no adult mortality was observed with adults exposed to spiromesifen 240 SC and acaropathogenic fungi *viz*., *B. bassiana* and *H. thompsonii* up to 48 hours of exposure. After 72 hours of application, neem oil (41 per cent) and *H. thompsonii* (31.98 per cent) emerged as the next leading treatments. *B. bassiana* and spiromesifen were found inferior with adult mortality of less than ten per cent (Table 1).

Fenazaquin is an acaricide which belongs to quinazoline class of chemicals which inhibits mitochondrial electron transport (MET) at complex I. It has high efficacy against eggs and motile stages of tetranychid mites (Marcic *et al*., 2011). The high level of egg and adult mortality exhibited by this chemical, in a very short period of exposure was also earlier reported by many workers. In a laboratory bioassay conducted to test the ovicidal activity against *T.urticae* on okra, Sangeetha and Ramaraju (2013) reported 81.25 per cent egg mortality for fenazaquin 10 EC at 125 g a.i. ha^{-1}. 90.52 per cent mortality of adults of *T. macfarlanei* was caused by Fenazaquin 10 EC (Patil, 2005).

The insecto-acaricide diafenthiuron, is a novel thiourea compound that disrupts oxidative phosphorylation by inhibition of the mitochondrial ATP synthase enzyme. It has been reported as effective against active stages of spider mites (Marcic *et al*., 2011). Similar results were also reported by Patil (2005) who found that use of diafenthiuron resulted in more than 96 per cent mortality of adult mites. The ovicidal activity of diafenthiuron was identified by Patil and Nandihalli (2007) who, based on their bioassay studies on *T. macfarlanei* infesting brinjal, reported that diafenthiuron caused more than 98 per cent egg mortality. The findings of the present study are in agreement with the above studies.

Spiromesifen, a tetronic acid derivative acts as inhibitor of acetyl-CoA-carboxylase, a key enzyme in fatty acid biosynthesis. It is highly toxic to eggs and immature stages of spider

mites, while it acts more slowly against adult females, causing reduction in fertility and fecundity (Marcic *et al*., 2011). In baseline susceptibility studies conducted by Nauen *et al*. (2005), spiromesifen did not have a marked effect against *T. urticae* adult females, but was highly toxic against eggs of the mite. Sato *et al*. (2011) observed that among the different developmental stages studied, the egg stage of *T. urticae* was found to be the most sensitive to spiromesifen. In the present study also spiromesifen recorded a higher reduction in egg count over untreated control compared to standard check, dicofol**.** Saryazdi *et al*. (2013) observed ovicidal activity as well as reduction in the survival rate, fecundity and egg hatching rate when spiromesifen was used. This peculiar growth regulatory effect of spiromesifen might be the reason for very low adult mortality as observed in the present study.

The acaricidal action of neem oil 2 per cent may be attributed to slow action of azadirachtin, which includes complete or partial antifeedant response, delayed and/or disrupted moulting and inhibited reproduction (Copping and Duke, 2007). The studies on spider mites indicate that azadirachtin, in addition to being toxic to various development stages, acts as antifeedant, reduces fecundity and fertility and shortens the life span of adult mite (Sundaram and Sloane, 1995). Umamahesheswari *et al*. (1999) noticed that among the different neem formulations and castor oil tested for their efficacy against red spider mite, following dip method in the laboratory, neem oil gave significantly higher mortality (79.60 per cent) compared to the other treatments tested. A moderate to high level of mortality was also observed in the present study.

Though *B. bassiana* has been reported as a promising fungal pathogen against spider mites by several workers, in the present study it was found inferior including *H. thompsonii*. This may be because this fungus require more time for its development to finally cause mycosis to the mites. The better performance of *H. thompsonii* may be related to the high relative humidity prevailed during the study which causes increased rate of infection (Gerson *et al.,* 1979). The findings of Aghajanzadeh *et al*. (2006) also proves the high virulence of this fungus against *T. urticae*. In the present study *H. thompsonii* was also found to have high ovicidal than adulticidal activity.

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