Acaricide resistance among broad mite (*Polyphagotarsonemus latus* **(Banks)) populations in Karnataka, India**

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The broad mite, *Polyphagotarsonemus latus* **(Banks), is a cosmopolitan pest that attacks a wide range of economically important crops like hot and sweet peppers, mulberry, jute, tea and several ornamentals. This study was undertaken to monitor the development of acaricide resistance, if any, in five representative field-collected populations of Karnataka, India. Bioassays were carried out against five acaricide chemistries, and resistance** ratios were calculated by comparing the LC₅₀ values of **field populations with the susceptible laboratory population (Pa-Lab). The resistance ratios varied from 26.03 to 81.16-fold for diafenthiuron, 27.35 to 83.47-fold for dicofol, 9.72 to 45.42-fold for fenazaquin, 8.77 to 16.84 fold for propargite and 48.37 to 163.39-fold for spiromesifen. Resistance to the acaricides was unstable in** *P. latus* **as a decline in resistance (14.11–102.53-fold) was observed over generations in the absence of selection pressure. The results suggest that acaricides should be sprayed at economic threshold levels or on a rotation basis for one or more seasons for better management of** *P. latus* **by delaying the development of resistance.**

Keywords: Acaricide, bioassay, *Polyphagotarsonemus latus*, stability, susceptibility.

MITE infestations on crops have become an alarming concern in recent years, especially in the tropics and subtropics¹. This can be attributed to several factors, viz. climate change, rapid host expansion capability, development of acaricide resistance, withdrawal of broad-spectrum pesticides from the markets that were used against the multi-resistant pests, or a combination of these factors. Specifically, the broad mite or yellow mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) has emerged as a major pest attacking several crops of agricultural and horticultural importance^{2,3}. Originally it was reported in 1890 from tea plants in Sri Lanka but later was distributed to other countries through trade and commerce. Currently, it is a well-established pest in all six zoogeographical realms of the world, including Australia, Asia, Africa, North America, South America and

the Pacific Islands⁴. It flourishes well and multiplies faster under tropical, subtropical and greenhouse habitats. The broad mites are known to damage over 250 host plants belonging to 57 families, including hot and sweet peppers, mulberry, jute, tea, sesame, cotton and several ornamentals where the feeding of this pest habitually compromises the $yield^{2,3,5-7}.$

The larval and adult stages feed on the sap from the young foliage and budding tips leaving the newest growths severely damaged and plant growth suppressed⁸. The typical symptoms noticed on chilli and capsicum are bronzing, crinkling and downward curling of the leaves, giving an inverted boat-shaped appearance, elongation of the petiole, crumpled apical shoot, development of abnormal side shoots, thickening of leaves and stunted growth⁹. The damage due to mites in chilli had been estimated to the tune of 60% and can cause 100% yield loss under polyhouse conditions¹⁰.

The utility of host plant resistance and biocontrol agents like predatory mites, fungi and bacteria against *P. latus* has been explored with little success. Hence, chemical control with synthetic acaricides is a common management strategy followed by farmers in India. More than two dozen acaricides/insecticides alone or in combination under 12 different modes of action are registered for official use in India to manage broad mites 11 . Due to its microscopic nature (<0.2 mm) and short generation time, the damage is caused even before the mite is detected. This necessitates the repeated application of pesticides that would lead to the rapid development of resistance. Hence, the present study examined the development of resistance to major acaricides, if any, and the resistance stability in *P. latus* populations collected from Karnataka, India.

Material and methods

Mite colony maintenance

A colony of *P. latus* (National accession number NBAIR-GR-TAR-01a) has been maintained at ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, since July 2020. The colony is maintained in an insect

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growth chamber with a temperature between $25^{\circ} \pm 5^{\circ}$ C and relative humidity (RH) of $65\% \pm 5\%$ on potted mulberry plants (variety V1) containing a mixture of farmyard manure (FYM) and red soil in the ratio 1 : 2. This mite population, designated as Pa-Lab, has been retained for more than 70 generations without any exposure to acaricides.

The species identity of the mites was confirmed both by morphological⁴ and molecular¹² methods. Bioassays were conducted with the Pa-Lab population at regular intervals to monitor the progression of susceptibility in the absence of selection pressure. The median lethal doses (LC_{50}) estimated at the 70th generation were used as a reference for comparing the response of field populations to acaricides.

In order to evaluate the status of acaricide resistance in the field populations of *P. latus*, roving surveys were conducted from July 2020 to March 2022 in the major chilli-growing areas of Karnataka, where acaricides and insecticides were being used indiscriminately. The data on the frequency of acaricide application and the percentage of farmers reporting control failures were collected from each location from at least ten farmers. Mite populations were collected from five locations, viz. Bangalore (13.0713°N, 77.5905°E), Chikkaballapur (13.1432°N, 77.6428°E), Haveri (14.6834°N, 75.3849°E), Tumkur (13.4489°N, 77.1678°E) and Ramnagara (12.6254°N, 77.2319°E). The collected mites were released on uninfested mulberry plants under growth-chamber conditions for mass multiplication. Adult female mites from the F_1 generation were used in resistance monitoring bioassays.

Acaricides and bioassays

Five acaricides approved by the Central Insecticides Board and Registration Committee, India, for controlling broad mites representing different chemical groups were chosen¹¹. Commercial formulations of diafenthiuron 50% WP, dicofol 18.5% EC, fenazaquin 10% EC, propargite 57% EC and spiromesifen 22.9% SC were procured from local retailers. Different concentrations of each chemical that bracketed 5–95% mortality were recognized on the basis of preliminary bioassay studies. A minimum of five required concentrations were prepared by serial dilution from stock solutions of acaricide and used for bioassays.

The leaf-dip bioassay (method no. 4) standardized by the Insecticide Resistance Action Committee was adopted with appropriate modifications¹³. Fresh mulberry leaf discs were immersed in the respective test chemical solutions for 30 sec and allowed to air-dry at room temperature. An untreated control was maintained by dipping the leaf discs in distilled water. The leaf discs were placed on wet cotton wads in Petri plates that were kept moistened to maintain leaf disc turgidity and to check for the escape of mites. Thirty adult female mites were gently transferred onto each leaf disc and kept in a BOD incubator at $25^{\circ} \pm 1^{\circ}$ C temperature and $65\% \pm 10\%$ RH. Each concentration and the respective control treatments were replicated thrice, and observations on mortality were documented after 24 h of acaricide treatment. Moribund mites that were unable to show any signs of movement when probed with a fine brush were considered dead.

Statistical analysis

The median lethal concentrations (LC_{50}) and their 95% confidential limits (CLs) were determined by Probit analysis¹⁴ using Polo Plus 2.0 software¹⁵. For the susceptible strain, susceptibility indices were calculated on the basis of LC_{50} values obtained for the F_1 and F_{70} generations¹⁶.

Susceptibility index $SI = LC_{50}$ of F_1/LC_{50} of F_{70} .

Other parameters, namely the rate of resistance decline (*R*) and the number of generations required for a tenfold decrease in LC₅₀ (*G*), were calculated as described by Tabashnik¹⁷.

Response to selection, $R = \lceil \log(\text{final LC}_{50}) \rceil$ $-\log$ (initial LC₅₀)]/*n*.

The rate of resistance decline that quantifies the rate of change in LC_{50} when the selection pressure is withdrawn was estimated using the number of generations not exposed to insecticide (n) , LC₅₀ of the parental generations before *n* generations (initial LC_{50}) and LC_{50} after *n* generations without selection (final LC_{50}).

The number of generations (*G*) required for a tenfold decrease in the LC_{50} values was calculated using the formula: $G = 1/R$.

For the field populations, lethal concentration ratios (LCRs) at LC_{50} (LCR 50) and their 95% CLs were calculated, and tests for the hypotheses of equality and parallelism were performed as given by Robertson *et al*. 18. Further, resistance ratios (RRs) were calculated by dividing the LC_{50} value of the corresponding field population by that of the susceptible population. Based on the RR values, the intensity of resistance was categorized as low $(RR < 10)$, moderate $(10 < RR < 40)$, high $(40 < RR < 160)$ and extremely high $(RR > 160)^{19}$. To ascertain cross-resistance, pairwise correlation coefficients between the LC_{50} values of field populations for acaricides were evaluated by Pearson's correlation analysis and visualized using GraphPad prism.

Results

Field surveys and pest identity

Field surveys indicated partial to total control failure using acaricides, which was highest for spiromesifen (66.67%), followed by diafenthiuron (63.33), fenazaquin (58.33%), dicofol (55.56%) and propargite. On average, inadequate control of the mite with the selected acaricides was

Acaricide	Generation	LC_{50} (95% CL)	$Slope \pm SEM$	χ^2 (df)	Heterogeneity
Diafenthiuron	F_1	34.48 (20.576-56.520)	1.47 ± 0.18	4.47(3)	1.49
	F_{20}	$11.84(9.491-14.455)$	1.50 ± 0.16	1.12(3)	0.37
	F_{40}	$0.69(0.567-0.828)$	1.40 ± 0.12	2.55(4)	0.64
	F_{60}	$0.45(0.374 - 0.527)$	2.07 ± 0.21	0.43(3)	0.14
	F_{70}	$0.40(0.339 - 0.465)$	2.02 ± 0.18	1.17(3)	0.39
Dicofol	F_1	37.10 (25.008-58.153)	1.40 ± 0.16	3.63(3)	1.21
	F_{20}	$9.72(7.999 - 11.772)$	1.55 ± 0.14	2.64(4)	0.66
	F_{40}	$1.52(1.259 - 1.801)$	1.76 ± 0.17	1.02(3)	0.34
	\mathcal{F}_{60}	$0.77(0.646 - 0.916)$	1.82 ± 0.18	0.25(3)	0.19
	F_{70}	$0.70(0.585 - 0.832)$	1.92 ± 0.16	2.42(3)	0.81
Fenazaquin	F_1	$17.03(14.071 - 20.514)$	1.77 ± 0.19	2.46(3)	0.82
	F_{20}	$3.76(3.123 - 4.484)$	1.74 ± 0.17	1.21(3)	0.40
	F_{40}	$0.74(0.615 - 0.876)$	1.75 ± 0.16	0.62(3)	0.21
	F_{60}	$0.42(0.309 - 0.548)$	2.02 ± 0.19	3.01(3)	1.00
	F_{70}	$0.44(0.370 - 0.513)$	1.84 ± 0.17	0.34(3)	0.11
Propargite	F_1	$5.25(3.537 - 7.351)$	1.55 ± 0.16	3.30(3)	1.10
	F_{20}	$2.11(1.471 - 2.986)$	1.35 ± 0.14	4.32(4)	1.08
	F_{40}	$0.84(0.632 - 1.090)$	1.12 ± 0.13	3.72(4)	0.93
	F_{60}	$0.39(0.317 - 0.472)$	1.54 ± 0.14	1.48(4)	0.37
	F_{70}	$0.37(0.233 - 0.546)$	1.20 ± 0.13	4.95(4)	1.24
Spiromesifen	F_1	59.16 (38.207-97.568)	1.64 ± 0.17	5.62(3)	1.87
	F_{20}	23.73 (19.568-28.719)	1.61 ± 0.17	1.71(3)	0.57
	F_{40}	$1.31(1.094 - 1.551)$	1.63 ± 0.13	1.29(4)	0.32
	F_{60}	$0.63(0.523 - 0.746)$	1.98 ± 0.20	0.50(3)	0.17
	F_{70}	$0.58(0.485 - 0.678)$	1.91 ± 0.18	0.60(3)	0.20

Table 1. Stability of resistance in *Polyphagotarsonemus latus* to different acaricides over generations in the absence of selection pressure

d*f*, Degrees of freedom.

Table 2. Acaricide selection response in *P. latus* over generations

Acaricide	Susceptibility index	Response to selection	Resistance stability
Diafenthiuron	86.41	-0.039	25.83
Dicofol	52.77	-0.034	29.00
Fenazaquin	38.88	-0.032	31.50
Propargite	14.11	-0.023	43.48
Spiromesifen	102.53	-0.040	24.89

reported by 57.86% of the farmers interviewed. The taxonomic identity of *P. latus* was confirmed both by morphological and molecular methods. A 631-bp long sequence was deposited in the NCBI-GenBank and BOLD databases (accession number: ON103156; BIN: AED8321).

Stability of acaricide resistance

Bioassays with Pa-Lab showed a decrease in resistance levels over the generations (Table 1). The initial LC_{50} values at the F_1 generation were 34.48 ppm for diafenthiuron, 37.10 ppm for dicofol, 17.03 ppm for fenazaquin, 5.25 ppm for propargite and 59.16 ppm for spiromesifen. At the 70th generation, the population was found to be highly susceptible to propargite with an LC_{50} value of 0.37 ppm, followed by diafenthiuron (0.40 ppm), fenazaquin (0.44 ppm), spiromesifen (0.58 ppm) and dicofol (0.70 ppm).

The LC_{50} values obtained for F_1 were compared with those of F_{70} to generate SI, which was observed to be the highest for spiromesifen (102.53) (Table 2). SIs for the other chemicals were found to be 86.4 (diafenthiuron), 52.77 (dicofol), 38.88 (fenazaquin) and 14.11 (propargite). Propargite recorded the highest number of generations required for a tenfold decrease in LC_{50} (43.48 generations), which was followed by fenazaquin (31.50), dicofol (29.00), diafenthiuron (25.83) and spiromesifen (24.89).

The values of response to selection (R) varied from -0.023 (propargite) to -0.040 (spiromesifen). The consistent negative value of *R* indicated a decline in resistance to acaricide over successive generations. The highest values for susceptibility index and response to selection in spiromesifen indicated a rapid reversion of the mite population to susceptibility for the chemical when there was no selection pressure.

Acaricide resistance in field mite populations

Significant differences were recorded in LC_{50} of the populations with respect to the susceptible population for all the tested chemicals, as the 95% CLs of their LCRs did not include 1.0 (If the 95% confidence limit includes 1, then the LC_{50} of the populations is not significantly different¹⁸.) (Table 3).

Observations on resistance of populations to diafenthiuron showed a high level of resistance development in Haveri (LC₅₀ of 32.38 ppm), Tumkur (LC₅₀ of 32.00) and

 h , Heterogeneity; LCR, Lethal concentration ratios at LC₅₀.

Figure 1. Resistance levels of field populations of *Polyphagotarsonemus latus* to major acaricides. Red horizontal lines denote the mean of resistance ratios of different field populations.

Chikkaballapur populations (LC_{50} of 26.00 ppm). Tests for the hypotheses of equality (of slopes and intercepts) and parallelism (of slopes) between the populations revealed that the regression lines were neither equal (χ^2 = 496; df = 10; $P < 0.05$) nor parallel ($\chi^2 = 15.48$; df = 5; $P = 0.01$).

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Three of the populations were highly resistant to dicofol, with the highest RR for the populations collected from Haveri (83.47-fold), followed by Chikkaballapur (49.29 fold) and Ramanagara (46.16-fold) (Figure 1). Bangalore and Tumkur populations recorded moderate levels of resistance with RRs of 28.47- and 27.35-fold respectively. The hypothesis of parallelism was accepted (χ^2 = 4.94; df = 5; $P = 0.42$), but that of equality was rejected ($\chi^2 = 467$; df= 10; $P < 0.05$).

The bioassays with fenazaquin indicated a low level of resistance in the Bangalore population (9.72-fold), moderate resistance in Ramanagara (18.62-fold), Tumkur (28.61 fold) and Chikkaballapur (33.42-fold) populations, and a high level of resistance in the Haveri population (45.42 fold). The tests for equality and parallelism hypotheses between the populations revealed that regression lines were not equal (χ^2 = 470; df = 10; *P* < 0.05) but were parallel (χ^2 = 6.47; df = 5; P = 0.26) for fenazaquin.

Results of dose-responses of *P. latus* populations to propargite revealed a relatively narrow range of LC_{50} values (3.26–6.27 ppm), and they differed significantly from the susceptible strain. The RRs varied from 8.77- to 16.84-fold, representing low to moderate resistance levels. The tests for hypotheses of parallelism and equality between the populations revealed that the regression lines were parallel $(\chi^2 = 4.24; df = 5; P = 0.52)$ but not equal $(\chi^2 = 266;$ $df = 10$; $P < 0.05$).

Field populations of *P. latus* showed wider variation in susceptibility to spiromesifen with LC_{50} ranging from 27.91

Figure 2. Pairwise correlation analysis of LC_{50} values for five acaricides in the field populations of *P. latus.* Values below the unfilled diagonal squares indicate correlation coefficient *r* and those above the unfilled diagonal squares indicate the corresponding *P* values. The scale colours of the filled boxes indicate the magnitude of correlation. *represents significant correlation at *P* < 0.05.

to 94.28 ppm. *P. latus* collected from the Haveri region showed an extremely high level of resistance (163.40-fold), followed by the populations obtained from Bangalore, Chikkaballapur, Ramanagara and Tumkur (55.15-, 93.41-, 48.37- and 101.29-fold respectively). The hypothesis of parallelism of slopes was accepted (χ^2 = 4.89; df = 5; $P = 0.43$), but that of equality of slopes and intercepts was rejected (χ^2 = 553; df = 10; P < 0.05).

Assessment of cross-resistance by pairwise correlation analysis

To assess cross-resistance among different classes of acaricides, Pearson's correlation analysis was performed (Figure 2). The perusal of data in Figure 2 reveals that resistance to spiromesifen has a positive and significant correlation with that of fenazaquin resistance (correlation coefficient $r = 0.929$, $P \le 0.05$). On the other hand, a positive but nonsignificant correlation is observed between resistance to all other acaricides in field populations of *P. latus* tested $(P > 0.05)$.

Discussion

The broad mite, *P. latus* is distributed worldwide, and its population build-up is strongly favoured by many intrinsic and extrinsic factors such as short life cycle, sex ratio, arrhenotokous reproduction, absence of effective natural enemies, host plants, temperature, humidity and other microclimatic conditions, especially under protected cultivation^{20–22}. The indiscriminate application of chemical pesticides in many crop ecosystems and under protected cultivation causes

the elimination of natural enemies and the development of resistance in many populations of Tetranychid mites like *Tetranychus urticae*, *Panonychus citri* and *Panonychus* $ulmi²³$. A similar situation was perceived in the case of the broad mite outbreak in red pepper and capsicum in the sampled areas in the present study, where inadequate control of the mite with any of the selected acaricides was reported by 57.86% of the farmers interviewed. However, compared to spider mites and other insect pests, there are no field studies on broad mite resistance to acaricides in India and abroad, despite frequent applications of heavy doses of various acaricides.

The reversal of field-evolved resistance over generations (14.11–102.53-fold) in the absence of selection indicates the recessive nature of the genes involved in resistance. The recessive inheritance of resistance could be easily tackled by temporarily withdrawing that particular acaricide from field usage for a few years 24 . Similar results were observed in the red spider mite *T. urticae*, where a significant decline in resistance in the absence of selection pressure under laboratory conditions was reported against acaricides like fenpyroximate²⁵, abamectin²⁶, spirodiclofen²⁷ and milbemectin²⁸. Specifically, susceptibility was restored to 282-, 89-, 31- and 221-fold respectively, for dicofol, fenazaquin, propargite and spiromesifen at the end of 91 generations of laboratory rearing²⁹.

In the present study, the susceptible population of the mite regained partial to full susceptibility to the tested acaricides by the 70th generation under laboratory conditions. Similarly, Mohin³⁰ has reported baseline LC_{50} values in *T. urticae* after the 128th generation, which were 0.18 ppm for fenazaquin, 0.20 ppm for propargite, 0.29 ppm for spiromesifen, and 0.30 ppm for diafenthiuron and dicofol. Naveena

*et al.*³¹ estimated the LC₅₀ values of the laboratory population of *T. urticae* at the 25th generation and observed the highest susceptibility to fenazaquin (LC_{50} of 0.11 ppm), followed by fenpropathrin (0.12 ppm), chlorfenapyr (0.15 ppm), diafenthiuron (0.22 ppm), propargite (0.91 ppm) and spiromesifen (2.00 ppm).

The tested field populations showed high to extremely high resistance to spiromesifen (up to 163.39-fold) (Figure 1). Being an insecticide-cum-acaricide, this chemical is frequently used against the sucking pests complex^{32,33}, especially in chilli and capsicum ecosystems, which might have accelerated resistance development to this chemical. Three populations showed significantly high resistance to diafenthiuron (mean RR of 56.21), which is another broad-spectrum insecticide–acaricide widely used against sucking pests in Karnataka. Furthermore, the lowest levels of resistance were observed in the case of propargite (mean 13.42).

In *T. urticae* populations of Karnataka, the levels of resistance were high to extremely high (143–1038-fold) for dicofol, moderate (15.65–32.83-fold) for propargite, moderate to high (12.02–75.00-fold) for fenazaquin while that for spiromesifen was extremely high $(431.26-969.10\text{-}fold)^{34}$. In Tamil Nadu, India, the resistance levels in *T. urticae* to major acaricides were monitored, and a low level of resistance (2.00–8.62-fold) to fenazaquin, low to moderate level of resistance to fenpropathrin (1.86–37.28-fold), moderate to a high level of resistance to diafenthiuron (15.81–50.53-fold), high level of resistance to propargite (45.16–65.10-fold) and extremely high level of resistance to spiromesifen $(193.04-452.61-fold)$ were recorded³¹. However, comparatively lower levels of resistance to the aforesaid acaricides have been reported previously in *T. urticae* populations of Punjab³⁵, Himachal Pradesh³⁶ and Kerala³⁷, India.

In the present study, the analysis methodology combining LCRs, their 95% CLs, and the tests of hypotheses of parallelism and equality was used where dose–response regressions were considered as evidence for acaricide resistance¹⁸. This method is more instructive and has greater statistical power. According to the tests for equality and parallelism hypotheses between the populations, the regression lines were parallel but not equal for all the tested chemicals except diafenthiuron. This indicates the heterogeneity of the experimental populations, which could imply that they are typical of both resistant and susceptible individuals. This might be attributed to the polyphagous nature of the pest and its inter-seasonal movement across different crops.

Studies on cross-resistance between pesticides are of utmost importance as alteration, rotation and mixing of pesticides are common strategies to delay or avoid the development of resistance³⁸. The pairwise correlation coefficients assessed between the LC_{50} values of the tested chemicals revealed a significant positive correlation between resistance to spiromesifen and fenazaquin. The lack of significant cross-resistance in other acaricides could guide their rotation and sequential application in the field.

Conclusion

The present study is a concerted effort to establish reference susceptibility data and monitor resistance and cross-resistance to major acaricides in *P. latus*. These are indispensable elements for devising a sustainable and economically viable management programme for this pest and can also aid in reducing pesticide load in the environment. The results of resistance studies revealed that field populations from five districts of Karnataka have developed resistance to acaricides, especially spiromesifen and diafenthiuron. Hence, there is an imperative requirement for rotation or alteration of chemicals, especially insecticide–acaricide compounds like spiromesifen and diafenthiuron, with others. The plausible biochemical and molecular bases of acaricide resistance in *P. latus* populations necessitate further research. Also, studies on the genetic and molecular aspects of *P. latus* must be extended to other hosts and agroecosystems to maintain the efficacy of currently available acaricides.

Conflict of interest: The authors declare that there is no conflict of interest.

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ACKNOWLEDGEMENTS. N.A. thanks the Department of Science and Technology, Government of India for the INSPIRE fellowship. We thank the Director, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru for providing infrastructure facilities.

Received 7 October 2022; revised accepted 28 February 2023

doi: 10.18520/cs/v124/i12/1462-1468