Indian Journal of Entomology 85(3): 605-609 (2023) DoI. No.: 10.55446/IJE.2022.657

DISSIPATION AND PERSISTENCE OF IMIDACLOPRID USED AS SEED TREATMENT IN PEAS

Dalvir Kataria[1], Kousik Mandal[1], Smriti Sharma[1]* and Balpreet Kaur Kang[1]

[1]Pesticide Residue Analysis Laboratory, Department of Entomology,
Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: smritisharma80@pau.edu (corresponding author)

ABSTRACT

Dissipation and persistence study of imidacloprid on peas as seed treatment revealed presence of 6.47 and 9.92 mg kg^{-1} residues on green pea leaves at a dose of 1.8 g and 3.6 g a.i. per kg of seed, respectively after 19 days of treatment. Residues of imidacloprid dissipated below limit of quantification of 0.01 mg kg^{-1} after 49 and 54 days of treatment. Residues of imidacloprid were found below limit of quantification of 0.01 mg kg^{-1} in immature pod with succulent seeds, succulent seeds (shelled), mature pod with seeds, mature seeds (shelled), soil and dry fodder (without root and pod).

Key words: Imidacloprid, seed treatment, peas, residues, persistence, leaves, seed, mature pod, fodder, QuEChERS method

Neonicotinoids have a novel mode of action by acting as agonists at the insect nicotinic acetylcholine receptor (Tomizawa and Casida, 2011), these have selective-toxicity (Jeschke et al., 2011), and have systemic action (Stoner and Eitzer, 2012). Neonicotinoid seed treatments have shown long-lasting residual activity against aphids (Zhang et al., 2016a,b) and thrips in soybean and groundnut (Reisig et al., 2012; Zidan, 2012; Nataraja et al., 2016). Peas is an ancient cultivated leguminous crop (Dahl et al., 2012), and it is attacked by many pests, out of which aphids, thrips, leaf miner and pea stem fly are the most destructive (Anonymous 2019). Pea aphid *Acyrthosiphon pisum* is a serious pest causing losses of up to 35.7% (Barlow et al., 1977); Bhatnagar 1996). Presence of pesticide residues on agricultural products is a cause of concern, and emphasis has been given to resolve problems associated with the pesticide residues, indiscriminate and irrational use of pesticides. Presence of pesticide residues in food commodities is of consumer health concern and many studies have reported presence of imidacloprid residues (Chen et al., 2014; Shi et al., 2019). Since, the magnitude of pesticide residues in the vegetable commodities at harvest is a cause of concern in many of the occasions, the present study focused on evaluating the persistence and dissipation of imidacloprid applied as seed treatment in pea crop.

MATERIALS AND METHODS

Field experiment was conducted during rabi 2019-20 at the Entomological Research Farm, Punjab Agricultural University, Ludhiana following standard agronomic practices (Anonymous, 2019). Pea (variety Punjab 89) seeds were treated with an aqueous slurry of imidacloprid 600 FS at recommended dose (1.8 g a.i.kg^{-1} seed) and double the recommended dose (3.6 g a.i.kg^{-1} seed). Green peas seeds were put in polythene bags separately for each treatment and thoroughly mixed with slurry made from pesticide and water with volume of 12 ml/ kg seed (pesticide + water) and then dried in shade before sowing. Urea and superphosphate fertilizers were applied as per the practices recommended for cultivation of peas in Punjab (Anonymous, 2019). Pea leaf samples were collected randomly from each plot on 19th day of sowing followed by 20, 22, 24, 26, 29, 34, 39, 44, 49, 54 days after sowing. Samples of immature pods and succulent seed (shelled) samples were also collected at the time of first picking i.e. 110 days after sowing, while mature pods with seeds, mature seeds (shelled), dry fodder (without root and pod) and soil samples were collected at the time of harvest (128 days after sowing). Immature pods with succulent seeds, succulent seeds (shelled), mature pods with seeds, mature seeds (shelled), dry fodder (without root and pod) and soil samples were prepared following QuEChERS method for the determination of residues of imidacloprid by the method suggested by Akoijam et al. (2015) with slight modifications.

A sub sample of 15 g was weighed into a 50 ml centrifuge tube and then 30 ml acetonitrile was dispensed into it while in case of dry fodder a sub sample of 5 g was weighed into a 50 ml centrifuge tube and then

20 ml acetonitrile was dispensed into it. In case of soil, 10 ml of distilled water was added in addition to 30 ml acetonitrile. The sample was homogenized using high speed homogenizer (Heidolph Silent Crusher-M®) for 3 min at 15,000 rpm. Sodium chloride (NaCl) @10± 0.1 g was added to homogenize the sample for phase separation. The contents were centrifuged at 2,500 rpm for 3 min. An aliquot of 15 ml acetonitrile layer was transferred over 10± 0.1 g sodium sulfate (Na_2SO_4) in a test tube. The acetonitrile extract was subjected to cleanup by dispersive solid phase extraction (DSPE). An aliquot of 6 ml acetonitrile was taken in a test tube containing 0.15± 0.01 g PSA sorbent, 0.90± 0.01 g anhydrous $MgSO_4$ and 0.05± 0.01g graphitic carbon black and the contents were thoroughly vortex on vortex shaker. 4 ml aliquot was taken for estimation of imidacloprid residues. Sodium chloride (NaCl) and anhydrous sodium sulfate (Na_2SO_4) was bought from Sisco Research Laboratories Pvt. Ltd., New Mumbai, India. Before use anhydrous sodium sulphate (Na_2SO_4) and anhydrous magnesium sulphate ($MgSO_4$) were activated for 4 h at 800°C in muffle furnace to remove impurities of phthalate. Primary Secondary amine (PSA) bondesil, anhydrous magnesium sulphate ($MgSO_4$) and graphitized carbon black (GCB) were procured from Agilent Technologies Ltd. Bangalore. During analysis, blank samples were also run along with sample to check the suitability of these solvents.

The estimation of imidacloprid residues was done by using high performance liquid chromatography (HPLC) system (Make: Shimadzu Corporation, Kyoto, Japan; Model: SIL 20A/20 AC) with double plunger pump, Rheodyne injector having 20 µl loop with PDA detector. The chromatographic separation was achieved on C_{18} reverse phase column (4×150mm) with a particle size of 5 µm. The mobile phase consisted of HPLC grade acetonitrile (Make: Qualichem Lab): water (30:70 v/v) with a constant flow rate of 0.3 ml/ min and UV detection set at 272 nm. The method of estimating pesticide residues was further validated in terms of linearity, efficiency, precision (repeatability), limit of detection and limit of quantification by spiking the samples at different fortification levels of imidacloprid to assess the reliability and validity of the analytical method (Akoijam et al., 2015). Samples were spiked at three different concentrations *viz*., 0.01, 0.05 and 0.1 mg kg^{-1} and imidacloprid residues were calculated by comparing the peak areas of the reference standards with that of the unknown or spiked samples run under identical working conditions of the instruments. The data was analyzed for mean and standard deviation.

RESULTS AND DISCUSSION

For the validation of the analytical method sensitivity, selectivity, calibration and recovery experiments were performed. Graphs were constructed from chromatograms by plotting the peak area of the signal response versus the concentration of the analyte to assess the linear response of imidacloprid. The control samples were fortified with standards at 0.01, 0.05, and 0.10 mg kg^{-1} concentration levels of imidacloprid standard. The residues were extracted, cleaned up as per methodology mentioned above and analyzed by using high performance liquid chromatography. The untreated control samples were also processed as above to find any interference caused by matrix/substrate and reagents. The analytical method was found to be suitable since it did not show any interference peaks at the retention time of imidacloprid and was sensitive in terms of response. The recovery percentage of imidacloprid on pea leaf and pod samples spiked at 0.01, 0.05 and 0.1 mg kg^{-1} was also found to be very suitable varying between 88.78-98.33% which was within the acceptable range. The limit of quantification (LOQ) was found to be 0.01 mg kg^{-1} and limit of detection (LOD) being 0.003 mg kg^{-1}. The data also revealed that the analytical method was reliable and valid, and on the extraction and cleanup procedures were also effective and hence could be presented as such without any correction factor. Residues of imidacloprid on green pea leaves sampled at 19th day after treatment was found to be 6.47 and 9.92 mg kg^{-1} at the dose of 1.8 and 3.6 g a.i.kg^{-1}, respectively, which decreased to5.83 and 8.71 mg kg^{-1} at 20th day after treatment with 9.89% and 12.19% reduction, respectively. Residues of imidacloprid dissipated below limit of quantification of 0.01 mg kg^{-1} at 49 and 54 days after treatment in both the tested doses. Residues of imidacloprid were below the limit of quantification of (0.01 mg kg^{-1}) in immature pod with succulent seeds, succulent seeds (shelled), mature pod with seeds, mature seeds (shelled), while the samples of soil and dry fodder (without root and pod) collected at harvest were free from imidacloprid residues (Table 1, Fig. 1).

Present results are in agreement with those of other researchers regarding dissipation rate of imidacloprid in brinjal, bengal gram and tomato. Imidacloprid residues have been reported to dissipate below the limit of quantification after 7 and 10 days in brinjal from initial deposits of 0.72 and 1.92 mg kg^{-1} following application of spinetoram at 12% + imidacloprid 12% @ 75 and 150 g a.i. ha^{-1} respectively on brinjal crop (Bhardwaj et al., 2016). Likewise in another set of

Table 1. Recovery (%) and residue estimation of imidacloprid (mg kg^{-1}) on pea crop and soil

Recovery of imidacloprid on different matrices of pea crop and soil		
Substrates	Level of fortification (mg kg^{-1})	Recovery (%)
Leaves	0.01	98.33± 3.31
	0.05	96.57± 3.47
	0.10	96.42± 2.84
Immature pods with succulent seeds	0.01	95.35± 3.56
	0.05	89.26± 2.98
	0.10	88.78± 1.82
Succulent seeds (shelled)	0.01	87.21± 3.00
	0.05	87.23± 1.93
	0.10	93.05± 2.24
Mature pods with seeds	0.01	94.92± 3.14
	0.05	91.57± 3.47
	0.10	87.99± 3.64
Mature seeds (shelled)	0.01	88.20± 3.85
	0.05	85.56± 2.95
	0.10	91.07± 3.15
Dry fodder (without root and pod)	0.01	96.52± 3.67
	0.05	94.46± 2.30
	0.10	86.49± 1.95
Soil	0.01	96.47± 3.12
	0.05	90.11± 1.26
	0.10	96.37± 1.86

Days after treatment	Residues of imidacloprid (mg kg^{-1}) after application @ 1.8 g a.i.kg^{-1} seed		3.6 g a.i.kg^{-1} seed	
	Mean± S.D.	Dissipation (%)	Mean± S.D.	Dissipation (%)
	Green pea leaves			
19	6.47± 0.23	-	9.92± 0.36	-
20	5.83± 0.35	9.89	8.71± 0.51	12.19
22	3.99± 0.10	42.50	6.32± 0.31	36.29
24	2.49± 0.38	61.51	4.25± 0.15	57.15
26	1.51± 0.11	76.66	3.17± 0.14	68.04
29	1.19± 0.13	81.60	1.90± 0.23	80.84
34	0.46± 0.03	92.89	1.28± 0.08	87.09
39	0.15± 0.04	97.68	0.44± 0.12	95.56
44	0.03± 0.01	99.53	0.07± 0.01	99.59
49	<LOQ	-	0.042± 0.00	-
54	<LOQ	-	<LOQ	-
	Immature pods with succulent seeds			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Succulent seeds (shelled)			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Mature pods with seeds			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Mature seeds (shelled)			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Dry Fodder (without root and pod)			
110	<LOQ	<LOQ	<LOQ	<LOQ
	Soil			
110	<LOQ	<LOQ	<LOQ	<LOQ

*Limit of quantification (LOQ) = 0.01mg kg^{-1}; *Data mean of three replications

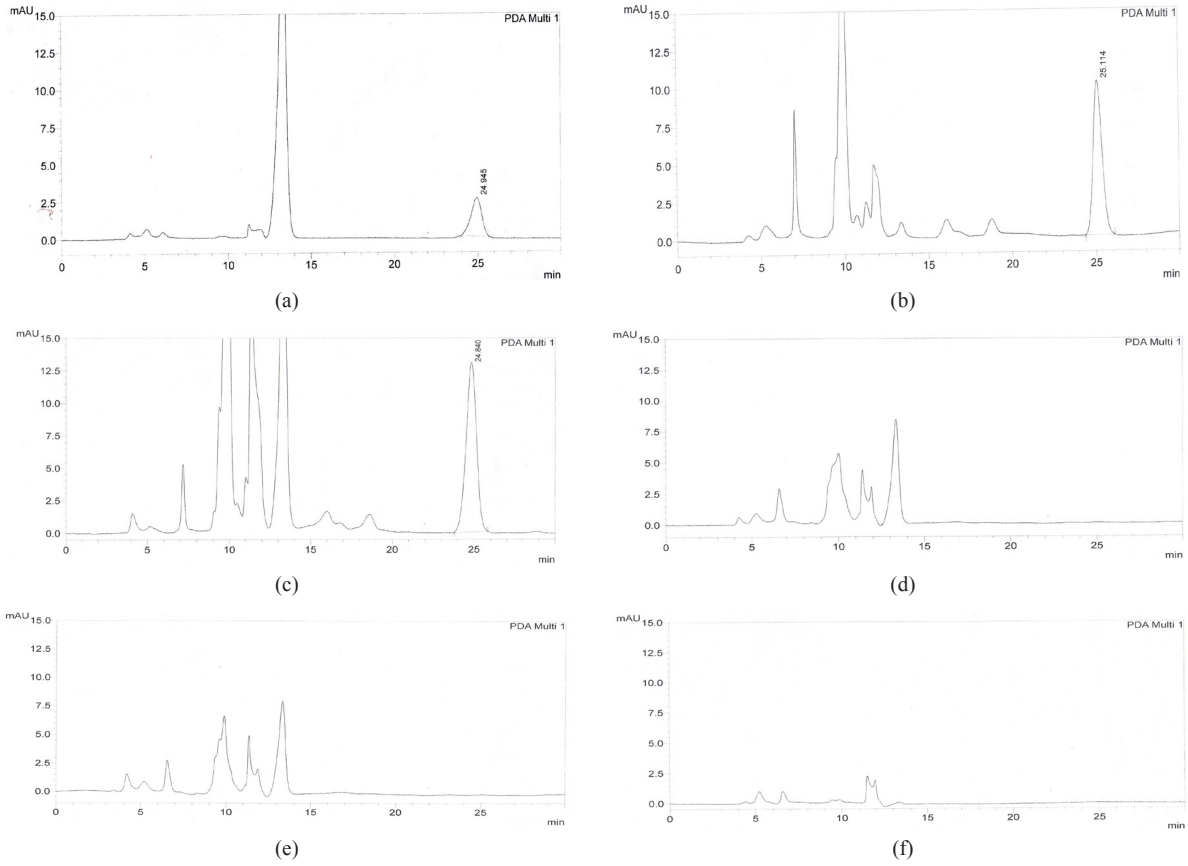

Fig. 1. Chromatograms of (a) imidacloprid standard (20 ng) (b) pea leaf sampled at 19 days after sowing (recommended dose) (c) pea leaf sampled at 19 days after sowing (double the recommended dose) (d) mature pod with seed samples (e) succulent seed samples and (f) soil samples

experiments were conducted by Gupta et al. (2005) to study the persistence of imidacloprid in gram following foliar application @ 20 and 40 g ai ha^{-1}. The results revealed that residues of imidacloprid persisted beyond 3 days but no residues were detected on 5th day and the residues were not detected in harvested seed and fodder samples. Earlier also, Romeh et al. (2009) have reported the dissipation of imidacloprid in tomato fruits by the application of imidacloprid 20 SC @ 10 g a.i. $feddan^{-1}$ (1 feddan = 4,200 m^2) and found average initial deposits of 4.22 and 1.95 mg kg^{-1} in the leaves and tomato fruits which dissipated to 0.44 and 0.075 mg kg^{-1} respectively 14 days after spraying. The variation in results on dissipation of a pesticide could be attributed to crop type, method of application, cropping season and other agroclimatic variations. In another study also, Sahoo et al. (2012) found that soil samples collected at 15 days did not show the presence of imidacloprid at their detection limit of 0.01 mg kg^{-1} after application of Solomon (β-cyfluthrin 9% + imidacloprid 21%) @ 60 and 120 g a.i. ha^{-1} in okra crop. It can be concluded that after seed treatment of peas with imidacloprid @ 1.8 and 3.6 g a.i.kg^{-1}, the initial deposits reached below the limit of quantification in pea leaves after 49 and 54 days at the respective doses and these residues were below the limit of quantification in immature pods, seeds and mature pods. Hence, seed treatment of peas with imidacloprid @ 1.8 and 3.6 g a.i.kg^{-1} seed does not result in residues above the quantification limit in pea seeds and could be integrated in IPM.

ACKNOWLEDGEMENTS

Authors thank the ICAR-All India Network Project on Pesticide Residues for providing financial support and Punjab Agricultural University, Ludhiana for providing facilities.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Akoijam R, Singh B, Mandal K. 2015. Development and validation of a quick, easy, cheap, effective, rugged and safe method for the determination of imidacloprid and its metabolites in soil. Journal of Chromatographic Science 53(4): 542-547.

Anonymous. 2019. Package of practices for cultivation of vegetables. Punjab Agricultural University. 190 pp.

Barlow C A, Randolph P A, Randolph J C. 1977. Effects of pea aphids, *Acyrthosiphon pisum* (Homoptera: Aphididae) on growth and productivity of pea plants, *Pisum sativum*. Canadian Entomologist 109: 1491-1502.

Bhardwaj U, Jyot G, Mandal K, Battu RS, Singh B. 2016. Residues of spirotetramat and imidacloprid in brinjal (*Solanum melongena* L.) and their risk assessment. Pesticide Research Journal 28: 35-41.

Bhatnagar A. 1996. Efficacy and economics of some insecticides and a neem formulation on incidence of pea aphid on pea. Annals of Plant Protection Science 4: 131-133.

Chen M, Tao L, McLean J, Lu C. 2014. Quantitative analysis of neonicotinoid insecticide residues in foods: implication for dietary exposures. Journal of Agricultural and Food Chemistry 62(26): 6082- 6090.

Dahl W J, Foster L M, Tyler R T. 2012. Review of the health benefits of peas. British Journal of Nutrition 108(S1): S 3-S10.

Gupta R K, Gupta S, Gajbhiye **V** T, Wiener H C, Singh G. 2005. Residues of imidacloprid, acetamiprid and thiamethoxam in gram. Pesticide Research Journal. 17(1): 46-50.

Jeschke P, Nauen R, Schindler M, Elbert A. 2011. Overview of the status and global strategy for neonicotinoids. Journal of Agricultural Food Chemistry 59(7): 2897-2908.

Nataraja M V, Harish G, Holajjer P, Savaliya S D. 2016. Efficacy of imidacloprid seed treatment for the control of leafhoppers and thrips in groundnut. Legume Research 39(2): 305-309.

Reisig D, Herbert D D A, Malone S. 2012. Impact of neonicotinoid seed treatments on thrips Thysanoptera: Thripidae) and soybean yield in Virginia and North Carolina. Journal of Economic Entomology 105(3): 884-889.

Romeh A A, Mekky T M, Ramadan R A, Hendawi M Y. 2009. Dissipation of profenofos, imidacloprid and penconazole in tomato fruits and products. Bulletin of Environmental Contamination and Toxicology 83(6): 812-817.

Sahoo S K, Chahil G S, Mandal K, Battu R S, Singh B. 2012. Estimation of β-cyfluthrin and imidacloprid in okra fruits and soil by chromatography technique. Journal of Environmental Science and Health, Part B 47(1): 42-50.

Shi Y H, Xiao J J, Liu Y Y, Fu Y Y, Ye Z, Liao M, Cao H Q. 2019. Interactions of food matrix and dietary components on neonicotinoid bioaccessibility in raw fruit and vegetables. Food and Functions 10(1): 289-295.

Stoner K A, Eitzer B D. 2012. Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*). PLoS One 7(6): e39114.

Tomizawa M, Casida J E. 2011. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annual Review of Entomology 48: 339-364.

Zhang P, Zhang X, Zhao Y, Wei Y, Mu W, Liu F. 2016a. Effects of imidacloprid and clothianidin seed treatments on wheat aphids and their natural enemies on winter wheat. Pest Management Science 72(6): 1141-1149.

Zhang Z, Zhang X, Wang Y, Zhao Y, Lin J, Liu F, Mu W. 2016b. Nitenpyram, dinotefuran, and thiamethoxam used as seed treatments act as efficient controls against *Aphis gossypii* via high residues in cotton leaves. Journal of Agricultural and Food Chemistry 64(49): 9276-9285.

Zidan L T M. 2012. Bio-efficacy of three new neonicotinoid insecticides as seed treatment against four early sucking pests of cotton. American-Eurasian Journal of Agricultural and Environmental Sciences 12(4): 535-540.

(Manuscript Received: March, 2022; Revised: July, 2022;
Accepted: July, 2022; Online Published: July, 2022)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e22190