

Persistence of atrazine in soil under fodder sorghum

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ABSTRACT

Atrazine was applied @ 1.5 kg ai/ha in soil before emergence of fodder sorghum (cv.PC-6) grown during kharif season in two successive years 2006 and 2007 under two different nitrogen sources viz., sole inorganic (T_2) and organic and inorganic in the ratio of 50:50 (T_4) keeping T_1 and T_3 as their respective controls. Samples collected at different intervals from two depths of soil i.e. 0-15 and 15-30 cm were analysed for atrazine residues in both the years. The average initial zero day deposition in 0-15 cm depth soil was 0.4779 and 0.4619mg/kg which dissipated by 62% (0.1823mg/kg) and 65% (0.1619mg/kg) in 15 days in T_2 and T_4 respectively. However, after elapse of 30 days about 86-88% residues disappeared in both the treatments. In samples of 15-30 cm depth the initial zero day deposits were 0.1935 and 0.2005 mg/kg in T_2 and T_4 respectively which dissipated down to 0.066mg/kg in 15 days in both the treatments. Residues were below detectable level in samples collected at 30 days from 15-30 cm depth and at 45 days from 0-15cm depth. In plant foliage collected at harvest we could detect traces of atrazine in few samples only in first year but in the second year's sample it was totally absent, thus the fodder at harvest was absolutely safe for animal feeding. The dissipation pattern followed first order rate kinetics with high correlation coefficients ($r = -0.98$ to -0.99). The statistically calculated half-life values were found to be 10.27, 9.35 days in 0-15 cm and 9.65, 9.38 days in 15-30 cm depth soil in T_2 and T_4 respectively.

Key Words: Atrazine, dissipation, fodder crops, persistence and sorghum

Sorghum or jowar [*Sorghum bicolor* (L.) Moench] is one of the most important fodder crops of the semi arid tropics. It is also grown for grain and dual purpose during summer and kharif season mainly in northern and central India. The crop is preferred as a fodder crop because it can be utilized as green fodder, stover and also for silage making. Atrazine (6-chloro-N²-ethyl-N⁴-isopropyl-1,3,5-triazine-2,4-diamine), a substituted s-triazine, selective herbicide is used for the control of grasses and broad leaved weeds in maize, sorghum, sugarcane, vines, fruit orchards and many others. It is recommended for pre emergent application in fodder sorghum for control of various weeds (Tomar *et al.*, 1999).

Presence of organic matter plays an important role in persistence, adsorption and degradation of herbicides in soil. The difference in adsorption of atrazine between soils has been attributed mainly to organic matter level, type of clay and pH in the immediate vicinity of clay surface (Anderson *et al.*, 1980, Raman *et al.*, 1988, Bhardwaj and Gehlan, 1986). Atrazine is absorbed through roots and foliage with translocation. So, it is important to know about the persistence of the herbicide in soil and its translocation to foliage of fodder crops because green fodders are straight way fed to animals after harvesting from the field having, therefore, very little chance of decontamination. If the fodder is contaminated then the residues present in it may be transferred to animal body from where subsequently it may translocate and contaminate milk in case lactating animals, which is absolutely undesirable. The contaminated fodder, therefore, should either be allowed to decontaminate to lower the residue level below the permissible level or discarded for the sake of safety of human and animal health.

Keeping these points in view, the present investigation was undertaken to observe the residues and difference in persistence pattern of atrazine from soil under fodder sorghum grown with two sources of nitrogen, i.e. sole inorganic and combination of organic and inorganic in 50:50 ratio. Another important objective of the experiment was to see the translocation of atrazine residues, if any, to sorghum foliage at the time of harvest.

MATERIALS AND METHODS

The experiment was conducted during kharif season of two successive years, 2006 and 2007, at the experimental central research farm of Indian Grassland and Fodder Research Institute, Jhansi. Fodder sorghum (cv PC-6) was sown in 4 x 3 square meter plots and pre emergence application of atrazine was done as per the following treatment schedule. Each treatment and control was replicated four times and the experiment was laid out in randomized block design. The treatments were :

- T_1 = No herbicide and total nitrogen applied through fertilizer (inorganic)
- T_2 = Atrazine @ 1.5 Kg ai/ha and total nitrogen applied through fertilizer (inorganic)
- T_3 = No herbicide and total nitrogen applied through FYM (organic) and fertilizer (inorganic) in 50:50 ratio
- T_4 = Atrazine @1.5 Kg ai/ha and total nitrogen applied through FYM (organic) and fertilizer (inorganic) in 50:50 ratio

Soil samples were collected from two depths viz. 0-15 and 15-30 cm from all the replicates at 0, 3, 7, 10, 15, 30 and 45 days after treatment and at harvest time. Sorghum foliage samples were also collected at harvest time which is about 90 days after treatment.

Extraction and clean up

Soil: Soil samples were collected with an auger from two depths 0-15 and 15-30cm. They were dried in air under shade, pulverized and screened through 2 mm sieve and stored at sub zero temperature. A representative of 50 g soil was taken in 150 mL of conical flask and extracted with 100 mL of dichloromethane for 2 hours in a mechanical horizontal shaker. The suspension was filtered through Whatman filter paper No.1 placed on a Buchner filtering funnel connected to an air suction pump to create vacuum. The extraction flask was washed with 2x50 mL dichloromethane which was transferred to same filter paper and filtered. The filtrate collected in the filtering flask was quantitatively transferred to a separating funnel and washed thrice with 3x50 mL distilled water, the aqueous fraction being discarded each time. The organic layer was finally passed through anhydrous sodium sulfate and evaporated to dryness in a rotary vacuum evaporator. The residue was dissolved in hexane and acetone (8:2 v/v) mixture and subjected to gas chromatographic (GC) analysis.

Plants: A representative of 50 g samples of sorghum foliage from each replicate collected at harvest i.e. after about 90 days after treatment were blended in a mixer grinder for two minutes each with 100 mL and 2x50 mL of acetone. The extract was then filtered through Buchner filtering funnel to a filtering flask. The residue on the funnel was washed 2-3 times with acetone and collected in the same flask. The combined acetone extract was placed in a 1 liter separating funnel and mixed with 100 ml. each of petroleum ether and dichloromethane. After partitioning the lower aqueous layer was transferred in a conical flask and upper organic layer was passed through anhydrous sodium sulfate. The aqueous layer was again transferred to the separating funnel and 5% sodium chloride and 100 ml. dichloromethane was added to it. After shaking the separating funnel for 2-3 times the lower organic layer was separated and collected in the same flask after passing through anhydrous sodium sulfate. The same process was repeated twice. The combined dichloromethane fraction was concentrated in rotary vacuum evaporator, the residue was dissolved in hexane and acetone (8:2 v/v) and subjected to column chromatography using silica gel, florisil and anhydrous sodium sulfate as adsorbents. The column was eluted with 100 ml. of hexane and acetone (8:2 v/v) and the eluate was concentrated to 10 mL for GC analysis.

Analysis in GC: The qualitative and quantitative determination was done in gas chromatograph on a Varian 3800 equipment fitted with TSD (Thermionic Specific Detector). The column used was WCOT fused silica chrompack capillary column having a

dimension of 10m x 0.53 mm id x 1 µm film thickness and CP-SIL 5CB coating at the temperature programming of 150°C (1min) to 200°C (0 min) at 10°C/min to 240°C (5 min) at 20°C/min. The injector and detector temperatures were kept at 250°C and 300°C respectively. Nitrogen was used as carrier gas with a flow rate of 2 ml/min through column and 30 ml/min as back up. The detector gas flow rates were 4 ml/min for hydrogen and 175 ml/min for air. The identification of peak and quantification of concentration in the samples was done on the basis of known concentration of external standard solution injected intermittently.

Recovery experiments were also conducted to test the efficiency of extraction, clean up and estimation procedure by spiking the untreated soil and sorghum foliage samples with known concentrations of atrazine. The average recovery in soil was between 92-95% while that in foliage samples was between 85-90%. The limit of determination was 0.015 mg/kg.

RESULTS AND DISCUSSION

Soils of the experimental plots were clayey in texture and neutral in reaction (pH around 7) and the electrical conductivity was low (Table-1). The organic carbon status was in the medium range. The data on residues and dissipation of atrazine in soil under fodder sorghum presented in Tables 1 and 2 are the average of two consecutive years study. The concentration and percent dissipation values reveal a slow but steady disappearance of the residues. In samples of 0-15 cm depth soil the initial deposits were around 0.48 and 0.46 mg/kg in treatments T₂ and T₄ which dissipated by 11-13% to 0.42 and 0.40 mg/kg, respectively, in initial three days. After 7 days the dissipation of residues was 23-26% while after 10 days about 36-38% of initial deposits were lost. Atrazine residue at a concentration of 0.23 mg/kg was detected at 4 days after treatment in soil samples of a maize field in Saldana, Tolima, Colombia after application of the herbicide @ 2.4 kg a.i./ha (Fuentes *et al.*, 2003).

The residue after 15 days of application was around 0.18 and 0.16 mg/kg in T₂ and T₄ respectively, thus measuring a dissipation of 62-65%. After elapse of 30 days the concentration of atrazine went below 0.1 mg/kg level and measured at 0.068 in T₂ and 0.054 mg/kg in T₄. The samples of 45 days had no detectable residues at all. The above data revealed that the dissipation of atrazine in soil was slow but steady and on almost uniform rate. There was not much variation in rate of dissipation during initial or later days which might be due to the fact that there was not much rainfall (~500 mm) during the study period as the area suffered from draught in both the years. So, the degradation and dissipation of residues occurred due to usual microbial activity in the soil.

In soils of 15-30 cm depth, initial atrazine concentration was around 0.2 mg/kg which dissipated by about 66-67% in 15 days to the level of 0.066 mg/kg in both the treatments T₂ and T₄ and in samples collected after 30 days no residues could be detected. The dissipation pattern of atrazine in soil followed first order kinetics as high coefficient of determination (R²) as observed from the semi logarithmic plot between mean residue level and days after application (Fig.1 and 2). This corroborate well with the reports of Sheets (1970) and Wood *et al.* (2005). In the 0-15 cm layer soil the statistically calculated half-life values were 10.27 days in T₂ and 9.35 days T₄ while the corresponding figures in 15-30 cm layer soil were 9.65 and 9.38 days respectively. Atrazine dissipation at all soil depths under study with a residual half-life of 15-28 days was also observed by Wood *et al.* (2005). But these half-life values were quite low as compared to reported value of 37-138 days (Erickson and Lee, 1989) or a half-life value of 23.8-39.4 days at 25°C reported by Xiongwu Qiao *et al.*, 1996) in certain acidic and alkaline soils. Warm and moist climate promote disappearance of atrazine herbicides from soils (Dowler *et al.*, 1968, Harrris *et al.*, 1969) and persistence is more prolonged in cold, dry climate (Adams, 1968, Burnside *et al.*, 1969).

It is also reported that atrazine decomposition in soil increased with temperature between 10-35°C (Burnside, 1965; Roeth *et al.*, 1969). Buchanon and Rogers (1963) reported that inactivation of simazine, ipazine, atrazine and atratone proceeded more rapidly at 45°C than at 25, 30 or 35°C. The prevailing hot and humid condition during *kharif* season and due to possible enhanced mineralization of atrazine in the rhizospheric and nearby lower zone under fodder sorghum helped in degrading and dissipating atrazine at a comparatively higher rate thus residual half lives becoming shorter than the reported values in other areas. Atrazine mineralization was the main dissipation mechanism in the superficial horizon of the Argiustoll because of microbial adaptation after repeated applications.

In contrast, little atrazine mineralization was found in the Haplustoll profile, and it decreased with depth as observed by Hang *et al.* (2005) in their investigation of atrazine behavior in the different

pedological horizons from profiles of two non-tilled soils in Argentina. Atrazine residues in the range of 0.02-0.10 mg/kg in 0-15 cm and up 0.05 mg/kg in 15-30 cm soil depending on soil type was reported from temperate country Serbia as a result of five year investigation of pollution of soil during 1995-1999 (Gasic *et al.*, 2002). On the other hand soil samples, collected from 20 locations at four depths (0-15, 15-30, 30-45 and 45-60 cm) from sugarcane growing areas of Nizamabad district of Andhra Pradesh where atrazine application was a regular practice for over 20 years, had no detectable residues (< 0.01 mg/kg) (Sannappa *et al.*, 1997).

A comparison of the percent dissipation of atrazine and the residual half-life values obtained in the experiment shows a slightly higher rate of dissipation in treatment T₄ than in T₂ which might be due to the presence of higher organic matter in the former since organic matter plays an active role in degradation of pesticides in soil. Another reason may be the increased formation of bound residues which can not be extracted by normal extraction procedure. Hang *et al.* (2005) reported that atrazine-bound residues depended on the soil organic matter content and the size of the fraction. Organic matter in the largest size fractions had a higher capacity to form atrazine-bound residues.

Sorghum plants harvested around 90 days after sowing were also analyzed to detect any residues of atrazine if translocated and still persisting in foliage. Although in the first year we could detect traces of atrazine in few plant samples but in the second year nothing could be detected in any sample. In the succeeding berseem fodder crop taken in rotation in following rabi season there was no carry over of atrazine residues or any initial phytotoxicity observed by us. Thus the fodder at harvest was absolutely safe for animal feeding as far as atrazine residue was concerned in sorghum as well as succeeding berseem crop.

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Table 1: Soil characteristics before and after the experiment

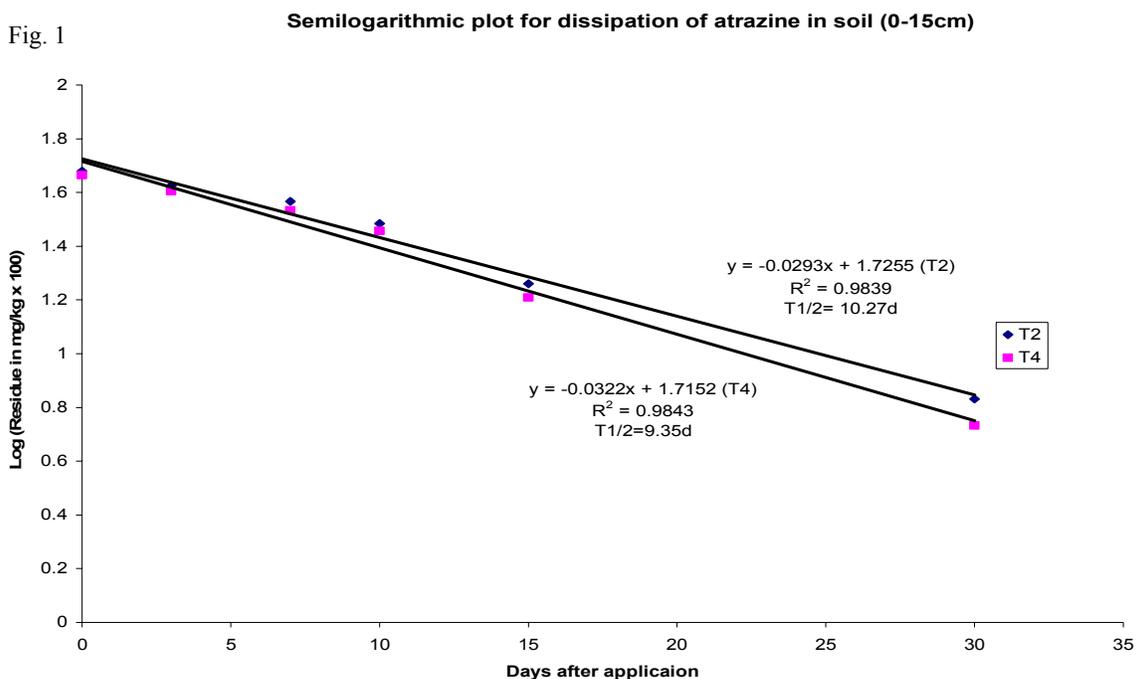
Soil parameter	Initial	Final
pH	7.19	7.20
EC (dS/m)	0.14	0.10
Texture	Clay	Clay
Organic carbon (%)	0.52	0.61
Total nitrogen (%)	0.08	-
Available nitrogen (Kg/ha)	133	236
Available phosphorus (Kg/ha)	12.7	17.5
Available potassium (Kg/ha)	272	311

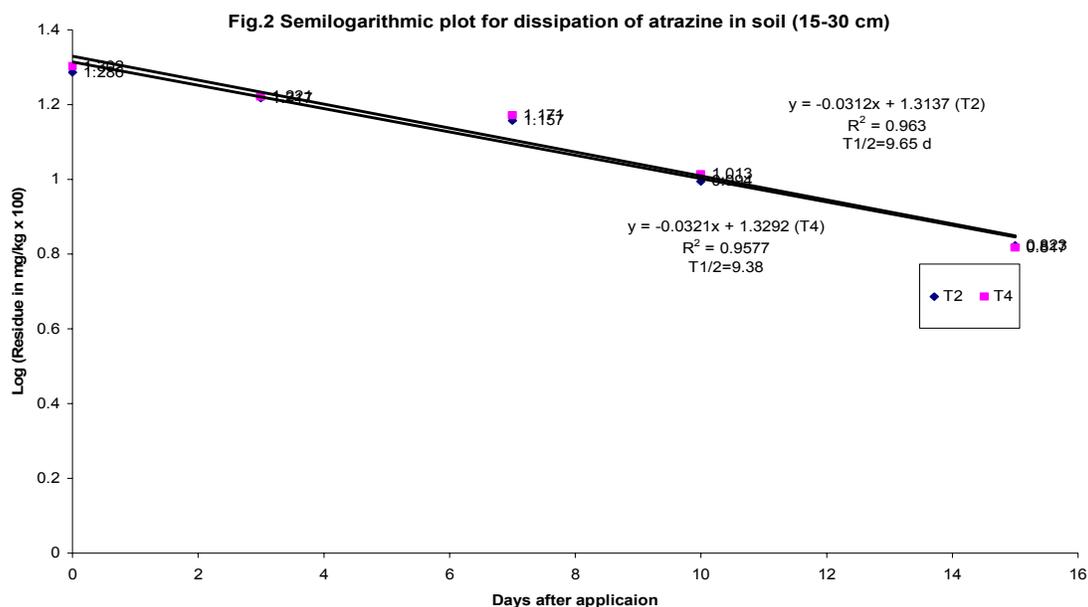
Table 2: Dissipation of atrazine residues in soil (0-15cm) after its pre emergence application @ 1.5 kg ai/ha.

Days after application	T ₂		T ₄	
	Residue in mg/kg (mean+s.d.)	Dissipation (%)	Residue in mg/kg (mean+s.d.)	Dissipation (%)
0	0.4779±0.042	-	0.4619±0.066	-
3	0.4218±0.104	11.74	0.4029±0.103	12.77
7	0.3692±0.063	22.75	0.3414±0.089	26.08
10	0.3060±0.048	35.97	0.2869±0.052	37.88
15	0.1823±0.033	61.85	0.1619±0.020	64.95
30	0.068 ±0.022	85.77	0.054 ±0.015	88.31
45	ND		ND	

Table 3: Dissipation of atrazine residues in soil (15-30cm) after its pre emergence application @ 1.5 kg ai/ha.

Days after application	T ₂		T ₄	
	Residue in mg/kg (mean+s.d.)	Dissipation (%)	Residue in mg/kg (mean+s.d.)	Dissipation (%)
0	0.1935±0.044	-	0.2000±0.038	-
3	0.165±0.082	14.73	0.1664±0.049	16.8
7	0.1438±0.051	25.68	0.1485±0.032	25.75
10	0.0987±0.034	48.99	0.1031±0.052	48.45
15	0.066±0.022	65.89	0.0657±0.010	67.15
30	ND		ND	





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The fate and behavior of *Imidacloprid 0.3% G* in water maintained at different pH and soils of different agro-climatic zones

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ABSTRACT

Imidacloprid 0.3% G is a new insecticide formulation having Imidacloprid [1-(6-chloro-3 pyridylmethyl) – N – nitroimida-zolidinimine] as active ingredient. A laboratory experiment was undertaken to determine the dissipation pattern as well as residue level of Imidacloprid 0.3% G exist in water maintained at different pH and soils of different agro-climatic zones following application at 1 ppm and 2 ppm. Samples were processed for analysis of Imidacloprid residues at intervals of 0 (2h after application), 5, 15, 30, 60 and 90 days after application. Imidacloprid 0.3% G was extracted from water by partitioning with dichloromethane. In case of soil samples the residue was extracted with acetonitrile: water (8:2) mixture. The extracts in acetonitrile were concentrated in rotary vacuum evaporator. The concentrated extract was subjected to solvent partitioning with dichloromethane. In both the cases, dichloromethane part after collection was evaporated to dryness in rotary vacuum evaporator. Final volume was reconstituted with acetonitrile for HPLC analysis. The residue of Imidacloprid 0.3% G in spiked water samples of pH 4.0, 7.0 and 9.2 as well as soils of different agro-climatic zones gradually decreased with time following first order kinetics in all the cases. The calculated half life ($T_{1/2}$) values in water were found to be in the range of 66.9 to 94.07 days and in case of different soils these values were range of 59.03-75.26 days. The dissipation of Imidacloprid 0.3% G appeared to be faster in acid medium than neutral but slower in alkaline medium.

Key Words: Behaviour, dissipation, fate and imidacloprid

Imidacloprid 0.3% G is a new insecticide formulation having Imidacloprid as active ingredient. Imidacloprid [1-(6-chloro-3 pyridylmethyl) –N–nitroimida-zolidinimine] is a ‘chloronicotiny’ group of insecticide having systemic, contact and stomach action. Imidacloprid, first introduced by Bayer, was very effective against resistant pests due to acting as an agonist of the nicotinyl acetylcholine receptor (Chalan and Subbratnam, 1999, Olsen *et al.*, 1996, Elbert and Nauen, 1996). It shows a high activity, especially against a sucking insects such as aphids, leaf and plant hoppers, thrips, whiteflies, soil insects, termites and some species of chewing insects as well as seed dressing, soil treatments and foliar treatments in different crops (Jarande *et al.*, 1994, Kumar and Santharam, 1999, Kumar *et al.*, 2001, Mote *et al.*, 1994, Sarkar *et al.*, 2001, Tomlin, 2000, Kanrar *et al.*, 2006). The present investigation was undertaken to determine the dissipation pattern as well as residue level of Imidacloprid 0.3% G (very recently introduced by M/S Excel crop care Ltd., Mumbai) in water maintained at different pH and soils of different agro-climatic zones of India under the laboratory simulated condition at Pesticide Residue Laboratory, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India.

MATERIALS AND METHODS

1. Sample preparation

1.1 Preparation of water sample with varied pH level

One buffer capsule of pH 4.0, 7.0 and 9.2 per 100 ml of distilled water was used for preparation of water

samples of the particular pH respectively. 200 ml distilled water was taken in each of a series of 250 ml conical flask and two buffer capsules were added to each of the conical flask. They were then left at room temperature for overnight for homogeneous mixing. The pH of water samples were checked intermittently during the entire study period.

1.2 Preparation of soil sample

Four types of agricultural field soils were collected from different agro-climatic zones, viz. i) New Alluvial soil from University Research Station, BCKV, Mohanpur, Nadia ii) Red and Lateritic soil from Regional Research Station, BCKV, Jhargram, Midnapore; and iii) Coastal Saline soil from Research Station of Central Soil Salinity Research Institute (ICAR) at Canning, 24- Parganas (S) iv) Black Soil from NRC Grapes, Pune, following the standard methodology of soil sampling. Soils were air dried, grinded and passed through 2 mm sieve and subsampled by the usual method of quartering. Selected physico-chemical properties of the soil are given in the Table 1. Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). Soil pH was measured in soil + deionised water (1+2.5 by weight) (Jackson, 1973). The organic carbon content of the soil was determined by Walkley and Black wet oxidation method (Nelson and Soemmers, 1982).

2. Fortification of samples with Imidacloprid 0.3% G

2.1 Water sample

2 ml and 4 ml from the 100 ppm stock solutions of Imidacloprid 0.3% G were added to each

conical flask containing 200 ml water of different pH. The initial concentrations become 1 ppm (T_1) and 2 ppm (T_2) respectively. For each treatment three replications were taken along with untreated control containing buffer solution.

2.2 Soil sample

Soil samples (50 g) were taken in 250 ml conical flasks to form a set for each type of soil and 10 ml of water was added to it. Then 1 ml and 2 ml of the 50 ppm stock solution of Imidacloprid were added to conical flasks separately containing 50 g of different soil samples. The initial concentrations become 1 ppm (T_1) and 2 ppm (T_2) respectively. The control soils (50 g) received 10 ml of water only. Three replicate flasks for each treatment were taken for analysis on each days of sampling along with untreated control. Samples (three replicates) were processed for analysis of Imidacloprid residues at intervals of 0 (2h after application), 5, 15, 30, 60 and 90 days after application.

2.3 Extraction and clean up

2.3.1 Extraction and clean up of water samples

Water sample (200 ml) after addition of 10 g NaCl, was taken in a 1 l separatory funnel and partitioned thrice with dichloromethane (100 + 50 + 50 ml). Organic phase was collected over anhydrous Na_2SO_4 . The combined dichloromethane fraction was evaporated to dryness in a rotary vacuum evaporator at 40°C using water suction. Final volume was reconstituted with HPLC grade acetonitrile.

2.3.2 Extraction and clean up of soil samples

Soil sample (50 g) was taken in a 250 ml conical flask and 150 ml Acetonitrile : water (8:2) mixture was added to it and kept overnight. Then the conical flasks were shaken with a mechanical shaker for 2 hours and subsequently filtered. The acetonitrile extract obtained from soil sample was concentrated in rotary vacuum evaporator at 40°C. After addition of 150 ml of distilled water and 10 g of NaCl the concentrated extract was subjected to solvent partitioning with dichloromethane for three times (100 + 50 + 50 ml). The dichloromethane fractions were collected through anhydrous sodium sulfate and concentrated to about 5 ml using rotary vacuum evaporator. The concentrated dichloromethane fraction was quantitatively transferred on a glass column packed with a mixture of 10 g of silica gel and 1 g of activated charcoal. 150 ml of dichloromethane was used as mobile phase to elute the residues of Imidacloprid. The cleaned up extract was collected and concentrated in rotary vacuum evaporator and finally the volume was reconstituted with HPLC grade acetonitrile.

2.4 Analysis of imidacloprid by HPLC

Imidacloprid was estimated by HPLC (Hewlett-Packard - Model 1050) equipped with

Variable Wavelength detector (Agilent 1100 series) and Agilent 1100 series software. Reverse Phase C_{18} column (250 x 4.6 mm; Thermo Hypersil ODS, 5 μ) was used for chromatographic separation with Acetonitrile: Water (9:1 for water and 7:3 for soil samples) as the mobile phase at a flow rate of 1 ml min^{-1} . Under these working conditions imidacloprid was detected (at $\lambda_{\text{max}} = 270$ nm) with the retention times of 2.8 and 3.1 min for water and soil samples respectively. The LOD and LOQ of the method were 0.01 ppm and 0.05 ppm respectively for both soil and water.

2.5 Recovery study

Recovery study was carried out in control samples (with no previous history of imidacloprid application) in order to establish the efficiency and reliability of the analytical method employed.

2.5.1 Recovery study for water samples

Distilled water samples (200 ml), maintained at pH 4.0, 7.0 and 9.2 levels, were fortified at the level of 0.05, 0.1 and 1.0 ppm with the standard solutions of Imidacloprid and were analysed as mentioned in 2.4. The average recovery was in the range of 91.67 to 96.67 % (Table 2).

2.5.2 Recovery study for soil samples

Soil samples (50 g) were fortified at the level of 0.05, 0.1 and 1.0 ppm with the stock solutions of Imidacloprid and were analysed following the method as mentioned in 2.4. The average recovery was in the range of 92.67 to 99.00% (Table 3).

RESULTS AND DISCUSSION

The results regarding the residue of Imidacloprid 0.3% G in spiked water samples of pH 4.0, 7.0 and 9.2 as well as soils of different agro-climatic zones are summarized in Tables 4 and 5. No residue was detected in the control samples during the entire study. The residue gradually decreased with time following first order kinetics in all the cases. The calculated half life ($T_{1/2}$) values in water were found to be in the range of 66.9 to 70.01 days, 81.36 to 88.54 days and 79.22 to 94.07 days in case pH 4.0, 7.0 and 9.2 respectively. In case of different soils these values were 59.03 days, 71.67 to 73.42 days 64.05 to 75.26 days and 66.9 to 71.67 days for Jhargram soil, Mohanpur soil, Canning soil and Pune soil respectively. In both the case Imidacloprid dissipated faster in acidic medium. The dissipation of Imidacloprid 0.3% G appeared to be faster in acid medium [*i.e.* in acidic water (pH 4.0) as well as in acidic soil (Jhargram soil)] than neutral but slower in alkaline medium. The result of present experiment is in well agreement with the result of the experiment entitled "Persistence and metabolism of imidacloprid in different soils of West Bengal" obtained by Sarkar *et al.*, 2001.