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Field efficacy and residue dynamics of azoxystrobin 11% + tebuconazole 18.3% SC on kalmegh against leaf spot

Dinesh Rai[1], Rimlee Das[1], Sanjay Kumar Sahoo[2]*, R K Ranjan[1] and A.K. Mishra[3]

[1]*Department of Plant Pathology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, 848125, Bihar, India*
[2]*Department of Entomology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, 848125, Bihar, India*
[3]*Department of Plant Pathology, College of Agriculture, TCA, Dholi, Dr. Rajendra Prasad Central Agricultural University, Pusa*

ABSTRACT

Kalmegh is susceptible to various diseases like other crops, which reduce the yield and quality. Fungicides are applied on crops mainly for protection of plants against various diseases. Toxic pesticide residues build up in agricultural produce as a result of frequent, indiscriminate fungicide usage. This fact has casual concern in society to assess health hazard more accurately. Public health is seriously endangered when pesticide residue is consumed in food. To maintain the safety of humans and other animals, it is crucial to research pesticide persistence in crops. In the course of the study on integrated management of Corynespora leaf spot in Kalmegh under pot conditions, application of Azoxystrobin + Tebuconazole @ 0.1% at the onset of disease development was found to be most effective, with 72.03% reduction observed over control. It was found that the residues of Azoxystrobin 11% + Tebuconazole 18.3% - 29.35 SC applied @ 146.5 g ai/ha in Kalmegh leaves reached LOQ of 0.05 µg/g after 5 days. The waiting period of 9 days can be suggested to be followed for the safe consumption and processing of Kalmegh leaves following the application of an approved dose of Azoxystrobin 11% + Tebuconazole 18.3% w/w SC.

Keywords: *Efficacy, Residue Dynamics, Leaf spot, Azoxystrobin, Tebuconazole, Kalmegh, Waiting period, Medicinal plant*

Introduction

Kalmegh (*Andrographis paniculata*) is an important medicinal crop indigenous to India and Sri Lanka that has been valued in ancient oriental and ayurvedic medicine for its immunopotenting ability against many diseases. Kalmegh is ranked 17th out of 32 prioritised medicinal plant lists presented by the Indian National Medicinal Plants Board [1]. All parts of the plant, such as leaves, roots, stems, etc., have value in the national and international markets. The presence of the active compounds andrographolide, isoandrographolide, and neoandrographolide, which are diterpenoids derivatives, gives *Andrographis paniculata* Nees. Plant its therapeutic significance. It is an effective scavenger of several reactive oxygen species, such as hydrogen peroxide, nitric oxide, and superoxide anion [2]. The World Health Organisation (WHO) asserts that herbal medications are essential for achieving the objective of "Health for All." Many medicinal plants are a gift from nature to humans since they can be utilized to treat a variety of illnesses and infections [3].

Kalmegh is also susceptible to various diseases like other crops, which reduce the yield and quality. Among different diseases, leaf spot disease caused by *Corynespora cassiicola* is an important disease of Kalmegh. Several fungicides have been recommended for the controlling of major diseases (leaf spot, wilt, and stem rot) of Kalmegh.

Fungicides are applied on crops mainly for protection of plants against various diseases. Toxic pesticide residues build up in agricultural produce as a result of frequent, indiscriminate fungicide usage. This fact has casual concern in society to assess health hazard more accurately. Public health is seriously endangered when pesticide residue is consumed in food. To maintain the safety of humans and other animals, it is crucial to research pesticide persistence in crops. Waiting period for fungicides has also been reported by various workers in various crops by 10-15 days. In the present study, suitable waiting period of Azoxystrobin and Tebuconazole was evaluated for harvesting residue free Kalmegh leaves which has not been investigated to date.

Materials and Methods

Effect of combi formulation of trifloxystrobin and tebuconazole against leaf spot disease of Kalmegh caused by Corynespora cassiicola under pot conditions

The effectiveness of putative biocontrol agents, endophyte and plant extract against *Corynespora cassiicola*on the widely grown Kalmegh local varietywas assessed in pot conditions by applying the subsequent treatments as following:

Experiment Details:

- Design: RBD
- Treatments: 9
- Replication: 3
- Variety: Local
- No. of seedlings / pot: 3
- Total pots: 27

*Corresponding Author: ***Sanjay Kumar Sahoo***

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S. No.	Treatments
T1	Soil application of*Trichoderma asperellum* @ 1% at the time of planting
T2	Soil application of*Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens* @ 1%at the time of planting
T3	Soil application of*Trichoderma asperellum* @ 1% at the time of planting + Foliar spray of Endophyte "KLENB-1" @ 1% at 35 DAP
T4	Soil application of*Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens* @ 1%at the time of planting + 2 Foliar Spray of Endophyte "KLENB-1" @ 1% at 35 DAP and 50 DAP respectively
T5	Soil application of*Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens* @ 1%at the time of planting + 2 Foliar Spray of leafextract of *Artemisia annua*@ 5% at 35 DAP and 50 DAP
T6	Soil application of*Trichoderma asperellum* @ 2% + *Pseudomonas fluorescens* @ 2% at the time of planting + 2 Foliar Spray of Endophyte "KLENB-1"@ 2%at 35 DAP and 50 DAP respectively.
T7	Foliar Spray with leaf extract of *Artemisia annua* @ 10% at 35 DAP
T8	Foliar spray of Azoxystrobin + Tebuconazole @ 0.1% at 35 DAP
T9	Control

To study dissipation and persistence of Azoxystrobin 11% + Tebuconazole 18.3% to evaluate suitable waiting period.

Preparation of standard solution of fungicides

Certified reference materials of Tebuconazole (purity 98.6%) and Azoxystrobin (purity 98.9%) were utilized in the experiment. The Certified Reference Materials (CRM)s were procured from Dr. Erhenstrofer, India. For use in the field, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC was obtained. Azoxystrobin and Tebuconazole standard stock solutions of 400 ppm concentration were prepared from their CRMs with HPLC grade acetonitrile. The two stock solutions were serially diluted to obtain working standards with various concentrations. In the refrigerator, both working and stock solutions were stored for further usage.

To check the linearity of the working solutions, 1 ml of various concentrations were placed in vials and injected into the UHPLC. This was followed by calculating the calibration curve of concentration VS response of the standard. A high regression coefficient that was close to one indicated that the solutions were linear.

Application of insecticide

Kalmegh plants were sprayed with Azoxystrobin 11% + Tebuconazole 18.3%w/w SC (Custodia $^{TM)}$ at three different doses. Spraying was done manually using anHand Knapsack Sprayer with 15 L capacity. Spraying was done 60 days after planting. The amount of volume used while spraying was 500 litres ha^{-1} with following treatments:

a) T_1 :Azoxystrobin + Tebuconazole @ 146.5 g ai/ha
b) T_2 : Azoxystrobin + Tebuconazole @ 219.75 g ai/ha
c) T_3:Azoxystrobin + Tebuconazole @ 293.00 g ai/ha
d) $T_{4:}$ Control

Sampling

100 g of Kalmegh leaves were randomly selected from each plot and placed in polythene bags for sampling. These polythene bags were brought to the lab on the same day of collection and properly labeled with the plot's treatment and replication numbers, as the sample. Kalmegh leaves samples were collected at 0 (2 hrs. after spray), 1, 3, 5, 7, 10 and 15 days after the last application.

Residue analysis of Azoxystrobin 11%+ Tebuconazole 18.3%:

Following sample collection, the "Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)" technique was used to prepare the samples for analysis, with a few minor adjustments as per Fig.1[4].

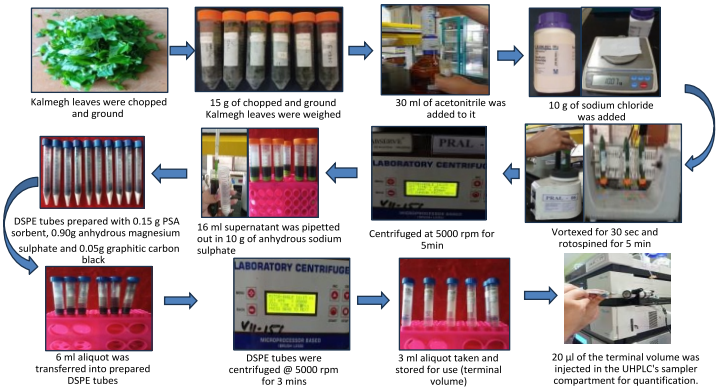


Fig. 1. Residue analysis of Azoxystrobin 11%+ Tebuconazole 18.3%

Chromatographic method for estimation of Azoxystrobin + Tebuconazole residues

Through the use of Dionex Ultimate 3000 Ultra High Performance Liquid Chromatography (UHPLC), it was possible to estimate minute amounts of Azoxystrobin + Tebuconazole residues in the sample extracts of Kalmegh leaves. A 150 mm long by 3 mm wide C18 column was employed for the stationary phase. The Photodiode Array Detector (PDA) was used to detect the fungicide with the following HPLC parameters:

Mobile phase	ACN : HPLC Water (70 : 30)
Flow rate	0.4 ml/min
UV wavelength	271 nm
Temperature	45 ºC
Run time	20 mins
Injected Volume	20µL

To determine if a residue was present, the "retention time" of working standards that had been previously or concurrently examined in the same sequence was compared to the resultant "retention time" from the analyzed leaves sample that contained residues of Azoxystrobin + Tebuconazole. Residues were quantified by comparing the "peak area" of the sample's chromatogram with the peak area of the standard, where both the samples and standards were analyzed using the identical set of instrument method parameters. Azoxystrobin and tebuconazole had a "retention time" of 10.580 + 13.350 minutes.

Results and discussions

Effect on management of Leaf Spot disease of Kalmegh caused by Corynespora cassiicola

Table 1 revealed that, all treatment combinations had

significant effect on percent disease index and percent disease reduction in management of the leaf spot of Kalmegh caused by *Corynespora cassiicola*. The minimum PDI was recorded in the treatment (T8) "Foliar spray of Azoxystrobin + Tebuconazole 0.1% which means T8 significantly caused 72.03 % disease reduction over control with PDI of 8.95%. This was followed by treatment (T5) comprising Soil application of *Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens* @ 1% at the time of planting + 2 foliar spray of plant extract of *Artemisia annua* @ 5% at 35 DAP and 50 DAP which registered PDI of 16.30% and percent disease reduction over control by 49.06%. The least effective combination was found to be the treatments (T1 and T7) comprising Soil application of *Trichoderma asperellum* @ 1% at the time of planting and foliar spray with plant extract of *Artemisia annua* @ 10% at 35 DAP respectively which recorded PDI of 24.50% and percent disease reduction over control by 23.43 %.

Table 1. Effect of different fungal, bacterial bioagents and plant extracts on growth and management of leaf spot disease of Kalmegh caused by Corynespora cassiicola under pot conditions

No.	Treatments	Plant height (cm)	Number of leaves/plant	Number of branches/ plant	Biomass weight (g)	Percent Disease Index	Per cent disease reduction over control
T1	Soil application of *Trichoderma asperellum* @ 1% at the time of planting	34.23	227.33	21.33	65.06	24.50	23.43
T2	Soil application of *Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens*@ 1% at the time of planting	38.67	237.33	23.00	64.10	23.50	26.56
T3	Soil application of *Trichoderma asperellum* @ 1% at the time of planting + Foliar spray of Endophyte "KLENB-1" @ 1% at 35 DAP	44.13	242.00	25.67	66.70	23.25	27.34
T4	Soil application of *Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens* @ 1% at the time of planting + 2 Foliar Spray of Endophyte "KLENB-1" @ 1% at 35 DAP and 50 DAP respectively.	47.66	246.00	26.33	71.00	22.50	29.68
T5	Soil application of *Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens*@ 1% at the time of planting + 2 Foliar Spray of Plant Extract of *Artemisia annua* @ 5% at 35 DAP and 50 DAP	38.96	240.66	25.66	69.46	16.30	49.06
T6	Soil application of *Trichoderma asperellum* @ 2% + *Pseudomonas fluorescens* @ 2% at the time of planting + 2 Foliar Spray of Endophyte "KLENB-1" @ 2% at 35 DAP and 50 DAP respectively.	52.63	258.00	30.66	75.83	20.50	35.93
T7	Foliar Spray with Plant extract of *Artemisia annua* @ 10% at 35DAP	29.26	219.67	15.00	59.16	24.50	23.43
T8	Foliar spray of Azoxystrobin + Tebuconazole @ 0.1%	47.30	224.67	20.66	62.26	8.95	72.03
T9	Control	27.50	215.00	14.33	55.33	32.00	0
	C.D. at 5%	3.09	6.74	4.44	3.31	1.79	
	SE(±m)	1.21	2.25	1.48	1.11	0.59	
	C.V. (%)	4.49	2.66	11.40	2.95	4.73	

The present finding are supported by AICRP (2019) where combined application of Tebuconazole and Trifloxystrobin @0.1% was found effective in management of leaf spots of *Andrographis paniculata* followed by treatment with *Pseudomonas fluorescens* @5 kg/ha + *Trichoderma viride* @5kg/ha with FYM @ 10 t/ha + Neem cake @ 1 t/ha [5]. Bairwa *et al*.(2022) reported that Azoxystrobin 23 SC @ 0.5 ml/lit was most efficient in lowering the disease severity of Corynespora leaf spot on mungbean by 57.7% and 67.7% [6]. As other researchers' studies focused on different crops and the use of different fungicides and bioagents, the results of the current experiment were only partially consistent with those of the cited researchers.

Estimation of Azoxystrobin residues in Kalmegh leaves

The results of quantitative estimation of Azoxystrobin residues after application of combi formulation of Azoxystrobin 11% + Tebuconazole 18.3%-29.3% SC at single dose, T1 (146.5 g a.i. /ha), 1.5 times of single dose, T2 (219.75 g ai/ha) and double dose, T3 (293 g a.i./ha) at different time intervals in Kalmegh leavesafter first application are expressed in table 2.

After the first application of Azoxystrobin11% +Tebuconazole18.3% - 29.3% SC @146.5 which correspondence to azoxystrobin @55 g ai/ha, the mean initial residuesof Azoxystrobin in Kalmegh leaves was found to be 5.20 mg/kg.The residues dissipated to a mean value of 2.17 mg/kg after one day of application, hence causing percent dissipation of58.26. After 3 and 5 days, the residue level reduced to 1.23 mg/kg with 76.34% dissipation and 0.29 mg/kg with 94.42% dissipation respectively. The residues reached below LOQ of 0.05 mg/kgafter 7 daysofapplication.

SprayingofKalmegh leaves with Azoxystrobin 11% +Tebuconazole18.3% - 29.3% SC @ 219.75 which correspondence to azoxystrobin @ 82.5 g ai/ha,assessed the mean initial residues of Azoxystrobin by 7.06 mg/ kg which dissipated to 3.07 mg/kg after 1 day with 56.51% dissipation. The average residue levels for Azoxystrobin at 3 and 5day were foundto be 1.83 and 0.57 mg/kg, therefore expressing 74.08 and 91.92% dissipationrespectively. The residues reached below LOQ of 0.05 mg kg^{-1} following 7 daysafter application.

Spraying of Kalmegh leaves with Azoxystrobin 11% +Tebuconazole18.3% - 29.3% SC @ 293 which correspondence to azoxystrobin @ 110 g ai/ha recorded the initial mean residue amount of Azoxystrobin to be 9.87 mg/kg. This amount dissipated to 4.21, 2.90, 1.08 and 0.16 mg/kg after 1, 3, 5 and 7 days with corresponding dissipation percentage of 57.34, 70.61, 89.05 and 98.37 %. Residue level reached below the level of quantification after 10 days.

Table 2. Residue of Azoxystrobin in Kalmegh leaves when applied with combination of Azoxystrobin 11 + Tebuconazole 18.3 - 29.3% SC

Days after treatment	@ 146.5 g ai/ha			@ 219.75 g ai/ha			@ 293 g ai/ha		
	Residue (mg/ kg)		Per cent dissipation	Residue (mg/ kg)		Per cent dissipation	Residue (mg/ kg)		Per cent dissipation
	Replicates	Mean ± SD		Replicates	Mean ± SD		Replicates	Mean ± SD	
Before spray	<LOQ	<LOQ	-	<LOQ	<LOQ	-	<LOQ	<LOQ	-
	<LOQ			<LOQ			<LOQ		
	<LOQ			<LOQ			<LOQ		
0 (2hrs after spray)	5.75	5.20 ± 0.73	-	8.23	7.06 ± 0.84	-	8.92	9.87 ± 0.74	-
	4.17			6.27			9.97		
	5.69			6.69			10.73		
1	1.92	2.17 ± 0.19	58.26	3.34	3.07 ± 0.49	56.51	3.45	4.21 ± 0.54	57.34
	2.38			3.49			4.69		
	2.23			2.37			4.48		
3	1.10	1.23 ± 0.26	76.34	1.84	1.83 ± 0.06	74.08	2.95	2.90 ± 0.19	70.61
	1.14			1.90			3.10		
	1.46			1.76			2.65		
5	0.44	0.29 ± 0.14	94.42	0.66	0.57 ± 0.07	91.92	1.45	1.08 ± 0.26	89.05
	0.11			0.56			0.84		
	0.32			0.49			0.96		
7	<LOQ	<LOQ	-	<LOQ	<LOQ	-	0.11	0.16 ± 0.03	98.37
	<LOQ			<LOQ			0.17		
	<LOQ			<LOQ			0.19		
10	<LOQ	<LOQ	-	<LOQ	<LOQ	-	<LOQ	<LOQ	-
	<LOQ			<LOQ			<LOQ		
	<LOQ			<LOQ			<LOQ		

LOQ = Limit of Quantification (0.05 mg/ kg)

The findings obtained from the present experiment were partially in agreement with reports of other researchers as they had their work done on other crops and with different doses of Azoxystrobin. Garau *et al.* (2002) analysed tomato samples and reported a mean Azoxystrobin residue of 0.15 mg/kg after seven days of application @ 15 g a.i./ha [7]. Bursic and Lazic (2012) reported that the residue concentration of Azoxystrobin in cucumber ranged from 3 mg/kg two hours after the deposit was dried to 0.1 mg/kg fourteen days after the treatment [8]. Ali *et al.* (2015)analysed tomato leaf samples and reported a mean Azoxystrobin residue of 8.417 mg/kg after seven days of application at the rate of 50ml/100 lit water [9].

Estimation of Tebuconazole residues in Kalmegh leaves

The results of quantitative estimation of Tebuconazole residues after application of combi formulation of Azoxystrobin 11% + Tebuconazole 18.3%- 29.3% SC at single dose, T1 (146.5 g a.i. /ha), 1.5 times of single dose, T2 (219.75 g ai/ha) and double dose, T3 (293 g a.i./ha) at different time intervals in Kalmegh leaves after first application are expressed in table 3. Representative chromatograms of T1, T2, and T3 at 0 day (2 hrs after spray) are presented in figure 2.

After the first application of Azoxystrobin 11% +Tebuconazole18.3% - 29.3% SC @ 146.5 which correspondence to Tebuconazole @ 91.5 g ai/ha, the mean initial residuesof Tebuconazole in Kalmegh leaves was found to be 2.47 mg/kg.The residues dissipated to a mean value of 0.95 mg/kg after one day of application, hence causing percent dissipation of61.53. After 3 and 5 days, the residue level reduced to 0.65 mg/kg with 73.68% dissipation and 0.16 mg/kg with 93.52% dissipation respectively. The residues reached below LOQ of 0.05 mg/kg after 7 days of application.

Spraying of Kalmegh leaves with Azoxystrobin 11% +Tebuconazole18.3% - 29.3% SC @ 219.75 which correspondence to Tebuconazole @ 137.25 g ai/haassessed the mean initial residues of Tebuconazole by 3.49 mg/ kg which dissipated to 1.79 mg/kg after 1 day with 48.71% dissipation. The average residue levels for Tebuconazoleat 3 and 5day were found to be 0.97 and 0.37 mg/kg, therefore expressing 72.20 and 89.39% dissipation respectively. The residues reached below LOQ of 0.05 mg/kgfollowing 7 daysafter application.

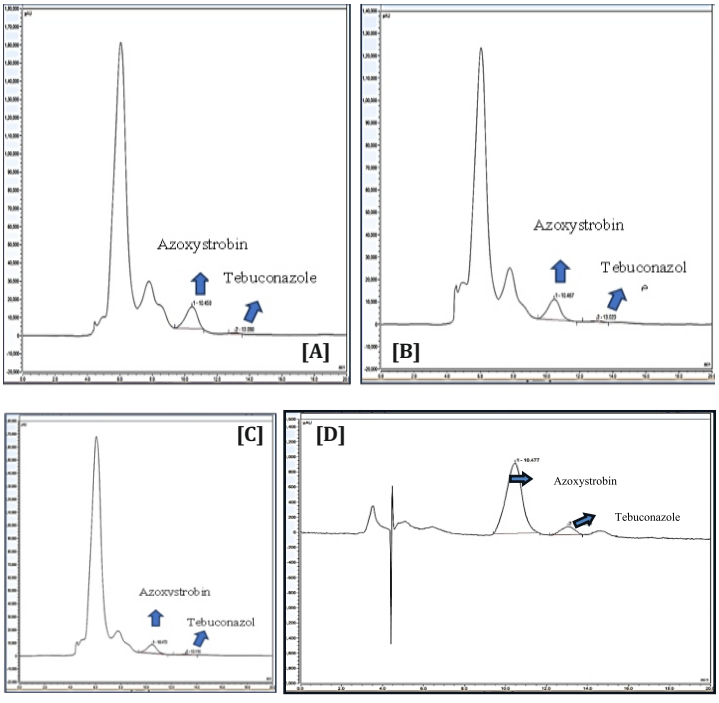
Spraying of Kalmegh leaves with Azoxystrobin 11% +Tebuconazole18.3% - 29.3% SC @ 293 which correspondence to Tebuconazole @ 183 g ai/harecorded the initial mean residue amount of Tebuconazole to be 5.35 mg/kg. This amount dissipated to 2.13, 1.35, and 0.49 mg/kg after 1, 3and 5 days with corresponding dissipation percentages of 60.18, 74.76 and 90.84 %. Residue level reached below the level of quantification after 7 days.

Table 3. Residue of Tebuconazole in Kalmegh leaves when applied with combination of Azoxystrobin 11 + Tebuconazole 18.3 - 29.3% SC

Days after treatment	@ 146.5 g ai/ha			@ 219.75 g ai/ha			@ 293 g ai/ha		
	Residue (mg/ kg)		Per cent dissipation	Residue (mg/ kg)		Per cent dissipation	Residue (mg/ kg)		Per cent dissipation
	Replicates	Mean±SD		Replicates	Mean±SD		Replicates	Mean±SD	
Before spray	<LOQ	<LOQ	-	<LOQ	<LOQ	-	<LOQ	<LOQ	-
	<LOQ			<LOQ			<LOQ		
	<LOQ			<LOQ			<LOQ		
0 (2hrs after spray)	2.41	2.47 ± 0.25	-	3.47	3.49 ± 0.36	-	5.23	5.35 ± 0.30	-
	2.79			3.94			5.77		
	2.20			3.05			5.04		
1	0.98	0.95 ± 0.07	61.53	1.77	1.79 ± 0.087	48.71	2.05	2.13 ± 0.06	60.18
	1.01			1.90			2.15		
	0.85			1.69			2.20		
3	0.54	0.65 ± 0.09	73.68	0.89	0.97 ± 0.09	72.20	1.23	1.35 ± 0.09	74.76
	0.62			1.10			1.47		
	0.78			0.93			1.36		
5	0.15	0.16 ± 0.04	93.52	0.43	0.37 ± 0.04	89.39	0.48	0.49 ± 0.03	90.84
	0.21			0.37			0.54		
	0.11			0.32			0.47		
7	<LOQ	<LOQ	-	<LOQ	<LOQ	-	<LOQ	<LOQ	-
	<LOQ			<LOQ			<LOQ		
	<LOQ			<LOQ			<LOQ		
10	<LOQ	<LOQ	-	<LOQ	<LOQ	-	<LOQ	<LOQ	-
	<LOQ			<LOQ			<LOQ		
	<LOQ			<LOQ			<LOQ		

LOQ = Limit of Quantification (0.05 mg/ kg)

The findings obtained from the present experiment were partially in agreement with reports of other researchers as they had their work done on other crops and with different doses of Tebuconazole. Mohapatra *et al.* (2011) did the residual analysis of Tebuconazole and Quinalphos on immature onion bulb with leaves at doses of tebuconazole @ 187.5 and 375 g a.i. ha−1; quinalphos @ 300 and 600 g a.i. ha^{-1} [10]. and showed that the mean initial residue of Tebuconazole at both doses are 0.628 and 1.228 mg kg−1. Jyot *et al.* (2010) reported that the mean initial residues of tebuconazole were 13.84 and 26.55 mg/kg at a single and double dose which dissipated to >90% after 10 days of application at both the dosages respectively, when foliar applications of Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) was applied on grapes @ 175 and 350 g/ha, resulting in active applications of tebuconazole @ 87.5 and 175 g a.i./ha [11]. Sahoo *et al.*(2012) studied the dissipation of trifloxystrobin and tebuconazole after two applications of Nativo 75 WG (trifloxystrobin25% + tebuconazole 50%) @ 250 and 500 g ha^{-1} at 10 days interval and found that Tebuconazole residues dissipated below its limit of quantification (LOQ) of 0.01 mg kg^{-1} after 7 and 10 days, respectively, at single and double dose [12].



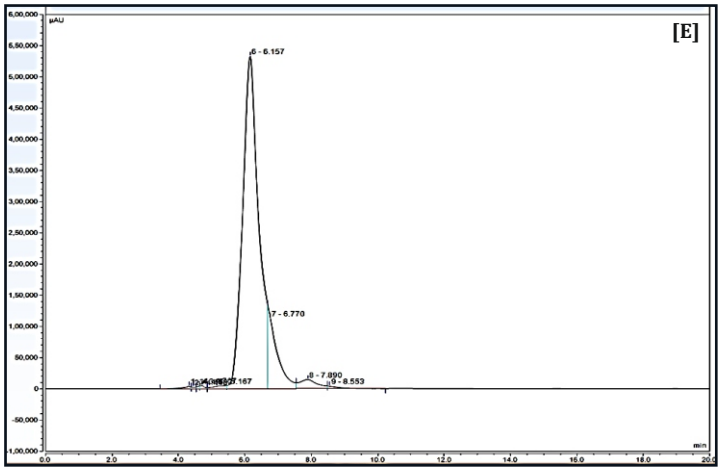


Fig. 2. HPLC chromatograms of Azoxystrobin 11% + Tebuconazole 18.3% forKalmegh leaves samples after 0 days (A) @146.5 g ai/ha; (B) @219.75 g ai/ha; (C) @293.00 g ai/ha (D) Azoxystrobin + Tebuconazolestandard @ 0.1µg/ml; (E) Control

Waiting period for Azoxystrobin and Tebuconazole in Kalmegh leaves:

Figure 3 and 4. illustrates semi-logarithmic graphs that represent the dissipation kinetics for azoxystrobin and tebuconazole residues in Kalmegh leaves. The semi-logarithmic graph led to a linear relationship for azoxystrobin by plotting the log of mean residue multiplied by 100 against days after treatment. It demonstrates that declination in azoxystrobin residues exhibits a first order kinetic process.

Azoxystrobin in Kalmegh leaves was shown to have half-lives of 1.29, 1.48, and 1.29 at doses of 146.5, 219.5, and 293.00 g a.i./ha, respectively. When applied at recommended dose of 146.5 g ai/ha, the initial mean residue deposits of azoxystrobin in Kalmegh leaves were recorded to reach below 0.05mg/kg after 8.46 days whereas, following application at 219.5 and 293.00 g a.i./ ha, residues found to be dissipated after 10.33 and 9.93 days (Table 4).

Tebuconazole in Kalmegh leaves was shown to have half-lives of 1.39, 1.61 and 1.60 days at doses of 146.5, 219.5, and 293.00 ga.i./ha, respectively. When applied at recommended dose of 146.5 g ai/ha, the initial mean residue deposits of Tebuconazole in Kalmegh leaves were recorded to reach below 0.05after 7.55 days whereas, following application at 219.5 and 293.00 g a.i./ ha, residues found to be dissipated after 9.66 and 10.31 days (Table 4.).

The current research therefore recommended a waiting period of 9 days when Kalmegh leaves were applied with the combi formulation of azoxystrobin 11% + tebuconazole 18.3% - 29.3 % SC @ 146.5 g ai/ha following GAP.

The results of the current experiment are partially in agreement with the findings of other researchers due to difference in target crop, formulation , dose, location and other factors. Half-life of Azoxystrobin was obtained at 5.90 days in tomato leaves and 4.07 days in tomato fruits as per the findings of Ali *et al.* (2015), hence a protection period of 12.94 days for leaves and 19.87 days for fruits was suggested [9]. Szpyrka *et al.* (2009) reported that the half-life of Azoxystrobin in tomato leaves was found to be 13 days on tomato plants [13] , Jyot *et al.* (2010) showed half-life of Tebuconazole residue in grape leaves for single and double dose to be 2.68 and 3.96 days, respectively when foliar applications of Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) was applied on grapes @ 175 and 350 g/ha, resulting in active applications of tebuconazole @ 87.5 and 175 g a.i./haand that of trifloxystrobin @ 43.75 and 87.5 g a.i./ha [11].

Table 4. Dissipation parameters of Azoxystrobin and Tebuconazole residue in Kalmegh leaves

Dissipation parameters	Azoxystrobin			Tebuconazole		
	Rate of application (g a.i. /ha) After 1st spray			Rate of application (g a.i. /ha) After 1st spray		
	@146.5	@219.75	@293.00	@146.5	@219.75	@293.00
K_1 (b)	-0.2329	- 0.2022	-0.2320	-0.2158	-0.1866	-0.1881
K_2 (a)	2.6690	2.7875	3.0024	2.328	2.5024	2.6383
$T_{1/2}$	1.29	1.48	1.29	1.39	1.61	1.60
T_{Tol}	8.46	10.33	9.93	7.55	9.66	10.31
R^2	0.9674	0.9667	0.9397	0.9408	0.9861	0.9511
Y	y = -0.2329x + 2.669	y = -0.2022x + 2.7875	y = -0.232x + 3.0024	y = -0.2158x + 2.328	y = -0.1866x + 2.5024	y = -0.1881x + 2.6383

K_1= "Slope of the regression line"
K_2= "Initial deposit obtained as in the regression equation"
$T_{1/2}$= "Residual half life (in days)"
T_{Tol}= "Time (in days) required for the pesticide residue to reach below the maximum residue limit of 0.05 mg/kg"
R^2= "Coefficient of determination"

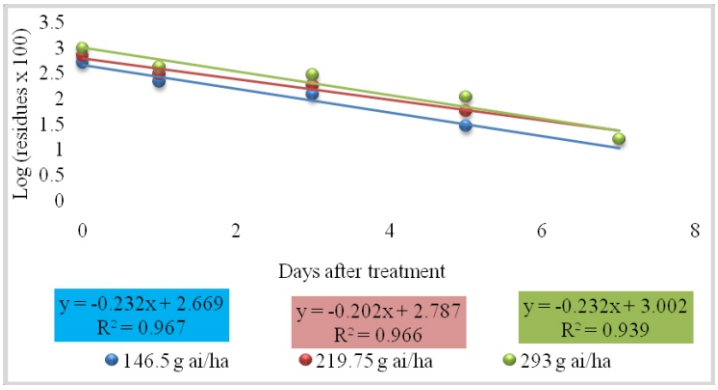


Fig. 3. Semi logarithmic graph showing dissipation kinetics of Azoxystrobin in Kalmegh leaves after 1st spray

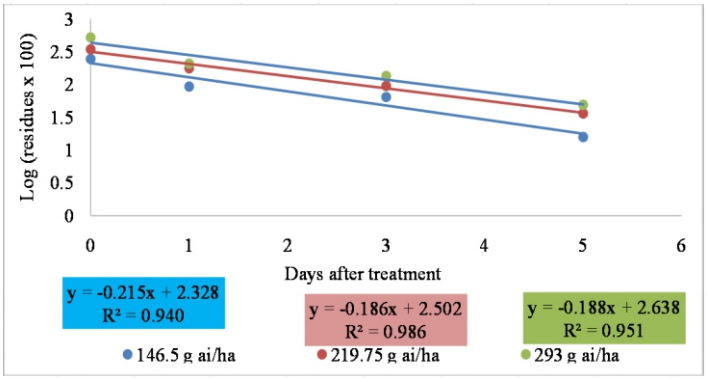


Fig. 4. Semi logarithmic graph showing dissipation kinetics of Tebuconazole in Kalmegh leaves after 1st spray

Conclusion

In course of study on integrated management of *Corynespora* leaf spot disease in Kalmegh under pot conditions, application of Azoxystrobin 11% + Tebuconazole 18.3% @ 0.1%was found to be significantly effectiveand PDI recorded was 72.03% However, among eco-friendly treatments, Soil application of *Trichoderma asperellum* @ 1% + *Pseudomonas fluorescens* @ 1% at the time of planting + 2 foliar spray of plant extract of *Artemisia annua* @ 5% at 35 DAP and 50 DAP was observed to be effective whichreduced 49.06 % of disease. The residues of Azoxystrobin and Tebuconazolein Kalmegh leaveswhen applied in combi formulation of Azoxystrobin 11% + Tebuconazole 18.3%- 29.3% SC at recommended dose of 146.5 g a.i. /ha reached LOQ of 0.05 µg/g after 5 days. The residual half life of Azoxystrobin and Tebuconazole in Kalmegh leaves when applied in combi formulation of Azoxystrobin 11% + Tebuconazole 18.3%- 29.3% SC at recommended dose of 146.5 g a.i. /ha ranged from 1.29 to 1.61 days. The waiting period of 9 days should be followed for safe consumption and processing of Kalmegh leaves following the application of approved dose (@146.5 g ai/ha) of Azoxystrobin 11% + Tebuconazole 18.3% w/w SC.

Future Scope of Study

The metabolism of fungicides applied may be studied in plants. The complete profiling of causal organism and its management at molecular level may be studied for its comprehensive management.

Conflict of Interest

Authors declare no conflict of interest

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