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Bioefficacy of imidacloprid against leafhopper on sunflower

G. SANTHARAM, D. SIVAVEERAPANDIAN, K. RAMESH BABU AND S. KUTTALAM
Department of Agricultural Entomology, Tamil Nadu Agrl. University, Coimbatore-641 003, Tamil Nadu

Abstract : Bioefficacy of imidacloprid against leafhopper, *Amrasca devastans* was evaluated on sunflower. Results of three season experiments revealed that seed treatment with imidacloprid 70 WS at 7g kg^{-1} protected the crop utpo 6 weeks against leafhopper. Foliar spray of imidacloprid 200 SL at 100ml ha-1 given at 3 weeks after sowing reduced the leafhopper population significantly and the effect persisted for 3 weeks. Imidacloprid 600 FS tested in the third experiments as seed treatment (5 ml kg^{-1}) protected the crop upto 6 weeks against leafhopper. There was no reduction in the honey bee activity in these treatments when the crop was in full bloom. Significant increase in grain yield was observed in the imidacloprid treated plots. Germination was not affected by seed treatment and there was no phytotoxic symptom on the treated plants even when applied at a higher dosage. Residue of imidacloprid in seed and oil was at below detectable level at harvest when applied as seed treatment at 20 g kg^{-1} or as foliar spray at 400 ml ha^{-1}.

Key words : Imidacloprid, Sunflower, Leafhopper, Bioefficacy, Honeybee, Phytotoxicity, Residue analysis.

Introduction

Sunflower, *Helianthus annuus* L. is an important oilseed crop in India. The crop is damaged by pests from seedling stage to harvest. Among the early stage pests, leafhopper, *Amrasca devastans* (Distant) (Cicadellidae:Hemiptera) is important. Due to sucking of sap by nymphs and adults from leaves, the plants lose vigour and become stunted in growth leading to severe yield loss. Systemic insecticides like dimethoate, methyl demeton and monocrotophos are advocated as foliar spray for the management of this pest (Chakkravarthy and Balasubramanian, 1986; Dhawan *et al.* 1979). Development of resistance to many insecticides by this pest has been reported by Chalam and Subbaratnam (1999). Hence there is an urgent need to find alternate insecticides which are less harmful to environment by selective application method and there is least chance for development of resistance among the target pests. In this context, a new insecticide molecule, imidacloprid belonging to chloronicotinyl group reported to be effective against sucking pests (Elbert *et al.* 1991) was evaluated for its bioefficacy against leafhopper on sunflower including estimation of residue in seed and oil at harvest.

Materials and Methods

Three field experiments were conducted during January 1999 to March 2000 at the College Farm of Tamil Nadu Agricultural University, Coimbatore to evaluate the bioefficacy of imidacloprid as seed treatment and foliar spray against the leafhopper on sunflower. The experiments were conducted in a randomized block design with three replications under irrigated conditions with cultivar CO 2 during three different periods, *viz.* January-April 1999, July-September 1999 and December 1999-March 2000. Seed treatment was done with imidacloprid 70 WS (Gaucho 70 WS) which contained 700g a.i. kg^{-1} of imidacloprid in all the three experiments at 5,7 and 10g kg^{-1} and in the third experiment imidacloprid 600 FS (Gaucho 600 FS) (600g a.i. litre^{-1}) at 5,9 and 12ml kg^{-1} was used. Seed treatment was done a day before sowing. For foliar treatments, imidacloprid 200 SL (Confidor 200 SL containing 200g a.i.litre^{-1}) was used at 100 and 200ml ha^{-1} three weeks after sowing when the incidence of leafhopper was observed. The test chemical was supplied by Bayer (India) Ltd. Methyl demeton 25 EC used as foliar spray at 500 ml ha^{-1} was the

Table 1. Bioefficiency of imidacloprid in the control of leafhopper on sunflower
Experiment - I, January-April, 1999
Population, number/15 leaves (Mean of 4 observations)

Treatments	Weeks after sowing (W)							
	2 W#	3W**		4W**	5W**	6W**	7W#	Yield** of seed (kg ha^{-1})
			3 DAT**	7 DAT**	14 DAT**	21 DAT**	28 DAT	
Imidacloprid 70 WS 5 g kg^{-1} ST	1.00 (1.18)	1.8 (1.49)^{a}	3.0 (1.85)^{c}	2.0 (1.56)^{c}	3.5 (1.99)^{c}	4.3 (2.17)^{a}	2.8 (1.79)	1463^{d}
Imidacloprid 70 WS 7 g kg^{-1} ST	0.8 (1.09)	1.5 (1.40)^{a}	2.3 (1.63)^{abc}	1.8 (1.49)^{bc}	3.3 (1.93)^{c}	4.3 (2.17)^{a}	3.0 (1.86)	1545^{cd}
Imidacloprid 70 WS 10 g kg^{-1} ST	0.8 (1.09)	1.3 (1.31)^{a}	1.3 (1.31)^{a}	1.3 (1.31)^{abc}	1.3 (1.31)^{ab}	3.5 (1.99)^{a}	2.8 (1.77)	1550^{cd}
Imidacloprid 200 SL 100 ml ha^{-1} FS	1.3 (1.31)	5.0 (2.33)^{b}	1.8 (1.47)^{abc}	1.0 (1.18)^{ab}	1.8 (1.47)^{b}	3.3 (1.92)^{a}	2.8 (1.78)	1756^{ab}
Imidacloprid 200 SL 150 ml ha^{-1} FS	1.0 (1.18)	5.8 (2.49)^{b}	1.5 (1.40)^{ab}	0.8 (1.09)^{a}	0.8 (1.09)^{a}	3.5 (1.99)^{a}	2.5 (1.72)	1857^{a}
Methyl demeton 25 EC 500 ml ha^{-1} FS	1.3 (1.31)	5.3 (2.39)^{b}	2.8 (1.78)^{bc}	2.3 (1.65)^{c}	2.8 (1.79)^{c}	4.0 (2.11)^{a}	3.0 (1.86)	1651^{bc}
Untreated check	1.3 (1.25)	6.0 (2.54)^{b}	6.3 (2.59)^{d}	5.5 (2.44)^{d}	6.5 (2.64)^{d}	5.5 (2.43)^{b}	3.3 (1.93)	1391^{d}

ST : Seed treatment; FS : Foliar spray
DAT - Days after treatment; foliar. #- Not significant; *Significant at P=0.05; **Significant at P=0.01
In a column means followed by a common letter are not significantly different by DMRT (P=0.05)
Values in parentheses are transformed values, $\sqrt{x + 0.05}$

standard check. Foliar treatments were given using a high volume sprayer (Aspee Backpack) in 500 litres of spray fluid per hectare^{-1}. The treatments included an untreated check for comparison.

Observations on the incidence of leafhopper were recorded from two weeks after sowing at weekly intervals until 7th week. For foliar treatments, pre and post treatment observations were recorded three and seven days after treatment (DAT) besides weekly observations with other treatments. Population of leafhopper was recorded on three leaves per plant, one each from top, middle and bottom region from five plants per plot selected at random leaving border rows. When the crop was in full bloom, nine weeks after sowing, observation on the bee activity was recorded in 10 heads/plot selected at random for five consecutive days at 0900 hr. Yield of seed was recorded at harvest.

Phytotoxicity studies

Along with bioefficacy studies, three field experiments were conducted separately to study the phytotoxic effect of imidacloprid formulations on sunflower plants. Seed treatment was given with imidacloprid 70 WS at 5,10 and 20g kg^{-1} (three experiments) while imidacloprid 600 FS was used at 10,20 and 40ml kg^{-1} in the third experiment. Foliar spray was given with imidacloprid 200 SL, three weeks after sowing at 100,200 and 400 ml ha^{-1} using a spray volume of 500 litres ha^{-1} in a Aspee Backpack high volume sprayer.

Observation on the germination of seeds was recorded 10 days after sowing. Thereafter the crop was observed on 1,3,5,7,10 and 20

Table 2. Experiment - II, July - September, 1999

(Mean of 3 observations)

Sl. No.	Treatments	Weeks after sowing (W)							Yield of seed (kg ha^{-1})
		2 W#	3W**	4W**		5W**	6W**	7W#	
				3 DAT**	7 DAT**	14 DAT**	21 DAT**	28 DAT*	
1	Imidacloprid 70 WS 5 g kg^{-1} ST	0.66 (1.07)	0.66 (1.07)^{a}	1.66 (1.46)^{a}	2.33 (1.67)^{c}	2.33 (1.67)^{b}	2.33 (1.67)^{a}	2.66 (1.77)^{bc}	1531^{d}
2	Imidacloprid 70 WS 7 g kg^{-1} ST	0.66 (1.07)	0.33 (0.89)^{a}	1.00 (1.22)^{a}	1.66 (1.46)^{abc}	1.30 (1.34)^{ab}	2.33 (1.67)^{a}	1.66 (1.47)^{ab}	1618^{cd}
3	Imidacloprid 70 WS 10 g kg^{-1} ST	0.66 (1.07)	1.00 (1.22)^{a}	0.66 (1.07)^{a}	1.00 (1.22)^{ab}	0.66 (1.07)^{a}	2.33 (1.67)^{a}	1.66 (1.47)^{ab}	1638^{cd}
4	Imidacloprid 200 SL 100 ml ha^{-1} FS	0.66 (1.07)	5.30 (2.40)^{b}	1.00 (1.22)^{a}	0.66 (1.07)^{a}	1.66 (1.47)^{ab}	2.66 (1.77)^{a}	2.00 (1.58)^{ab}	1871^{ab}
5	Imidacloprid 200 SL 150 ml ha^{-1} FS	1.00 (1.22)	6.00 (2.55)^{b}	0.66 (1.07)^{a}	0.66 (1.07)^{a}	0.66 (1.07)^{a}	2.00 (1.58)^{a}	1.33 (1.34)^{a}	1938^{bc}
6	Methyl demeton 25 EC 500 ml ha^{-1} FS	1.33 (1.34)	6.66 (2.67)^{b}	1.00 (1.22)^{a}	2.00 (1.58)^{bc}	2.33 (1.67)^{b}	2.33 (1.67)^{a}	2.66 (1.77)^{bc}	1751^{b}
7	Untreated check	1.00 (1.22)	7.00 (2.74)^{b}	6.33 (2.60)^{b}	6.66 (2.67)^{d}	7.33 (2.79)^{c}	6.33 (2.60)^{b}	3.33 (1.95)^{c}	1398^{a}

ST : Seed treatment; FS : Foliar spray
DAT - Days after treatment; foliar. #- Not significant; *Significant at P=0.05; **Significant at P=0.01
In a column means followed by a common letter are not significantly different by DMRT (P=0.05)
Values in parentheses are transformed values, $\sqrt{x + 0.05}$

days after emergence for phytotoxicity symptoms like injury to leaf tip and leaf surface, wilting, vein clearing, necrosis and epinasty and hyponasty. Similarly observations were made for foliar treatments.

Residue determination

Samples of seed were collected at harvest from the phytotoxicity studies experiment. Residue of imidacloprid was determined from the three field experiments in seed and oil. Samples of 20g of seeds for each analysis in seed and in oil in four replicates were taken from each treatment.

Seed

20 g seed sample was soaked overnight in acetonitrile-water, blended, filtered and the filtrate was evaporated to near dryness in a rotary vacuum evaporator and the remainder was treated with 50ml of saturated sodium chloride and 150ml of hexane in a separating funnel. Lower aqueous phase was collected and 100ml of hexane: ethyl acetate (98:2 v/v) was added and shaken well. Lower aqueous phase was collected and partitioned with dichloromethane and the extract was poured through anhydrous sodium sulphate. The extract was evaporated to near dryness and the aqueous remainder was dissolved in ethyl acetate. The extract was cleaned up in Florisil column and the elute was concentrated to near dryness. The residue was dissolved in acetonitrile and fed into HPLC.

Oil

Seed sample of 25 g collected for oil residue analysis was blended, placed in a soxhlet apparatus and ran for 6-8hr in hexane. Hexane portion was collected and to this 5 ml acetonitrile

Table 3. Experiment - III, December 1999 - March 2000

(Mean of 3 observations)

Treatments	Weeks after sowing (W)						Yield** of seed (kg ha^{-1})
	3W**	4W**		5W**	6W**	7W#	
		3 DAT**	7 DAT	14 DAT	21 DAT	28 DAT	
Imidacloprid 70 WS 5 g kg^{-1} ST	1.67 (1.46)^a	1.00 (1.17)^a	0.33 (0.88)^a	0.67 (1.05)^a	1.33 (1.34)^a	0.67 (1.05)	1115^d
Imidacloprid 70 WS 7 g kg^{-1} ST	1.33 (1.34)^a	1.00 (1.23)^a	1.00 (1.23)^a	0.33 (0.88)^a	1.33 (1.34)^a	0.33 (0.88)	1125^d
Imidacloprid 70 WS 10 g kg^{-1} ST	1.00 (1.23)^a	0.33 (0.88)^a	0.33 (0.88)^a	0.33 (0.88)^a	1.00 (1.23)^a	0.67 (1.05)	1260^{bc}
Imidacloprid 600 FS 5 ml kg^{-1} ST	1.00 (1.17)	1.33 (1.34)^a	0.67 (1.00)^a	0.67 (1.05)^a	1.33 (1.34)^a	0.33 (0.88)	1175^{cd}
Imidacloprid 600 FS 9 ml kg^{-1} ST	1.00 (1.17)^a	0.67 (1.05)^a	0.33 (0.88)^a	0.33 (0.88)^a	1.33 (1.34)^a	0.67 (1.05)	1200^{cd}
Imidacloprid 600 FS 12 ml kg^{-1} ST	0.67 (1.05)^a	0.33 (0.88)^a	0.33 (0.88)^a	0.33 (0.88)^a	1.00 (1.17)^a	1.00 (1.23)	1225^{cd}
Imidacloprid 200 SL 100 ml ha^{-1} FS	6.67 (2.65)^a	1.00 (1.23)^a	0.33 (0.88)^a	0.33 (0.88)^a	1.67 (1.46)^a	0.67 (1.05)	1360^{ab}
Imidacloprid 200 SL 100 ml ha^{-1} FS	6.67 (2.67)^b	0.33 (0.88)^a	0.33 (0.88)^a	0.33 (0.88)^a	1.33 (1.34)^a	0.67 (1.05)	1375^a
Methyl demeton 25 EC 500 ml ha-1 FS	9.00 (3.07)^b	3.00 (1.86)^b	1.33 (1.34)^a	1.00 (1.23)^a	2.00 (1.56)^a	1.67 (1.46)	1125^d
Untreated check	8.00 (2.90)^b	7.67 (2.84)^c	7.33 (2.79)^b	4.67 (2.26)^b	4.67 (2.27)^b	1.67 (1.46)	860^e

ST : Seed treatment; FS : Foliar spray

DAT - Days after treatment; foliar. #- Not significant; *Significant at P=0.05; **Significant at P=0.01

In a column means followed by a common letter are not significantly different by DMRT (P=0.05)

Values in parentheses are transformed values, $\sqrt{x + 0.05}$

and 50ml of sodium chloride (3%) was added: lower aqueous phase was collected and to this 100 ml of hexane: ethyl acetate (98:2 v/v) was added. Further partitioning and clean up was done as described for seed sample. End analysis was done in HPLC.

HPLC conditions :

Liquid chromatograph	: Hitachi l 6200
Column	: ODS_2
Injection volume	: 20 µl
Detection (wave length)	: 270 nm
Mobile servant	: Acetonitrile : Water (35:65 v/v)
Flow rate	: 1 ml/min
Retention time	: 2.69 min
Sensitivity	: 0.5 µg
Determinability level	: Seed : 0.125 µg
	Oil : 0.250 µg

Recovery studies were conducted with fortified samples in seed and oil at 1 and 2 ppm level. Results showed recovery ranging from 87 to 92 per cent.

Results and Discussion

Results on the bioefficacy of three experiments are furnished in Table 1,2 and

3. Seed treatment with imidacloprid 70 WS at 5,7 and 10g kg^{-1} recorded significantly less leafhopper population when compared to untreated check. The effect persisted from three to six weeks. Population of leafhopper ranged from 1.00 to 4.3 per 15 leaves at 5g kg^{-1} while it was 0.8 to 4.3 at 7g kg-1 and 0.8 to 3.5 at 10g kg-1 in the first experiment. In the untreated check it was 1.3 to 6.5. A similar trend was observed in the second and third experiments. Imidacloprid 600 FS tested in the third experiment at 5,9 and 12 ml kg^{-1} was also superior to untreated check and was equal to imidacloprid 70 WS in the bioefficacy.

Foliar spray of imidacloprid 200 SL at 100 and 150 ml ha^{-1} reduced the leafhopper population significantly three days after treatment from 5.8 to 1.8 and 5.8 to 1.5 per 15 leaves, respectively. The effect persisted for three weeks. These treatments were significantly superior to standard, methyl demeton 25 EC at 500ml ha^{-1}. Though there was an increase in leafhopper population two weeks after treatment, still they were significantly superior to untreated check and equal to the standard. Similar superior performance of imidacloprid 200 SL treatments were observed in second and third experiment also.

In the yield of seed, all the treatments were significantly superior to untreated check. Among the imidacloprid formulations, foliar treatments recorded significantly higher yield than seed treatments, it was 1756 kg ha^{-1} at 100ml ha^{-1} while the seed treatment at 10g kg^{-1} yielded 1550 kg in the first experiment. Similar increased yield was observed in the foliar treatments in the second and third experiments.

Superior performance of imidacloprid 70 WS seed treatments in sunflower against leafhopper have been reported earlier by Men *et al.* (2001); Satpute *et al.* (2001) and Promod Katti (2001). In the present study also, seed treatment of imidacloprid 70 WS at 7g kg^{-1} and imidacloprid 600 FS at 5 ml kg^{-1} protected the crop upto 6 weeks from leafhopper. Efficacy of foliar spray of imidacloprid 200 SL against *A.devastans* on cotton was reported earlier by Kumar (1998). Ramesh Babu and Santharam (2000) evaluated the bioefficacy of imidacloprid 200 SL against *Empoasca kerri* Pruthi on groundnut and found that the effect persisted for 21 days when applied at 100ml ha^{-1}. Kumar (1998) reported increased seed cotton yield in imidacloprid treatments and attributed this to the control of sucking pests and also to the phytotonic effect of the insecticide. In the present study also, imidacloprid 200 SL at 100 ml ha^{-1} reduced the leafhopper population significantly and resulted in increased seed yield.

Imidacloprid seed treatments and foliar spray have not affected the honeybee activity when the crop was in full bloom, nine weeks after sowing and six weeks after foliar application. There was no difference in the number of bees visiting the flowers for collection of pollen and nectar between imidacloprid treatments and the untreated check. The number ranged from 8-12 $head^{-1}$ $minute^{-1}$ in all the five days of observations. The species of honey bees observed were rock bee, *Apis dorsata* F., little bee, *A.florea* F., Indian honey bee *A.cerana indica* F., Italian bee *A.mellifera ligustica* L. and the dammer bee *Trigona iridipennis* Smith. Schmuck *et al.* (2001) studied in detail with radio labelled imidacloprid 70 WS as seed treatment in sunflower and the resulting of residues in nectar and pollen. They found that the lethal food concentration (LD50) to *A. mellifera* was between 0.14 and 1.57 mg kg^{-1}. But the parent compound detected in nectar and pollen was below 0.001 mg kg^{-1} (limit of detection<0.001 mg kg^{-1}). They concluded that sunflower seed dressing with imidacloprid poses no risk to honey bees.

In the present study also, no adverse effect was observed in the number of bees visiting the sunflower in seed treatment as well as foliar application treatments with imidacloprid.

Phytotoxicity studies

Seed treatment with either imidacloprid 70 WS at 5,10 or 20g/kg^{-1} or imidacloprid

00 FS at 10,20 or 40ml kg^{-1} had no adverse effect on germination of sunflower seeds. The mean germination in these treatments was 97.69 per cent while it was 97.59 in the untreated. No phytotoxic symptom was observed on the plants in these treatments. Similarly no phytotoxic symptom was observed in the foliar treatment of imidacloprid 200 SL when applied at 100, 200 or 400ml ha^{-1}. Similar results were reported earlier by Kumar (1998) on cotton.

Residue analysis

Results of the residue analysis in all the three experiments revealed that the residue of imidacloprid was at below detectable level in seed and oil when applied as seed treatment with 70 WS formulation at 5,10 or 20g kg^{-1} and 600 FS formulation at 10,20 or 40ml kg^{-1} or when applied as foliar spray with 200 SL formulation at 100, 200 or 400ml ha^{-1}. Schmuck *et al.* (2001) determined the residue of imidacloprid in nectar and pollen of sunflower by the imidacloprid 70 WS seed-treated plants and found that the residue was less than the detectable level of 0.001 mg kg^{-1}. Results of the present study also show that the imidacloprid residue was at below detectable level even when applied at a higher dosage.

Considering the results of the three field experiments on the bioefficacy, safety to honeybees, phytotoxicity, residue and yield, we can advocate either seed treatment with imidacloprid 70 WS at 7g kg^{-1} or imidacloprid 600 FS at 5ml kg^{-1} or foliar spray of imidacloprid 200 SL at 100ml ha^{-1} to manage the leafhopper on sunflower.

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