Evaluation of selected acaropathogenic fungi, botanicals and new acaricide molecules against *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) on okra

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Abstract

Field study was conducted to evaluate two acaropathogenic fungi (*Hirsutella thompsonii* and *Beauveria bassiana*), two botanicals [neem oil 2 per cent and neem seed kernel extract (NSKE)5 per cent] and three new acaricide molecules (Fenazaquin 10 EC, Diafenthiuron 50 WP and Spiromesifen 240 SC) along with a standard check (Dicofol 18.5 EC) and an untreated control against *Tetranychus urticae* on okra during two seasons *viz.,* February–May, 2012 and November, 2012–February, 2013. The new acaricide molecules fenazaquin 10 EC, diafenthiuron 50 WP and spiromesifen 240 SC exhibited high efficacy against *T. urticae* and significant reduction in mite population was observed ten days after treatment application. Among the botanicals evaluated, neem oil 2 per cent was found to be superior to NSKE 5 per cent during the second season, though on par with each other during the first season of study. Among the acaropathogenic fungi, *B. bassiana* was found to perform better than *H. thompsonii* during first season and was on par with each other during the second season. The study identified three novel acaricide molecules for the effective management of *T. urticae* though the bio-agents and botanicals did not give consistent results during the two seasons of study.

Keywords: Acaropathogenic fungi, Botanicals, Diafenthiuron, Fenazaquin, Spiromesifen, *Tetranychus urticae.*

Introduction

India is the second largest producer of vegetables in the world, next to China, with an estimated production of about 90 million tonnes, which accounts for about 15 per cent of world's vegetable production (Patil, 2005). Okra (*Abelmoschus esculentus* (L.) Moench) is one of the major vegetable crops in India which is cultivated throughout the year. One of the important limiting factors in the cultivation of okra is insect and mite pest incidence. About 72 species of pests have been recorded on okra (Rao and Rajendran, 2003). Of these, the red spider mite, *Tetranychus urticae* Koch is considered as a major pest on okra grown in summer season. The problem of mite has gained momentum because of the change in climatic conditions and over-use of plant protection chemicals. Farmers depend mostly on synthetic acaricides for managing mite problems because of instant spectacular knock down effects. However, this may often lead to several problems such as resurgence and the development of wide spread resistance to acaricides. The development of safer alternate management strategies against mite pests is warranted. In this context, the present study was undertaken.

Materials and Methods

Field study was conducted to evaluate the efficacy of two acaropathogenic fungi *viz*., *Hirsutella*

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Table 1.Treatments evaluated against *Tetranychus urticae* on okra

Treatments	Remarks
T1: *Hirsutella thompsonii* @ 10^7 spores ml^{-1}	NBAII liquid formulation
T2: *Beauveria bassiana* @ 10^7 spores ml^{-1}	NBAII talc based formulation
T3: Neem oil 2%	Freshly prepared
T4: NSKE 5%	Freshly prepared
T5: Spiromesifen 240 SC @ 100g a.i. ha^{-1}	Oberon
T6: Fenazaquin 10 EC @ 125g a.i. ha^{-1}	Magister
T7: Diafenthiuron 50 WP @ 400g a.i. ha^{-1}	Pegasus
T8: Standard Check (Dicofol 18.5 EC @ 250g a.i. ha^{-1})	Hilfol
T9: Untreated Control	-

thompsonii Fisher and *Beauveria bassiana* (Balsamo) Vuillemin, two botanicals *viz*., neem oil 2 per cent and neem seed kernel extract (NSKE) 5 per cent and three novel acaricide molecules *viz*., fenazaquin 10 EC, diafenthiuron 50 WP and spiromesifen 240 SC along with a standard check, dicofol 18.5 EC and an untreated control (Table 1) against *T. urticae* on okra, variety 'Arka Anamika'. The experiments were carried out at College of Horticulture, Vellanikkara during two seasons *viz*., February–May, 2012 (Season I) and November, 2012 – February, 2013 (Season II). The crop was raised in the field as per the Package of Practices- Recommendations (KAU, 2011) at a spacing of 60 x 30 cm in plots of 3 x 3 m size. The experimental layout was Randomized Block Design with three replicates per treatment. Mites were released on six weeks old plants by stapling mite infested okra leaf bits of 5 cm^2 size carrying an average of 32 mites per bit at the rate of one bit per plant on the top leaf.

Treatments were imposed three weeks after the release of mites. Spray solution was prepared by thorough mixing of measured quantity of the insecticide and required amount of water to form a uniform mixture. The treatments were applied using a hand operated high volume knapsack sprayer. The population counts of active stages of *T. urticae* were observed on three windows of 1 cm^2 each from three leaves per plant representing the top, middle and bottom canopy. The population counts were recorded one day before spraying and 3rd, 7th and 10th day after spraying. The mean mite count was worked out and analyzed statistically.

Data on mean population of active mites was transformed using square root transformation. Population differences on three, seven and ten days after treatment application were first tested by one way ANOVA. Subsequently the transformed data were analyzed by analysis of covariance (ANOCOVA), taking population counts prior to the first application as covariate and ANOCOVA was done for three, seven and ten day's observations. The result obtained was subjected to DMRT (Duncan's Multiple Range Test). The mean per cent reduction in population over untreated control of mites was also worked out ten days after treatment application.

Results and Discussion

Efficacy of treatments against T. urticae on okra in Season I

The results of the field experiment to evaluate the efficacy of different treatments against *T. urticae* in season I (February–May, 2012) are presented in Table 2. The mean population of active mites one day before imposing the treatments ranged from 21.96 to 68.48 cm^{-2} leaf area. At three and seven days after treatment application, the new acaricide molecule fenazaquin recorded the lowest mite population of 1.63 and 1.96 mites cm^{-2} leaf area respectively. The treatments, diafenthiuron (3.52 and 2.55 mites cm^{-2} leaf area), spiromesifen (2.67 and 3.33 mites cm^{-2} leaf area) and standard check dicofol (2.55 and 2.67 mites cm^{-2} leaf area) were

Table 2. Efficacy of treatments against active stages of *T. urticae* on okra during season I

Treatments	Mean no. of mites cm^{-2} leaf area				Mean
	1 DBT †	3 DAT ††	7 DAT ††	10 DAT††	reduction (%)
Hirsutella thompsonii @10^7spores ml^{-1}	35.11(5.92) ^{a}	39.44(6.32)^{ab}	36.56(6.01) ^{a}	17.28(4.21)^{ab}	24.57
Beauveria bassiana @10^7spores ml^{-1}	34.18(5.76) ^{a}	18.59(4.28)^c	16.89(4.12)^b	30.63(5.07)^{ab}	46.54
Neem oil 2%	39.78(5.79) ^{a}	12.33(3.55)^{cd}	27.30(5.12)^{ab}	37.96(6.02)^{ab}	37.26
NSKE 5%	33.78(5.83) ^{a}	28.04(5.11)^{bc}	23.15(4.84)^{ab}	57.22(7.26) ^{a}	12.33
Spiromesifen 240 SC @ 100 g a.i. ha^{-1}	21.96(4.64) ^{a}	2.67(1.69)^{de}	3.33(2.12)^c	13.26(3.34) ^{b}	84.43
Fenazaquin 10 EC @ 125 g a.i. ha^{-1}	28.41(5.37) ^{a}	1.63(1.38)^e	1.96(1.60) ^{c}	6.70(2.63) ^{b}	91.68
Diafenthiuron 50 WP @ 400 g a.i. ha^{-1}	68.48(7.83) ^{a}	3.52(1.98)^{de}	2.55(1.36) ^{c}	6.85(2.83) ^{b}	89.55
Standard Check (Dicofol 18.5 EC @ 250 g a.i. ha^{-1})	32.34(5.71) ^{a}	2.55(1.64) ^{de}	2.67(1.74) ^{c}	6.56(2.65) ^{b}	90.47

DBT = Day Before Treatment; DAT = Days After Treatment ; Means followed by same letters do not differ significantly by DMRT (p = 0.05) ; Mean reduction- Mean reduction over untreated control; †- Values in the parenthesis are square root transformed values, ††- Values in the parenthesis are adjusted means of square root transformed values based on ANOCOVA

found to be on par with fenazaquin. Similar trend was observed at 10 days after treatment application, where the chemical acaricides continued to record lower mite population, with dicofol recording the lowest count (6.56 mites cm^{-2} leaf area). Fenazaquin (6.70 mites cm^{-2} leaf area), diafenthiuron (6.85 mites cm^{-2} leaf area) and spiromesifen (13.26 mites cm^{-2} leaf area) were found to be on par with dicofol. Three days after treatment, neem oil recorded a lower mean population of 12.33 as against 28.04 mites per cm^2 leaf areain case of NSKE. However both the botanicals were on par with each other. The mite population was found to be significantly lower in *B. bassiana* treated plots (18.59) as against 39.44 per in *H. thompsonii* treated plots and was on par with the botanicals. At seven and ten days after spraying both botanicals and acaropathogens were found on par with the untreated control. Ten days after treatment, fenazaquin recorded the highest per cent reduction in the mite population (91.68%), closely followed by dicofol (90.47%) and diafenthiuron (89.55%) The next best treatment was spiromesifen with a mean per cent reduction of 84.43 over untreated control. The botanicals, neem oil and NSKE recorded 37.36 per cent and 12.33 per cent reduction in mite population, while bioagents, *B. bassiana* and *H. thompsonii* reduced the mite population to the level of 46.54 and 24.57 per cent respectively.

Efficacy of treatments against T. urticae on okra in Season II

The mean population of active stages of *T. urticae* before imposing the treatments ranged from 44.67

Table 3. Efficacy of treatments against active stages of *T. urticae* on okra during season II

Treatments	Mean no. of mites cm^{-2} leaf area				Mean
	1DBT†	3DAT††	7DAT††	10DAT††	reduction (%)
Hirsutella thompsonii @10^7spores ml^{-1}	44.67(6.70) ^{a}	26.77(4.69)^{bc}	29.30(5.44)^{ab}	17.78(4.21)^{ab}	51.62
Beauveria bassiana @10^7spores ml^{-1}	71.48(8.47) ^{a}	31.00(5.75)^b	26.41(5.17)^{ab}	11.33(3.30)^{bc}	54.96
Neem oil 2%	64.71(8.02) ^{a}	18.89(4.29)^{cd}	7.59(2.83)^c	2.29(1.65)^d	81.15
NSKE 5%	71.85(8.29) ^{a}	33.74(5.89)^b	20.33(4.53)^b	18.00(4.29)^{ab}	52.78
Spiromesifen 240 SC @ 100 g a.i. ha^{-1}	76.26(8.75) ^{a}	18.19(4.49)^{bc}	1.67(1.43) ^{c}	2.41(1.64) ^{d}	85.41
Fenazaquin 10 EC @ 125 g a.i. ha^{-1}	63.86(7.93) ^{a}	17.63(4.15) ^{cd}	1.15(1.24)^c	1.04(1.24) ^{d}	87.01
Diafenthiuron 50 WP @ 400 g a.i. ha^{-1}	59.33(7.59) ^{a}	14.63(3.60)^{cd}	1.78(1.51) ^{c}	1.15(1.31) ^{d}	88.50
Standard Check (Dicofol 18.5 EC @ 250 g a.i. ha^{-1})	69.07(8.08) ^{a}	8.11(2.89)^d	5.81(2.32)^c	3.37(1.83) ^{cd}	88.67
Untreated Control	78.59(8.80) ^{a}	73.70(8.84)^a	45.56(6.58)^a	33.37(5.67)^a	-

DBT = Day Before Treatment; DAT = Days After Treatment ; Means followed by same letters do not differ significantly by DMRT (p = 0.05) ; Mean reduction- Mean reduction over untreated control; †- Values in the parenthesis are square root transformed values, ††- Values in the parenthesis are adjusted means of square root transformed values based on ANOCOVA to 78.59 cm^{-2} leaf area. The results of the field experiment to evaluate the efficacy of different treatments against active stages of *T. urticae* in season II (Nov. 2012–Feb. 2013) are presented in Table 3. At three days after treatment application the lowest mite population of 8.11 cm^{-2} leaf area was recorded in dicofol followed by diafenthiuron (14.63), fenazaquin (17.63) and spiromesifen (18.19). However these chemicals were also on par with each other. At seven and ten days after treatment application, fenazaquin recorded the lowest mite population, (1.15 and 1.04 mites cm^{-2} leaf area) with diafenthiuron (1.78 and 1.15 mites cm^{-2} leaf area), spiromesifen (1.67 and 2.41 mites cm^{-2} leaf area) and dicofol (5.81, and 3.37 mites cm^{-2} leaf area) recording mite population on par with fenazaquin. At three, seven and ten days after treatment application the botanical, neem oil continued to record lower mite population (18.89, 7.59 and 2.29 mites cm^{-2} leaf area) compared to NSKE (33.74, 20.33 and 18.00 mites cm^{-2} leaf area) and was also on par with the new acaricide molecules and standard check.

However both the botanicals were superior over untreated control. At three days after spraying, the mean mite population was 26.77 in case of *H. thompsonii* as against 31.00 in *B. bassiana* and were superior over untreated control. However at seven and ten days days after spraying, the acaropathogens *H. thompsonii* (29.30 and 17.78 mites cm^{-2}) and *B. bassiana* (26.41 and 11.33 mite cm^{-2}) were found to be on par with each other and untreated control. The greatest reduction in mite population over untreated control was recorded by dicofol (88.67%) followed by diafenthiuron (88.50%), fenazaquin 10 EC (87.01%) and spiromesifen (85.41%). Among the botanicals, highest reduction in mite population was recorded by neem oil (81.15%) as against NSKE (52.78%). The acaropathogens reduced mite population to the tune of 54.96 per cent in case of *B. bassiana* and 51.62 per cent in case of *H. thompsonii*.

Though the new acaricide molecules, diafenthiuron 50 WP, fenazaquin 10 EC, and spiromesifen 240 SC showed a similar trend during the two seasons of study, the acaropathogenic fungi and botanicals differed in their performances in the two seasons tested against *T. urticae* and hence could not obtain consistent results. This may be due to the environmental factors that modify the microclimate of the crop, either favouring or adversely affecting the performances of the same. 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). Fenazaquin 10 EC was reported to cause 90.52 per cent mortality of adult mites of *T. macfarlanei* (Patil, 2005). In the present study, fenazaquin recorded significantly lower mite population for up to ten days after treatment application. In strawberry, fenazaquin was found to cause significantly higher mortality of *T. urticae* three days after the spray. After seven days, the same proved highly effective by maintaining the mite population under control, which extended up to 15 days of application (AINPAA, 2011). A similar trend in the efficacy of fenazaquin was evidenced in the present study also.

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 to be effective against active stages of spider mites (Marcic et al., 2011). Patil, (2005) found that use of diafenthiuron resulted in more than 96 per cent mortality of adult mites. Onkarappa and Puttaswamy (1999) reported that under polyhouse condition, diafenthiuron (300, 450 and 600 g a.i. ha^{-1}) could effectively reduce the population of *T. urticae* infesting okra which supports the findings of the present study. In both seasons, diafenthiuron consistently recorded higher per cent reduction in mite over untreated control showing its high efficacy against various active stages of the mite.

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). During the two seasons of study, spiromesifen consistently recorded lower mite counts up to ten days. Sato et al. (2011) reported that under field condition, a complete suppression of population of *T. urticae* could be achieved in ten days time using spiromesifen. 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 have contributed to its high efficacy in the field as observed in the present study.

In the study, new acaricide molecules have exhibited acaricidal activity on par with dicofol 18.5 EC. This indicates that in the present scenario of phasing out of toxic chemicals which are not safe to non-target organisms and environment, these new molecules can substitute dicofol with rotational use to avoid development of resistance and ill effects on environment.

Among the botanicals evaluated, the acaricidal activity of neem oil 2 per cent and NSKE 5 per cent on motile stages of *T. urticae* lasted only up to three days of treatment during season I. However, both neem oil 2 per cent and NSKE 5 per cent were found to perform better during season II. During this period, the acaricidal activity of neem oil was superior to that of NSKE and was on par with that of chemicals. The acaricidal activity of neem oil 2 per cent and NSKE 5 per cent on motile stages of *T. urticae* lasted up to ten days of treatment application. The varied effects of the active ingredient of neem based botanicals, *viz.,* azadirachtin, include 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). Among many botanicals tested in the polyhouse against *T. urticae* on rose, neem oil 2 per cent was found to be the most effective while NSKE 5 per cent was only moderately effective (Kumar, 2007) as was also found in the second season trial of present study. Since no consistent results could be drawn on these botanicals from the present study, further trials should be undertaken to obtain conclusive results. *Hirsutella thompsonii* is an acarinemyco pathogen which penetrates the mite's integument mainly through the legs and forms hyphal bodies in chains in the haemolymph (Gerson et al., 1979). *Beauveria bassiana*, is a filamentous entomopathogenic fungus, which causes white muscardine disease by invading directly through the cuticle (Sandhu et al., 2012). During both the seasons of study comparatively lower performance was observed in case of these pathogens and initiation of epizootics by these fungi. The prevalence of high temperature during the period of study averaging from 33-35^{0}C (meteorological data recorded at College of Horticulture, Vellanikkara) might have had a negative influence on the same which has been observed in previous studies as well (Bugeme et al., 2008; Gerson, et al., 1979). As a result, no consistent results have been obtained on their efficacy against *T. urticae* in the field.

Thus the present study highlighted that the new acaricide molecules *viz.,* fenazaquin 10 EC @ 125 g a.i. ha^{-1}, diafenthiuron 50 WP @ 400 g a.i. ha^{-1} and spiromesifen 240 SC @ 100 g a.i. ha^{-1} were highly effective against *T. urticae*, bringing significant reduction in mite population. Among the botanicals evaluated, neem oil 2 per cent was found to be superior to NSKE 5 per cent during the second season though on par with each other during the first season of study. Among the acaropathogenic fungi, *B. bassiana* was found to perform better than *H. thompsonii* during first season and was on par with each other during the second season.

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