Persistence studies of Cyazofamid in potato plant soil and water

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Abstract: Persistence of Cyazofamid was studied in potato plant, in four types of soil viz., black, clay, sandy loam and loamy sand soils and in three types of water viz., acidic (pH 4.0), natural (pH 7.0) and basic (pH 9.0). All the matrixes were treated with Cyazofamid so as to get concentrations of 1 & 2 ppm. These samples of soil and water were kept in laboratory at ambient temperature. Potato plants were collected from the experimental field. The periodic samples in triplicate were drawn at intervals of 0, 3,7,15 & 30 days after treatment and were processed for analysis. The analysis of plant samples on 0 day (after 2 hrs. of application) showed initial deposition of 1.601 & 2.827 ppm of Cyazofamid when sprayed @ 80 g a.i./ha & @ 160 a.i./ha with half life of 1.74 days to 1.89 days respectively. No residue of Cyazofamid could be detected (detection level 0.02 ppm) on 10th day after application in both the treatments. The analysis of soil samples on 0 day showed mean initial deposition of Cyazofamid as 0.816, 0.817, 0.831 and 0.817 ppm when treated with 1 ppm concentration and 2.704, 1.785, 1.753 and 1.706 ppm when treated with 2 ppm in black, clay, sandy loam and loamy sand soils. The half life varied from 4.86-5.04 days in black soil, 3.02-3.21 days in clay soil, 4.15-4.30 days in sandy loam soil and 3.67-3.90 days in loamy sand soil at 1 and 2 ppm respectively. The analysis of water samples on 0 day showed initial deposition of Cyazofamid as 0.872, 0.8769 and 0.850 ppm in acidic, neutral and basic water respectively at 1 ppm and 1.703, 1.712 and 1.759 ppm in acidic, neutral and basic water respectively at 2 ppm. The half life values varied from 5.7 & 6.1 at 1 and 2 ppm level respectively in acidic water; 4.2, 4.5 days at 1 & 2 ppm level respectively in neutral water and 3.8 and 3.9 at 1 & 2 ppm level respectively in basic water.
[Vibha Doshi, V.P. Gupta and Meenakshi Dheer. **Persistence studies of Cyazofamid in potato plant soil and water.** Academia Arena, 2011;3(12):25-30] http://www.sciencepub.net. 6

Key words: Cyazofamid, persistence, Potato plant, soil, water

Introduction

Cyazofamid a new fungicide is used for the control of disease caused by Oomycetes and Plasmodiophoromycetes fungi. The biochemical mode of action to Cyazofamid is by inhibition of all stages of fungal development. It is commonly used to control early and late blight of tomatoes and potatoes and downy mildew of cucurbit vegetables. No doubt use of pesticides which include insecticides, fungicides and herbicides or any other substance used to control pests improve the crop production by protecting them but residue of these chemicals have negative effects on human health. United State EPA and European commission strictly regulates the level of pesticide residue in various food commodities through maximum residue limit. Therefore, it is very important to determine the persistence of Cyazofamid in plant water and soil which may contribute to residue carry over problem and hence the present studies was carried out to detect level of residue persisted at different time intervals.

Material & Methods

Chemicals and reagents

All the solvent and water used were of HPLC grade. The chemicals used were of analytical grade. The Cyazofamid standard obtained from United Phosphorus Limited Mumbai, India was 99.3 per cent pure. One hundred ppm stock solution of Cyazofamid was prepared in Acetonitrile and serial dilutions of desired concentration were prepared using mobile phase.

Extraction and clean up of samples were done as per AOAC QuEChERS 2007. 01 method by Steven J. Lehotay, (2007).

Persistence in soils

Collection of soil samples

Four types of cultivable field soils were collected from different locations *viz.* (i) Black soil from Regional Research Station, JNKVV, Khandwa, M.P, India (ii) Clay soil from Agriculture Research Station, MPUAT, Kota, Rajasthan, India (iii) Sandy loam soil from Agriculture Research Station, RAU, Durgapura Rajasthan India and (iv) Loamy sand soil from Agriculture Research Station, RAU, Sri Ganganagar, India following standard methodology of soil sampling. The air dried soils were grounded and passed through 1 mm sieve and sub-sampled by the usual method of quartering. The physico-chemical properties of soils (Table 1.) are as under:

Table 1.Physico-chemical properties of soils

Location	Texture	pH	Bulk Density (g/cm^3)	Organic carbon (%)
JNKVV, Khandwa	Black soil	7.81	1.56	0.69
MPUAT, Kota	Clay soil	8.12	1.58	0.68
ARS, RAU, Durgapura	Sandy loam soil	7.20	1.27	0.87
ARS, RAU Sri Ganganagar	Loamy sand soil	6.20	1.62	0.70

Fortification of soil samples

Weighed 50 g of soil samples and transferred to 250 ml beakers separately and fortified at 1 and 2 ppm levels by adding 5 ml of 10 and 20 ppm stock solution of Cyazofamid. In control, 5 ml of water was added. Three replicate flasks for each treatment were taken for analysis on each sampling day along with untreated control. During entire study period, soil moisture was maintained at one third of soil water holding capacity by adding distilled water on regular intervals and stored at room temperature. Samples were then processed for analysis of Cyazofamid residues at intervals of 0 (2h after application), 3, 7, 15 and 30 days after application.

Extraction and Cleanup of soil sample

5.0 g of thoroughly comminuted samples and 10 ml water was taken into Teflon centrifuge tubes. The Teflon centrifuge tubes were covered with black paper to avoid light exposure. 15 ml of 1% Acetic acid in Acetonitrile per 15 g sample was then added in each tube using the solvent dispenser. The tubes were then kept in cold at 4 degree C overnight. 6 g anhydrous MgSO4, and 1.5 g anhydrous sodium acetate per 15 g sample was added to the tubes. The tubes were vigorously shaken by hand for 1 min ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are broken up sufficiently during shaking. The tubes were then centrifuged at >1500 rcf for 10 min. 6 ml of the Acetonitrile extracts (upper layer) was transferred to the centrifuge tubes containing 50 mg PSA sorbent and 150 mg MgSO4 per mL extract. The tubes were sealed well and shaken for 30 seconds. the tubes were again centrifuged at >1500 rcf for 10 min. The final extract was filtered through Axiva 0.2 µm nylon syringe filter and transferred to HPLC vial for analysis. All the processes were completed in dark.

Validation of Method

Recovery studies were carried out in order to establish the analytical method and to know the efficiency of extraction and clean up steps employed for the present study by fortifying the representative samples with analytical standard of Cyazofamid at 0.02, 0.1 and 0.2ppm level. The results of recovery studies are presented in table 2.

Table 2 Recovery of Cyazofamid from different soil samples

Matrix	Amount fortified (µg/g)	Mean Per cent recovery
Black soil	0.02	92.9
	0.10	91.7
	0.20	91.9
Clay soil	0.02	92.1
	0.10	92.0
	0.20	93.2
Sandy loam soil	0.02	91.7
	0.10	92.8
	0.20	92.8
Loamy sand soil	0.02	92.6
	0.10	92.8
	0.20	93.5

Persistence in water

Preparation of water samples

Sample of water at different pH level *i.e.,* Basic (9.0 pH), Neutral (7.0 pH) and Acidic (4.0 pH), were prepared using buffer capsules of pH 9.0, 7.0 and 4.0 to set up the pH of water. One buffer capsule is required for 100 ml of distilled water to set up respective pH level. In a series of 250 ml conical flask, 200 ml distilled water was added and two capsules were added to each of the conical flask. The conical flasks were then left at room temperature for overnight for homogeneous mixing.

Fortification of water samples

22.5 ml of water samples were transferred to 100 ml beakers separately and fortified at 1 and 2 ppm level by adding 2.5 ml of 10 and 20 ppm stock solution for Cyazofamid prepared from Cyazofamid. In control, 2.5 ml of water was added. Samples were stored at room temperature. Three replicate flasks for each samples were processed for analysis of Cyazofamid residues at intervals of 0 (2h after application), 3, 7, 15 and 30 days after application.

Extraction and Cleanup of water sample

15.0 g of water samples was taken into Teflon centrifuge tubes and processed as per method mentioned in the extraction and cleanup of sample in soil

Validation of Method

Recovery studies were carried out in order to establish the analytical method and to know the efficiency of extraction and clean up steps employed for the present study by fortifying the representative samples with analytical standard of Cyazofamid at 0.02, 0.1 and 0.2ppm level. The results of recovery studies are presented in table 3.

Table 3 Recovery of Cyazofamid from different water samples

Matrix	**Amount fortified (μg/g)**	**Mean Per cent recovery**
Acidic water	0.02	93.4
	0.10	94.5
	0.20	94.5
Neutral water	0.02	95.3
	0.10	95.4
	0.20	95.6
Basic water	0.02	93.8
	0.10	93.7
	0.20	94.9

Persistence in plant

Preparation of plant Sample

Homogenized the sample and took 15 gms of representative plant sample for extraction.

Extraction and Cleanup of sample:

15.0 g of thoroughly comminuted plant samples was taken into Teflon centrifuge tubes and processed as per method mentioned in the extraction and cleanup of sample in soil

Validation of Method

Recovery studies were carried out in order to establish the analytical method and to know the efficiency of extraction and clean up steps employed for the present study by fortifying the potato plant samples with analytical standard of Cyazofamid at 0.02, 0.1 and 0.2ppm level. The results of recovery studies are presented in table 4

Table 4 Recovery of Cyazofamid from potato plant

Matrix	Amount fortified (ppm)	Mean % Recovered
Potato Plant	0.02	91.1
	0.1	91.5
	0.2	92.1

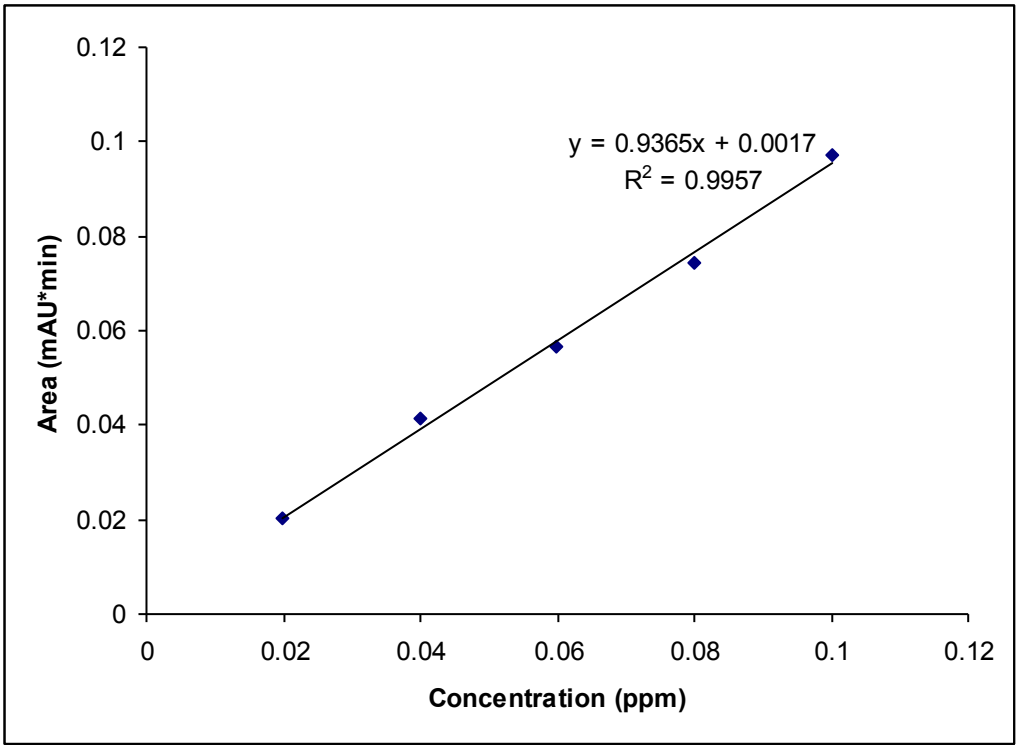
Linearity check study:

Different known concentrations of Cyazofamid standards (0.02, 0.04, 0.06, 0.08 and 0.10 ppm) were prepared in mobile phase and injected 20 µl of std. solution into HPLC system & measured the peak area (Table 5). A standard calibration curve was plotted for concentration of standard vs area measured and curve was found linear within concentration range for Cyazofamid

Table 5 Linear Dynamic Range Data of Cyazofamid Standard

Concentration (ppm)	Area (mAU*min)
0.02	0.0202
0.04	0.0412
0.06	0.0566
0.08	0.0743
0.10	0.0973

Standard Curve:



Tale 6.Persistence of Cyazofamid in soil, water and potato plant

Treatment	days	Residue ppm								Dissipation							
		Soil				Water			Plant	Soil				Water			Plant
		Black	Clay	Sandy Loam	Loamy Sand	Acidic	Neutral	Basic		Black	Clay	Sandy Loam	Loamy Sand	Acidic	Neutral	Basic	
Cyazofamid 1.0 ppm	0	0.817±0.005	0.817±0.005	0.831±0.010	0.817±0.005	0.872±0.008	0.869±0.003	0.850±0.019	1.601	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.668±0.030	0.547±0.022	0.581±0.011	0.586±0.012	0.677±0.005	0.638±0.040	0.588±0.015	0.940	18.2	33.0	30.2	28.3	22.4	26.6	30.9	41.3
	7	0.307±0.011	0.169±0.018	0.262±0.018	0.223±0.011	0.375±0.019	0.274±0.007	0.239±0.024	0.105	62.4	79.3	68.5	72.7	57.0	68.5	71.9	93.5
	15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-
	30	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-
Half Life (Days)		4.86	3.02	4.15	3.67	5.7	4.2	3.8	1.74								
Cazofamid 2.0 ppm	0	1.733±0.021	1.744±0.029	1.753±0.010	1.764±0.042	1.726±0.017	1.712±0.022	1.759±0.038	2.827	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	1.235±0.052	1.078±0.026	1.178±0.016	1.166±0.029	1.318±0.041	1.315±0.112	1.204±0.064	1.276	28.7	38.2	32.8	33.9	23.6	23.2	31.5	54.9
	7	0.690±0.011	0.495±0.026	0.601±0.009	0.592±0.021	0.792±0.027	0.599±0.031	0.565±0.054	0.223	60.2	71.6	65.7	66.4	54.1	65.0	67.9	92.1
	15	0.226±0.013	0.072±0.016	0.161±0.016	0.127±0.017	0.320±0.046	0.183±0.007	0.125±0.019		87.0	95.9	90.8	92.8	81.4	89.3	92.9	
	30	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-
Half Life (Days)		5.04	3.21	4.30	3.90	6.1	4.5	3.9	1.89	-	-	-	-	-	-	-	-
Untreated control	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-
	3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-
	7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-
	15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-
	30	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	-	-	-	-	-	-

BDL: Below determination limit=0.02ppm (MRL of Cyazofamid is 0.02ppm as reported by Pest Management Regulatory Agencies2011 Canada)

HPLC analysis

All the determinations were performed using Dionex Ultimate 3000 HPLC with DAD and Acelaim 120.C-18,5um, 120A, 4.6x150mm. HPLC column. The temperature was ambient, mobile phase used was a mixture of Acetonitrile, methanol and HPLC water (pH-4 with acetic acid) in ratio of 52:65:63 (v/v/v). The UV wavelength was 280 nm with run time 15 minute at flow rate 1 ml/min

Results and discussion

Persistence in soil

Data regarding the initial deposition, per cent dissipation and half life value of Cyazofamid in different soil after treatments at the rate of 1 ppm and 2 ppm have been presented in table 6 The analysis of soil samples on 0 day (after 2 hrs of application) showed mean initial deposition of Cyazofamid as 0.816, 0.817, 831 and 0.817 ppm in black, Clay, sandy loam and loamy sand, respectively at 1 ppm and 1.704, 1.785, 1.753 and 1.706 ppm in black, Clay, sandy loam and loamy sand, respectively at 2 ppm. However, Cyazofamid dissipated to below detectable level on 15 day and 30 days at 1 ppm and 2 ppm level respectively in all four types of soil (black, Clay, sandy loam and loamy sand). The half life ($T_{1/2}$) values varied from 4.86-5.04 days in black soil, 3.02- 3.21 days in clay soil, 4.15- 4.30 days in sandy loam soil and 3.67- 3.90 days in loamy sand soil at 1 and 2 ppm levels, respectively. Similar results of cyazofamid degradation in various types of soil (ranging between 3.5-15.1 days) have been mentioned in review report issued by European Commission (2002). Data from table 6 reveals that

half life of cyazofamid was slightly shorter when fortified in lower concentrations (1 ppm) than that of higher concentration (2 ppm) in all the four types of soils. Fomsgaard *et. a*l.(2004) and Weidenhamer and Romeo (2004) also reported slower degradation of chemicals when present in lower concentration as compared to higher.

Persistence in Water

Data regarding the initial deposition, per cent dissipation and half life value of Cyazofamid in different water after treatments at the rate of 1 ppm and 2 ppm have been presented in table 6. The analysis of water samples on 0 day (after 2 hrs of application) showed mean initial deposition of Cyazofamid as 0.872, 0.869 and 0.850 ppm in acidic, neutral and basic water, respectively at 1 ppm and 1.703, 1.712 and 1.759 ppm in acidic, neutral and basic water, respectively at 2 ppm. However, Cyazofamid dissipated to below detectable level on 15 day and 30 days at 1 ppm and 2 ppm level respectively in all three types of water (acidic, neutral and basic water). The half life ($T_{1/2}$) values varied from 5.7 and 6.1 days at 1 and 2ppm level, respectively in acidic water, 4.2 and 4.5 days at 1 and 2 ppm level, respectively in neutral water and 3.8 and 3.9 days at 1 and 2 ppm level, respectively in basic water .These results are in close confirmation to the results reported In pesticide fact sheet of cyazofamid issued by United State EPA (2004), where pesticide degraded in different types of water ranging between 10.8 to 12.9 days at 25 degree centigrade which is slightly higher than our studies probably because of higher temperature in our conditions

Persistence in potato plant

The analysis of plant sample on 0 days (after 2 hrs of application) showed initial deposition of 1.601 and 2.827 ppm for Cyazofamid from UPF 206 @ 80g a.i./ha (200ml/ha) and160 g a.i./ha (400ml/ha) dose treatments respectively. Cyazofamid dissipated to below detectable level at 10 days after application of UPF 206 @ 80 g a.i./ha (200ml/ha) and 160 g a.i./ha (400ml/ha) with the half life of 1.74 days and 1.89 days, respectively(Table 6).Jerome (2009) studied the dissipation of cyazofamid in turfgrss and found half life of 18-19 days.

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12/1/2011