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Establishment of baseline toxicity data to thiamethoxam for adult black legume aphid, *Aphis craccivora* Koch. by leaf dip bioassay method

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Abstract
Generating the baseline toxicity data for any insecticide is an important requirement for insecticide resistance monitoring. It also provides guide for formulating resistance management strategies in Integrated Pest Management**.** The present study on "Establishment of baseline toxicity data to thiamethoxam for adult black legume aphid, *Aphis craccivora* Koch. by leaf dip bioassay" was carried out at the Insecticide Toxicology Laboratory, Department of Entomology, College of Agriculture, OUAT, Bhubaneswar, Odisha during 2017-2018. The LC_{50} for the susceptible strain was found to be 2.62 ppm with fiducial limits of 1.91 – 3.43 ppm and the LC_{95} was found to be 27.73 ppm with fiducial limits of 16.77 – 63.72 ppm. Other findings and supporting reports are discussed.

Keywords: *Aphis craccivora* Koch, baseline toxicity, thiamethoxam, leaf-dip method

1. Introduction

The black cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae) is a serious pest of many crops throughout the world [8, 4]. Cowpea is one of the economically important vegetable crops attacked by *A. craccivora.* It not only cause damage by sucking sap from plants, but also indirectly damage involving in transmission of viral diseases such as cowpea mosaic virus (CMV). The current aphid management strategies solely rely on the use of various synthetic chemical insecticides such as organophosphates, carbamates, pyrethroids, and neonicotinoids [13, 20]. Thiamethoxam-a neonicotinoid insecticide provides excellent control of a broad range of economically important pests, such as aphids, whiteflies, thrips, rice hoppers, Colorado potato beetle, flea beetles, wireworms, leaf miners as well as some lepidopterous species [16]. The extensive use of neonicotinoids in pest control and the reduced availability of insecticides with other modes of action to rotate with the neonicotinoids could create conditions that favour the development of neonicotinoid resistance in aphids [21]. Although many insect species are still successfully controlled by neonicotinoids, their popularity has imposed a mounting selection pressure for resistance, and in several species resistance has now reached levels that compromise the efficacy of these insecticides [3]. The indiscriminate and large-scale use of synthetic chemical insecticides to control aphid pests has resulted in the development of insecticide resistance [10, 7]. Increasing levels of resistance to the most commonly used insecticides have resulted in increased human health and environmental concerns [9]. These problems necessitate establishing an efficient resistance management strategies based on information available about the extent and nature of resistance. Therefore, in the present study an attempt has been made to find out the baseline toxicity data of *A. craccivora* to thiamethoxam which would be useful information for the evaluation of standard for resistance monitoring in the future.

2. Materials and Methods

2.1 Collection and Rearing of *Aphis craccivora*

Field strain of *A. craccivora* were collected from cowpea crop grown without insecticide protection from the Central Research Station field of OUAT, Bhubaneswar (20.2647° N, 85.8141° E) and reared in insectary in the same host which was unprotected. Aphids were reared in well ventilated cages and the insects were kept on cowpea seedlings grown in plastic pots (15 cm diameter) filled with soil. This strain was reared continuously without being exposed to any insecticides for few generations and bioassay was conducted in each generation using leaf – dip method to obtain a susceptible population.

2.2 Leaf-dip bioassay and data analysis
Baseline toxicity to thiamethoxam was determined using leaf dip method of bioassay [18] (method No. 019) developed and recommended by the Insecticide Resistance Action Committee (IRAC) [12]. At first, preliminary assay was done by taking arbitrary concentrations of insecticide without any replication to know in between which concentrations the aphid mortalities range between zero to hundred per cent. Then, between those two concentrations seven concentrations of thiamethoxam were freshly prepared through serial dilution method in water from a stock solution of 1% thiamethoxam based on the preliminary and repeated response of adult aphids and then kept in labeled beakers. For each concentration 3 replications were made. Freshly excised cowpea leaves dipped in water only, served as the controls. In different concentrations of thiamethoxam, cowpea leaves were dipped for 10 seconds and then the leaves were dried for 30 seconds and were kept upside down inside plastic containers (60 mm diameter) with wire mesh caps and lined with moist blotting paper. Freshly collected 10 adult aphids were placed carefully on treated leaves in the containers using camel hair brush. The containers with treated insects on cowpea leaves were kept in a B.O.D. incubator under laboratory set of conditions (28 ± 2ºC; 75-80% RH). The mortality was counted after 24 hours. Aphids which are unable to right themselves within 10 seconds once turned on their back were considered as dead.

3. Statistical Analysis
The mortality data were corrected using Abbott's formula [1] when any control mortality was observed and LC_{50} was calculated following Probit Analysis [6] using Ldp line software. The Abbott's formula is as follows –

Corrected percent mortality = (T-C/100-C) X 100
Where, T = Treatment mortality, C = Control mortality

4. Results and Discussion
The observed mortality response of adult aphids ranged from 17.24 per cent in 0.5 ppm to 93.10 per cent in 10 ppm thiamethoxam. After correction of the mortalities using Abbott's formula the mortality response became 12.37 per cent in 0.5 ppm which increased to 82.46 per cent in 10 ppm (Table 2). Utilizing the log dose - probit mortality (Ldp) software the linear probit was found to range between 3.84 in 0.5 ppm concentration to 5.93 in 10 ppm concentration of thiamethoxam. Using the same software the LC_{50} of thiamethoxam to adult *A. craccivora* was determined to be 2.62 ppm with 1.91 ppm as fiducial lower limit and 3.43 ppm as fiducial upper limit (Table 1). The 'b' value or the slope was found to be 1.60 ± 0.22. The regression equation was Y= 1.60X+4.327. The chi square (X^2) test revealed the calculated value to be 10.13 with tabulated value 11.1. From the same Ldp line (Fig. 1), on extrapolation, the LC_{95} was found to be 27.73 ppm with fiducial limits of 16.77 – 63.72 ppm of thiamethoxam.

Various authors have earlier reported baseline susceptibility data for *A. craccivora* to thiamethoxam. According to Abdallah *et al.*, (2016), the LC_{50} value of the susceptible population of *A. craccivora* to thiamethoxam was 0.079 mg/l with a fiducial limit of 0.034 – 0.134 mg/l. According to Mokbel *et al.* [17], the baseline toxicity of *A. craccivora* to thiamethoxam was 0.44 ppm with a fiducial limit of 0.29 – 0.60 ppm at 95 per cent confidence level using a leaf dip bioassay. The susceptibility (LC_{50}) of *A. craccivora* to another chloronicotinyl neonicotinoid insecticide imidacloprid was found to be 6.33 ppm with fiducial limits of 3.88-8.82 ppm [22]. In the above reports, different values in LC_{50} obtained may be due to the differential responses of susceptible populations and genetic variability existing among the treated population coupled with the environmental influences. Besides bioassay, exposure time to insecticide is another important factor affecting bioassay against aphids [11, 5]. Most insecticides usually give stable mortality after 48 or 72h [11]. Considering the views of the above authors we have chosen 24h exposure to score mortality.

Some of the workers have determined the LC_{50} value of thiamethoxam to another common aphid *i.e.* cotton aphid, *Aphis gossypii* using the same leaf dip bioassay method. Pan *et al.*, [19] found the LC_{50} value of thiamethoxam at 95 per cent confidence level to susceptible *A. gossypii* as 2.85 mg/l with a fiducial limit of 1.74-4.24 mg/l. Koo *et al.*, [14] reported that the LC_{50} of thiamethoxam to susceptible strain of cotton aphid *A. gossypii* was 0.14 mg/l with a fiducial limit of 0.015 – 1.21 mg/l at 95 per cent confidence level using leaf-dip bioassay. Using a different modified dipping method, Chen *et al.* [5] reported that the LC_{50} of thiamethoxam to *A. gossypii* was 1.88 ug/ml with a fiducial limit of 1.52-2.20 ug/ml. Wei *et al.*, [23] found the LC_{50} for susceptible strain of *A. gossypii* to related neonicotinoid insecticide imidacloprid to be 1.13 ppm.

5. Conclusion
It may be concluded from the present investigation that LC_{50} for the susceptible strain of *Aphis gossypii* was found to be 2.62 ppm with fiducial limits of 1.91 – 3.43 ppm. This basic susceptible baseline toxicity data will be required for insecticide resistance monitoring in future. It will also provide guide for formulating resistance management strategies in Integrated Pest Management programmes for this sucking pest.

Table 1: Baseline toxicity data of thiamethoxam to *Aphis craccivora* Koch.

Insecticide	N[a]	Intercept	slope±SE	X^2	X^2 tab	LC_{50} (ppm)	LL	UL	LC_{95} (ppm)	LL	UL	Regression equation
Thiamethoxam	420	4.327	1.60±0.22	10.13	11.1	2.62	1.91	3.43	27.73	16.77	63.72	Y= 1.60X+4.327

[a] number of adult aphids assayed

Table 2: Log concentration and linear probits for susceptible strain of *A. gossypii* to thiamethoxam

Conc. (ppm)	Conc.×10	Log (Conc.×10)	Treated	Observed response (%)	Linear response (%)	Linear probit
0.50	5	0.70	30	17.24	12.37	3.84
1.00	10	1.00	29	28.66	25.03	4.33
3.00	30	1.48	30	41.38	53.70	5.09
5.00	50	1.70	30	51.72	67.35	5.45
7.00	70	1.85	30	72.41	75.31	5.68
9.00	90	1.96	30	89.66	80.49	5.86
10.00	100	2.00	30	93.10	82.46	5.93

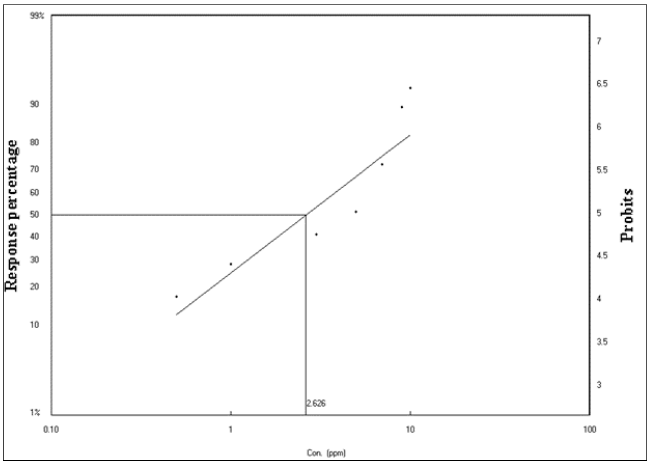

Fig 1: Log Conc. vs Mortality of *A gossypii* to thiamethoxam by leaf-dip method

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