



# Bioassays of Fenazaquin 18.3% w/w (200 SC) against Two Spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae): Effect of Test Method, Exposure Period and Mortality Criterion on the Precision of Response Estimates

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## Article Information

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## ABSTRACT

Quinazoline class of pesticide fenazaquin 18.3% w/w (200 SC) (IUPAC name: 4-tert-butylphenethyl quinazolin-4-yl ether) is used to manage mites and insects by interfering with the biochemistry of the pests' mitochondria. Three different bioassay methods viz., leaf dip, slide dip, and residual film

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were evaluated using fenazaquin 18.3% w/w (200 SC) at various doses on adults of *Tetranychus urticae* along with two standard checks (fenazaquin 10 EC and spiromesifen 22.90% SC) and water as control. After 24 h of exposure, fenazaquin 18.3% w/w (200 SC) @ 1.25 and 1.60 ml/l exhibited 100% mortality which was comparably higher than the standard checks. The results revealed that all the doses of fenazaquin 18.3% w/w (200 SC) were superior for the management of two spotted spider mites.

**Keywords:** Bioassays; fenazaquin 18.3% (200 SC); *Tetranychus urticae*; mortality.

## 1. INTRODUCTION

*Tetranychus urticae* Koch (Acari: Tetranychidae), often known as the two spotted spider mite is regarded as a significant pest as it affects hundreds of wide varieties of crops [1,2,3,4]. They feed on the epidermal cells of the leaves which lower the amount of chlorophyll, stomatal conductance and photosynthetic rate [5]. Under severe infestations, each of these variables results in chlorotic areas, tanning and leaf abscission [6,7]. The two spotted spider mites led to a yield reduction of 7 to 48% was recorded in various crops such as bhendi [8,9], brinjal [10,11], cucumber [12], tomato [13] and potato [14]. The two spotted spider mites caused havoc both in protected areas and in field conditions [15]. On a global scale, the two spotted spider mite population has the capacity to quickly evolve pesticide resistance was well documented [16]. *T. urticae* has traits of rapid population expansion, high fecundity and haplo-diploid sex determination that favours the risk of pesticide resistance [3,17].

Despite other management approaches, synthetic acaricides are still a crucial and more reliable component for *T. urticae* management [18]. It is critical to select and apply ecologically selective acaricides and rotate acaricides with different classes of chemistry that can manage the two spotted spider mites and delay the development of resistance. Bioassay is a procedure used to determine the relationship between a physiologically active agent and its effect on living organisms [19,20]. It is essential to adopt bioassay methods based on the pest and criteria to be measured to

superimpose the results for the field conditions [21].

This research is an initial attempt to assess the effectiveness of fenazaquin 18.3% w/w (200 SC) against two spotted spider mites under laboratory conditions.

## 2. MATERIALS AND METHODS

### 2.1 Rearing of Two Spotted Spider Mites

*Tetranychus urticae* collected from infested castor plants in Sivapuri village (Lat 11.3838092°N and Long 79.7092702°E) were reared on 30-day-old cowpea (*Vigna unguiculata* L. cv. Paiyur 1) seedlings at 25 ± 1°C and 75 ± 5% and used as a stock culture. Fresh cowpea seedlings were added at weekly intervals to ensure the continuous supply of test mites [22,23].

### 2.2 Bioassays

#### 2.2.1 Leaf dip bioassay

Tomato leaves dipped in each treatment for 30 secs were allowed to dry at room temperature and placed in petri dishes (9 cm diameter) containing the moistened cotton pad to maintain the turgidity. Ten adult mites were released onto the surface of each leaf under a stereo zoom microscope with a fine camel hair brush (size: 000). A barrier composed of damp cotton wool was arranged around the leaf in each petri dish to confine the mites on the leaf. Each treatment was replicated four times. Mortality was recorded 24, 48 and 72 hours after treatment (HAT). The mortality criterion used for this method was an inability to move when lightly prodded [24,25].

**Table 1. The following treatments were used in this experiment**

S. No.	Treatments	Dose (ml/l)	Source
1.	Fenazaquin 18.3% w/w (200 SC)	1.00	Gowan India Pvt. Ltd, Gurugram
2.	Fenazaquin 18.3% w/w (200 SC)	1.25	Gowan India Pvt. Ltd, Gurugram
3.	Fenazaquin 18.3% w/w (200 SC)	1.60	Gowan India Pvt. Ltd, Gurugram
4.	Standard Check-1 Fenazaquin 10 EC	2.50	Corteva Agriscience, Hyderabad
5.	Standard Check-2 Spiromesifen 22.90% SC	0.80	Bayer Crop Science, Mumbai
6.	Control (Water)	-	-

## 2.2.2 Slide dip bioassay

The slide dip bioassay method proposed by Chillar et al. [24] was followed with minor modifications. A piece of double-sided adhesive tape (size: 2 Sq.cm) was affixed to the right-side corner of the glass slides. Ten adult mites were adhered dorsally on each slide under a stereo zoom microscope using a fine camel hair brush (size: 000). Slides adhered with mites were immersed in each treatment for 5 secs. Excess fluid on both the glass slides and adhesive tape was wiped with absorbent paper. Slides were dried at room temperature for 15 min and transferred to a controlled environment chamber (25 ± 1°C, 75 ± 5% relative humidity and 16:8 hours light: dark photoperiod). Mortality was recorded 24 HAT of treatment and each treatment was replicated four times. The mortality criterion used for this method was an inability to move a leg when lightly prodded [24].

## 2.2.3 Residual film bioassay

One ml of the respective treatments was placed in each petri dish (9 cm diameter). The Petri dish was closed tightly and swirled for 5 secs in both upright and inverted positions. Excess fluid was drained off and allowed to dry at room temperature for an hour. Ten adult mites were transferred to each petri dish under a stereo zoom microscope using a fine camel hair brush (size: 000) and placed in a controlled environment chamber (25 ± 1°C, 75 ± 5% relative humidity and 16:8 hours light: dark photoperiod). Each treatment was replicated four times and mortality was recorded 24 HAT using a stereo zoom microscope. The mortality criterion used for this method was an inability to move when lightly prodded [20,24].

## 2.3 Statistical Analysis

Mortality data were corrected using Abbott's formula [26] Data were evaluated using analysis of variance (ANOVA) under a completely randomized design (CRD). The values in the parentheses were arc sine transformed. The significant differences among means were determined by Duncan's Multiple Range Test (DMRT) [27,28]. Statistical analyses were performed using CCARI-ICAR WASP 2.0.

$$\text{Corrected per cent mortality} = \frac{Po - Pc}{100 - Pc} \times 100$$

Where;

Po- Observed mortality in the treatment

Pc- Observed mortality in the control

## 3. RESULTS

### 3.1 Effect of Leaf Dip Bioassay on Adults of *T. urticae*

Maximum reduction in the survival rate of *T. urticae* was noticed at higher doses of fenazaquin. Fenazaquin 18.3% w/w (200 SC) @ 1.60 and 1.25 ml/l resulted in 100% mortality after 24 h of exposure, followed by standard check-1 fenazaquin 10 EC @ 2.50 ml/l (92.50%), fenazaquin 18.3% w/w (200 SC) @ 1.00 ml/l (85%) and standard check-2 spiromesifen 22.9 % SC @ 0.80 ml/l (70%). The mite mortality increased in all the treatments after 48 h of exposure. After 48 h of exposure, fenazaquin 18.3% w/w (200 SC) @ 1.00 ml/l recorded 97.50% mortality, which was comparable to standard check-1 fenazaquin 10 EC @ 2.50 ml/l (95%). Whereas mortality in the control was mere 2.50% up to 48 h of treatment. After 72 h of exposure, fenazaquin 18.3% w/w (200 SC) @ 1.00 ml/l recorded 100% mortality, compared to 10% with control (Table 2).

The order of efficacy of the treatments was fenazaquin 18.3% w/w (200 SC) @ 1.60 ml/l = fenazaquin 18.3% w/w (200 SC) @ 1.25 ml/l > standard check-1 fenazaquin 10 EC @ 2.50 ml/l > fenazaquin 18.3% w/w (200 SC) @ 1.00 ml/l > standard check-2 spiromesifen 22.9 % SC @ 0.80 ml/l (Table 2).

### 3.2 Effect of Slide Dip Bioassay on Adults of *T. urticae*

After 24 h of exposure, fenazaquin 18.3% w/w (200 SC) @ 1.60 and 1.25 and 1.00 ml/l and standard check-1 fenazaquin 10 EC @ 2.50 ml/l resulted in 100% mortality followed by standard check-2 spiromesifen 22.9% SC @ 0.80 ml/l (80%) and control (10%) (Table 3).

The order of efficacy of the treatments was fenazaquin 18.3% w/w (200 SC) @ 1.60 ml/l = fenazaquin 18.3% w/w (200 SC) @ 1.25 ml/l = fenazaquin 18.3% w/w (200 SC) @ 1 ml/l = standard check-1 fenazaquin 10 EC @ 2.50 ml/l > standard check-2 spiromesifen 22.9 % SC @ 0.80 ml/l (Table 3).

**Table 2. Response of *T. urticae* adults on various exposure times for fenazaquin 18.3% w/w (200 SC) in leaf dip bioassay**

S. No.	Treatments	Dose (ml/l)	*Adult Mortality (%)			Mean (%)
			24 HAT	48 HAT	72 HAT	
T <sub>1</sub>	Fenazaquin 18.3% (200 SC)	1.00	85.00 (70.38) <sup>bc</sup>	97.50 (85.18) <sup>a</sup>	100 (89.71)	94.17
T <sub>2</sub>	Fenazaquin 18.3% (200 SC)	1.25	100.00 (89.71) <sup>a</sup>	100.00 (89.71) <sup>a</sup>	100 (89.71)	100.00
T <sub>3</sub>	Fenazaquin 18.3% (200 SC)	1.60	100.00 (89.71) <sup>a</sup>	100.00 (89.71) <sup>a</sup>	100 (89.71)	100.00
T <sub>4</sub>	Standard Check – 1 Fenazaquin (10 EC)	2.50	92.50 (81.48) <sup>ab</sup>	95.00 (83.14) <sup>a</sup>	100 (89.71)	95.83
T <sub>5</sub>	Standard Check – 2 Spiromesifen (22.90% SC)	0.80	70.00 (56.95) <sup>c</sup>	90.00 (76.57) <sup>a</sup>	97.5 (85.18)	85.83
T <sub>6</sub>	Control (Water)	-	2.50 (4.82) <sup>d</sup>	2.50 (4.82) <sup>b</sup>	10.00 (15.93)	5.00
CD (p=0.05)			14.64	14.45	8.70	-
SE(d)			7.00	6.88	4.14	-

HAT – Hours After Treatment; \*Mean of four replications; Figures in parentheses are arc sine transformed values; In a column, means followed by the same letters do not differ significantly by DMRT ( $p=0.05$ )

**Table 3. Response of *T. urticae* adults for fenazaquin 18.3% w/w (200 SC) in slide dip bioassay**

S. No.	Treatments	Dose (ml/l)	* Adult Mortality (%)
			24 HAT
T <sub>1</sub>	Fenazaquin 18.3% w/w (200 SC)	1.00	100 (89.71) <sup>a</sup>
T <sub>2</sub>	Fenazaquin 18.3% w/w (200 SC)	1.25	100 (89.71) <sup>a</sup>
T <sub>3</sub>	Fenazaquin 18.3% w/w (200 SC)	1.60	100 (89.71) <sup>a</sup>
T <sub>4</sub>	Standard Check - 1 Fenazaquin (10 EC)	2.50	100 (89.71) <sup>a</sup>
T <sub>5</sub>	Standard Check - 2 Spiromesifen (22.90% SC)	0.80	80 (63.81) <sup>b</sup>
T <sub>6</sub>	Control (Water)	-	10 (18.44) <sup>c</sup>
CD (p=0.05)			3.67
SE(d)			1.75

HAT – Hours After Treatment; \*Mean of four replications; Figures in parentheses are arc sine transformed values; In a column, means followed by the same letters do not differ significantly by DMRT ( $p=0.05$ )

**Table 4. Response of *T. urticae* adults for fenazaquin 18.3% w/w (200 SC) in residual film bioassay**

S. No.	Treatments	Dose (ml/l)	*Adult Mortality (%)
			24 HAT
T <sub>1</sub>	Fenazaquin 18.3% w/w (200 SC)	1.00	92.50 (76.10) <sup>bc</sup>
T <sub>2</sub>	Fenazaquin 18.3% w/w (200 SC)	1.25	100.00 (89.71) <sup>a</sup>
T <sub>3</sub>	Fenazaquin 18.3% w/w (200 SC)	1.60	100.00 (89.71) <sup>a</sup>
T <sub>4</sub>	Standard Check - 1 Fenazaquin (10 EC)	2.50	97.50 (85.18) <sup>ab</sup>
T <sub>5</sub>	Standard Check - 2 Spiromesifen (22.90% SC)	0.80	87.50 (69.53) <sup>c</sup>
T <sub>6</sub>	Control (Water)	-	7.50 (13.90) <sup>d</sup>
CD (p=0.05)			9.85
SE(d)			4.69

HAT – Hours After Treatment; \*Mean of four replications; Figures in parentheses are arc sine transformed values; In a column, means followed by the same letters do not differ significantly by DMRT ( $p=0.05$ )

### 3.3 Effect of Residual Film Bioassay on Adults of *T. urticae*

Fenazaquin 18.3% w/w (200 SC) @ 1.60 and 1.25 ml/l resulted in cent per cent mortality after 24 h of exposure, followed by standard check-1 fenazaquin 10 EC @ 2.50 ml/l (97.50%) and fenazaquin 18.3% w/w (200 SC) @ 1 ml/l (92.50%) and standard check-2 spiromesifen 22.9 % SC @ 0.80 ml/l (87.50%) with 7.50 per cent mortality in control (Table 4).

The order of efficacy of the treatments was fenazaquin 18.3% w/w (200 SC) @ 1.60 ml/l = fenazaquin 18.3% w/w (200 SC) @ 1.25 ml/l > standard check-1 fenazaquin 10 EC @ 2.50 ml/l > fenazaquin 18.3% w/w (200 SC) @ 1 ml/l > standard check-2 spiromesifen 22.9 % SC @ 0.80 ml/l (Table 4).

### 4. DISCUSSION

Fenazaquin exhibited a significant effect on tetranychid adults [29]. Fenazaquin 20% SC @ 0.335 ml/l resulted in 100% mortality against *T. urticae* after 24 h of treatment [30]. Fenazaquin 10 EC @ 1.7 ml/l recorded 100% mortality of *T. urticae* after 24 h of exposure [31]. Chlorfenapyr 10 SC @ 1.5 ml/l reported up to 100% mortality after 72 h of treatment [32,33]. The mortality rate of *T. pueraricola* treated with 6.67 g/L GC16 (a novel pesticide) was greater than 80% in the slide dip method and was significantly higher compared to the leaf-dip method [34]. In contrast the population of *T. urticae* survived 48 h of residual contact in the slide dip method with 10,000 ppm (10 ml/l) of dicofol [35]. Mites exposed for more than 24 h had high mortality in control which may be due to a lack of food [20]. In our experiment, 10% mortality was detected in the control after 24 h of exposure in the slide dip method. Residual film bioassay was introduced more than three decades before and found more reliable indicators of mite survival under field circumstances in comparison to topical bioassays like the slide dip [36,37,38,39]. Permethrin @ 50 ppm resulted higher mortality in the residual film (76%) than the slide dip (53%) [40]. In contrast, our findings revealed that higher mortality was noticed in the slide dip method as compared to the residual film method in various treatments. Because, the toxicity of miticides was method dependent. Fenpyroximate @ 50g/l recorded higher mortality in slide dip bioassay than residual film bioassay after 24 hours of exposure [41] which was in line with our research.

### 5. CONCLUSION

The present findings unambiguously demonstrated that, though the various doses of fenazaquin evaluated exhibited higher mortality of *T. urticae*, suspension concentrate formulation was found superior over emulsifiable concentration. The behaviour of SC and EC formulations are different as the particle size of each formulation is different. The fenazaquin 18.3% w/w (200 SC) formulations are a suspension of fenazaquin particles in water whilst the fenazaquin 10 EC is dissolved in hydrocarbon solvents. Since this study was conducted in laboratory, further research is needed to verify these results under field conditions.

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### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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