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**Research Article****Toxicity of selected neonicotinoid insecticides against *Phenacoccus solenopsis* infesting ornamental hibiscus plants****Muhammad Saqib<sup>1</sup>, Munir Ahmad<sup>1</sup>, Farid Asif Shaheen<sup>1</sup>, Qaiser Hussain<sup>2</sup>**<sup>1</sup>Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Punjab, Pakistan.<sup>2</sup>Institute of Soil and Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Punjab, Pakistan.**ABSTRACT**

The cotton mealybug, *Phenacoccus solenopsis*, poses a significant threat to economically important crops. Insecticide application is an effective and quick method for pest control and stresses continuous search for number of insecticides to be available for alternate use. Current research focuses the toxicity comparison of acetamiprid, imidacloprid, clothianidin, thiamethoxam and emamectin benzoate using leaf dip method under controlled laboratory conditions. Among these, clothianidin exhibited the highest toxicity with LC<sub>50</sub> values of 0.26 and 0.19 ppm after 72 hours for Rawalpindi and Islamabad populations, respectively. Thiamethoxam followed with LC<sub>50</sub> of 0.41 and 0.49 ppm for these populations, respectively. Emamectin benzoate, however, was the least toxic. This study suggests neonicotinoids like clothianidin and thiamethoxam for effective mealybug management.

**Keywords:** Toxicity; *Phenacoccus solenopsis*; chemical insecticides; leaf dip method; neonicotinoids.

**INTRODUCTION**

Chinese *Hibiscus* are extensively grown in gardens, parks and indoor for their captivating flowers and lush foliage which significantly enhance the aesthetic appeal of a variety of landscapes (Pounders & Sakhanokho, 2016; Magdalita & San Pascual, 2022). The genus *Hibiscus*, classified under the Malvaceae family, is renowned for its extensive diversity, comprising an estimated 250 distinct plant species across various regions worldwide (Jadhav et al., 2009; Khristi & Patel, 2016; Mandaji et al., 2022) with health benefits (Jeffery & Richardson, 2021).

Cotton mealybug (*Phenacoccus solenopsis*) is one of the most devastating sap sucking invasive and polyphagous insect pests of cotton in Pakistan and India (Hodgson et al. 2008) and on *Hibiscus rosa-sinensis* species threatening its flowers and plant aesthetic look. Because of its rapid spread and destructive feeding habits, it is considered important for horticulturists and plant enthusiasts to manage it in time (Prishanthini & Vinobaba, 2014). It pierces plant tissues and extract sap causing wilting, stunted growth and chlorosis. The honeydew, a sugary substance that promotes the growth of sooty mold, excreted from its body make the plant's photosynthetic ability and overall health weak eventually causing the infested plants to decline and possibly die (Puspitasari et al., 2023). Synthetic insecticides of different groups have played a significant role in its management (Fatima et al., 2016). The development of various insecticides especially neonicotinoids as preventive measures has led to the control of cotton mealybug infestation and the preservation of the health of plants. To ensure effective pest control, it is crucial to make the right insecticide choice (Noureen et al., 2016). The present study was

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conducted to trace out the best insecticide and most effective concentration for controlling cotton mealybugs.

## MATERIALS AND METHODS

### Insect Collection and Rearing

*Phenacoccus solenopsis* was collected from infested *Hibiscus* plants from Rawalpindi and Islamabad. In small 5 cm diameter Petri dishes, collected insects from Rawalpindi and Islamabad were raised separately on fresh *Hibiscus* plant leaves while adult mealybugs were allowed to lay eggs. After 4-5 days, there were enough second instar larvae to be used for bioassays. Leaves were cut in a circle to fit each petri dish with moist filter paper beneath to avoid desiccation. Under standardized laboratory conditions with a temperature of 25°C, relative humidity of 50%, and a photoperiod of 10/14 (D/L) hours, leaves were changed after three days (Saddiq et al., 2017).

### Insecticides

Commercial formulation of selective new chemistry insecticides were used for bioassay. These were acetamiprid (Acelan, 20%SL, FMC Pakistan), imidacloprid (Confidor 20%SL, Bayer Crop science Pakistan), clothianidin (Parlor 20%SC, Green Let International Private Limited), thiamethoxam (Confest 25%WG, Alnoor Agro Chemicals) and emamectin benzoate (1.9%EC, Alnoor Agro Chemicals).

### Bioassay of Insecticides

The leaf dip method described by Afzal et al. (2015) was followed for toxicity bioassays of the tested insecticides. Six concentration levels, including the control, were used in the experiment, with four replications for each treatment. Fresh, untreated *Hibiscus* plant leaves were dipped for 10 seconds in each concentration level and allowed to air dry. Twenty cotton mealybug nymphs of second instar were slowly dropped to each petri dish with help of fine camel hairbrush. The procedure was performed for both populations of Rawalpindi and Islamabad separately. *Hibiscus* leaves were only dipped in distilled water for the control of each treatment. For observation of mortality, if an insect did not move after being gently touched, it was assumed to be dead.

### Statistical Analysis

The data regarding mortality as the endpoint were collected at three times intervals of 24, 48 and 72 hours post-application. To analyze the data and obtain key parameters such as 95% fiducial limits (FL), slope, chi-square value, standard error, and LC<sub>50</sub> values, POLO-Plus software was used (Finney, 1971).

## RESULTS AND DISCUSSION

This study focused on evaluating the toxicity of five insecticides (acetamiprid, imidacloprid, clothianidin, thiamethoxam, and emamectin benzoate) against the second instar nymphs of cotton mealybug (*P. solenopsis*) under laboratory conditions.

LC<sub>50</sub> of acetamiprid were 1.70, 1.49 and 1.07 ppm and LC<sub>90</sub> were 19.8, 11.7 and 7.90 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of imidacloprid were 8.60, 2.49 and 1.47 ppm and LC<sub>90</sub> were 313, 131 and 46.7 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of clothianidin were 2.04, 0.97 and 0.26 ppm and LC<sub>90</sub> were 13.8, 4.96 and 3.97 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of thiamethoxam were 1.59, 0.66 and 0.41 ppm and LC<sub>90</sub> were 90.9, 29.2 and 13.0 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of emamectin benzoate were 39.8, 21.8 and 8.96 ppm and LC<sub>90</sub> were 1147, 728 and 304 ppm after 24, 48 and 72 hours, respectively (Table 1).

After 72 hours of Rawalpindi population, clothianidin was more toxic with LC<sub>50</sub> value of 0.26 ppm followed by thiamethoxam with LC<sub>50</sub> value of 0.41 ppm. Both clothianidin and thiamethoxam have not much difference between their effectiveness after 72 hours. Third toxic insecticides after thiamethoxam were acetamiprid with LC<sub>50</sub> value of 1.07 ppm after 72 hours. Imidacloprid was also found toxic after 72 hours with LC<sub>50</sub> value of 1.47 ppm which have not much difference in their effectiveness than acetamiprid. The least toxic insecticide than other insecticides after 72 hours was emamectin benzoate with LC<sub>50</sub> value of 8.96 ppm.

According to LC<sub>90</sub> value after 72 hours, clothianidin was the most toxic with LC<sub>90</sub> value of 3.97 ppm followed by acetamiprid with LC<sub>90</sub> value of 7.90 ppm. Third and fourth toxic insecticides after acetamiprid were thiamethoxam and imidacloprid with LC<sub>90</sub>s value of 13.0 ppm and 46.7 ppm after 72 hours, respectively. The least toxic insecticide was emamectin benzoate with LC<sub>90</sub> value of 304 ppm.

After 48 hours, thiamethoxam was found more toxic than clothianidin with LC<sub>50</sub> value of 0.66 ppm and 1.49 ppm, respectively. Acetamiprid was more toxic than imidacloprid and emamectin benzoate with LC<sub>50</sub> value of 1.49, 2.49 and

21.8 ppm, respectively. According to LC<sub>90</sub> value after 48 hours, clothianidin was more toxic than acetamiprid, thiamethoxam, imidacloprid and emamectin benzoate with LC<sub>90</sub> value of 11.7, 29.2, 131 and 728 ppm, respectively. After 24 hours, thiamethoxam was more toxic than acetamiprid, clothianidin, imidacloprid and emamectin benzoate with LC<sub>50</sub> value of 1.59, 1.70, 2.04, 8.60 and 39.8 ppm, respectively. On the other hand, clothianidin was toxic according to LC<sub>90</sub> value of 13.8 ppm followed by acetamiprid, thiamethoxam, imidacloprid and emamectin benzoate with LC<sub>90</sub> value of 19.8, 90.9, 313 and 1147 ppm, respectively.

Table 1. Effect of toxicity of selective insecticides against cotton mealybug of Rawalpindi population.

Insecticides	Hours	LC <sub>50</sub>	95% FL (Fiducial Limits)	LC <sub>90</sub>	95% FL (Fiducial Limits)	Slope ± SE	χ <sup>2</sup>	p
Acetamiprid	24	1.70	1.21-2.20	19.8	12.4-42.8	1.20±0.163	0.33	0.95
	48	1.49	1.07-1.93	11.7	8.20-20.3	1.43±0.18	0.42	0.93
	72	1.07	0.72-1.42	7.90	5.72-12.8	1.47±0.19	0.71	0.87
Imidacloprid	24	8.60	6.01-14.3	313	100-3509	0.82±0.15	1.01	0.79
	48	2.49	1.26-3.77	131	47.5-1378	0.74±0.15	0.28	0.96
	72	1.47	0.65-2.31	46.7	22.9-203	0.85±0.16	0.29	0.96
Clothianidin	24	2.04	1.35-3.49	13.8	6.55-83.7	1.54±0.21	3.11	0.37
	48	0.97	0.78-1.19	4.96	3.62-7.81	1.81±0.19	1.25	0.74
	72	0.26	0.04-0.50	3.97	1.91-39.0	1.09±0.17	3.74	0.29
Thiamethoxam	24	1.59	0.97-2.37	90.9	28.5-1344	0.72±0.15	1.26	0.73
	48	0.66	0.27-1.05	29.2	12.3-202	0.77±0.16	0.81	0.84
	72	0.41	0.15-0.69	13.0	6.87-49.6	0.85±0.16	0.58	0.9
Emamectin Benzoate	24	39.8	28.1-66.9	1147	395-9883	0.87±0.15	1.72	0.63
	48	21.8	15.2-32.0	728	270-5601	0.84±0.15	2.22	0.52
	72	8.96	1.34-17.4	304	89.0-94830	0.83±0.16	3.60	0.30

For Islamabad population, the tested insecticides evaluated against the second molt of cotton mealybug (*P. solenopsis*) showed following results.

LC<sub>50</sub> of acetamiprid were 1.50, 1.10 and 0.77 ppm and LC<sub>90</sub> were 13.5, 8.24 and 3.68 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of imidacloprid were 3.81, 1.29 and 0.71 ppm and LC<sub>90</sub> were 62.9, 52.5 and 14.9 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of clothianidin were 0.99, 0.26 and 0.19 ppm and LC<sub>90</sub> were 10.7, 4.04 and 3.02 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of thiamethoxam were 1.82, 0.71 and 0.49 ppm and LC<sub>90</sub> were 27.0, 16.6 and 8.09 ppm after 24, 48 and 72 hours, respectively. LC<sub>50</sub> of emamectin benzoate were 20.2, 8.30 and 5.66 ppm and LC<sub>90</sub> were 392, 153 and 100 ppm after 24, 48 and 72 hours, respectively (Table 2).

Clothianidin was the most found toxic insecticide against second instar of cotton mealybug for Islamabad population with LC<sub>50</sub> value of 0.19 ppm followed by thiamethoxam with LC<sub>50</sub> value of 0.49 ppm after 72 hours of application.

Imidacloprid and acetamiprid have resulted approximately same toxicity with LC<sub>50</sub> of 0.71 and 0.77 ppm, respectively. Emamectin benzoate resulted least toxic than other insecticides with LC<sub>50</sub> value of 5.66 ppm. According to LC<sub>90</sub> value after 72 hours for Islamabad population, clothianidin was found most toxic with 3.02 ppm than acetamiprid, thiamethoxam, imidacloprid and emamectin benzoate with 3.68, 8.09, 14.9 and 100 ppm, respectively.

After 48 hours of application against *P. solenopsis*, clothianidin was found more toxic with LC<sub>50</sub> value of 0.26ppm followed by thiamethoxam with LC<sub>50</sub> value of 0.71 ppm but more toxic than acetamiprid, imidacloprid and emamectin benzoate which has LC<sub>50</sub>s value of 1.10, 1.29 and 8.30ppm, respectively. According to their LC<sub>90</sub> after 48 hours, clothianidin was more toxic than acetamiprid with 0.04 and 8.24ppm, respectively and thiamethoxam was less toxic than both but more toxic than imidacloprid with LC<sub>90</sub> value of 16.6 and 52.5ppm, respectively. Less effective insecticides resulted emamectin benzoate with LC<sub>90</sub> value of 153 ppm after 48 hours.

After 24 hours for Islamabad population, clothianidin resulted more toxic with LC<sub>50</sub> value of 0.99 ppm and LC<sub>90</sub> value of 10.7 ppm. It followed acetamiprid with LC<sub>50</sub> value of 1.50 ppm and LC<sub>90</sub> value of 13.5 ppm. Thiamethoxam was effective than imidacloprid with LC<sub>50</sub> value of 1.82 ppm and LC<sub>90</sub> value of 27.0 ppm while imidacloprid has LC<sub>50</sub> value of 3.81 ppm and LC<sub>90</sub> value of 62.9 ppm. Emamectin was least toxic than other insecticides with LC<sub>50</sub> value of 20.2 ppm and LC<sub>90</sub> value of 392 ppm after 24 hours.

Table 2. Effect of toxicity of selective insecticides against cotton mealybug of Islamabad population.

Insecticides	Hours	LC <sub>50</sub>	95% FL (Fiducial Limits)	LC <sub>90</sub>	95% FL (Fiducial Limits)	Slope ± SE	χ <sup>2</sup>	p
Acetamiprid	24	1.50	1.10-1.92	13.5	9.24-24.4	1.34±0.16	1.15	0.76
	48	1.10	0.78-1.42	8.24	6.02-13.1	1.47±0.18	0.57	0.90
	72	0.77	0.53-0.99	3.68	2.92-5.12	1.89±0.24	1.88	0.59
Imidacloprid	24	3.81	2.75-5.04	62.9	33.4-191	1.05±0.15	1.45	0.69
	48	1.29	0.49-2.10	52.5	24.3-282	0.79±0.16	0.80	0.84
	72	0.71	0.25-1.23	14.9	9.43-35.2	0.97±0.17	0.74	0.86
Clothianidin	24	0.99	0.45-2.20	10.7	3.75-904	1.23±0.16	7.26	0.06
	48	0.26	0.14-0.38	4.04	2.55-9.02	1.08±0.17	1.67	0.64
	72	0.19	0.09-0.30	3.02	1.97-6.37	1.08±.17	2.96	0.39
Thiamethoxam	24	1.82	1.37-2.40	27.0	14.4-79.2	1.09±0.15	0.48	0.92
	48	0.71	0.38-1.04	16.6	8.81-55.9	0.93±0.16	1.68	0.64
	72	0.49	0.24-0.74	8.09	5.01-19.1	1.05±0.17	2.65	0.44
Emamectin Benzoate	24	20.2	14.9-27.6	392	186-1536	0.99±0.15	1.32	0.72
	48	8.30	5.00-11.6	153	85.7-438	1.01±0.16	1.19	0.75
	72	5.66	2.89-8.44	100	59.7-256	1.02±0.17	0.31	0.95

The current study demonstrated that under laboratory conditions, mealybug populations obtained from ornamental *Hibiscus* plants in Rawalpindi and Islamabad showed no variation in their susceptibility to the tested insecticides. Clothianidin consistently demonstrated the highest toxicity against both populations of the studied insecticides. Thiamethoxam, acetamiprid, and imidacloprid then showed nearly similar toxicity values. Emamectin benzoate, on the other hand, shown the least toxicity. Overall, there were no statistically significant changes between the two populations and the pesticides provided similar levels of effectiveness.

Similar study was conducted on cotton mealybug, *P. solenopsis* under laboratory conditions using the Insecticide Resistance Action Committee (IRAC) leaf dip bio assay technique, evaluated the efficacy of seven insecticides acetamiprid, imidacloprid, thiamethoxam, lambda cyhalothrin, profenofos, spinosad and buprofezin. Among these tested insecticide profenofos, acetamiprid, buprofezin, imidacloprid, thiamethoxam were more toxic, respectively while spinosad and lambda cyhalothrin were least toxic than other insecticides (Padaliya et al., 2022). This showed that neonicotinoids tested as in our studies proved efficient to manage this pest in toxicity response.

Comparable findings were reported by Liang et al. (2014) who identified thiamethoxam the most effective insecticide using leaf dip method than other tested neonicotinoid insecticides (acetamiprid, imidacloprid and nitenpyram). Similarly, Waqas et al. (2019) evaluated that acetamiprid resulted more effective than imidacloprid against *P. solenopsis* using leaf dip method. Field evaluation conducted by Hamed et al. (2022) also demonstrated that profenofos and thiamethoxam were highly effective while flonicamid, methomyl, and alpha-cypermethrin showed moderate effectiveness. Mamoon-ur-Rashid et al. (2011) reported thiamethoxam the most toxic against second instar nymphs of cotton mealybug under laboratory conditions and caused maximum (82%) mortality). In contrast, Kz-oil and detergent soap had low effectiveness, and emamectin benzoate showed the lowest effectiveness. Most of the previous literature supported the efficient and positive role of neonicotinoid insecticides for this pest which strengthen the present findings of better mealybug mortality of the study.

## CONCLUSION

We observed that the tested insecticides worked consistently against Rawalpindi and Islamabad mealybug populations in the laboratory. Clothianidin was the most effective insecticide followed by thiamethoxam, acetamiprid and imidacloprid while emamectin benzoate showed the lowest toxicity.

## AUTHOR'S CONTRIBUTION

Dr. Munir Ahmad planned and supervised the research and Muhammad Saqib conducted the experimentation and early draft of the manuscript. Dr Farid Asif Shaheen and Dr Qaiser Hussain provided technical assistance during experimentation.

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## AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article in the form of tables.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not required for this study, as it performed under control environment and did not include human or sensitive animal subjects.

## CONSENT FOR PUBLICATION

Not applicable. This study does not involve any individual person's data in any form. All authors are agreed for its publication.

## CONFLICT OF INTERESTS

All authors attest to the validity of manuscript contents and agree for submission.

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