Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
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http://ansinet.com

Pakistan Journal of Plant Pathology, Volume 1 Number 2-4, 76-77, 2002

Fate and Residue Analysis of Isoproturon Applied to Control Jangli Jai (*Avena fatua*) in Wheat (*Triticum aestivum*)

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Abstract: The study was conducted to determine the fate of herbicide (isoproturon) in the grain, straw and soil samples for the control of jangli jai in the wheat fields. Arelon (45% isoproturon) at a dose of 3.7 L ha^{-1} to control jangli jai was sprayed. The wheat control rating of isoproturon in control and treated plots were observed as highly significant ($P<0.05$). Similarly, isoproturon application has also been found to be highly significant ($P<0.05$) in increasing the grain yield of treated plots. The residue concentration in the whole plants of treated plot was 0.66 and 0.71 ppm, 0.25 and 0.26 ppm in grain, 0.37 and 0.36 ppm in wheat straw and the soil collected from treated plots contained 1.74 and 1.76 ppm residue of isoproturon determined by thin layer chromatography and spectrophotometric techniques, respectively. Grain and wheat straw samples from control plots contained no residues but soil samples contained 0.014 and 0.018 ppm of isoproturon.

Key words: Isoproturon, TLC, spectrophotometry, wheat straw, grain, soil, Jangli Jai

Introduction

Intensive crop production technology involving high yielding varieties, multiple cropping system, heavy inputs of fertilizers and irrigation have led to a serious weed problems in field crops. The weeds reduce the crop yield to a great extent and the loss in grain yield of wheat due to weed competition may be as high as 15-50%. To combat weed problem, both mechanical and chemical methods are employed, of which, use of herbicide have been found to be most efficient and economical method (Ashiq *et al.*, 2000).

Wheat *(Triticum aestivum),* among the cereals is the main staple food in the Pakistan. The annual production of wheat is 2, 107, 800,0 tons and total area under cultivation is 8, 575,000 ha (Anonymous, 2000).

Phenyl urea is one of the major group of herbicides widely used in agriculture. One member of this group, isoproturon, is most commonly used. Isoproturon @ 1 kg ha^{-1} provides 47% control of *Avena fatua* (Jangli Jai) (Kumar and Singh, 1997). Phenyl urea herbicides are also toxic to human beings and animals. The residual effect has been viewed with alarm throughout the world.

The objective of this study was to determine isoproturon residues in wheat grains, straw and soil samples. The study also conduct to determine the reproducibility and comparability of TLC and spectrophotometric techniques for the analysis of isoproturon.

Materials and Methods

Wheat variety Inqalab was obtained from Nuclear Institute for Agriculture and Biology (NIAB) Seed Farm and sown in plot size of 0.404 ha, seed rate was 125 kg ha^{-1}. Arelon (45% isoproturon) at a dose of 3.7 L ha^{-1} to control *Avena fatua* (Jangli Jai) was sprayed in three parts of the plot leaving one part as untreated after 28-30 days of sowing.

Samples: Wheat grains, straw and soil samples were collected from 4 different places of wheat field after harvesting for arelon estimation.

Extraction of isoproturon from wheat grains, straw and soil samples

Grains: Grinded 50 g of wheat grains, added 50 ml ethyl acetate shacked it for one hour and filtered. Collected the residue and added in it 20 ml ethyl acetate, shacked and filtered. Took I & II filtrate, (i.e. 65-70 ml). Passed this filtrate through a column of 10 g anhydrous Na_2SO_4 activated at 600°C at a flow rate of 1 ml/min. After cleanup this filtrate was evaporated up to complete dryness with rotary evaporator.

Straw: In 8g plant sample, added 25 ml distilled water and homogenized it by magnetic stirrer for 2 min. Washed the contents with 15 ml distilled water added 0.8 ml conc. HCl, reflexed at 100°C for 1 hour. Then centrifuged the homogenate at 5000 rpm for 10 min. Collected the supernatant layer and added 15 g NaCl and shacked it. Extracted the aliquot three times with 20, 20 and 10 ml portions of methylene chloride and passed through anhydrous Na_2SO_4. Evaporated it up to complete dryness.

Soil: In 60g of soil samples added 100 ml methanol: water (90:7) and shacked it at a rate of 150 rev/min and filtered. Took the filtrate added 15 g NaCl and shacked. Extracted the filtrate with 150 ml of methylene chloride and passed through anhydrous Na_2SO_4 and evaporated it up to complete dryness

All the samples (grains, straw and soil) were reconstituted in l ml acetone for thin layer chromatography (TLC) and in 1 ml methanol for spectrophotometric analysis.

Analytical technique: The extracted isoproturon (IPN) is analyzed by using two techniques.

1. Thin layer chromatography (TLC) (Asi *et al.*, 2000)
2. Spectrophotometric method (Raju *et al.*, 1990)

Determination of isoproturon by TLC: Wheat leaves (30 g) were grounded with 3 ml glycerol for the extraction of chloroplast. Added 15 ml distilled water. Ten ml chloroplast extract was mixed with 14 ml dichlorophenol indophenol (DCPIP) reagent solution (containing 200 ml DCPIP mixed with 500 ml borax buffer solution) to prepare spray reagent solution for TLC.

After the preparation of TLC plates with silica gel (60 G) activated them for 30 min at 105°C. Carefully spotted the standard and sample developed the plate in ethyl acetate, marked position of solvent and dried at room temperature. Then sprayed the plate with spray reagent. Marked the spots and noted their R_f values.

For different concentrations of isoproturon known quantity of standard and samples were applied on TLC plates and results were noted.

Determination of isoproturon by spectrophotometric method: The stock solution contained 0.1 mg/ml of isoproturon was prepared in methanol. From this standards concentrations of 0.05, 0.1, 0.5 ,1.0 , 1.5 , 2.0, 2.5 and 3.0μg/ml for spectrophotometric and TLC analysis were prepared.

Pipetted 1 ml of each standard isoproturon solutions in duplicate into separate 10 ml calibrated flasks. Solvent was evaporated to dryness on water bath after which 1.0 ml of 0.5 M methanolic sodium hydroxide solution was added and the flasks were set

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aside for 5 min for complete hydrolysis. After evaporating the solvent again, 2.5 ml of 5 % paradimethyl aminobenzyldehyde (PDAB) solution and 3.0 ml of 0.2 M HCl acid were added and the mixture was heated on a water-bath for 80 min. Then cooled at room temperature for 15 min. and then solutions diluted to 10 ml with methanol. The absorbance of the solution was measured at 438 nm, against a reagent blank using spectrophotometer. Similar procedure was adopted for extracted samples (grain, straw and soil), noted the absorbance and calculated the amount of IPN with the help of standard curve.

Results and Discussion

Isoproturon applied at a dose of 3.7 l/ha effectively control the growth of *Avena fatua* (Jangli Jai) a weed which has been reported by various workers to compete with wheat crop for soil moisture, nutrient and sunlight (Eshel, 1978; Bernal, 1982; Bhardwaj, 1981). *Avena fatua* (Jangli Jai) incidence in the treated plots was of wheat controlled significantly ($P<0.05$) in comparison to the untreated plot (Table 1) which ultimately increase grain yield. Uncontrolled weeds caused 49% reduction in grain yield of wheat (Klisochowski and Beyer, 1979). Isoproturon application has highly increased the grain yield of treated plot and yield was more as compared to control plots (Table 2). Gill *et al.* (1982) and Rastogi *et al.* (1984) also reported the same trend in yield.

Table 1: Weed (*Avena fatua*) control rating of isoproturon applied on wheat crop

	No. of plants of Avena fatua			
Treatments	A	B	C	D
Control	34	46	42	38
Treated	8	13	10	11

Plot size 5 x 5m, t = 60.800**, df = 3

Table 2: Grain yield of wheat crop treated with isoproturon

	Yield (kg/plot)			
Treatments	A	B	C	D
Control	149	165	150	142
Treated	360	372	349	350

Plot size 3509.68m^2, t = 13.20**, df = 3

Table 3: Comparison of techniques for the residue analysis of isoproturon

Samples	TLC (ppm)	Spectrophotometric (ppm)	Control (ppm)
Whole plant	0.66	0.71	0
Wheat straw	0.37	0.36	0
Grain	0.25	0.26	0
Soil	1.74	1.76	0.014 TLC 0.018 spectro.

** Highly significant, A,B,C,D = Replications

The amount of isoproturon residues decreased successively from whole wheat plant (0.66, TLC; 0.71ppm spectrophotometric method) to wheat straw (0.37, 0.36ppm) and wheat grains (0.25, 0.26ppm) but it increased successively in soil of treated plot upto the level of 1.74 and 1.76ppm TLC and spectrophotometric method. Similar results were also reported by Randhawa and Sandhu (1997). This is because during spray on plant a significant amount was not sprayed properly on plant and got waste on soil, so more concentration of isoproturon was detected in soil samples as compared to others.

It was also recorded that the presence of isoproturon determined in the whole plants is to be concerned when the plants were of three months old and the spray of the herbicide was done at the age of one month as the plant at this maturity is also consumed as animal fodder. The residue level was upto 0.7 ppm. The residues of isoproturon remained undetected in whole wheat plant, wheat grains and wheat straw of controlled plot while in soil of control plot, it was detected by both methods and that was 0.014 and 0.018ppm determined by TLC and spectrophotometer respectively.

So, in conclusion the recommended dose of manufacturer and amended dose reported in the present study have not been successful to control completely the *Avena fatua* in the wheat fields. It controlled 40 % weed. Results (Table 3) indicated that the residue level determined in wheat grains, straw, whole plant and soil does not exceed permissible level recommended by FAO/WHO but it was high in the soil samples. The availability of herbicide residues in the soil is of concern because of its take up in follow up crop and its possible entry into the sub soil water during leaching at the time of raining and irrigation.(Mouvet *et al.*, 1997). It is also concluded that TLC and spectrophotometric techniques are reproducible and comparable for the determination of isoproturon residues in wheat grains, straw, whole plant and soil samples.

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