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Persistence and dissipation of forchlorfenuron residues in soybean

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Abstract
An experiment was conducted on research field at A block, Director of Farm, MPKV, Rahuri to study the persistence and dissipation of plant growth regulator (PGR), forchlorfenuron in/on soybean. Single application of forchlorfenuron was given at the pod initiation stage at different concentrations i.e. 1.25 ppm, 2.50 ppm and 5.0 ppm by knapsack sprayer fitted with flood jet nozzle. The samples of leaves, green pods, dry pods, seed straw and soil were analysed and residues were estimated on HPLC with PDA detector at AINP on Pesticide Residues, Department of Entomology, MPKV, Rahuri (Maharashtra). The results revealed that mean initial residues of forchlorfenuron @ 1.25, 2.50 and 5.00 ppm were found to be 0.119, 0.224 and 0.390 mg/kg. Initial residues dissipated to BQL on 3, 5 and 7 days at 1.25, 2.50 and 5.00 ppm dose, respectively. Forchlorfenuron residues were found to be BQL in green pods, dry pods, seed, straw and soil samples

Keywords: Forchlorfenuron, plant growth regulator, soybean, dissipation

1. Introduction
Globally, soybean (*Glycine max* (L.) Merr) ranks first as an oilseed crop. It has remarkable value in agriculture as a source of high quality plant protein and vegetable oils. Soybean seed contains 40-45% protein, 20-26% carbohydrate, 20-22% oil and a high amount of Ca, P and vitamins (Rahman *et al*., 2011). Soybean contributes significantly to the Indian edible oil pool. Presently soybean contributes 43 % of the total oilseeds and 25% of the total oil production in the country. Currently, India ranks fourth with respect to production of soybean in the world. The soybean crop earned valuable foreign exchange of Rs. 62,000 millions in 2012-13 by way of soya meal exports (Anonymous, 2013).

Production of soybean in India is dominated by Maharashtra and Madhya Pradesh which contributes 89 per cent of the total soybean production in country. However, in farmer's field its average yield is much lower due to lack of improved agricultural practices of which application of different bioregulators is an important determinant for better production of soybean.

Plant growth regulators whether endogenously produced by plants themselves or applied externally, play a vital role in regulation of physiological processes such as cell division, enlargement, differentiation, senescence, ripening, germination, reproduction and protective response of plants against stress. (Davis 1995, Han *et al*, 2012)

Forchlorfenuron is a plant growth regulator and work as an excellent tool to increase the fruit size and getting good market price. It promotes cell division, differentiation and development, it induces budding of callus and controls apical dominance, breaks dormancy of lateral buds and promotes germination, Further, it delays the aging process and maintains chlorophyll in excised leaves regulates the transport of nutrients and promotes seed formation. Frequent applications of these plant growth regulators result in accumulation of residues at harvest which could be a food safety issue. Keeping this in view the studies were carried out to evaluate the harvest time residues of forchlorfenuron in/on soybean.

2. Material and Methods
2.1 Field experiment
The supervised field experiment was conducted at A block, Director of Farm, MPKV, Rahuri to study the persistence and dissipation of forchlorfenuron in/on soybean. The experiment was arranged in Randomized Block Design (RBD) with four treatments i.e. untreated control, forchlorfenuron @ 1.25 ppm, forchlorfenuron @ 2.50 ppm and forchlorfenuron @ 5.00 ppm.

All the recommended agronomic practices were followed for raising the cop. The soybean crop at pod initiation stage was sprayed with forchlorfenuron at different concentrations i.e. @ 1.25 ppm, 2.50 ppm and 5.00 ppm. The control plots were left unsprayed. Three replicates were made for each treatment.

2.2 Chemicals
Certified Reference Material as well as commercial formulation of forchlorfenuron was obtained from concerned firm. HPLC grade solvents of methanol, formic acid and acetone were obtained from M/s. Rankem Fine Chemicals, Ltd., New Delhi. PSA was procured from Agilent Technology, Bangalore. Working standards were prepared by dissolving reference standards in methanol.

2.3 Residue analysis
2.3.1 Standard preparation: Primary stock solution, intermediate standard and working standards were prepared by dissolving reference standards in methanol.

2.3.2 Method validation: Prior to analysis of samples, linearity of forchlorfenuron was established on HPLC. Accuracy and precision of the method was determined by per cent mean recovery and per cent relative standard deviation. Linearity was studied by injecting standard solution of forchlorfenuron at five linear concentrations i.e. 0.05, 0.10, 0.25, 0.40 and 0.50 mg/kg. The linearity curve was established with concentration of the standard and corresponding peak area. Recovery studies were carried out in order to establish the reliability of the method of analysis by fortifying leaves, green pods, dry pods, seeds, straw and soil at 0.05 (LOQ), 0.25(5LOQ) and 0.5(10LOQ) mg/kg fortification levels with forchlorfenuron standard.

2.3.3 Sample collection: Approximately 250 g leaves were collected at 0,1,3,5,7 and 10 days after application whereas green pods were collected at 10,20 and 30 days after application**.** Dry pods, seed, straw and soil were collected at harvest.

2.3.4 Extraction and Clean up
2.3.4.1 Green leaves: Weighed 10g homogenized sample in a 50 ml polypropylene tube and the tube was kept in deep freezer for 10 min. To this, added 10 ml acidified methanol containing 1 % formic acid, hand shaked vigorously and centrifuged the content at 3500 rpm for 5 min. Transferred 2 ml supernatant to 15 ml tube containing 50 mg PSA. The content was vortexed for 30 Sec and then cetrifuged at 2500 rpm for 2 min. The supernatant was filtered through 0.2 micron filter and performed HPLC analysis.

2.3.4.2 Dry pods and Seed: Weighed 5 g homogenized sample in a 50 ml polypropylene tube and the tube was kept in deep freezer for 10 min. To this, added 10 ml acidified methanol containing 1 % formic acid, hand shaked vigorously and centrifuged the content at 3500 rpm for 5 min. Transferred 2 ml supernatant to 15 ml tube containing 50 mg PSA. The content was vortexed for 30 Sec and then cetrifuged at 2500 rpm for 2 min. The supernatant was filtered through 0.2 micron filter and performed HPLC analysis.

2.3.4.3 Straw: Weighed 10g homogenized sample in a 50 ml polypropylene tube and the tube was kept in deep freezer for 10 min. To this, added 10 ml acidified methanol containing 1 % formic acid, hand shaked vigorously and centrifuged the content at 3500 rpm for 5 min. Transferred 2 ml supernatant to 15 ml tube containing 50 mg PSA. The content was vortexed for 30 Sec and then cetrifuged at 2500 rpm for 2 min. The supernatant was filtered through 0.2 micron filter and performed HPLC analysis.

2.3.4.4 Soil: Weighed 10 g homogenized sample in a 50 ml polypropylene tube and tube was kept in deep freezer for 10 min. To this, added 10 ml acidified methanol containing 1 % formic acid, hand shaked vigorously and centrifuged the content at 3500 rpm for 5 min. Transferred 2 ml supernatant to the 15 ml tube containing 50 mg PSA. The content was vortexed for 30 Sec and then cetrifuged at 2500 rpm for 2 min. The supernatant was filtered through 0.2 micron filter and performed HPLC analysis.

2.3.5 Chromatographic Separation Parameters: Forchlorfenuron residues were estimated on HPLC-PDA (Model HPLC-LC-20AT) equipped with LC solution data software. Chromatographic conditions are mentioned in Table1.

Table 1: HPLC Conditions

Column	Purospher@STAR, RP-18, Hibar 150-4,6 (5 µm)
Mobile phase	Methanol: Water (70:30 v/v)
Flow rate	0.8 ml/min
Wavelength	272 nm
Retention time	5.0 min

3 Results and Discussion
3.4.1 Linearity
Output of linearity studies in terms of peak area (Table 2 and Fig.1) indicated that response was linear over the range tested and the regression coefficient (R^2) was greater than 0.99 over the range tested. The details are as under.

Table 2: Corresponding peak area of different concentrations of forchlorfenuron.

Compound	Corresponding peak area				
	50 mg/kg	100 mg/kg	250 mg/kg	400 mg/kg	500 mg/kg
Forchlorfenuron	27470	56303	114277	192782	259825

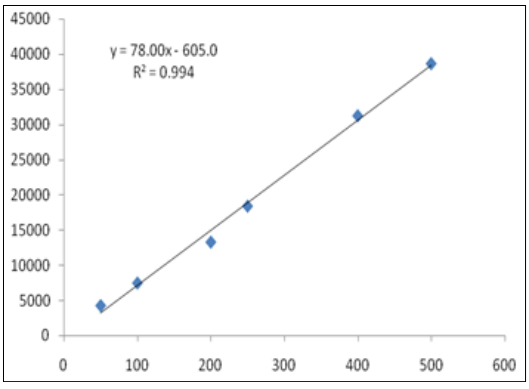

Fig1: Linearity Curve

3.4.2 Recovery
Accuracy of the analytical method was determined by recovery studies. The per cent recovery is mentioned in Table 3. The samples from control plots were used for recovery studies. Homogenized sample each of leaves, green pods, dry pods, seed, straw and soil was taken in 15 ml polypropylene tubes. The samples were spiked separately with different concentrations *viz.* 0.05, 0.25 and 0.5 mg/kg in triplicate. The extraction and cleanup of different substrates were performed as described earlier.

Table 3: Recovery of forchlorfenuron in soybean.

Substrate	Fortification Level	Recovery (%)			
		R-I	R-II	R-III	Mean
Green leaves	0.05 mg kg^{-1}	102.15	90.92	84.45	92.51
	0.25 mg kg^{-1}	88.29	84.55	90.20	87.68
	0.50 mg kg^{-1}	113.57	115.53	107.86	112.32
Dry pods/ Seeds	0.05 mg kg^{-1}	87.51	79.75	93.21	86.82
	0.25 mg kg^{-1}	90.69	85.85	91.14	89.23
	0.50 mg kg^{-1}	91.21	84.65	82.68	86.18
Straw	0.05 mg kg^{-1}	86.31	91.76	86.68	88.25
	0.25 mg kg^{-1}	84.77	91.79	86.58	87.71
	0.50 mg kg^{-1}	113.57	107.54	115.21	112.11
Soil	0.05 mg kg^{-1}	78.57	95.94	82.44	85.65
	0.25 mg kg^{-1}	88.29	88.40	73.13	83.27
	0.50 mg kg^{-1}	104.73	104.38	93.97	101.03

Table 4: Residues of forchlorfenuron in soybean

Parameters	Control	Forchlorfenuron @ 1.25 ppm		Forchlorfenuron @ 2.50 ppm		Forchlorfenuron @ 5.00 ppm	
Leaves		Residues (mg/kg)	Dissipation (%)	Residues (mg/kg)	Dissipation (%)	Residues (mg/kg)	Dissipation (%)
0 day	ND	0.119	--	0.224	--	0.390	--
1 day	ND	0.052	57.30	0.123	45.10	0.187	52.05
3 day	ND	BQL	--	0.076	66.10	0.110	71.74
5 day	ND	BQL	--	BQL	--	BQL	84.10
7 day	ND	BQL	--	BQL	--	BQL	--
10 day	ND	BQL	--	BQL	--	BQL	--
15 day	ND	BQL	--	BQL	--	BQL	--
20 day	ND	BQL	--	BQL	--	BQL	--
$RL_{50\ (days)}$	-	0.84	--	2.02	--	2.01	--
Green pods	ND	BQL	--	BQL	--	BQL	--
Dry pods	ND	BQL	--	BQL	--	BQL	--
Seed	ND	BQL	--	BQL	--	BQL	--
Straw	ND	BQL	--	BQL	--	BQL	--
Soil	ND	BQL	--	BQL	--	BQL	--

*ND-Not Detected, BQL= Below Quantification Limit, LOQ 0.05 mg/kg

3.4.3 Residues of forchlorfenuron in soybean

Mean initial residues of forchlorfenuron @ 1.25, 2.50 and 5.00 ppm were found to be 0.119, 0.224 and 0.390 mg/kg. Initial residues dissipated to BQL on 3, 5 and 7 days with half-life of 0.84, 2.02 and 2.01 days at 1.25, 2.50 and 5.00 ppm dose, respectively. Forchlorfenuron residues were found to be BQL in green pods, dry pods, seed, straw and soil samples. No residues were detected in the untreated control samples. Ugare *et al* (2013) reported that initial residues of forchlorfenuron in grape berries were dissipated within 7 days by 57 and 59 per cent at recommended and double the recommended dose, respectively. These results are in agreement with present findings.

It could be seen that dissipation of forchlorfenuron was faster at recommended dose of 1.25 ppm (57.30) as compared to other two higher doses. Forchlorfenuron persisted for a day in soybean at recommended dose of 1.25 ppm as against 3 days and 5 days at 2.50 ppm and 5.00 ppm, respectively. The present half-life calculated in soybean is much lower as compared to those reported in grape by Ugare *et al* (2013) and in citrus by Chen *et al* (2013) [2]. Present findings in soybean can't be discussed due to lack of available literature in soybean.

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5. References

1. Anonymous. Report on evaluation of the PPPIAD project on soybean. 2013.
2. Chen W, Jiao B, Su X, Zhao Q, Qin D, Wang C. Dissipation and residue of forchlorfenuron in citrus fruits. Bull Environ Contam Toxicol. 2013; 90(6):756-60.
3. Chudhary NY, Khan A. Effect of growth hormones i.e., A3, IAA and kinetin on shoot of *Cicer arietinum* L. Pak J Biol Sci. 2000; 3(8):1263-1266.
4. Davis PJ. Plant hormones: physiology, biochemistry and molecular biology (2nd) Dordrecht, Netherlands: Kluwer. 1995, 1-2.
5. Han Z, Lin G, Rao Q, Bai B, Zhao Z, Lin H. A liquid chromatography- tandem mass spectrometry method for simultaneous determination of acid/ alkaline phytohormones in grapes. Journal of chromatography. 2012; 881-882:83-89.
6. Hernandez P. Morphogenesis in sunflower as affected by exogenous application of plant growth regulators. Agri Scientia. 1997; 13:3-11.
7. Kabar K. Comparison of kinetin and gibberellic acid effects on seed germination under saline conditions. Phyton (Horn., Austria). 1990; 30(2):291-298.
8. Lee JH, Jin ES, Kim WT. Inhibition of auxin-induced ethylene production by SA in mungbean hypocotyls. J Plant Biol. 1999; 42:1-7.
9. Rahman MM, Hossain MM, Anwar MP, Juraimi AS. Plant density influence on yield and nutritional quality of soybean seed. Asian Journal of Plant Sciences. 2011; 10(2):125-132.