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RESEARCH ARTICLE

EFFICACY OF FENAZAQUIN AGAINST SPIDER MITES AND ITS POTENTIAL TOXICITY TO NON-TARGET SPECIES

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Abstract: Fenazaquin is a new acaricide of the quinazoline group. It could be more hazardous to non-target species. Very scanty information is available on the efficacy of fanazaquin towards controlling spider mites and its toxicity effects over non-target species present in the environment. Present study was aimed to decipher information over these crucial aspects. Okra crop was grown in field following recommended agronomical practices. Fenazaquin was sprayed at recommended dose of 125 g a.i. ha^{-1} and double dose of 250 g a.i. ha^{-1}. It was observed that the efficacy of fenazaquin was significant in controlling the infestation of spider mites *Tetranychus urticae* Koch in okra crop at both applied doses. However, double dose showed significantly more detrimental toxicity effects towardscoccinellid beetlepredator*Stethorus punctillum*, pollinators *Apis mellifera*and soil acarine population. Recommended dose caused less toxicity to non-target species. It was found that fenazaquin application at recommended dose could provide effective control over *Tetranychus urticae* along-with maintaining good environmental health.

Keywords: Fenazaquin, Toxicity, *Stethorus punctillum*, *Apis mellifera*, Population

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) belongs to family malvaceae, is an important vegetable grown extensively in India. However, its export is very low because of increased domestic requirement and other limitations due to pest menace causing on an average 40% yield losses. The crop is attacked by a number of insect pests, diseases and mites, out of which, two spotted mite (*Tetranychus urticae* Koch) is major constraint in getting higher yields. Phytophagous mites due to their small size, high reproductive potential (*tetranychids*), survival on small quantity of food and habitat in small niches, increase their chances of survival and protect them against biotic/ abiotic stresses. Out of 73 phytophagous mite species, 28 species are considered as major and 45 species as minor pest status in various agricultural crops (Chhillar *et al*., 2007).

Plant feeding mites belong to five families namely *Tetranychidae*, *Tenuipalpidae*, *Eriophyidae*, *Tarsonemidae* and *Tuckerellidae*. These plant feeders cause various types of direct damages like loss of chlorophyll, appearance of stippling or bronzing of foliage, stunting of growth, severe defoliation and reduction in yield/ marketable produce and indirect damage by acting as vectors of plant diseases especially members of *Tetranychidae* and *Eriophyidae*, causing more loss to growers. The two spotted spider mite, *T. urticae* (Acari: Tetranychidae) is a cosmopolitan species found on a wide range of hosts including vegetable (Nandagopal and Gedia, 1995). It is reported to cause 10-15% loss in vegetables, among which okra is worst sufferers with 23-25% yield losses (Singh and Mukherjee, 1991; Gupta, 2003).The potentiality of this mite is increasing day by day. Fenazaquin 4[[4 (1,1dimethylethyl) phenyl] ethoxy]quinazoline, is a low dose emerging acaricide used to control mites attack in okra. It is very effective for the control of insect/mites' pests of okra crop. Its acute oral and dermal LD_{50} value for rat is 134/138 mg/kg body weight (male/female) respectively. Its maximum residue limit (MRL) for okra is 0.010ppm. Persistence of fenazaquin residues may cause sever toxicity in non-target species and associated environment. The extensive use of fenazaquin and its mammalian toxicity warrants for persistence of its toxic residues in open environment. It is a biologically active chemical and through movement in environment, it may contaminate ground and surface water bodies. Entering in the food chain, it may pose severe toxicity to humans, cattle, aquatic species, wild life and other non-target organisms.

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To achieve effective and eco-friendly control of mite populations, the application of fenazaquin should be safe for the predators too. A coccinellid beetle, *Stethorus punctillum* Weise, is a voracious feeder of *T.urticae* infesting okra. *S. punctillum* has been found highly susceptible to other pesticides (Antonelli *et al*., 1997).Honeybees, are important pollination vectors in okra and are responsible for 5-20% cross-pollination (Franklen and Galun, 1977). The pesticides, in general, are dangerous to honey bees as it drastically reduces the number of natural and managed bee pollinators, resulting in quality and quantity of crop yield, brood area and strength of bees in the colonies (Johanson *et al*., 1983). Similarly, pesticide application also leads to decline in soil acarine fauna, which are instrumental in the breakdown of litter and releaseof minerals in the soil (Crossley, 1977). Very scanty information is available onefficacy of fenazaquin against spider mites and its toxicity effects in non-target organisms.Therefore, the objectives of the present research were to evaluate the efficacy of fenazaquin in controlling spider mites and assessing its toxicity effects over non-target species like coccinellid beetle *S. punctillum*, honey bees and soil acarine fauna.

MATERIALS AND METHODS

Determination of fenazaquin efficacy against spider mites

A field trial was conducted to evaluate the effectiveness of fenazaquinagainst *T. urticae andS. punctillum*in okra crop under field conditions. Seeds of okra (cv. *Varsha Uphar*) were sown in three plots (3m x 3m) following recommended agronomical practices in randomized block design. One plot per treatment was maintained as control. The plant to plant and row to row spacing was maintained at 30 cm distance. Fenazaquin was sprayed at the application rate of 125 g a.i. ha^{-1} as single dose (T_1) and at 250 g a.i. ha^{-1} (T_2) as double dose for controlling two spotted mite i.e. *T.urticae* and predatory beetle i.e. *S. punctillum* on okra crop in different plots. Water treated plots acted as control. Before spraying, pretreatment count of *T. urticae* and *S. punctillum* was recorded from ten randomly selected plants/ plot. Ten okra plants were randomly selected from each of the three plots, every week in the field. Under stratified sampling, each plant was divided into three vertical strata i.e. top, middle and bottom strata. Two leaves from each strata per plant were collected in different labelled polybags, one bag for each plant. Observations on the number of live mites were recorded per square centimeter of leaf area whereas number of egg/ grub/ pupae/ beetle was counted per leaf from both dorsal and ventral surface of leaf. Mixed population was counted with the help of hand lens from two leaves each from top, middle and bottom strata.Post treatment observations were recorded after 1, 7 and 15 days on the number of live *T. urticae* mites, its predator *S. punctillum.*

Determination of fenazaquin toxicityagainst soil acarine fauna

To know the toxicity of fenazaquin against soil acarine fauna, three plots of size 3 x 3m were taken. Fenazaquin (Magister 10EC) was sprayed with the hand spray directly on the ground of the plot at an application rate of 125 g a.i. ha^{-1}(T_1) and 250 g a.i. ha^{-1} (T_2). Untreated plot was used for the control sampling. Post treatment observations was recorded after 0 (1h after treatment) and 72 hof spray for *Cryptostigmata, Mesostigmata, Astigmata and Prostigmata* spp. Composite soil samples were collected/ plot and the number of mites was expressed per 250 g soil. The number of mites was estimated through standard Berlese funnel method. Each soil sample was spread individually on 20 mesh metallic sieve kept on glass funnel and it was processed for 24h under 60 W electric bulb. Mites shifted to water containing glass vials placed below the glass funnel because of their photonegative behavior. Mites were counted under a stereo binocular microscope. Population fluctuation of soil mites were monitored in sprayed and unsprayed plots. With the help of trowel, three sub samples of soil measuring 100 g each were collected randomly from each of the three plots each measuring 3 × 3 m. All sub samples from one plot were pooled in enameled tray and mixed thoroughly with hands. 100 g of mixed soil was taken out and further processed for mite extraction.

Determination of fenazaquin toxicity against Apis mellifera

Okra was grown at the Research Farm, Department of Entomology, CCS Haryana Agricultural University, Hisar following normal recommended agronomical practices. The experiment was conducted in a randomized block design, replicated thrice in three plots, each of 9 sq. meters in size. Crop was sprayed with fenazaquin at an application rate of 125 g a.i. ha^{-1} as T_1 and at an application rate of 250 g a.i. ha^{-1}as T_2. One plot per treatment was maintained in the field. Third plot was sprayed with water to serve as control. Three foragers each of ten *A. mellifera* spp. were confined in wooden cages (15 x15 x 15 cm) fitted with wire mesh and placed among the crop plants. A sliding wooden plate acted as top cover of the cage. Cotton swab soaked in 50% sugar solution was also placed in each cage, which acted as food source for the base. The mortality of the bee foragers during insecticide treatment was recorded from such cages at interval of 0, 2 and 4 hours of application.

Contact and residual toxicity of fenazaquin in A. mellifera

Contact and residual toxicity of fenazaquin was estimated against pollinators, *A.mellifera* following the standard method Gulati *et al*., 2004.For assessing the residual toxicity, two methods were subjected for standardized to estimate the fenazaquin residues in

honey bees by performing recovery experiments. In first method, 10 g of honey bees were taken and homogenized using 5g of Na_2SO_4.The homogenized samples were transferred into the 250 mL Erlenmeyer flask followed by addition of 50 mL acetone and shaken over mechanical shaker for an hour for thorough extraction of residues from the honey bees. After shaking, the supernatant extract was filtered through Buchner funnel. The extract was concentrated to 10 mL on rotary vacuum evaporator. Concentrated extract was subjected to liquid-liquid partitioning after diluting with 100 mL of 4% aqueous solution of sodium sulphate and partitioned thrice with 40, 30 and 20 mL ethyl acetate. The organic phase was filtered through 5 cm layer of anhydrous sodium sulphate and further concentrated to 5 mL. For sample clean-up, a glass column (60 cm x 22mm i.d.) was compactly packed with florisil and silica gel (1:1 w/w) in between 1cm layer of anhydrous sodium sulphate. The glass columns were prewetted with hexane before loading concentrated filterate and elution of the column with 10 mL hexane: acetone (3:7 v/v). The eluate was concentrated first on rotary vacuum evaporator followed by gas manifold evaporator. Final volume was made to 2 mL in n-hexane for GC analysis.

In second method, Sep- Pack cartridges were used for standardizing the effective method for estimation of fenazaquin residues in honey bees. Representative 5 g sample of honeybees was homogenized with acetone and filtered through Whatmman filter paper No. 1. Pre conditioned the Sep- Pack cartridges (C-18 and HLB Envicarb) with methanol. Passed the filterate through conditioned cartridge and eluted with 5 mL dichloromethane. The eluate was concentrated to near dryness on gas manifold evaporator, made the final volume to 2 mL in n-hexane and analysed on GC-NPD system.

Quantification of Residues

Estimation of micro quantities of fenazaquin residues in honey bees was carried out by using a gas chromatograph (GC) (Shimadzu Model 2010) equipped with a nitrogen phosphorus detector (NPD) and fitted with HP-1, capillary column (30 m x 0.32 mm x 0.25 µm film thickness) of 5% diphenyl and 95% dimethyl polysiloxane. The operating parameters were as follows: column, temperature 100 ºC (1 min hold) was increased at 10 ºC min^{-1} to 200 ºC (0 min hold) followed by an increase in temperature at 20 ºC min^{-1} to 260 ºC (3 min hold). Injection port temperature was 270 ºC; whereas, detector temperature was maintained at 275 ºC. The carrier gas (N_2) flow was maintained at2 mL min^{-1} through the column with split ratio of 1: 10. The retention times observed for fenazaquin was 14.38 min.

RESULTS AND DISCUSSION

Standardization of analytical techniques for the estimation of fenazaquin residues

Before estimation of micro-quantities, the fenazaquinfenazaquinwas standardized prior to taking up analysis of test samples. The calibration curve of fenazaquin (peak area versus concentration) was found to be linear in the concentration range of 0.0001 to 1.0 µgmL^{-1}, with R^2> 0.976. With a %RSD of less than 5, ten successive injections of each concentration indicated outstanding repeatability. The limit of detection (LOD) and limit of quantification (LOQ) were 0.0005and 0.001 µg/g, respectively. The matrix effect (1.08–2.11%) was found to be less than the indicated maximum threshold limit of 20%. Recoveries of fenazaquin in honey bees fortified at 0.25 and 0.50 µgmL^{-1}levels by using method Iwere 50.02 ± 3.20 and 62.58 ± 2.15%, respectively with overall recovery of 56.19% and correction factor 1.77. Keeping in view the analytical standards, the recoveries were not considered to be satisfactory.In method II, average recoveries of fenazaquin in honey bees fortified at 0.25 and 0.50 µgmL^{-1} levels were 76.19 ± 1.20 and 98.37 ± 1.50%, respectively with overall recovery of 87.28% and correction factor 1.14.The recovery methods used for final sample preparation meet the standards outlined in the analytical method recommendations of the European Union Reference Laboratories for Pesticide Residues, EURL.

Efficacy against T. urticae

The evidence of acaricidal property of fenazaquin was given by the fact that both the treatments were effective in reducing the *T. urticae* population (Table 1) as compared to water spray which acted as control. The pretreatment count was 19.6 to 20.6 mites sq.cm^{-1} leaf in both the treatments. Spraying with 125 and 250 g a.i ha^{-1} of fenazaquin resulted in 100% reduction up to seven and fifteen days after spray, respectively. Among the treatments, significantly higher reduction was recorded at 250 g a.iha^{-1} followed by 125 g a.i ha^{-1}. In all the treatments, post treatment count was significantly lower than the pretreatment count at all the durations, however, the number of *T. urticae* recorded were statistically comparable at 1 and 7 days after treatment. Significant differences in number of live mites were observed after 15 days of treatment (CD= 0.18; p=0.05) as compared to other durations.Statistical analysis done through ANOVA suggested that interaction between treatment and duration was non-significant.

Devine *et al*., (2001);Mahajan and Nath, (2002); Kim *et al*., (2004); Ashley *et al*., (2006); Kim *et al*., (2006) reported toxicity of several acaricides to *Panonychus ulmi, T. urticae* and the predatory mite, *Amblyseius longispinosus*. Fenazaquin was found to be most toxic to both species of mites. The toxicity persisted for 15 days under field conditions. There

were variations in acaricide toxicities to different populations of mite showing built up of resistance and differences in the two species of mite. Mahajan and Nath, (2002) studied intrinsic toxicity of some acaricides against two spotted spider mite, European red mite and a predatory mite, *A. longispinostus*. Agaisnt adult *T. urticae*, fenazaquin was found to be most toxic and the order of toxicity was fenazaquin > garlic oil > dicofol > chlorpyriphos > azadirachtin > monocrotophos > bifenthrin > fenpyroximate > endosulfan by leaf dip and slide dip methods. Fenazaquin and dicofol were highly toxic to the eggs of both *T. urticae* and *P. ulmi*. The toxicity of different acaricides by leaf dip method to adults of predatory mite, *A. longispinosus* showed that fenazaquin and endosulfan were slightly toxic; whereas, bifenthrin, chlorpyriphos, fenpyroximate, monocrotophos, lndane and dicofol were highly toxic. This specific acaricide/insecticide has generally no effect on beneficial insects including predaceous mites (Hollingworth *et al*., 1992) and thus offers a desirable reason for its use in developing new strategies of integrated pest management in tea. Thus, the present results corroborate the earlier findings.

Table 1. Efficacy of fenazaquin against two spotted mite, *T. urticae* Koch

Dose	Average number of mites /leaf (sq. cm) after days of treatment				Mean (Reduction after treatment)
	Pre-treatment	1DAT	7DAT	15DAT	
125 ga.i. ha^{-1}	20.6	0	0	0.6	(2.33)
(T_1)	(4.74)	(1.41)	(1.41)	(1.77)	
250 ga.i. ha^{-1}	19.6	0	0	0	(2.25)
(T_2)	(4.63)	(1.41)	(1.41)	(1.41)	
Mean	(4.69)	(1.41)	(1.41)	(1.65)	

DAT: Days after treatment; Figures in parentheses are √n+1 transformation
CD (p=0.05): Dose=NS; Time: 0.189; Dose x Time = NS

Efficacy against S. punctillium

The effect of two doses of fenazaquin was also tested against its predator, *S. punctillum* (Table 2). The natural population of *S. punctillum* in different treatments was inthe range of 2.4 to 3.0 beetles per leaf. Among the treatments, *S. punctillum* population at single dose was significantly reduced to 0.20, 1.40 and 1.40 beetle per leaf withinone, seven and fifteen days after treatment, respectively in comparison to 1.13 beetles per leaf in control treatment. Likewise at higher dose, number of beetles/leaf was reduced to 0.40, 0 and 0 at 1, 7 and 15 days after treatment, respectively. The difference among the treatments were statistically significant (CD=0.078; p=0.05) which suggested significant reduction in beetle population at higher dose (250 g $a.i.ha^{-1}$) than at lower dose (125 g a.i. ha^{-1}). Interaction between dose and time was also significant.

Stethorus spp. is the predators of tetranychid mites so their seasonal abundance is directly correlated with the incidence of mite pests. As tetranychid mites flourish well in summer season, so is the beetle. From the present results, it becomes clear that application of fenazaquin remained effective up to 15 days particularly at T_2. Contrary to present results, *S. punctillum* was found highly susceptible to pesticides/acaricides like permethrin, dicofol and fenvelarate (Hull *et al*., 1985), bifenthrin (Antonelli *et al*., 1997). Abamectin, fenoxycarb, methomyl and teflubenzuron were found to be toxic to different developmental stages of *S. punctum* but it was tolerant to all organophosphate insecticides tested (Biddinger and Hull, 1995). Malathion had low toxicity to *S. punctillum* (Antonelli *et al*., 1997).

Table 2. Efficacy of fenazaquin against coccinellid predator *S. punctillium*

Dose	Average number of mites /leaf (sq. cm) after days of treatment				Mean (Reduction after treatment)
	Pre-treatment	1DAT	7DAT	15DAT	
125 ga.i. ha^{-1}	3.00	0.20	1.40	1.40	(2.17)
(T_1)	(2.23)	(2.14)	(2.14)	(2.18)	
250 ga.i. ha^{-1}	2.40	0.40	0.00	0.00	(1.61)
(T_2)	(2.09)	(1.54)	(1.41)	(1.41)	
Mean	(2.16)	(1.84)	(1.77)	(1.80)	

DAT: Days after treatment; Figures in parentheses are √n+1 transformation;
CD (p=0.05): Dose=0.078; Time: 0.111; Dose x Time = 0.156

Efficacy against A. mellifera

Fenazaquin at 250 ga.i.ha^{-1} dose was more detrimental to honeybees *A. mellifera* as it caused higher mortality (44.86%) within 4h of spray in comparison to 33.56% mortality at T_1 dose of 125 g a.i.ha^{-1} (CD:9.48;p=0.05) (Table 3). Initial mortality (just after spray) was significantly lower (7.99%) as compared to honeybee mortality at 2h (51.89%) and 4h (57.76%).Honeybee mortality at 2 and 4 h after spray was comparable with each other (CD=11.61; p =0.05). Lower dose (T_1) did not cause mortality immediately after spray but mortality increased to 45.4 and 72.2% after 2 and 4 h of spray. In case of T_2, 7.6 % mortality was recorded immediately after spray which increased to 76.9 to 100% after 2 and 4 h of spray.

Systemic insecticides, those that are incorporated by treated plants, can contaminate nectar or pollen, and kill bees in the hive. Stark *et al*. (1995) and Suchail *et al*. (2000) reported LD_{50} values for imidacloprid in the range of 6.7–23.8 ng/bee, Senn *et al*. (1998) estimated the LD_{50} for thiamethoxam as 24 ng/bee, and Elbert *et al*. (2000) reported that the LD_{50} for thiacloprid was 24.2 µg/bee. Sharma *et al*., 2000; Gulati and Kalra, (2003) reported heavy loss of forager population due to pesticide application on flowering crops.

Kumari *et al*., (2000) also reported mortality of oxydemeton-methyl, phosphamidon (insecticide/ acaricide) and dimethoate (insecticide /acaricide) from 46.4 to 64.5% within 20 h of spray and from 97.1 to 100% in 68 h of application of the mentioned pesticides. Residues of fenazaquin were found below detectable limit of 0.0005 µg/g in honey bees. However, method II may be more useful for sample preparation and residue analysis in honeybees. It could help researchers during future analysis.

Table 3. Efficacy of fenazaquin against honey bees *(A. mellifera)*

Dose	% Mortality			Mean (Dose)
	0 h	2 h	4 h	
125 ga.i. ha^{-1} (T_1)	0	45.4	72.2	(33.56)
250 ga.i. ha^{-1} (T_2)	7.6	76.9	100	(44.86)
Mean	(7.99)	(51.89)	(57.76)	

CD (p=0.05) dose= 9.48; time=11.61; dose x time=N.S.

Effect of fenazaquin on soil acarine fauna

The results (Table 4 and 5) clearly indicated that the acaricidal nature of fenazaquin as no mite were recorded within one hour of application at higher dose (250g a.i. ha^{-1}) in the soil as compared to 21 mites /100 g soil in pretreatment count. No mite was recorded after 72 h of soil application of fenazaquin. Lower dose (T_1) also significantly reduced the number of mites to 6 and 5 mites /100 g soil after 1 and 72 h of soil application (CD=1.08; p=0.05).At T_1(125 g a.i.ha^{-1}), mites recovered (10.0 mites/100g soil) were more in number as compared to the mites (7.0 mites/100g soil) recovered at T_2(250 g a.i.ha^{-1}) (CD=0.62; p=0.05). Duration wise, significant reduction in number of mites recorded were noticed irrespective of the dose (CD=0.76; p=0.05). After 1 and 72 h of soil application, number of mites was reduced to 3 and 2.5 mites/100 g soil in comparison to 21 mites/100g soil in pre-treatment count which was significantly higher than post-treatment count. After treatment, mites reduced significantly. Number of mites recovered after 1 and 72 h were statistically comparable with each other. Interaction between dose and time was significant.

Several workers (Banerjee and Roy, 1981; Banerjee, 1984) have also recorded maximum number of mites in soil. In present study, maximum population belonged to *Cryptostigmata* mite and the minimum population belonged to *Prostigmata*. Among the acarine fauna distribution of *Cryptostigmata* and *Prostigmata* constituting 62 and 26%, respectively of the total population has been reported by Harding and Stuttard (1974).

Table 4. Effect of fenazaquin on different species of soil acarine fauna

Treatment	Number of mites/100g soil				Total
	Cryptostigmata	Mesostigmata	Astigmata	Prostigmata	
Pre treatment	12	4	4	1	21

T_1 (125g a.i. ha^{-1}) (after 1h)	5	0	0	1	6 (28.57)
T_2 (250g a.i. ha^{-1}) (after 1h)	0	0	0	0	0 (100.00)
T_1 (125g a.i. ha^{-1}) (after 72h)	5	0	0	0	5 (23.80)
T_2 (250g a.i. ha^{-1}) (after 72h)	0	0	0	0	0 (100.00)

Figures in parentheses are % loss in soil acarine fauna

Table 5. Effect of fenazaquin on % mortality of soil acarine fauna

Hours after treatment	Mites (%)/100g soil		
Pretreatment	125 g a.i. ha^{-1} T_1	250 g a.i. ha^{-1} T_2	Mean
1h	21.00 (27.25)	21.00 (27.25)	21.00 (27.25)
72h	6.00 (14.15)	0.00 (0.00)	3.00 (7.07)
Mean	(18.09)	(9.08)	

Figures in parenthesis are angular transformed values for %
CD (p=0.05) dose=0.625; time=0.766; dose x time=1.08

CONCLUSION

Higher dose of fenazaquin at 250 g a.i. $ha^{-1}$was significantly more detrimental to mites, predators and other non-target organisms like honeybees and soil acarine population. However, recommended dose are comparatively less fatal for non-target species. Following good agricultural practices and application of fenazaquin at recommended dose of 125 g a.i. ha^{-1} could provide effective control against spider mites in okra along-with preventing toxicity to non-target organisms and associated environment.

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