Effect of different water pH and soil conditions on dissipation pattern of a new fungicide mixture (Tricyclazole 33.3% + Azoxystrobin 16.7%) SC under laboratory condition

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ABSTRACT

A laboratory study has been experimented to determine the persistence nature of a new ready mix fungicide formulation (tricyclazole 33.3% + azoxystrobin 16.7%) SC in both aqueous systems of different pH and soil samples collected from four different agro climatic regions at single dose (T_1) of 1 ppm and double dose (T_2) of 2 ppm of each active ingredient under laboratory simulated condition. All samples were collected at specific interval after treatments and processed for residue analysis of both tricyclazole and azoxystrobin. Extraction and clean up procedure was developed and their residue were analyzed by LC-MS/MS. The recovery % of both analyte in every case was 85% or more than that. Dissipation was approximated to first order kinetics in every condition. It is seen that the degradation nature of both of the compound were quicker in alkaline condition followed by neutral and least in acidic condition.

*Keywords***:** *Azoxystrobin, LC-MS/MS, Recovery, Residue, Tricyclazole.*

Tricyclazole {5-methyl-1,2,4-triazolo (3,4-b) benzothiazole} and azoxystrobin {Methyl (2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyprop-2-enoate}-3-methoxyacrylate}, both are systemic fungicides belonging to the chemical class of thiazole and strobilurin respectively. The first fungicide is responsible to control the blast disease of rice caused by the fungus *Pyricularia oryzae*. Whereas, The second one with another specific mode of action is useful for controlling sheath blight disease of rice which is caused by the fungus *Rhizoctonia solani* (Tokousbalides and Sisler, 1978; Bhuvaneswari and Raju, 2012). Tricyclazole 33.3% + azoxystrobin 16.7% is a new mixture formulation in suspension concentrate (SC) form of the previously mentioned two different chemical compounds used to control such diseases at a time. Specific fungicides having specific properties were regarded as a good use in crop protection. Crop protection is our main objective for agricultural yield improvement by reducing pest and disease infestation. Being a mixture one it lowered not only the dose rate but also the number of applications per season. But the widespread use of pesticide simultaneously leads to environmental contamination in different ways. So it is necessary to find out their residual fate in a different environmental component like water, soil etc. for observing their persistent nature and half-life values. This type of research work becomes more effective when a new pesticide or formulation is first time entered in a new area because this study will help to take precautionary activities to reduce hazardous effect after its application in the environment. There are different earlier reported research experiments by several scientists including the degradation nature of tricyclazole and azoxystrobin under both field and laboratory condition (Vogue *et al*., 1994; Padovani *et al*., 2006; Meenakshi *et al.*, 2007; Phong *et al*., 2009; Szpyrka and Sadlo, 2009; Singh *et al.*, 2010; Tsochatzis *et al.*, 2013). But till date, no systematic work has been performed to study the dissipation pattern of this new combo formulation product in soils and water for impact assessment under such condition. Therefore, the present study has been carried out to understand the dissipation behaviour of (tricyclazole 33.3% + azoxystrobin 16.7%) SC in the water of three different pH and soils collected from four different agro-climatic zones of India under laboratory simulated condition and also to find out their probable effect on the stability of tricyclazole and azoxystrobin coming from this formulation.

MATERIALS AND METHODS

Certified reference material (CRM, analytical grade) of tricyclazole (99.7% purity) was employed from Sigma Aldrich Company and analytical grade standard of azoxystrobin (98.2% purity) and the formulation (tricyclazole 33.3% + azoxystrobin 16.7%) SC was supplied by M/s Crystal Crop Protection Pvt. Ltd., Delhi.

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Acetonitrile (HPLC grade) and dichloromethane were procured from Rankem used for this experiment. Other inorganic reagents which were used in the total experiment are of laboratory grade. Distilled water used for the entire study was obtained from the laboratory distillation unit system. To prepare a standard stock solution (100 ppm) of tricyclazole and azoxystrobin, 10.03 mg of analytical tricyclazole and 10.17 mg of analytical azoxystrobin was dissolved separately in two different volumetric flasks containing 100 mL acetonitrile. The working and calibration standards of both were prepared from these standard stock solutions by dilution in acetonitrile.

For the preparation of different buffer solutions, buffer capsules of pH 4.0, 7.0 and 9.2 procured from Rankem were used. One buffer capsule is effective to prepare a 100 mL buffer solution. In a series of 250 mL, conical flask 100 mL distilled water was taken and one capsule was added to each of the conical flasks to prepare three different pH solutions. The conical flasks were then left at room temperature for overnight to get a homogeneous mixture.

All the gross soil samples were collected from different agro-climatic zones of India such as New alluvial soil from Mohanpur (22°59´N/88°29´E), Lateritic soil from Jhargram (22°27´N/86°59´E), Coastal saline soil from Canning (22°19´N/88°40´E) and Black soil from Pune (18°32´N/73°52´E) from the agricultural field with no previous history of the application of this new mixture fungicide formulation. Thus, in the current study, soils with various Physico-chemical properties were considered for laboratory experiment of new mixture formulation of tricyclazole and azoxystrobin. Soil samples were collected from 0 to 15 cm depth with the help of a soil auger from at least ten numbers of spots in each case following the 'zig-zag' pattern of soil sampling. Then, the representative portion of the gross field sample was packed and taken to the laboratory. Then the samples were air-dried under shade, ground and passed through a 0.2 mm sieve. From this, sub-samples were taken by the quartering technique to prepare laboratory sample for analytical purpose. To maintain 60% maximum water holding capacity, distilled water was added to the soil sample from time to time. The physico-chemical properties of different soils were measured by the following methods: soil texture was determined by the hydrometer method (Gee and Bauder, 1986). Soil pH was determined by soil: deionised water (1: 2.5 by weight) (Jackson, 1973). The organic carbon content of the soil was tested by Walkley and Black wet oxidation method (Nelson and Sommers, 1982) and summarized in Table 1.

Ten-gram sodium chloride (Merck) was thoroughly mixed with 100 mL water and taken into a separatory

funnel and agitating with 100 mL dichloromethane for two minutes. After collecting the organic portion into a separate conical flask, the remaining aqueous layer was further partitioned with 50 mL of dichloromethane two times. After combining all the organic phases, it was passing through anhydrous sodium sulphate (SRL) for removing the water stress and evaporated using a rotary vacuum evaporator below 40ºC temperature and volume was made up with 10 mL HPLC grade acetonitrile. Then, 2 mL of the sample was filtered through a 0.22μ membrane filter and analyzed by liquid chromatography-tandem mass spectroscopy (LC-MS/MS) to analyze the residue of tricyclazole.

A similar method was adopted for measuring the residue of azoxystrobin from water samples of different pH. Water samples were extracted by partitioning with dichloromethane three times and then combined dichloromethane portion passed through anhydrous sodium sulphate and evaporated using rotary vacuum evaporator below 40ºC temperature and volume was made up with 10 mL HPLC grade acetonitrile. Then, 2 mL of the sample was filtered through a 0.22μ membrane filter and analyzed by LC-MS/MS.

A Ten-gram soil sample was taken in a 50 mL centrifuge tube and 10 mL water was mixed and vortexed for one minute. After that 10 mL acetonitrile, 4 g of activated magnesium sulphate and 1 g of sodium chloride were added to the mixture and vortex for two minutes. Then the sample was centrifuged for five minutes at 5000 r.p.m speed. After centrifugation, about 2 mL supernatant organic phase was collected with the help of micropipette to a polypropylene centrifuge tube containing 25 mg PSA (Varian) and 25 mg florisil (Acros Organics) and vortex for 30 sec and centrifuge for five minutes @ 6000 r.p.m. speed. After that, the clear extracts were filtered with a syringe filter through 0.22 μ nylon filter paper and transferred into the vials and analyzed in LC-MS/MS. In the case of azoxystrobin, similar extraction and clean up procedure had been performed as follows in the case of tricyclazole and also analyzed in LC-MS/MS.

The chromatographic separation of both of the fungicide was performed by using LC-MS/MS (Waters, Milford, MA, USA). Quattro Micro triple-quad spectrometer with an electrospray ionization source (ESI) was used for their detection and quantification. The detailed information regarding chromatographic and spectroscopic parameters for the detection and quantification of tricyclazole and azoxystrobin is presented in Table 2.

For checking the linearity, calibration standards of tricyclazole and azoxystrobin injected in LC-MS/MS in their respective HPLC and mass spectroscopy condition were mentioned earlier. The linearity check was performed by calibration curve plotting taking five

calibration points for each compound *viz.*, 0.01, 0.02, 0.05, 0.10 and 0.25 ppm for tricyclazole (Fig. 1) and 0.005, 0.01, 0.02, 0.05, 0.10 ppm for azoxystrobin (Fig. 2). The correlation coefficient (R^2) of both of the calibration curve was 0.99 which indicated good linearity and reliability. The limit of detection (LOD) was determined as 0.01 ppm for tricyclazole and 0.005 ppm for azoxystrobin by considering a signal-to-noise ratio of 3 regarding the background noise (Dureja *et al.*, 2015). The limit of quantifications (LOQs) of tricyclazole and azoxystrobin were determined as the lowest concentration of the analytes in soil and water that can be identified and quantified with satisfactory relative standard deviation (RSD). Here the LOQ level was set at 0.02 ppm and 0.01 ppm for tricyclazole and azoxystrobin respectively.

For performing recovery study, tricyclazole and azoxystrobin separately fortified from their respective working standard solution prepared from their respective 100 ppm analytical stock into untreated 100 mL water sample of different pH and untreated 10 g soil samples collected from different agro-climatic zones at 0.02 (LOQ level), 0.05 and 0.25 ppm level and 0.01(LOQ level), 0.02 and 0.10 ppm for tricyclazole and azoxystrobin respectively with three replications in each case. Other untreated control samples of each substrate were also simultaneously kept for better comparison. After spiking the standards, the samples were allowed to stay for 2 hrs. Then the sample extraction and clean-up were done by the above mention method. The precision along with repeatability was measured with the help of relative standard deviation. The trueness of the method was determined by recovery percentage and relative standard deviation (RSD).

The result of the recovery experiment is furnished in Table 3 and Table 4. It was observed that the recovery of both of the compounds is 85% or above in every case which complies with SANCO guidelines (Anon, 2013). Method accuracy in terms of mean recoveries and precision (RSD) was within satisfactory range i.e. 70–110% and ≤20% respectively as per that guideline. No residue of both of the fungicides was detected in any control sample of water and soil in the present experiment. So, the above-mentioned extraction, clean up and instrumental parameters were adopted for the residue analysis of tricyclazole and azoxystrobin from water and soil samples in the following experiment.

Stock solution (100 ppm) of both the compound were prepared separately from the mixture formulation taking 30.03 mg (for tricyclazole) formulation in a 100 mL volumetric flask and 59.88 mg (for azoxystrobin) of fungicide formulation in another one for the experiment. The volume was made up of distilled water to prepare a 100 ppm stock solution of tricyclazole and azoxystrobin respectively by dissolving them in distilled water. The

fortification was done on the experimental water samples of different pH individually from this 100 ppm stock solution separately in such a way that gives a final concentration of 1 ppm (T_1) and 2 ppm (T_2) of tricyclazole and azoxystrobin. Similarly, to study the fate of fungicides in different soils, for every set of 10 g of each type of soil in 100 mL conical flask separately fortified from the same stock solution which results from final concentration of soil sample as 1 ppm and 2 ppm of a.i. of tricyclazole and azoxystrobin as single and double dose respectively. The water and soil sample of both of the doses had three replications for each experimental day. Throughout the experimental period, one set of untreated control was simultaneously maintained for all kinds of soil and water samples. The experimental flasks were kept at 28 ± 2 °C temperature throughout the study. After application of the formulation to different soils and water system, the samples were collected separately and periodically for residue analysis of both tricyclazole and azoxystrobin at an interval of 0 (2 h after fortification), 3, 7, 15, 30, 60, and 90 days after treatment. Then they were extracted and analyzed by the same procedure and same instrumental condition as mentioned above like recovery experiment.

RESULTS AND DISCUSSION

The outcomes of this experiment are presented in Table 5 and Table 6. The dissipation of both of the fungicides in water and soil samples followed the first-order reaction kinetics. The half-life was calculated by the following formulae: $t_{1/2} = \log 2 / k$ (Hoskins, 1961). No residue of tricyclazole and azoxystrobin was detected in any control sample throughout the study.

Dissipation of tricyclazole and azoxystrobin in different water system

The initial concentration of the residues of tricyclazole ranged from 0.89-0.93 ppm and 1.78-1.83 ppm for a single dose (T_1) and double dose (T_2) respectively in all types of the water sample. More than 50% of tricyclazole residue was disappeared 30 days after application (DAA) irrespective of doses in the water of pH 4.0 and pH 7.0 and in the case of the water sample having the pH value of 9.2, it was dissipated at 15 DAA in both the cases. The residue of tricyclazole was found up to 60 DAA in a single dose and 90 DAA in double dose in water samples of pH 4.0 and pH 7.0 whereas residue of tricyclazole was disappeared faster in alkaline water condition as compare to other which is 30 DAA and 60 DAA for single and double dose respectively. Calculated half-life values of tricyclazole are lowest at alkaline pH (16.72-20.07days) as compared to neutral pH (23.16-25.09 days) and acidic pH (25.09-27.37days). From the results, it appears that the dissipation rate of tricyclazole is faster in alkaline pH

Table 1: Physico-chemical characteristics of different soils

Physico-chemical properties of soil	New alluvial soil	Lateritic soil	Coastal saline soil	Black soil
Soil order	Mollisol	Aridisol	Alfisol	Vertisol
Location	Mohanpur	Jhargram	Canning	Pune
pH	7.02	5.45	7.60	8.14
Organic Carbon (%)	1.00	0.64	1.03	0.67
Sand (%)	12	54	50	16
Silt (%)	65	16	23	24
Clay (%)	23	30	27	60

Table 2: Instrumental parameters

HPLC parameters

Column (for resolution of tricyclazole)	Waters Symmetry C_{18}, 5μm i.d., 2.1 x100mm
Column (for resolution of azoxystrobin)	Waters Xterra, 3.5μm i.d.; 2.1 X 50 mm
Eluent	A: H_2O + 5mM CH_3COONH_4 + 0.1% CH_3COOH
	B: MeOH + 5mM CH_3COONH_4 + 0.1% CH_3COOH
Elution	Gradient
Flow rate	0.3 mL min^{-1}
Injection volume	20 μL

Gradient programming for tricyclazole	Time (minute)	%A	%B
	0	90	10
	2	10	90
	8	90	10
	10	90	10

Gradient programming for azoxystrobin	Time (minute)	%A	%B
	0	95	05
	2	05	95
	6	95	05
	10	95	05

Retention time (R_t) of tricyclazole	5.84±0.2 mimute
Retention time (R_t) of azoxystrobin	5.10±0.2 mimute

Mass spectroscopic parameters

Ionization mode	ESI +ve mode
Scan type	MRM (Multiple Reaction Monitoring)
Cappilary voltage	0.50 kV
Cone voltage	45 V (for tricyclazole) 22 V (for azoxystrobin)
Extractor	2 V
Source temperature	120 ºC
Desolvation temperature	350 ºC
Desolvation gas flow (nitrogen)	650.0 (L hr^{-1})
Collision gas flow (argon)	25.0 (L hr^{-1})
Molecular ion	189.84 (tricyclazole) 403.84 (azoxystrobin)
MRM (for tricyclazole)	189.84 > 135.85 (used for quantification)
	189.84 > 162.88
	189.84 > 64.34
MRM (for azoxystrobin)	403.84 > 371.92 (used for quantification)
	403.84 > 328.94
	403.84 > 343.93

Table 3: Recovery of tricyclazole in different substrates

	Recovery % in different substrates (RSD)						
Spiked level (ppm)	Water sample (pH 4.0)	Water sample (pH 7.0)	Water sample (pH 9.2)	New Alluvial soil	Lateritic soil	Coastal saline soil	Black Soil
0.020	88.33 (6.54)	96.67 (7.90)	85.00 (5.88)	86.67 (8.81)	91.67 (11.35)	88.33 (8.65)	93.33 (8.18)
0.050	91.33 (4.56)	87.33 (4.77)	86.00 (2.72)	90.00 (5.88)	92.67 (4.49)	85.33 (4.88)	86.67 (3.53)
0.250	89.33 (6.84)	90.27 (4.74)	92.40 (11.72)	86.53 (5.48)	92.80 (4.25)	84.00 (8.30)	88.00 (4.55)

*Average of three replicates; RSD: Relative Standard Deviation

Table 4: Recovery of azoxystrobin in different substrates

	Recovery % in different substrates (RSD)						
Spiked level (ppm)	Water sample (pH 4.0)	Water sample (pH 7.0)	Water sample (pH 9.2)	New Alluvial soil	Lateritic soil	Coastal saline soil	Black Soil
0.010	96.67 (5.97)	86.67 (6.66)	86.67 (6.66)	96.67 (5.97)	93.33 (6.19)	90.00 (11.11)	96.67 (5.97)
0.020	88.33 (3.27)	86.67 (8.81)	88.33 (8.65)	90.00 (5.56)	91.67 (8.33)	100.00 (5.00)	86.67 (6.66)
0.100	93.33 (4.46)	97.33 (2.14)	89.33 (5.05)	89.67 (3.59)	88.00 (6.91)	92.67 (2.25)	89.33 (5.74)

*Average of three replicates; RSD: Relative Standard Deviation

Table 5: Determination coefficient (R^2), regression equation, half-life ($t_{1/2}$) of tricyclazole and azoxystrobin at different pH

Sample	Dose	Tricyclazole			Azoxystrobin		
		R^2	Regression equation	Half-life ($t_{1/2}$)	R^2	Regression equation	Half life ($t_{1/2}$)
pH 4.0	T_1	0.979	y = 2.920-0.012x	25.09	0.983	y = 2.887-0.024x	12.54
	T_2	0.992	y= 3.243-0.011x	27.37	0.988	y= 3.178-0.017x	17.71
pH 7.0	T_1	0.982	y= 2.942-0.013x	23.16	0.978	y= 2.946-0.030x	10.03
	T_2	0.987	y= 3.186-0.012x	25.09	0.985	y= 3.216-0.021x	14.33
pH 9.2	T_1	0.985	y= 2.914-0.018x	16.72	0.990	y = 2.907-0.031x	9.71
	T_2	0.983	y= 3.207-0.015x	20.07	0.969	y= 3.148-0.029x	10.38

Table 6 : Determination coefficient (R^2), regression equation, half-life ($t_{1/2}$) of tricyclazole and azoxystrobin at different soils

| Sample	Dose	Tricyclazole			Azoxystrobin		
		R^2	Regression equation	Half-life ($t_{1/2}$)	R^2	Regression equation	Half-life ($t_{1/2}$)
New alluvial soil	T_1	0.996	y = 2.915-0.015x	20.07	0.996	y = 2.926-0.031x	9.71
	T_2	0.985	y= 3.183-0.012x	25.09	0.943	y= 3.111-0.021x	14.33
Lateritic soil	T_1	0.989	y= 2.925-0.012x	25.09	0.993	y= 2.947-0.025x	12.04
	T_2	0.977	y= 3.205-0.010x	30.10	0.987	y= 3.171-0.019x	15.84
Coastal saline soil	T_1	0.962	y = 2.888-0.016x	18.81	0.991	y = 2.890-0.030x	10.03
	T_2	0.997	y= 3.237-0.015x	20.07	0.979	y= 3.188-0.024x	12.54
Black soil	T_1	0.960	y = 2.906-0.025x	12.54	0.992	y = 2.868-0.032x	9.41
	T_2	0.992	y= 3.227-0.018x	16.72	0.993	y= 3.172 -0.024x	12.54
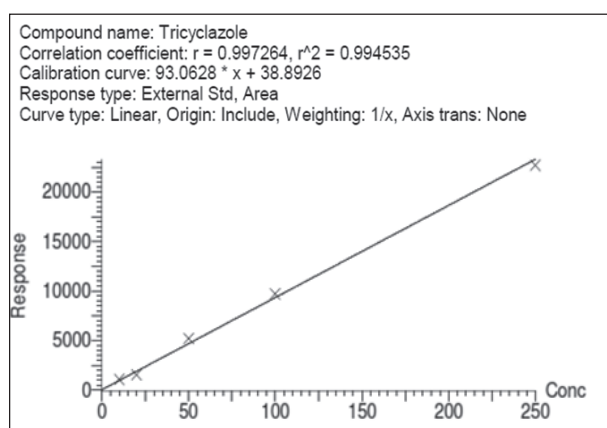
Fig. 1: Calibration curve of tricyclazole
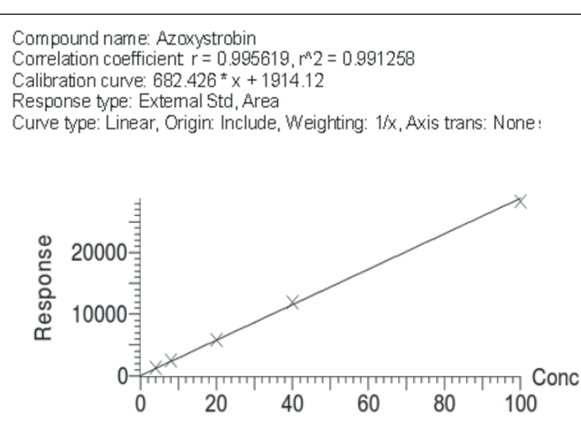

Fig. 2: Calibration curve of azoxystrobin

than neutral pH and the least in acidic pH in the aqueous medium. Tsochatzis *et al.*(2013) observed that the half-life of tricyclazole in normal water under laboratory condition was 16.4 days which is to some extent similar to our observation.

On the other side, the initial concentration of the residues of azoxystrobin ranged from 0.86-0.90 ppm and 1.71-1.87 ppm for a single dose (T_1) and double dose (T_2) respectively in all types of the water sample. More than 50% azoxystrobin residue was dissipated within 15 DAA irrespective of doses in the water of pH 4.0 and pH 7.0 and in the case of water sample having the pH value of 9.2, more than 50% residue was dissipated at 7 DAA irrespective of doses. The residue of azoxystrobin was persisted up to 30 DAA in a single dose and 60 DAA in double dose in water samples of pH 4.0 and pH 7.0 whereas it was disappeared in alkaline water within 30 DAA irrespective of doses. Calculated half-life values of azoxystrobin were lowest at alkaline pH (9.71-10.38 days) as compared to neutral pH (10.03-14.33 days) and acidic pH (12.54-17.71 days). From the results, it appears that the dissipation rate of azoxystrobin is faster in water with an increase in pH. Singh *et al.* (2010) also observed that degradation of azoxystrobin was faster in alkaline water as compared to neutral and acidic condition.

Dissipation of tricyclazole and azoxystrobin in the different soil system

The initial concentration of tricyclazole was found to vary from 0.91-0.93 ppm and 1.81-1.87 ppm respectively for single and double dose irrespective of soil types. In the case of a single dose, more than half of the tricyclazole residue was dissipated at 15 DAA in New alluvial and Coastal saline soil whereas, in the case of Lateritic soil and Black soil, it was 30 DAA and 7 DAA respectively. The residue of tricyclazole was detected up to 90 DAA in New alluvial soil (pH 7.02) and Lateritic soil (pH 5.45) irrespective of doses. In

Coastal saline soil (pH 7.60) tricyclazole residue was observed up to 90 DAA only in double dose condition. In Black soil (pH 8.14) residue of tricyclazole was present up to 30 DAA and 60 DAA at single and double dose respectively. The calculated half-life values of tricyclazole for Lateritic soil was found in the range of 25.09-30.10 days followed by New alluvial soil where half-life values were 20.07-25.09 days. The half-life values were to some extent lower in Coastal saline soil which was in the range of 18.81-20.07 days. Stability was lowest in Black soil, where half-life values were in the range of 12.54-16.72 days. Padovani *et al.* (2006) reported that half value of tricyclazole was 4–17 month under laboratory condition. Phong *et al.* (2009) reported the half-life of tricyclazole in the soil of 203–407 days under laboratory condition which were much longer than the ones reported here. Another source, Vogue *et al*. (1994) cited a soil half-life of only 21 days for tricyclazole under laboratory condition.

In the case of azoxystrobin, the initial residue was found to vary from 0.82-0.93 ppm and 1.69-1.86 ppm respectively for single-dose and double dose irrespective of soil types. Except for Black soil, more than half of the residue of azoxystrobin was degraded at 15 DAA irrespective of doses. In Black soil, azoxystrobin residue was persisted up to 7 DAA and 15 DAA for single and double dose respectively. The calculated half-life values of azoxystrobin were highest in Lateritic soil (12.04-15.84 days) followed by New alluvial soil and Coastal saline soil where the half-life values were 9.71-14.33 days and 10.03-12.54 days respectively. Stability was to some extent lower in Black soil where half-life values were in the range of 9.41-12.54 days. Meenakshi *et al*. (2007) also reported that the rate of degradation of azoxystrobin increased with an increase in soil pH.

From the above discussion, it is seen that some of the observation reported earlier is similar to our findings whereas in some cases their observation differs from our result. This difference could be attributed to the

different mode of application and formulation used. Saha *et al*. (2018), Sarkar *et al*. (1999), Zavala *et al*. (2004) reported in their experiments that persistence behaviour of the same active constituent was influenced by the formulation type. Most of the cases were studied by us; tricyclazole and azoxystrobin were used from a single pesticide formulation whereas, in our experiment, a new mixture formulation of tricyclazole and azoxystrobin was used. Besides this, the study was performed in laboratory simulated condition, other environmental factors like sunlight, rainfall etc. does not affect the degradation of both of the fungicide soil. So, it can be easily considered that the pH of soil and water plays an important role in the degradation of the fungicides. The microbial population also was responsible for their degradation because any soil or water sample was not sterilized before the study.

The present experiment exhibits the dissipation behaviour of the new mixture fungicide of tricyclazole and azoxystrobin. The pH is an important factor that affects the degradation nature of tricyclazole and azoxystrobin in water and soil. The result of this current experiment indicates that the rate of degradation of both compounds was faster in the alkaline condition in both soil and water. Besides this, it is coming out from the above experiment that tricyclazole is more persistent than azoxystrobin in every case. The outcomes of our study may help for assessing environmental safety due to the application of this new ready mix fungicide formulation (tricyclazole 33.3% + azoxystrobin 16.7%) SC in the agricultural field under different agro-climatic conditions in India.

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REFERENCES

Anonymous 2013. Guidance document on residue analytical methods. European Union Commission Directorate of General Health and Consumer Protection. SANCO/12571/2013 rev. 0, dated 19 November, 2013

Bhuvaneswari, V. and Raju, S.K. 2012. Efficacy of new combination fungicide against rice sheath blight caused by *Rhizoctonia solani* (Kuhn). *J. Rice Res.*,**5**: 57-61

Dureja, P., Singh, S.B. and Parmer, B.S. 2015. Pesticide maximum residue limit (MRL): background, Indian scenario. *Pestic. Res. J.*,**27**: 4-22

Gee, G.W. and Bauder, J.W. 1986. Particle size analysis. *In*: *Methods of soil analysis: Part-1- Physical and mineralogical methods*(2ⁿᵈEdn, Ed. by Klute A.,)ASAMadison, Wiskinson, USA: pp. 383-09

Hoskins, W.M. 1961. Mathematical treatment of the rate of loss of pesticide residues. *FAO Plant Prot. Bull.*,**9**:163-168

Jackson, M.L. 1973.*Soil Chemical Analysis*. Prentice-Hall of India Private Limited, New Delhi.

Meenakshi, S.N., Jeyaramraja, P.R and Manian, R. 2007. Degradation of fungicides azoxystrobin and difenoconazole in soil and their influence on soil microbial activity. *Pest Technol.*, **1**: 133-38

Nelson, D.W. and Sommers, L.E. 1982.Total carbon, organic carbon and organic matter.*In*.*Methods of soil analysis: Part-2- Physical and Mineralogical methods* (2ⁿᵈEdn, Ed. by Page A.L.,) ASA, Madison, Wiskinson, USA: pp. 539-579

Padovani, L., Capri, E., Padovani., C., Puglisi, E. andTrevisan, M. 2006. Monitoring tricyclazole residues in rice paddy watersheds. *Chemosphere*,**62**:303–314

Phong, T.K., Nhung, D.T.T., Motobayashi, T., Thuyet, D.O., and Watanabe, H. 2009. Fate and transport of nursery-box-applied tricyclazole and imidacloprid in paddy fields. *Water Air Soil Pollut*.,**202**: 3-12

Saha, S., Majumder, S., Das, S., Das, T.K., Bhattacharyya, A. and Roy, S. 2018. Effect of pH on the transformation of a new readymix formulation of the herbicides bispyribac sodium and metamifop in Water. *Bull. Environ.Contam.Toxicol.*,**100**: 548-552

Sarkar, M.A., Biswas, P.K., Roy, S., Kole, R.K. and Chowdhury, A. 1999. Effect of pH and type of formulation on the persistence of imidacloprid in water. *Bull. Environ.Contam.Toxicol.*,**63**: 604–609

Singh, N., Singh, S.B., Mukerjee, I., Gupta, S.,Gajbhiye,V.T., Sharma, P.K., Goel, M., and Dureja, P.2010. Metabolism of ¹⁴C-azoxystrobin in water at different pH.*J. Environ. Sci. Health*, **45**: 123-127

Szpyrka, E. and Sadlo, S. 2009.Disappearance of azoxystrobin, cyprodinil, and fludioxonil residues on tomato leaves in a greenhouse. *J. Plant Prot. Res.*,**49**: 204-208

Tokousbalides, M.C. and Sisler, H.D. 1978. Effect of tricyclazole and secondary metabolism in *Pyriculariya oryzae*. *Pestic. Biochem. Phys*., **8**: 26-32

Tsochatzis, E.D., Tzimou-Tsitouridou, R., Menkissoglu-Spiroudi, U., Karpouzas, D.G. and Katsantonis, D. 2013. Laboratory and field dissipation of penoxsulam, tricyclazole and profoxydim in rice paddy systems. *Chemosphere*,**91**:1049-1057

Vogue, P. A., Kerle, E. A. and Jenkins, J. J. 1994. OSU Extension pesticide properties database, National pesticide information centre*, Oregon State University http://npic.orst.edu/ppdmove.htm accessed on 15 January 2020

Zavala, M.A.L., Funamizu, N. and Takakuwa, T. 2004. Temperature effect on aerobic biodegradation of feces using sawdust as a matrix. *Water Res.*,**38**: 2406–2416