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Effect of Soil Type, Soil pH, and Microbial Activity on Persistence of Clodinafop Herbicide

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Pesticides have become an integral part of today's agriculture. In wheat, one of the most important cereals worldwide and in India, for the last 15 years, littleseed canarygrass (*Phalaris minor* Retz.) has been effectively controlled by the use of herbicide isoproturon. Recently, several reports stated that some biotypes of *Phalaris minor* are showing resistance to isoproturon (Malik and Singh, 1995). To cope up with this situation, alternate herbicides are being tried (Saini and Singh, 2001; Chhokar and Malik, 2002) and the Government of India has given provisional registration to clodinafop along with sulfosulfuron, fenoxaprop and tralkoxydim (Pool et al, 1995; Singh et al, 1998; Dhaliwal et al, 1998; Brar et al, 1999). Clodinafop-propargyl [prop-2-ynyl (R)-2-[(5-chloro-3-fluoro-2-pyridyloxy) phenoxy] propanoate] (1, Figure 1) is a herbicide from the aryloxyphenoxy propionate group of chemistry ("fops"). It can be used at a very low rate (30-80 g a.i. ha^{-1}) to control a wide range of important grass weed species at various growth stages. The bioefficacy of this herbicide is widely reported in wheat (Walia et al, 1998; Darwent and Moyer, 1999; Blackshaw et.al, 1998; Afentoli and Eleftherohorinos, 1999; Airoldi et al, 1997; Punia etal, 2003). Environmental fate of any pesticide in soil depends mainly on soil type, pH and microbial activity (Kulshrestha and Singh, 1989; Singh and Kulshrestha, 2000). Chemical analysis of fop herbicides to ascertain environmental fate is complex, involving both the parent ester and the acid moiety (the active ingredient). The purpose of this study was to evaluate the effect of soil type, soil pH and microbial activity on persistence of clodinafop.

MATERIALS AND METHODS

Clodinafop-propargyl (97.8%, analytical) obtained from Novartis India Limited was crystallized from hexane and purity was checked by HPLC before use. Distilled analytical grade ethyl acetate was used for quantitative recovery of the herbicide from different matrices. Anhydrous sodium sulphate was used as a drying agent for different samples. For HPLC, methanol and water were of HPLC grade.

Alluvial soil was collected from the fields of Indian Agricultural Research Institute (IARI), Delhi. Black and red soil were collected from Nagpur and

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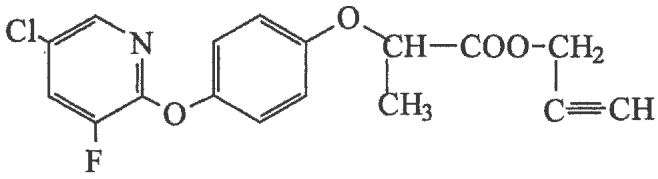

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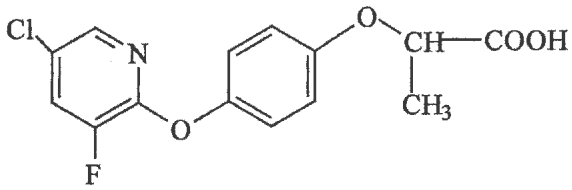

(2)

Figure 1. Chemical structures of Clodinafop propargyl (1) and Clodinafop acid (2)

Mysore region of India. Physicochemical properties of three soils are described in Table 1. Delhi (alluvial) soil was used in the laboratory experiments for effect of microbial activity.

Table 1. Physicochemical properties of soils.

Region	Delhi	Nagpur	Mysore
Common class	Alluvial soil	Black soil	Red soil
Sand (%)	63.80	51.60	79.39
Clay (%)	17.50	26.40	14.61
Silt (%)	18.70	22.00	6.00
Moisture (%)	16.00	18.00	12.00
Organic carbon (%)	0.5	0.84	0.4
pH	8.1	7.5	6.64
Textural class	Sandy loam	Sandy clay loam	Loamy sand

To study the effect of pH on clodinafop persistence, three major Indian soils of different pH were used. Twenty eight Erlenmeyer flask were taken for each soil type. Twenty grams of soil was added to each of 28 flasks and field moisture capacity condition was maintained throughout the experiment. Out of 28 flasks, 21 flasks per soil type were fortified with 2μg g^{-1} clodinafop propagryl. The remaining 7 flasks for each soil type were kept as controls. Four samples (1 control and 3 treated) for each soil type were drawn at regular intervals, extracted, and analysed by HPLC.

For effect of microbial activity a total of 48 Erlenmeyer flasks each containing 20g of IARI alluvial soil, were used. Out of them, 24 flasks were sterilized by autoclaving (130°C and 15 atmospheres) for 1 h for three consecutive days. From

them 18 sterilized soil samples were fortified with clodinafop propargyl (2μg g^{-1}); 6 flasks were kept as untreated controls. A comparable set of 24 samples was prepared with nonsterile soil. All flasks were kept in B.O.D. incubator at 30±2°C. Moisture was maintained by adding water at regular intervals. For sterilized flasks, sterilized water was added under laminar flow. Four samples (1 control and 3 treated) for each soil type (sterile and nonsterile) were drawn at regular intervals (0, 1, 5, 10, 15 days) and samples were extracted and analysed for herbicide content.

The samples were extracted by acid quantification method (Roy and Singh, 2005). Sieved and air-dried soil (50 g samples in Erlenmeyer flasks) was brought to field capacity by addition of distilled water. After 1 h, 3-4 drops of ammonium hydroxide were added to each flask and stirred with glass rod. The flasks were kept undisturbed until the smell of ammonia disappeared. One hundred milliliters of ethyl acetate was added to the flask and shaken on a horizontal shaker for 30 min. The contents of the flask were allowed to settle and the supernatant phase was filtered through Whatman no. 1 filter paper using Buchner funnel with water pump. The ethyl acetate extraction was repeated twice (50 ml each time) and again filtered. The combined filtrate was then concentrated by evaporating the solvent on a rotary vacuum evaporator at 35-40 °C to dryness. The residue was dissolved in 50 ml of 0.1N KOH (aqueous) and the content of the flasks were heated at 60°C on a water bath for 30 min. After cooling, the mixture was neutralized (pH 7) by addition of 1N HCl. The neutralized mixture was diluted with water (100 ml) and transferred to a 250ml separatory funnel. The aqueous solution was partitioned with ethyl acetate (3 × 50 ml). The organic layer was dried (anhydrous Na_2SO_4) and the solvent evaporated to dryness on rotary vacuum evaporator. The residue was finally dissolved in methanol for HPLC analysis. Control soil samples were also processed in the same manner.

A reverse-phase high performance liquid chromatographic technique was used for quantitative analysis. The Hewlett Packard series 1100 HPLC instrument was equipped with degasser, quarternary pump, diode-array detector, Rheodyne injection system, and Vectra computer (model) was used for analysis. The stainless steel column (250mm×4mm i.d) contained Lichrospher RP-8 as stationary phase. Methanol: water (4:1) at 1ml min^{-1} flow rate was used as mobile phase. The analysis was performed at λ_{max} 240 nm. Chromatograms were processed by a Windows NT-based HP Chemstation programme.

The authentic sample of clodinafop acid (2, Fig 1), required for the entire study, was prepared by alkaline hydrolysis of clodinafop propargyl according to the method described (Roy and Singh, 2005). The structure of this compound was elucidated by IR, ^{1}H-NMR spectroscopy and comparison with literature m.p.

To determine the efficacy of the analytical procedure undertaken during the experiment, a recovery experiment was conducted in triplicate. The recovery from soil was standardized at two levels of fortification i.e. 0.5 and 1 μg g^{-1}. The

fortified samples were processed by acid quantification method as described above.

RESULTS AND DICCUSSION

Clodinafop propargyl ester and clodinafop acid gave sharp peaks at 3.91 and 1.31 min under the described conditions of HPLC. The calibration curve was linear from 0.5 to 10 μg ml^{-1}. Instrumental detection limit (IDL) for clodinafop ester and acid was found to be 0.25 and 0.1 μg ml^{-1} with sensitivity of 5 and 2 ng respectively. Recovery of clodinafop ester by acid quantification method ranged between 81.3 - 83% at 0.5 and 1.00 μg g^{-1} fortification level. The method showed no interfering peak from the soil matrix. The ester generally converts in soil to clodinafop acid, a major metabolite and also herbicidal in action. Acid quantification method is useful for the estimation of total herbicidally active compound whether in the form of acid or ester.

The results of soil type and pH effect on the persistence of clodinafop are shown in Table 2. The results indicated that dissipation of herbicide clodinafop was not affected by specific pH and soil type. In three soils e.g alluvial, black and red soil having pH 8.1, 6.6 and 7.2 respectively the dissipation pattern was almost similar.

Table 2. Persistence of clodinafop in soil of different pHs.

Time (Days after Application)	[a]Herbicide amount remaining (μg g^{-1}) ±SD		
	Alluvial soil (pH – 8.1)	Red soil (pH –6.64)	Black soil (pH –7.5)
0	1.733±0.047(0)	1.722±0.010(0)	1.717±0.004(0)
1	1.312±0.014(24.28)	1.427±0.012(17.13)	1.410±0.035(18.05)
3	0.965±0.010(44.30)	0.992±0.022(42.39)	0.931±0.029(50.90)
5	0.543±0.026(68.66)	0.620±0.033(63.90)	0.530±0.050(88.21)
10	0.169±0.020(90.20)	0.169±0.004(90.41)	0.143±0.014(91.07)
15	0.093±0.014(94.00)	0.097±0.002(94.02)	0.081±0.001(95.30)

[a]Average of three replicates, Figure in parenthesis indicates % dissipation, SD – Standard deviation

The logarithmic plots indicated that the rate of dissipation fitted a first order kinetics decay (r = -0.99). The regression equation and half-lives are given in Table 3.

Table 3. Half-life, correlation coefficient and regression equations

Substrate	Half life (days)	Corr. coeff. (r)	Regression eq.
Black soil	3.43	0.99	y = -0.0887x+0.2401
Red soil	3.20	0.99	y = -0.094x+0.2284
Sterile soil (alluvial	4.61	0.99	y = -0.0746x+0.2903
Non-sterile soil (alluvial)	3.44	0.98	y = -0.0873x+0.2014

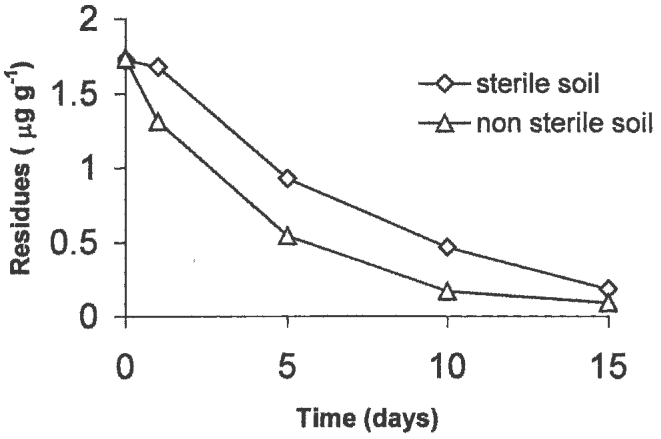

Figure 2. dissipation of clodinafop in sterile and non sterile soil.

Sterile and non-sterile soils are the representative factors of microbial transformation under field conditions. To have a complete picture of the factors influencing dissipation this study was carried out. The results of this study are given in Table 4. Initially a slow degradation was found in case of sterile soil. On first and fifth day 18.5 and 46.3% herbicide degraded under sterile conditions versus 24.2 and 68.6% in non-sterile soil respectively. At each interval more degradation was found in non-sterile soil confirming that microbial degradation is also participating in dissipation of clodinafop along with other factors (Fig 2).

Table 4. Persistence of clodinafop in sterile and non sterile soil

Time (Days after Application)	[a]Herbicide amount remaining (μg g^{-1}) ±SD	
	Sterile soil	Non sterile soil
0	1.727±0.022 (0)	1.733±0.007 (0)
1	1.679±0.012 (18.50)	1.312±0.019 (24.29)
5	0.928±0.009 (46.32)	0.543±0.012 (68.68)
10	0.467±0.004 (72.99)	0.169±0.003 (90.20)
15	0.183±0.010 (89.41)	0.093±0.008 (94.00)

[a]Average of three replicates, Figure in parenthesis indicates % dissipation, SD – Standard deviation

Persistence of clodinafop in soil was influenced by both biotic and abiotic factors. Studies in soil of different pH showed a similar dissipation pattern indicating similar degradation pattern under acidic or alkaline pH. Studies for the effect of microbial population on persistence of clodinafop revealed that the half-life in sterile soil (4.6 days) was higher than the non-sterile soil ($t_{1/2}$ = 3.4 days) indicating a role of soil microbes in the dissipation of clodinafop. Studies on photodegradation showed that the role of sunlight (Roy and Singh, 2005a) is another prominent factor in the dissipation of this herbicide as 41% of the applied

compound degraded in 15 days. Under actual field conditions (Roy and Singh, 2005b) the clodinafop dissipated with a half-life of 2.9-3.0 days in comparison to 4.6 days in sterile soil indicating role of light and microbial activity in its dissipation. The low persistence, rapid degradation by sunlight and microbes, excellent bioefficacy and low mammalian toxicity of clodinafop may provide the solution for an effective alternative for the control of isoproturon resistant *P. minor* biotypes to combat the weed flora shift (an unintended byproduct of the Green Revolution) in wheat fields of India.

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REFERENCES

Afentouli CG, Eleftherohorinos IG (1999) Competition between wheat and canarygrass biotypes and their response to herbicides. Weed Sci 47: 55-61.

Airoldi M, Alberti UD, Gut H, (1997) Topik R: New postemergence graminicide for wheat. Informatore Fitopatologico 47: 57-60.

Blackshaw RE, Semach G, Entz T(1998) Post emergence control of foxtail barley (*Hordeum jubatum*) seedlings in spring wheat (*Triticum aestivum*) and flax (*Linum usitatissmum*). Weed Technol 12: 610-616.

Brar LS, Walia US, Dhaliwal BK (1999) Bioefficacy of new herbicides for the control of resistant *Phalaris minor* in wheat. Pestic Res J 11: 177-180.

Chhokar RS, Malik RK (2002) Isoproturon-resistant littleseed canarygrass (*Phalaris minor*) and its response to alternate herbicides. Weed Technol 16: 116-123.

Darwent AL, Moyer JR (1999) Control of wild oat in the year of smooth brome grass establishment and its effect on the yield and quality of subsequent seed crops. Canadian J Plant Sci 79: 447-453.

Dhaliwal BK, Walia US, Brar LS (1998) Response of *Phalaris minor* Retz biotype to various herbicides. Indian J Weed Sci 30: 116-120.

Kulshrestha G, Singh SB (1992) Influence of moisture and microbial activity on pendimethalin degradation. Bull Environ Contam Toxicol 48: 269-274..

Malik RK, Singh S (1995) Littleseed canarygrass (*Phalaris minor*) resistance to isoproturon in India. Weed Technol 9: 419-425.

Pool CF, Villiers BL de, Smit HA de, Villiers BL (1995) Influence of growth stage on the efficacy of three wild oat herbicides applied to *Panicum schinizii*. Appl Plant Sci 9: 23-25.

Punia SS, Rathee SS, Malik RK (2003) Effect of fenoxaprop and clodinafop alone and in combination with isoproturon in controlling weeds infesting wheat (*Triticum aestivum*). Indian J Agric Sci 73: 232-234.

Roy S, Singh SB (2005) Liquid chromatographic method for the micro-quantitative determination of clodinafop in soil, wheat and *Phalaris minor*. J Chromatog 1065: 199-206.

Roy S, Singh SB (2005a) Phototransformation of clodinafop propargyl herbicide, J Environ Sci Health 40: 525-534.

Roy S, Singh SB (2005b) Persistence of clodinafop in soil, wheat and *Phalaris minor*. Pestic Res J (In publication).

Saini JP, Singh KP (2001) Efficacy of new herbicides against grass weeds in wheat (*Triticum aestivum*) under mid-hill conditions of Himachal Pradesh. Indian J Agronomy 46: 233-238.

Singh S, Kirkwood RC, Marshall G, Singh S (1998) Control of isoproturon-resistant biotypes of *Phalaris minor* by chlorotoluron and clodinafop propargyl. Resist Pest Manage 10: 15-18.

Singh SB, Kulshrestha G (2000) Effect of pH on the hydrolysis of chlorimuron ethyl, a sulfonyl urea herbicide. Pestic Res J 12: 235-238.

Walia US, Brar LS, Dhaliwal BK(1998) Performance of clodinafop and fenoxaprop-p-ethyl for the control of *Phalaris minor* in wheat. Indian J Weed Sci 30: 48-50.