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In vitro Evaluation of Persistent Toxicity of Imidacloprid 600 FS against Brown Plant Hopper and Green Leaf Hopper and its Impact on Germination and Seedling Vigour in Rice

S. Sangamithra*, K. Samiayyan and S. Chandrasekaran

Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India

**Corresponding author*

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A B S T R A C T

The laboratory experiment was conducted to investigate the persistence of imidacloprid 600 FS against brown plant hopper and green leaf hopper, to standardize the incubation period of paddy seeds before seed treatment and impact on germination and seedling vigour. The results revealed that twenty hours incubation period before treatment is finest and does not affect germination percentage. Moreover seed treatment did not influence seedling length and vigour. The imidacloprid treated plants @ 2.5 ml kg^{-1} seed exhibited higher persistence up to 50 days against brown plant hopper and green leaf hopper.

Introduction

Imidacloprid 1[(6-chloro-3-pyridynyl)methyl] – 4 – 5 – dihydro – N – nitro – 1H – imidazole – 2 amine] is a neonicotinoid insecticide belonging to the chloronicotinyl subfamily (Kagabu *et al.,* 1992). The discovery of imidacloprid by Shinzo Kagabu, and its subsequent market introduction in 1991, started the era of the neonicotinoid class of insecticides (Tomizawa and Casida, 2011). It is the most important neonicotinoid used primarily as a systemic compound against sucking and some biting insect pests (rice hoppers, aphids, thrips, whitefly, termites, etc. (Sur and Stork, 2003). Their physicochemical characteristics, mainly assessed in terms of their octanol water partition coefficient (K_{ow}) and dissociation constant (pKa), enable their entrance into plant tissues and their translocation to all its parts (Bromilow and Chamberlain, 1995; Bonmatin *et al.,* 2014). Regardless of the manner of application and route of entry to the plant, they translocate throughout all plant tissues making them toxic to any insects (and potentially other organisms) that feed upon the plant.

In Asia, major large-scale applications of neonicotinoids include spraying of rice fields and other crops (Taniguchi *et al.,* 2012), as well as granular applications (Thuyet *et al.,* 2011, 2012) and seed coatings. By far, the largest and most popular application in crop protection is the prophylactic seed coating. It is an a prior treatment against target pests that may decrease production yields. During germination and growing, the active substance in the seed coating is taken up by the roots and translocated to all parts of the crop, making the crop toxic to insects that attempt to feed upon it (Van der Sluijs *et al.,* 2013). Nevertheless, this group have a wide array of application modes as soil, seed and foliar treatments, seed treatment attains predilection since for convenience and safety for agriculturists. Besides copious merits of seed treatment over foliar spray, impact of seed treatment on germination, seedling vigour and its persistence in plant system is not comprehensible. Keeping this scientific scope, the present experiment was undertaken in laboratory condition, TNAU, Coimbatore to study the effect in germination, seedling vigour and persistence against brown plant hopper and green leaf hopper.

Materials and Methods

Evaluation of persistent toxicity of imidacloprid 600 FS

Pot culture experiments were conducted in the glass house at the insectary of Dept. of Agrl. Entomology, TNAU in order to assess the persistent toxicity of imidacloprid 600 FS as seed treatment against brown plant hopper and green leaf hopper

Insect culture maintenance

The culturing of plant hoppers was done as per the method described by Heinrichs et al (1985) Nucleus population of brown plant hopper (BPH) *Nilaparvata lugens* Stal. and sgreen leaf hopper (GLH), *Nephotettix virescens* Distant. were collected from the fields of Paddy Breeding Station, TNAU, Coimbatore from unsprayed fields. In order to maintain a uniform and continuous culture of the hoppers for the use in experiments, the insects were mass cultured in potted TN_1 rice plants. Thirty days old rice seedlings, in which outer leaf sheaths and dried leaf parts were removed to destroy any possible oviposition by other insects were planted in plastic pots (10 cm height and 11.5 cm diameter). The potted plants were transferred to a large wooden cage and adult insects collected from field were released into the cage for mass multiplication. During the whole process of culturing, the potted plants were placed in the plastic basins with 10-15 cm depth of water. By this way, the relative humidity to the growing plants was maintained. The plants showing wilting symptoms due to nymphal feeding were replaced regularly with healthy seedlings of paddy from the rearing cages.

Method of assessment

The healthy and viable paddy seeds of ADT 43 for persistent toxicity study were obtained from Paddy Breeding Station, TNAU, Coimbatore. The experiment was conducted in a completely randomized design with five replications. The treatments included for the study were as follows

No.	Treatments	Dose (ml kg^{-1})
1	Imidacloprid 600 FS	1.5
2	Imidacloprid 600 FS	2.0
3	Imidacloprid 600 FS	2.5
4	Imidacloprid 600 FS	2.0 + 10 g *P.flourescens*
5	Thiamethoxam 300 FS	3.0
6	Untreated control	0.0

Fifty gram of seeds were soaked in 500 ml of water for 24 hours. The homogenous slurry of respective treatment was prepared by using 1 ml of distilled water which was equivalent to the doses mentioned above. The pre germinated seeds were treated with above prepared slurry and sown in the plastic pot (10 cm height and 11.5 cm height). The pots were placed in the plastic tray with 10-15 cm depth of water throughout the experiment. When the seedlings were 20-25 days old, plants were covered with cylindrical mylar film cages (38 cm height and 14.2 cm dia) with fine mesh screen windows. Ten individuals of third instar BPH and GLH were released separately in each pot.

The number of surviving insects in each pot was counted at 48 hours interval and fresh set of 10 individuals were released on each day of count after removing the insects released on the previous day. The procedure was followed, till no mortality on the treated plants was observed. In both the cases, mortality data were corrected with Abbott's formula (Abbott, 1925).

$$\text{Corrected per cent mortality} = \frac{Pt - P_c}{100 - P_c} \times 100$$

Where,

Pt – Observed per cent mortality in treatment

P_c – Observed per cent mortality in untreated check

Persistence toxicity= Average residual toxicity x Period for which the toxicity persisted (days).

Standardization of incubation hours for pre germination

The seeds of ADT 43 for the experiment were procured from Paddy Breeding Station, TNAU, Coimbatore. Five hundred gram of seeds were soaked for 24 hours. After soaking, seeds were incubated in darkness. Different hours of incubation were considered as factor with eight replications and experimental details are enclosed hereunder.

No.	Treatment (Incubation time)
1	24 hours incubation
2	48 hours incubation
3	72 hours incubation

Germination percentage

Four replicates of 100 seeds each were selected at random from each treatment and placed for germination in roll towel medium at uniform spacing and kept in the germination room maintained at of 25±1°C and 95±3 % RH and the seedlings were evaluated on the 14th day by enumerating all the normal seedlings and expressed as germination percentage (ISTA, 1999).

In vitro evaluation of imidacloprid 600 FS on germination and seedling vigour

The effect of imidacloprid 600 FS on germination and seedling vigour at standardised hour of incubation was evaluated with eight replications and treatment details are given hereunder. Dry seed treatment was maintained as absolute control. The dosage used for this experiment was 2.5 ml kg^{-1} seed. In case of dry seed treatment, seed without soaking and incubation, was mixed with imidacloprid and subjected to germination.

No.	Treatment
1	24 hours incubation + imidacloprid treatment
2	Imidacloprid treatment + 24 hours incubation
3	Absolute control

Germination Percentage

As per the method described in 2(a).

Root length

At final count (14th day), ten normal seedlings in each replication were chosen at random and length of root was measured from collar region to tip of the primary root and the mean values were expressed in centimeter.

Shoot length

The same normal ten seedlings in which root length was measured were used to measure shoot length.

The length of shoot was measured from the base of the shoot to tip of the primary leaf and the mean values were expressed in centimeter.

Vigour index

The vigour index of the seedling was computed using the formula suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

Vigour index =

Germination (%) x Mean seedling length (cm)

Statistical analysis

The data on germination percentage of each treatment were subjected to arcsine transformation for statistical analysis while data of root and shoot length were analysed as same without any transformation.

Critical difference values were calculated at five per cent probability level and mean values were compared by using Duncan's Latin Square Design (1951).

Results and Discussion

1) Persistent toxicity

Ninety per cent mortality of BPH was observed up to 13 days of observation in 2.5 ml kg^{-1} and up to 11 days in 2.0 ml kg^{-1} of imidacloprid. There was no marked difference of BPH mortality observed in the treatment combination *viz.,* imidacloprid 2.0 ml + 10 g *Pseudomonas flourescens* and imidacloprid alone at 2.0 ml kg^{-1}. The standard check thiamethoxam (3.0 ml kg^{-1}) and lowest dose of imidacloprid (1.5 ml kg^{-1}) registered mortality range of 81-89 per cent up to 13 days . Thiamethoxam (3.0 ml kg^{-1}) recorded 85 per cent mortality while it was 81 per cent in imidacloprid (1.5 ml kg^{-1}). Above fifty per cent mortality was observed up to 21 days in imidacloprid (2.5 ml kg^{-1}) while it was 19 days in imidacloprid (2.0 ml kg^{-1}) and 17 days with thiamethoxam (3.0 ml kg^{-1}) and imidacloprid (1.5 ml kg^{-1}). The mortality of BPH was there up to 31 days with imidacloprid (2.5 ml kg^{-1}). The order of relative efficacy (ORE) of the insecticides based on the persistent toxicity index (PTI) was: imidacloprid at 2.5> 2.0 ml kg^{-1}> 2.0 ml + 10 g *Pseudomonas flourescens* kg^{-1} > thiamethoxam at 3.0 ml kg^{-1}> imidacloprid at 1.5 ml kg^{-1} (Table 3).

Regarding green leaf hopper results observed was similar with BPH. The results Ninety per cent mortality was observed up to 11 days of observation in higher dose of imidacloprid at 2.5 ml kg^{-1} seed and eighty per cent mortality was recorded up to 15 days. This was followed by imidacloprid at 2.0 ml kg^{-1} which registered eighty per cent mortality up to 13 days. There was no pronounced significant effect in mortalty between the imidacloprid at 2.0 ml kg^{-1} alone and combination of imidacloprid at 2.0 ml + 10 g *P. flourescens* kg^{-1}. Above fifty per cent mortality was observed up to 23 days in imidacloprid at 2.5

ml kg^{-1} while it was 21 days in imidacloprid at 2.0 ml kg^{-1} and imidacloprid at 2.0 ml + 10 g *P. flourescens* kg^{-1} and 19 days with imidacloprid at 1.5 ml kg^{-1} and standard check thiamethoxam at 3.0 ml kg^{-1}. In the higher dose of imidacloprid *viz.,* 2.5 ml kg^{-1}, mortality persisted up to 31 days (Table 4).

2) Standardisation of incubation hours for pregermination

The results of the standardisation of incubation hours are shown in the Table 1. The higher germination percentage was recorded after 24 hours incubation (96 per cent) followed by 48 hours incubation (90 per cent). Comparatively germination percentage was lower with 72 hours incubation (81 per cent). Hence 24 hours incubation was choosen as a standard for subsequent study.

3) *In vitro* evaluation of imidacloprid 600 FS on germination and seedling vigour

The influence of imidacloprid 600 FS on germination and seedling vigour was not statistically significant at CD (P= 0.05). However, twenty four hours incubation after imidacloprid treatment exhibited trivial lofty root and shoot length of 18.46 and 8.52 cm, respectively. Besides, it did not affect germination percentage (97 per cent) (Table 2).

Table.1 Standardisation of incubation hours for pre germination of rice

Sl.No.	Treatments (Incubation time)	Rice seed Germination percentage* (%)
1	24 hrs	96^{a} (80)
2	48 hrs	90^{b} (71)
3	72 hrs	81^{c} (64)
4	**CD (P=0.05)**	**4.01**

*Values of rice germination percentage are mean value of eight observations
Values in parentheses are arc sine transformed values of eight observations

Table.2 Invitro evaluation of imidacloprid 600 FS on germination and seedling vigour of rice

Sl.No.	Treatments	Germination percentage (%)	Root length (cm)	Shoot length (cm)	Vigour index
1	24 hrs incubation + imidacloprid treatment	96 (82)	18.04	7.92	2503
2	Imidacloprid treatment + 24 hrs incubation	97 (81)	18.46	8.52	2603
3	Absolute control (Dry seed treatment)	96 (78)	17.35	7.48	2377
4	**CD (P=0.05)**	**NS**	**NS**	**NS**	**NS**

Values in parentheses are arc sine transformed values of eight observations

Table.3 Persistent toxicity of imidacloprid 600 FS to brown planthopper, *Nilaparvata lugens* (Stal) on rice

Treatment	Dose (ml/g kg^{-1} seed)	Corrected per cent mortality / Period (Days After Treatment)										
		1	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**	**21**
Imidacloprid 600 FS	1.5	87.6	87.5	87.3	87.3	87.1	85	81.2	69.4	53.2	46.3	39.7
Imidacloprid 600 FS	2.0	90.3	90.1	90.1	90.0	90.0	90.0	88.3	77.2	65.3	50.5	48.2
Imidacloprid 600 FS	2.5	91.2	91	91	91	91	90.7	90.0	80.3	71.0	55.1	51.4
Imidacloprid 600 FS+ *P. fluorescens*	2.0 + 10g	90.0	90.0	89.8	89.7	89.7	89.5	88.0	76.8	64.1	51.3	45.1
Thiamethoxam 300 FS	3.0	89.4	89.1	89.1	89.1	89.0	88	85	72.1	59.7	48.4	43.6

Treatment	Dose (ml/g kg^{-1} seed)	Corrected per cent mortality / Period (Days After Treatment)									
		23	**25**	**27**	**29**	**31**	**33**	**P**	**T**	**PTI**	**ORE**
Imidacloprid 600 FS	1.5	21.4	15.6	4.1	0	0	0	27	50.16	1354.32	**5**
Imidacloprid 600 FS	2.0	34.0	21.5	7.3	1.4	0	0	29	54.95	1593.55	**2**
Imidacloprid 600 FS	2.5	38.7	25.2	13.0	5.2	2.1	0	31	57.52	1783.12	**1**
Imidacloprid 600 FS+ *P. fluorescens*	2.0 + 10g	33.5	19.3	6.7	2.0	0	0	29	54.44	1578.76	**3**
Thiamethoxam 300 FS	3.0	27.6	11.1	5.4	0	0	0	27	52.15	1408.05	**4**

P – Period of persistence (days) **PTI** – Persistent Toxicity Index **T** – Mean per cent mortality **ORE** – Order of Relative Efficacy

Table.4 Persistent toxicity of imidacloprid 600 FS to Green leafhopper, *Nephotettix virescens* (Dist.) on rice

Treatment	Dose (ml/g kg^{-1} seed)	Corrected per cent mortality / Period (Days After Treatment)										
		1	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**	**21**
Imidacloprid 600 FS	1.5	88.0	87.2	86.8	85.2	85.0	82.0	80.4	70.1	65.6	50.1	41.2
Imidacloprid 600 FS	2.0	90.4	90.2	89.7	89.1	89.1	87.0	83.3	76.2	72.5	65.1	54.1
Imidacloprid 600 FS	2.5	91.3	91.3	91.0	90.7	90.3	88.7	85.1	80.0	75.3	69.3	58.2
Imidacloprid 600 FS+ *P. fluorescens*	2.0 +10g	90.1	90.0	89.8	88.7	88.6	86.6	83.0	75.3	71.6	64.3	53.5
Thiamethoxam 300 FS	3.0	89.2	89.0	88.1	87.3	87.1	85.1	81.7	72.0	68.1	62.1	43.2

Treatment	Dose (ml/g kg^{-1} seed)	Corrected per cent mortality / Period (Days After Treatment)									
		23	**25**	**27**	**29**	**31**	**33**	**P**	**T**	**PTI**	**ORE**
Imidacloprid 600 FS	1.5	29.2	16.2	4.1	0	0	0	27	51.24	1485.96	**4**
Imidacloprid 600 FS	2.0	49.1	31.6	17.6	4.2	0	0	29	58.19	1687.51	**2**
Imidacloprid 600 FS	2.5	50.1	38.1	25.4	12.0	3.4	0	31	61.19	1896.89	**1**
Imidacloprid 600 FS+ *P. fluorescens*	2.0 +10g	48.3	32.0	16.2	4.7	0	0	29	57.81	1676.49	**3**
Thiamethoxam 300 FS	3.0	30.0	19.3	6.1	0	0	0	27	53.43	1442.61	**5**

P – Period of persistence (days) **PTI** – Persistent Toxicity Index **T** – Mean per cent mortality **ORE** – Order of Relative Efficacy

Persistent toxicity

The results on persistent toxicity of imidacloprid as seed treatment revealed that the chemical persisted for 51 days at 2.5 ml kg^{-1}; 49 days at 2.0 ml kg^{-1} and in combination of 2.0 ml +10 g *P. flourescens* kg^{-1}; 47 days at both 1.5 ml kg^{-1} and standard check thiamethoxam 3.0 ml kg^{-1} against brown planthopper. Similar range of persistence was recorded against green leafhopper also. Present results are in agreement with the work of Zhang et al (2011) who reported that thiamethoxam (Cruiser ®) at 0.342 mg a.i. $seed^{-1}$ and imidacloprid (Gaucho Grande ®) at 0.375 mg a.i. $seed^{-1}$ exhibited similar efficacy against *Bemisia tabaci* (Gennadius) in cotton for up to 45 days after planting in the laboratory and greenhouse experiments and up to 56 days under field conditions. Kumar (1998) revealed persistence for foliar spray of imidacloprid 200 SL at 100 and 150 ml ha^{-1} as 22 days against aphid and 30 days against leafhopper in cotton.

The longer persistence of imidacloprid and thiamethoxam may be due to the metabolic products of active ingredient that also enhanced toxicity against the pest.

Seed treatments are often preferable to foliar sprays because they are safer, easy to handle and allow more precise targeting of the insecticidal active ingredient with the pest organism by forming a protective zone of active ingredient around the seed grain against insect pests. The coating of active ingredient can be easily taken up by the roots during plant establishment in nursery, hence uptake into plant tissues is increased and residual activity against pests is enhanced. The preceding concept can be evidenced by findings of Sur and Stork (2003), in which uptake, translocation and metabolism of imidacloprid has been deliberated. The results of translocation experiments for imidacloprid with different application types show, that there is a good acropetal translocation of the active substances to shoots and leaves (excellent xylem mobility) on the one hand and on the other hand a poor basipetal translocation to sinks, i.e. storage organs, roots and fruits (negligible phloem mobility). Three common metabolic pathways of conversion of imidacloprid in plant system had been observed, *viz.*, transformation of active substance into olefin, nitrosamine and 6 chloronicotinic acid. Nevertheless, irrespective of mode of application, they share the common metabolism and translocation pathways, the uptake after soil or seed treatment is about 5% of the applied dose and the a.s. shows good acropetal mobility within the xylem and poor basipetal translocation within the phloem. A quick degradation of the active substance was observed after root uptake of the active substance in seed treatment or soil application. In the case of spray application only a part of the active substance is translocated into the plant and metabolized there, so the degree of metabolism tends to be lower in this case.

In vitro evaluation of imidacloprid on germination and seedling vigour

The results of the germination percentage observed at different incubation hours in darkness revealed that higher germination was observed after 24 hours incubation (96 per cent). Hence 24 hours incubation is suggested before imidacloprid treatment wherein no deleterious effect on the seedling establishment was observed. The present findings are in line with Stevens et al (2008) who showed imidacloprid (2000 mg a.i. l^{-1}) had no adverse effects on post germination growth if applied to pre germinated rice shortly before sowing and even up to 4000 mg a.i. l^{-1}. Imidacloprid had no significant influence on germination, seedling length and

vigour index after treatment. The parameters like germination percentage, root and shoot length and vigour index were recorded from imidacloprid treatment after 24 hours incubation, 24 hours incubation after treatment with imidacloprid and absolute control (Dry seed treatment). All these parameters were on par in the above three cases and were not statistically significant. However, trivial increase in seedling length and vigour index was observed in case of 24 hours seed incubation after treatment with imidacloprid.

Similar findings were reported by Sajjan et al (2010) in sunflower. As per the study, in all the concentrations of imidacloprid 600 FS (2.5, 5.0, 7.5, 10.0 and 12.5 ml/kg seeds) the germination level was above the minimum seed certification standards (more than 70%) up to eighth month of storage and at ninth month after storage the germination was below 70 per cent. In contrast, the seedling length and vigour index were also significantly influenced by the imidacloprid concentrations. The mean seedling length and vigour index after nine month storage in imidacloprid 600 FS @ 2.5 ml / kg seed was 31.4 cm and 2540, respectively as against in untreated check (32.6 cm and 2748, respectively). However, seedling length and vigour index was observed to reduce with the increased dosage of imidacloprid.

Hence to conclude, seed treatment with imidacloprid 600 FS @ 2.5 ml kg^{-1} seed was persistent up to 50 days in laboratory condition. However, under field condition, this finding may not be correlated, since plants are exposed to UV and other abiotic factors, degradation of metabolites will be rapid that may cause reduced persistence. Incubation period of 24 hours in darkness is recommended before seed treatment with imidacloprid 600 FS to attain better efficiency and this treatment does not have any significant influence on seedling establishment and vigour.

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