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Persistence and Dissipation of Linuron (Afalon–50wp) in Pea Cropped Soil and Its Effect on Soil Microorganisms

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India is the third largest consumer of pesticides in the world and highest among the south Asian countries. Upto 1995-96, the major group of chemicals used in agriculture was insecticides (80%), followed by fungicides (10%), herbicides (7%) and others (3%). Thereafter, the consumption of insecticides declined with simultaneously increase in the consumption of herbicide and fungicides (Dhaliwal & Arora 2002). Linuron (3-(3,4 dichlorophenyl) -1- methoxy-1-methylurea) is a selective systemic urea group of herbicides absorbed principally by the roots and also by the foliage, with translocation primary acropitally in the xylem, inhibits photosynthesis and introduced by Dupont (Rengasamy and Dureja 2000). This herbicide is effective against annual grass and broad leaved weeds of potato, peas, soybean, onion, garlic, beans, cereals, oilseeds etc. (Rengasamy and Dureja 2000; Bhattacharya et al 1997; Prostko et al 1996). The degradation of linuron in soil in laboratory condition and also in field condition has been studied (Durand and Barcelo 1992; Blanco and blanco 1997). Phototransformation of linuron in aqueous solution has been investigated (Faure & Boule 1997). The uptake, translocation and metabolism of linuron in various crop has also been investigated (Salzman et al 1992). Roberts et al 1991 isolated bacterial culture for degradation of linuron in soil. A mixed bacterial culture isolated from soil also capable for degradation of linuron The culture was able to utilize linuron as a source of both nitrogen and carbon and mineralized linuron completely without formation of linuron in crops ((Roberts et al 1993). Sawicka et al 1996 reported that the herbicide stimulated development of bacteria and actinomycetes and inhibited the growth of fungi in legume cropped soil and it also causes a decrease in bacteria root nodule nitrogenase activity. Persistence of linuron in soil and various crops has been investigated (Blanco et al 1988; Cesana 1991; Crum et al 1998). The effect of linuron on various crops has also been reported (Salzman et al 1992; Ahmed & Kandel 1991). With this background the present field experiment was conducted to understand the persistence behaviour of linuron in soil, residue in harvested samples in pea and the effect of linuron on soil microorganism.

MATERIALS AND METHODS

Field experiment was conducted at Kalyani soil under pea cultivation in district seed farm BCKV, Kalyani, West Bengal. The experiment was started in the first week of

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December and continued till the 1st week of March or till harvest during the three consecutive year (2001-2004). Pea variety Arkel sown at approximately 20 cm row to row and 10 cm plant to plant spacing. All the recommended cultural practices were followed. The experiments were conducted in 5m × 5m plot with three replications and one untreated control. Randomised Block Design (RBD) was followed. On the two days after planting (8.12.2001 for first year, 18.12.2002 for second year and 12.12.2003 for 3rd year) commercially available linuron (Afalon-50WP) was applied as pre-emergent herbicide on soil with two treatment doses (T_1= 1000g a.i/ha, recommended and T_2= 2000 g a.i /ha double the recommended doses with T_0 = untreated control), However T_3 = 625 g a.i/ ha was applied for studying the effect of soil microorganism. Water was added 500 l/ha.

Soil samples were collected randomly near the rhizosphere using soil auger at the depth of 0-15 cm from five places from each plot and mixed thoroughly to prepare one homogeneous composite samples. Samples were drawn on 0 (2hr after application), 5, 10, 20, 30, 40, and 50 days after spraying. Residue analysis of harvest pea pods and soil were also carried out. Samples were brought to the laboratory in ice box and analyse immediately. The samples were air dried ground and passed through 2 mm sieve.

50 gm of each soil sample was transferred to a 250 ml conical flask and dipped overnight in 150 ml acetone. It was then shaken for 2 hr in a reciprocating shaker and kept aside for flocculation of soil particles. The supernatant was filtered under vacuum through a Whatman No.1 filter paper using 50 ml acetone as washing solvent.

50 gm each pea pod sample was mecerated and blended with 200 ml acetone for 2 minutes. Then it was filtered under vacuum using acetone (2 × 50 ml) as washing solvent. It was then concentrated and then water is added.

The clean up of extracts was performed by solvent partitioning followed by column. chromatography. The concentrated extract was then partitioned in a 500 ml separatory funnel with 2 × 100 ml hexane - dichloromethane (1:1). The hexane: dichloromethane layer was then separated. The aqueous layer was then again partitioned with 2 × 75 ml dichloromethane with 15 ml saturated NaCl solution. Combined the hexane - dichloromethane and dichloromethane layer was passed through anhydrous sodium sulphate. The combined n-hexane-dichloromethane layer was then concentrated in a rotary vacuum evaporator. The extract was then dissolved in n-hexane (5 ml) and subjected to a florisil (10 g) column chromatography. The column was eluted with 75 ml of hexane: dichloromethane (70: 30 V/V) followed by hexane: acetone (85:15 V/V). The hexane: acetone (85:15 V/V) fraction was concentrated and volume was made up with methanol for final analysis by HPLC. The residues of Linuron were estimated by HPLC (Model- 1050, Hewlett Packard,

USA) equipped with an UV/VIS detector at λ_{max} = 249 nm coupled with Chemito - 5000 Data Processor. Shandon Hypersil (250 × 4.6 mm ODS 5μ) reverse phase C_{18} (cartridge) column was used with Acetonitrile : water (7 : 3 V/V, HPLC grade) as the mobile phase at a flow rate of 1 ml min^{-1}.

Soil and pea pod samples each of 50g were fortified in triplicate at the levels of 0.25, 0.5 and 1.0 μg g^{-1} using standard solution (in methanol) containing 100 μg ml^{-1} of Linuron. The samples were analyzed by HPLC following the methods as described. The average recovery percentage was found to be 88 and 87.6 for soil and pea pod samples respectively (Table 1) and the method was adopted for estimation of Linuron from the above mentioned substrates.

Table 1. Recovery of linuron (Afalon-50WP) from soil and pea pod samples.

Substrate	Sl. No.	Amount of Linuron added (μg g^{-1})	Amount of Linuron recovered (μg g^{-1})*	% Recovery	Average % Recovery
Soil	1	1	0.86	86	88
	2	0.5	0.45	90	
	3	0.25	0.22	88	
Pod	1	1	0.89	89	87.6
	2	0.5	0.43	86	
	3	0.25	0.14	85.6	

*average of three replication

Soil sample were collected randomly from 8 spots from each plot. Collected soil samples were ground and homogenised. One gram of such sample was taken for microbial assay. The number (colony) of total Bacteria, Fungi and Actinomycetes was counted on Agar plates containing appropriate medium following serial dilution technique and pour plated method. Serial dilution was continued till 10 level using water as blank (sterilized). 1 ml soil extract was plated in 15 ml of (sterilized) Agar medium (5 replications). The plates were incubated for 7 days at 28 ± 2°C. Periodical counts were made on 3, 5, & 7 days of incubation. Final counts after 7 days were recorded per gram of soil. Thronton's Agar medium was used for bacteria, Martin's Rose Bengal Streptomycin agar medium for fungi and Jensen's Agar medium for Actinomycetes.

RESULTS AND DISCUSSION

The initial deposits varied from 0.69 - 0.79 μg g^{-1} in T_1 treatment in soil. The residue level declined and dissipated gradually with time recorded 25.32 - 30.1 % dissipation in 5 days, 50.6 - 55.07 at 10 days, 69.56 - 69.8% at 20 days, 84.6-84.9% at 30 days and 87.67-91.9 % in 40 days (Table 2). However no residues were detected in soil after 50 days of application irrespective of any year.

Table 2. Persistence and dissipation of linuron in pea cropped soil (T_1 dose).

Days	Treatment Dose-1000g a.i/ha (T_1)		
	*Mean residue in μg g^{-1} ± SD (% Dissipation)		
	2001-2002	2002-2003	2003-2004
0	0.69 ± 0.021	0.73 ± 0.008	0.79 ± 0.008
5	0.51 ± 0.021 (26.1)	0.51 ± 0.015 (30.10)	0.59 ± 0.012 (25.32)
10	0.31 ± 0.008 (55.07)	0.34 ± 0.012 (53.4)	0.39 ± 0.012 (50.6)
20	0.21 ± 0.024 (69.56)	0.22 ± 0.012 (69.8)	0.24 ± 0.012 (69.6)
30	0.106 ± 0.012 (84.6)	0.11 ± 0.008 (84.9)	0.129 ± 0.014 (83.7)
40	0.07 ± 0.012 (89.85)	0.09 ± 0.012 (87.67)	0.069 ± 0.008 (91.9)
50	BDL	BDL	BDL
	Y= 3.05-0.89X $T_{1/2}$= 6.5 days T_{MRL}=29.57 days	Y= 3.06-0.04X $T_{1/2}$= 6.69 days T_{MRL}=30.44 days	Y= 3.13-0.047X $T_{1/2}$= 6.4 days T_{MRL}=30.63 days

* Average of 3 replication

The dissipation followed first order reaction kinetics (Figure 1, 2 & 3) irrespective of any doses. The half-life ($T_{1/2}$) values in the present experiment in T_1 doses have been found to varied 6.4-6.69 days with average of 6.53 days. Similar observation were made by Blanco et al 1988 who reported lower rates of linuron tended to persist for shorter period in soybean cropped soil showing low half-life value. However Crum et al 1998 found 7.2-11.8 days of half-life of linuron in ditches. The safe waiting period (T_{MRL}) varied from 29.57-30.63 days with an average of 30.21 days (Table 2).

Table 3. Persistence and dissipation of linuron in pea cropped soil (T_2 dose).

Days	Treatment Dose-2000g a.i/ha (T_2)		
	*Mean residue in μg g^{-1} ± SD (% Dissipation)		
	2001-2002	2002-2003	2003-2004
0	1.22 ± 0.029	1.58 ± 0.025	1.25 ± 0.016
5	1.08 ± 0.043 (11.5)	1.25 ± 0.012 (20.8)	1.09 ± 0.029 (12.8)
10	0.93 ± 0.078 (23.8)	0.94 ± 0.012 (33.9)	0.87 ± 0.036(30.4)
20	0.48 ± 0.025 (60.66)	0.616 ± 0.021 (61.01)	0.54 ±0.021 (56.8)
30	0.27 ± 0.012 (77.9)	0.33 ± 0.008 (79.1)	0.307 ± 0.012 (75.8)
40	0.10 ± 0.012 (91.8)	0.14 ± 0.016 (91.1)	0.07 ± 0.008 (94.4)
50	BDL	BDL	BDL
	Y= 3.45-0.051X $T_{1/2}$= 5.92 days T_{MRL}=34.51 days	Y= 3.52-0.051X $T_{1/2}$= 5.90 days T_{MRL}=35.88 days	Y= 3.46-0.52X $T_{1/2}$= 5.79 days T_{MRL}=34.04 days

* Average of 3 replication

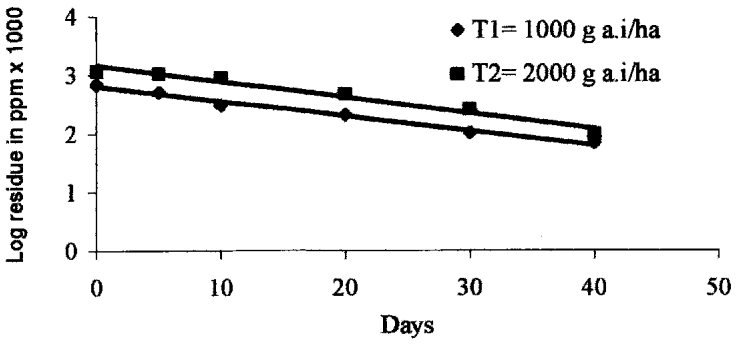

Figure 1. Linear plot of linuron in pea soil (2001-02)

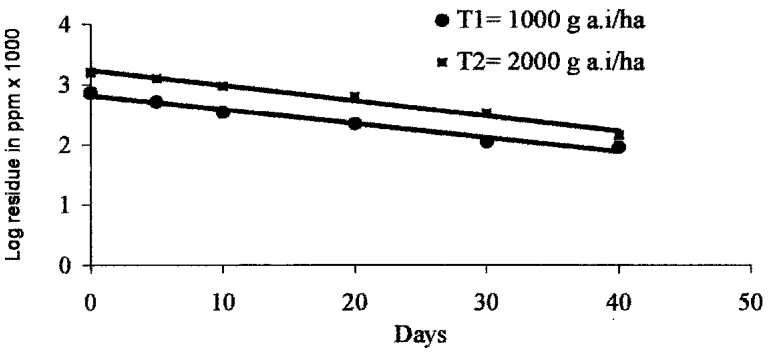

Figure 2. Linear plot of linuron in pea soil (2002-03)

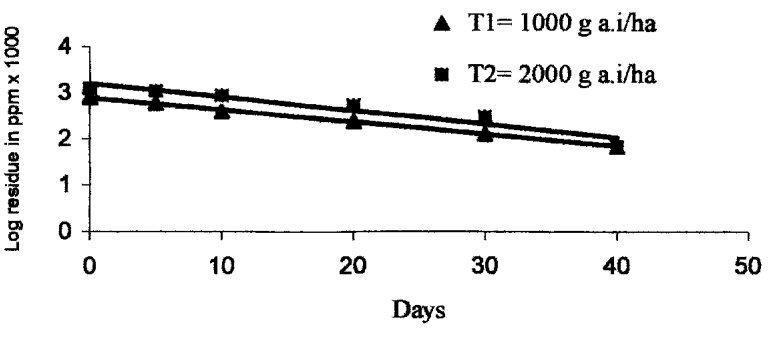

Figure 3. Linear plot of linuron in pea soil (2003-04)

Table 4. Effect of Linuron (Afalon -50 WP) on soil microflora on pea crop during 2001-2002 (Season - I) in per gram soil.

Treatment	Bacterial population ($\times 10^5$)		Fungal population ($\times 10^4$)		Actinomycetes population ($\times 10^5$)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
T_0	172.5	173.7	17.6	18.7	3.7	3.8
T_1	162.2	172.5	16.2	16.8	3.7	3.9
T_2	167.3	171.2	17.3	16.6	4.0	3.7
T_3	169.5	167.2	16.7	17.2	3.9	4.3
T_4	165.3	167.5	16.7	17.3	4.1	3.2

Table 5. Effect of Linuron (Afalon -50 WP) on soil microflora on pea crop during 2002-2003 (Season – II) in per gram soil.

Treatment	Bacterial population ($\times 10^5$)		Fungal population ($\times 10^4$)		Actinomycetes population ($\times 10^5$)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
T_0	170.3	170.6	17.8	16.9	3.5	3.7
T_1	170.3	168.9	17.2	16.8	4.1	3.9
T_2	166.2	162.8	16.9	15.8	4.2	3.8
T_3	166.7	165.9	16.8	16.2	3.8	4.0
T_4	172.5	171.9	17.2	16.8	3.7	4.0

The initial deposits varied from 1.22 – 1.58 $\mu g\ g^{-1}$ in soil when applied at double the recommended dese (T_2=2000 g a.i/ha) of Linuron in pea cropped soil (Table 3). The residue level declined with time showing 1.08-1.25 $\mu g\ g^{-1}$ in 5 days, 0.87-0.94 in 10 days, 0.48-0.616 in 20 days, 0.27-0.33 $\mu g\ g^{-1}$ in 30 days and 0.07-0.14 $\mu g\ g^{-1}$ in 40 days. However no residues were detected in soil after 50 days of application irrespective of any year.

The half-life values in the present experiment in T_2 doses have been found to varied 5.79 – 5.92 days with average of 5.87 days. The safe waiting period (T_{MRL}) varied from 34.04 – 35.88 days with an average of 34.81days (Table 3). The dissipation followed first order reaction kinetics (Figure 1, 2 and 3)

The rate of dissipation was slower in the soil with the highest dose (T_2) in comparison to the lower dose. The similar type of observation has been reported by Crum et al 1998. The more safe waiting period observed in T_2 treatment. However no residue has been detected in the harvested samples of pea cropped soil as well as pods.

Table 6. Effect of Linuron (Afalon -50 WP) on soil microflora on pea crop during 2003-2004 (Season-III) in per gram soil.

Treatment	Bacterial population ($\times 10^5$)		Fungal population ($\times 10^4$)		Actinomycetes population ($\times 10^5$)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
T_0	171.8	171.5	17.9	17.5	3.7	3.8
T_1	171.7	169.6	17.5	16.9	4.3	4.1
T_2	165.4	164.7	15.7	15.1	4.1	4.0
T_3	168.6	168.9	15.7	15.3	3.9	4.1
T_4	172.9	172.4	17.6	169	3.9	4.1

From Table 4, 5, and 6 it has been found that all the treatment T_0, T_1, T_2, T_3, and T_4 shows more or less similar type of results. All the treatment has neither any detrimental nor any influencial effect on the soil microflora in pea cropped soil irrespective of any season. They also caused a decrease in bacteria root nodule nitrogenase activity. So linuron (afalon 50WP) as a pre-emergent herbicide is a safe for the soilmicrobial functions essential for maintaining soil fertility and productivity.

From the progressive reduction of half- life value it has been found that 5-7 days time period was required for 50% dissipation of the initial deposits of Linuron (Afalon 50%WP) in soil irrespective of any treatment doses and year. 90% of the initial deposits of linuron had been dissipated within 40 days of application. No residue was found in harvested samples. It has no positive or negative effect on microorganisms under pea cropped soil.

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