

Research Article

## Susceptibility baselines for the invasive mealybugs *Phenacoccus manihoti* and *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in cassava ecosystem against selected neonicotinoid insecticides

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### Abstract

In recent years, an invasive cassava mealybug *Phenacoccus manihoti* has been threatening cassava cultivation alongside another invasive papaya mealybug *Paracoccus marginatus* which invaded the country more than a decade ago. In order to evaluate their responses against the commonly used neonicotinoid insecticides: thiamethoxam 25 WG and imidacloprid 17.8 SL, acute toxicity experiments to determine the susceptibility baselines in populations of two invasive mealybugs in the cassava agro-ecosystem, namely, cassava mealybug *P. manihoti* and papaya mealybug *P. marginatus* were performed upto 15 generations. A systemic uptake method was used for the bioassay. The LC<sub>50</sub> values of thiamethoxam for F<sub>1</sub> generation were 3.298 ppm whereas it was 1.066 ppm for F<sub>15</sub> in cassava mealybug. The LC<sub>50</sub> values of F<sub>1</sub> generation were 2.014 ppm and that of F<sub>15</sub> generation was 1.384 ppm when tested with imidacloprid. In the case of papaya mealybug, the LC<sub>50</sub> values ranged from 6.138 ppm (F<sub>1</sub>) to 2.503 ppm (F<sub>15</sub>) for thiamethoxam and 7.457 ppm (F<sub>1</sub>) to 3.231 ppm (F<sub>15</sub>) for imidacloprid. All the susceptibility indices calculated were less than threefold. The rate of resistance development was negative in all cases showing that none of the tested populations harboured any resistance without insecticidal selection pressure. Tentative discriminating doses were fixed for both chemicals with the help of LC<sub>95</sub> values obtained from the bioassay experiments, namely five ppm for both thiamethoxam and imidacloprid in the case of cassava mealybug and 10 ppm and 15 ppm, respectively, for thiamethoxam and imidacloprid in the case of papaya mealybug.

**Keywords:** Baseline toxicity, Discriminating dose, Neonicotinoids, *Paracoccus marginatus*, *Phenacoccus manihoti*

### INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Family: Euphorbiaceae) is a tuber crop that serves as a primary source

of nutrition for a wide range of people throughout the globe, specifically from developing countries (Bellotti *et al.*, 1999). In India, the crop is cultivated primarily in Tamil Nadu (51.9% of the total area, 57.8% of total

production) and Kerala (31.7% of the total area and 34.9% of total production) (Anonymous, 2018). Value-added cassava products like sago and starch are also in high demand throughout the country and generate a lot of income (Srinivas, 2007). Cassava is a hardy crop that needs only a minimum level of intervention during cultivation. A wide range of insect pests is found to be infesting the crop. Nonetheless, the crop is usually tolerant to pests and diseases and most of these insects, except for the major pests, do not affect the tuber yield (Bellotti *et al.*, 2012).

Cassava mealybug *Phenacoccus manihoti* Matile-Ferrero is an exotic pest that originated from Brazil. The insect is notorious for its ability to invade and spread across continents with high host specificity. It has already invaded cassava plantations in Africa and parts of Asia. This transboundary pest was reported for the first time in India in the year 2020 (Joshi *et al.*, 2020). Ever since its entry, the insect has been reported in all major cassava-growing areas of the country (Sampathkumar *et al.*, 2021). Yield loss of about 80% has been reported from cassava fields infested by this mealybug (Nwanze, 1982). This pest exists in the cassava ecosystem with other mealybugs, the major pest being papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink.

Papaya mealybug was introduced into India in 2008 and is known to infest various agricultural crops, including papaya, cassava, cotton, mulberry, etc. Three encyrtid parasitoids imported from Puerto Rico, namely *Acerophagus papayae*, *Anagyrus loeckii*, *Pseudoleptomastix mexicana* have been keeping the pest under check since 2010 (Shylesha *et al.*, 2010). An encyrtid parasitoid *Anagyrus lopezi* has been introduced in India for the management of the cassava mealybug (Mansion and Kumar, 2023). Neonicotinoids like imidacloprid and thiamethoxam have been in place for mealybug management in cassava ever since the arrival of papaya mealybug (Tanwar *et al.*, 2010).

The invasion provides a blank canvas for drafting pest management strategies, as the emphasis is placed on selecting and integrating all the facets of pest management in a sustainable and eco-friendly fashion. This forges a perfect opportunity to generate baseline data as the pests have yet to be exposed to insecticidal selection pressure. Therefore, changes in insecticidal susceptibility of the populations are not too drastic. Baseline data is a benchmark for monitoring insecticide resistance in insect populations (Cook *et al.*, 2004). In the case of an existing pest like papaya mealybug, susceptibility baselines need to be redefined every once in a while, to monitor any alarming changes in susceptibility levels for a future event of resistance outbreak and refining management.

With this in mind, the present investigation was designed to generate the toxicity baselines of cassava

mealybug and papaya mealybug against select insecticides. Experiments were performed to establish discriminating doses of test neonicotinoid insecticides (thiamethoxam 25 WG and imidacloprid 17.8 SL) against these mealybug pests.

## MATERIALS AND METHODS

### Test insects

Colonies of cassava mealybug *P. manihoti* were collected from an infested cassava field at Poolampatti village, Salem district, Tamil Nadu (11°40'05.1"N, 7°47'12.9"E) in July 2021. The cassava mealybugs were mass cultured on potted cassava plants using the methodology described by Odebiyi and Bokonon-Ganta (1986) at the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore (28±2°C; RH 60-70%; Photoperiod 10:14h light and dark). The mealybugs were reared on the plants for 15 generations without insecticides.

### Insecticides used

The study used two formulated neonicotinoid insecticides: Thiamethoxam 25 WG (Actara®, M/s Syngenta India Limited) and imidacloprid 17.8 SL (Tataimida®, M/s Rallis India Limited). Based on the recommended field rate, required bioassay concentrations with each insecticide were calculated and serial dilutions were made accordingly. Preliminary range-finding tests were conducted with each chemical to find out the range of concentrations causing 20-80% mortality, within which 5-6 concentrations were chosen for each target insecticide to test their acute toxicity.

### Bioassay

Laboratory bioassays were conducted to detect the baseline toxicity of cassava mealybug *P. manihoti* and papaya mealybug *P. marginatus* at the Department of Agricultural Entomology, Coimbatore, India. Free-moving, second instar nymphs of mealybugs were selected from the mealybug culture and used for bioassays. Systemic uptake method (Prabhaker *et al.*, 2012) with slight modifications was used for bioassay. Required dilutions of insecticidal solutions were prepared with distilled water and taken in 20 ml test tubes. Cassava leaves from plants of the variety Mulluvadi were collected with 10-15 cm of their petiole intact. Second instar cassava mealybug nymphs were released onto the leaves and contained with the help of foam clip-on cages as described by Haas *et al.* (2018). The petioles of the leaves were then inserted into the test tubes containing dilutions of insecticides. Three test tubes were maintained for each dilution, which acted as replications; ten mealybug nymphs were introduced per replicate. A test tube containing distilled water was used as the control. The bioassay setup was maintained in the

laboratory at 25±2°C with an RH of 70-75% with a photoperiod of 10:14h (light: dark). Observations on mortality of mealybug nymphs (both *P. manihoti* and *P. marginatus*) were made at 24 and 48 hours after release. Insects found unresponsive while gently prodding with a soft brush were counted dead.

### Statistical analysis

Observations from the acute toxicity bioassay were subjected to Abbott's correction (Abbott, 1925) and the corrected mortality values were analysed with the help of the software PoloPlus version 2 (Leora, 1987). The difference in LC<sub>50</sub> and LC<sub>95</sub> values of the same insecticides were considered to be statistically significant if the 95% confidence interval limits showed no overlapping. Based on the LC<sub>50</sub> values of the F<sub>15</sub> generation of the laboratory population, parameters like susceptibility index (SI), rate of resistance decline (R) and the number of generations (G) required for a ten-fold decrease in the median lethal concentrations (LC<sub>50</sub>) were calculated with the formulae as given by Regupathy and Dhamu (1990) as follows:

Eq.1: Susceptibility index (SI)=(LC<sub>50</sub> of F<sub>1</sub> generation) / (LC<sub>50</sub> of F<sub>n</sub> generation)

Eq.2: Rate of resistance decline (R) = (log (final LC<sub>50</sub>) / log (initial LC<sub>50</sub>))/n

Where, n is the number of generations not exposed to pesticides

Eq.3: Number of generations for a ten-fold decrease in the LC50 value (G) = R<sup>-1</sup>

Tentative discriminating doses for each insecticide were fixed based on the LC<sub>95</sub> value of F<sub>15</sub> laboratory population, as there was no previously established baseline data for the cassava mealybug against the test chemicals.

## RESULTS

### Baseline susceptibility of cassava mealybug *Phenacoccus manihoti*

The results of the bioassay experiments showing mortality response of *P. manihoti* to thiamethoxam and imidacloprid are shown in Table 1. The LC<sub>50</sub> value of thiamethoxam for F<sub>1</sub> generation was 3.298 ppm, whereas it was 1.066 ppm for F<sub>15</sub>. Similarly, a reduction in LC<sub>50</sub> values after subsequent rearing without insecticides was observed in imidacloprid (LC<sub>50</sub> of F<sub>1</sub> - 2.014 ppm and LC<sub>50</sub> of F<sub>15</sub> - 1.384 ppm). The susceptibility index of thiamethoxam was 3.094, whereas the F<sub>15</sub> population showed 1.451-fold susceptibility compared to F<sub>1</sub> in the case of imidacloprid. The rate of resistance decline recorded was -0.490 in the case of thiamethoxam and -0.163 for imidacloprid. It was also observed that the number of generations required for a 10-fold reduction in LC<sub>50</sub> of thiamethoxam and imidacloprid were 2.039 and 6.138, respectively. The tentative discriminating

doses fixed based on LC<sub>95</sub> were 5 ppm for both thiamethoxam and imidacloprid (Table 2).

### Baseline susceptibility of papaya mealybug

#### *Paracoccus marginatus*:

The outcomes of acute toxicity experiments on *P. marginatus* with thiamethoxam and imidacloprid are summarised in Table 1. A similar trend as cassava mealybug was noticed in papaya mealybug where a decrease in median lethal concentration of F<sub>15</sub> when compared to F<sub>1</sub> was observed in both thiamethoxam (F<sub>1</sub> - 6.138 ppm and F<sub>15</sub> - 2.503 ppm) and imidacloprid (F<sub>1</sub> - 7.457 ppm and F<sub>15</sub> - 3.231 ppm). F<sub>15</sub> population exhibited a 2.452-fold susceptibility compared to F<sub>1</sub> in case of thiamethoxam and 2.281-fold susceptibility for imidacloprid. The rate of resistance decline was -0.390 for thiamethoxam and -0.363 for imidacloprid. It was also observed that 2.567 generations are needed for a 10-fold decrease in LC<sub>50</sub> for thiamethoxam whereas it was 2.753 generations for imidacloprid. The tentative discriminating doses fixed based on LC<sub>95</sub> were 10 ppm and 15 ppm for thiamethoxam and imidacloprid, respectively (Table 2).

## DISCUSSION

In an experiment, Dai *et al.* (2021) observed that the LC<sub>50</sub> of imidacloprid against *Bemisia tabaci* was 13.62 ppm which was higher than the values in our study suggesting *B. tabaci* might have developed some resistance against the insecticide. Routray *et al.* (2019) observed LC<sub>50</sub> of 2.62 ppm for thiamethoxam against a susceptible population of *Aphis gossypii*, which is in a similar range as the current study. Similarly, the LC<sub>50</sub> values of imidacloprid and thiamethoxam were 1.000 ppm and 3.186 ppm, respectively, against *A. gossypii*, which was also in line with present findings.

Reduction in LC<sub>50</sub> values during culturing papaya mealybug without neonicotinoid insecticides subsequently for 6 generations was observed by Alexander *et al.* (2013) in the case of imidacloprid (reduction from 13.5002 to 7.9037 ppm) and thiamethoxam (from 6.0956 to 3.8811 ppm). Similarly, Preetha *et al.* (2014) in cotton leaf hopper *Amrasca biguttula biguttula* noticed that after 4 generations of culture without insecticide intervention, the LC<sub>50</sub> values of imidacloprid dropped from 0.069 to 0.059 ppm, thiamethoxam from 0.007 to 0.006 ppm, acetamiprid from 0.009 to 0.008 ppm and thiacloprid from 0.160 to 0.151 ppm. These findings align with our experiment's results, where a drop in LC<sub>50</sub> values was observed after 15 generations for both cassava and papaya mealybugs proving changes in susceptibility towards both the neonicotinoids.

Changes in susceptibility between generations can often be non-significant, whose significance can only

**Table 1.** Acute toxicity of thiamethoxam 25 WG and imidacloprid 17.8 SL to cassava and papaya mealybugs by systemic uptake method

Particulars	Genera- tion	Regression equation	Calculated $\chi^2$	LC <sub>50</sub> (ppm)	Fiducial limits		LC <sub>95</sub> (ppm)	Fiducial limits		SI	RRD	G
					LL	UL		LL	UL			
Cassava mealybug - Thiamethoxam 25 WG	1	y = 3.96+2.00x	3.550	3.298	2.380	4.580	21.828	7.950	59.950	3.094	-0.490	2.039
	15	y = 4.92+2.85x	1.171	1.066	0.868	1.310	4.024	2.724	5.944	-	-	-
Papaya mealybug - Thiamethoxam 25 WG	1	y = 3.09+2.42x	4.258	6.138	4.908	7.676	29.368	15.005	57.480	2.452	-0.390	2.567
	15	y = 3.76+3.12x	0.928	2.503	2.036	3.076	8.415	6.042	11.721	-	-	-
Cassava mealybug - Imidacloprid 17.8 SL	1	y = 4.11+ 2.94x	0.780	2.014	1.710	2.380	7.307	3.940	13.540	1.451	-0.163	6.138
	15	y = 4.44+ 4.00x	2.210	1.384	1.184	1.618	3.568	2.669	4.768	-	-	-
Papaya mealybug - Imidacloprid 17.8 SL	1	y = 3.28+1.98x	2.585	7.457	5.641	9.856	50.746	17.567	146.594	2.281	-0.363	2.753
	15	y = 3.75+2.45x	2.586	3.231	2.604	4.010	15.122	8.718	26.229	-	-	-

All regression lines are significantly a good fit ( $p < 0.05$ ); SI – Susceptibility index; R - Rate of resistance decline; G - Number of generations required for 10-fold decrease in LC<sub>50</sub>

be confirmed if the fiducial limits, otherwise known as the 95% confidence intervals of the sublethal doses, do not overlap (Senthilkumar & Regupathy, 2004). In the current study, the 95% confidence intervals of LC<sub>50</sub> for F<sub>1</sub> and F<sub>15</sub> were not overlapping in cassava mealybug populations and in papaya mealybug populations for both insecticides showing that the difference in susceptibility was significant though very small. All of them equal to or less than 3-fold. Zhao and Graffius (1993) stated that such small differences in susceptibility might not be alarming in field resistance monitoring.

Furthermore, the 95% confidence intervals (CI) of LC<sub>95</sub> of thiamethoxam in the present experiment were not overlapping in the case of both insects, confirming a small but significant change. But that was not the case with imidacloprid where the 95% CIs were overlapping in both cassava mealybug (95% CI of LC<sub>95</sub> of F<sub>1</sub> - 3.940 - 13.540 ppm and F<sub>15</sub> - 2.669 - 4.768 ppm) and papaya mealybug (95% CI of LC<sub>95</sub> of F<sub>1</sub> - 17.567 - 146.594 ppm and F<sub>15</sub> - 8.718 - 26.229 ppm) therefore no significant difference was observed between the LC<sub>95</sub> values. Notably, in a study by Preetha *et al.* (2014), a significant difference in susceptibility of *A.biguttula biguttula* against thiamethoxam was observed while comparing their F<sub>1</sub> and F<sub>4</sub> generations because of overlapping fiducial limits of LC<sub>50</sub> but the LC<sub>95</sub> values showed no overlapping. Conversely, the same study showed complete overlapping of CIs at both LC<sub>50</sub> and LC<sub>95</sub> levels of F<sub>1</sub> and F<sub>4</sub> for thiamethoxam showing no difference in susceptibility levels. These findings do not necessarily disagree with the findings of the current study but could rather be attributed to the fact that the insecticides produce differential responses in insects and Stanley *et al.* (2009) opined that such differential susceptibility within the same species of insect may also be recorded, which might arise due to difference in pesticide usage pattern.

The susceptibility index (SI) of F<sub>6</sub> over F<sub>1</sub> generation based on LC<sub>50</sub> and LC<sub>95</sub> for all the molecules tested was more than one in the case of papaya mealybug (Alexander *et al.*, 2013), which was in line with the results of the current investigation indicating that the F<sub>n</sub> generation in both the studies was more vulnerable to insecticides than the F<sub>1</sub> generation.

A negative rate of resistance decline was observed in case of neonicotinoids (-0.0388 for imidacloprid and -0.0327 for thiamethoxam) against *P.marginatus* by Alexander *et al.* (2013) and *A.biguttula biguttula* by Preetha *et al.*, (2014) (-0.017 for imidacloprid and thiamethoxam) both of which were in conjuncture with our results. Preetha *et al.* (2014) also reported that the populations needed 58.82 generations to record 10-fold less LC<sub>50</sub> values for both imidacloprid and thiamethoxam, indicating that the F<sub>4</sub> populations showed high relative susceptibility. Similarly, Alexander *et al.* (2013)

**Table 2.** Tentative discriminating doses of thiamethoxam 25 WG and imidacloprid 17.8 SL against cassava and papaya mealybugs by systemic uptake method

Particulars	Recommended field rate (g/ml per hectare)	Label rate concentration (ppm)	Tentative discriminating dose (ppm)
Cassava mealybug - Thiamethoxam 25 WG	300 g	150	5
Papaya mealybug - Thiamethoxam 25 WG			10
Cassava mealybug - Imidacloprid 17.8 SL	300 ml	107	5
Papaya mealybug - Imidacloprid 17.8 SL			15

recorded that 25.77 generations are needed in the case of imidacloprid and 30.58 generations were needed in case of thiamethoxam for a 10-fold drop in  $LC_{50}$ . In the current study, 10-fold drop in  $LC_{50}$  was observed for both cassava and papaya mealybugs in less than 10 generations showing that the field-collected populations ( $F_1$ ) showed some differential susceptibility when reared in laboratory subsequently in the absence of insecticides ( $F_{15}$ ), thus strengthening our findings based on  $LC_{50}$  values and their fiducial limits.

The present study established a susceptibility baseline for cassava mealybug, served by establishing tentative discriminating doses of 5 ppm for both thiamethoxam and imidacloprid. While Alexander *et al.* (2013) proposed tentative discriminating doses of 144 and 465 ppm for imidacloprid and thiamethoxam, the present study identified 95% of susceptible populations of papaya mealybug were found to be eliminated at the doses 10 ppm and 15 ppm for thiamethoxam and imidacloprid respectively. Multiple factors, such as differences in methodologies used for monitoring, variations in formulations of chemicals used, changes in pesticide usage patterns over the years, can lead to such differential response. It could also be due to the successful suppression of mealybugs over the years through effective parasitoids. In such cases, recalibrating the existing methods to better suit the current resistance situation can help in timely detection, which is pivotal for a successful management programme.

## Conclusion

The present investigation, intent on generating susceptibility baselines for imidacloprid and thiamethoxam against the two devastating mealybug species, namely, cassava mealybug *P. manihoti* and papaya mealybug *P. marginatus* in cassava, showed a change in susceptibility of populations to an extremely low degree which is typical of populations reared without insecticide. The susceptibility index was also not more than 3.1-fold for all the cases, which showed no alarming resistance development. Evaluation of insecticides on a regular basis will help in the identification of changes in the

susceptibility of the insecticide populations, which is of prime interest in resistance monitoring programmes.

## Conflict of interest

The authors declare that they have no conflict of interest.

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