Research Article

Assessment of Trifloxystrobin 25% + Tebuconazole 50%-75 WG Bioefficacy, Safety and Residue Dynamics against Leaf Spot of Cabbage

Sujoy Saha*[1], Sandip Hingmire[1], T.P. Ahammed Shabeer[1], Kaushik Banerjee[1], Nikhil Ashtekar[1], Ashlesha Patil[1] and A. B. Rai[2]

[1]ICAR-National Research Centre for Grapes, Pune, India
[2]ICAR-Indian Institute of Vegetable Research, Varanasi, India

Abstract

This paper reports the *in vitro* and *in vivo* bioefficacy of combination fungicide trifloxystrobin (25%) + tebuconazole (50%) against leaf spot disease of cabbage (*Brassica oleracea* var. *capitata*) caused by *Alternaria brassicae* and their corresponding pre-harvest intervals (PHI) with reference to the maximum residue limits (European Union). Under *In vitro* conditions, the test fungicide manifested the best control (80.83%) at 300 mg Kg^{-1} against 69.01% untreated control under field conditions. A sample preparation method based on ethyl acetate extraction and estimation by LC-MS multiple reaction monitoring (MRM) was validated in cabbage heads with Limit of Quantification (LOQ) at 0.005 and 0.01mg $Kg^{-1}$for trifloxystrobin and tebuconazole, respectively and dissipation studies were conducted in field at single and double doses. The residues of both the compounds on all the sampling days were below the European Union maximum residue limits (EU-MRLs) and the maximum permissible intakes (MPIs) were calculated on the basis of prescribed acceptable daily intake (ADI). The combined bioefficacy and residue dynamics information will support label-claim of this fungicide combination for the management of leaf spot of cabbage.

Keywords: Dissipation, early blight, bioefficacy, PHI, combination fungicide

***Correspondence**
Author: Sujoy Saha
Email: sujoyta@gmail.com

Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is a leafy vegetable grown as an annual crop in India. It comprised about 5.5% of the total vegetable production in the country with production of 9.04 MT from an approximate cultivated area of 0.04 million hectare [1]. The vegetable is consumed either as raw salad or by steaming or cooking it. It is an excellent source of vitamin B_6, C and K and play an important role in lowering the blood cholesterol levels [2]. Export of cabbage from India has been showing an increasing trend since last few years, with major export destination being Pakistan, United Arab Emirates, Sri Lanka, Maldives and Nepal [1].

The sustainable production of cabbage is severely affected by leaf spot disease caused by *Alternaria brassicae* (Berk.) Sacc., both in India as well as in the global paradigm and the extent of damage is assessed to be around 3.1 to 70.0% [3, 4]. Lack of effective resistance genes in cabbage has limited the efficacy of resistance breeding in management of leaf spot disease [5] and hence chemical interventions via fungicides became the major arsenal to combat the disease. Several conventional fungicides have been recommended for the purpose [6, 7] but, limited control of the disease, resistance and environmental issues have brought to the fore the mandate to search for new and safer fungicides.

This study was envisaged for evaluating the bioefficacy, understanding the residue dynamics, and assessing the safety of a combination fungicide involving methoxyimino acetate strobilurin compound *viz.* trifloxystrobin (methyl (*E*)-methoxyimino-{(*E*)-*α*-[1-(*α*,*α*,*α*-trifluoro-*m*tolyl) ethylideneaminooxy]-*o*-tolyl}acetate) and a triazole compound *viz.* tebuconazole (1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol) for two consecutive seasons against the leaf spot disease of cabbage.

The residue dissipation behavior of these chemicals in the field conditions was also investigated to evaluate consumer safety. These chemicals do not have recommended pre-harvest intervals (PHIs) for cabbage due to lack of information regarding their residue dissipation kinetics under field conditions. This might result in apprehension of food safety issues associated with their usage for domestic consumption as well as export. To ensure food safety to the consumers, the residues of these chemicals in the European Union (EU) are regulated at the maximum residue limit (MRL) of 1.0 mg $Kg^{-1}$for tebuconazole and 0.5 mg $Kg^{-1}$for trifloxystrobin [8] and hence it is of profound importance to establish their individual PHI so as to minimize accumulation of their residues below the respective MRLs at the stage of harvest there by to ensure smooth trade and consumer safety.

Experimental Methods

In vitro evaluation of the test fungicide against Alternaria brassicae (Berk.) Sacc

An *in vitro* experiment, with five doses of the combination fungicide and one dose of each of the triazole component and the strobilurin component had been evaluated against *A. brassicae*. Five different concentrations (100, 150, 200, 250 and 300 mg Kg^{-1}) of the test fungicide mixture were prepared along with 600mg $Kg^{-1}$of tebuconazole 250 EC and 150mg Kg^{-1} of trifloxystrobin 50 WG. Hexaconazole 5EC was used as a check fungicide at 500mg Kg^{-1} and the fungicides were evaluated by poisoned food technique [9]. Aforementioned concentrations of the required fungicides were added separately into molten and cooled Potato Dextrose Agar (PDA) medium and 20mL of the poisoned medium was poured into sterile petri plates. Mycelial discs of 5 mm from actively growing culture of the fungus were cut out by the sterile cork borer and one such disc was placed at the centre of each agar plate. Similarly, untreated control was maintained without adding any fungicide to the medium. Each treatment was replicated five times. The plates were incubated at 25°C for 12 days and radial colony growth was measured. The efficacy of the fungicide was expressed as per cent inhibition of the mycelia growth over control, which was calculated by using the formula;

$$I = C - T/C \times 100 \text{ [10]}$$

I = percent inhibition; *C*= radial growth in control;
T = radial growth in treatment

The percent values were converted to arc sine values before statistical analysis.

On field efficacy of the test fungicide against leaf spot of Cabbage

Field experiments were conducted at the research farm of the ICAR-Indian Institute of Vegetable Research located at Varanasi (latitude: 25.28°N, longitude: 82.99°E, elevation: 82 m MSL), India during two seasons (2011–12 and 2012–13). Twenty eight days old seedlings of cabbage (variety Golden Acre) were transplanted in plots of 20 square meters (5 meters X 4 meters) with a plant to plant and row to row spacing of 45 cm each and standard package of practices were followed to raise the crop. Seven treatments comprising of trifloxystrobin 25% + tebuconazole 50% (Nativo 75WG, a product of Bayer Crop Science (Monnheim, Germany) at 200, 250 and 300 and 350g ha^{-1}, tebuconazole 250 EC at 600mLha^{-1} trifloxystrobin 50WG at 150g ha^{-1}, hexaconazole 5EC at 500mL $ha^{-1}$and untreated control were laid down in the randomized block design (RBD) with three replications. Fungicide application was commenced with the visibility of initial symptoms (30 days after transplanting) and repeated after 20 days using knapsack sprayer. Ten plants from each replication except the border rows were taken at the beginning of each spray and 15 days after the second spray and scored for disease incidence using 0–5 scale of [11], (0 = no infection; 1 = 1–20% infection; 2 = 21–40% infection; 3 = 41–60% infection; 4 =61–80% infection; 5=>80% infection). The percent disease index (PDI) was calculated based on the last observation using the formula [12] where,

$$PDI = (\text{The sum of numerical values})/(\text{Number of plants observed} \times \text{maximum disease rating}) \times 100$$

After maturity, the heads were harvested and fruit yield was calculated in quintals per hectare. All data obtained were subsequently analyzed statistically.

Field study for persistence and residue dissipation

The field applications of the combination fungicide (trifloxystrobin 25% + tebuconazole 50%) for persistence and residue dissipation study were carried out at the location described above as per the EU guidelines for crop Field trials (http://ec.europa.eu/food/plant/protection/resources/app-b.pdf). The dose rates were, T_1: 250 gha^{-1} (standard dose), T_2: 500 g ha^{-1}(double dose), and T_3: control. The cabbage samples (2 kg) were collected at random from each replicate of the treated and control plots separately at regular time interval on 0 (2 h after spraying), 1, 3, 5, 7 and 10 days after the application. Heads showing signs of infestation of insect pests, diseases or any physiological disorders were not included in sampling. The cabbage heads were directly used for residue analysis without any washing or any kind of pre-treatments.

Residue analysis and dissipation study

Sample preparation

The entire laboratory sample was crushed thoroughly in a blender (GX7, Bajaj India Ltd. Mumbai, India) after adding

deionised water (1:1, sample:water). Approximately 200 g of the crushed sample was further homogenized (Homogenizer, Diax 900, Heidolph, Germany). From this, 10 g of subsample was drawn in a 50 mL of PTFE centrifuge tube. Then, 10 mL ethyl acetate and 10 g anhydrous sodium sulfate were added and the mixture was homogenized for 2 min followed by centrifugation (Centrifuge, Remi, Mumbai, India) at 5000 rpm for 5 min. Dispersive clean-up of 5 mL of the extract was performed using 25 mg PSA + 150 mg Na_2SO_4. 4 mL of the cleaned supernatant was evaporated to near dryness under N_2 gas in a low volume concentrator (TurboVap LV, Caliper Life Sciences, USA) at 35^{o}C in the presence of 200 µL of 10% diethylene glycol. The residue was re dissolved in 1 mL methanol + 1 mL 0.1% acetic acid, filtered through 0.2 µm nylon membrane filter and analyzed by LC-MS/MS.

LC-MS/MS analysis

An LC-MS/MS system comprising Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, USA) hyphenated to API 4000 hybrid triple quadrupole/linear ion trap (QqQLIT) mass spectrometer (Sciex, Foster City, CA, USA) was used for determination of the residues. The data acquisition and processing were carried out using Analyst® (Version 1.5) software. The chromatographic separation was carried out using a Purospher® STAR C18 Column (5 µm, 150 mm length, 4.6 mm i.d. Merck, Darmstadt, Germany). The mobile phase composition was (A) water and (B) methanol with 10 mM ammonium formate, and 0.1% formic acid each. The mobile phase flow rate and column temperature were maintained at 0.75 mL per min and 35°C, respectively. The mobile phase gradient program was 0-0.5 min 90% A, 0.5-2 min 90-40% A, 2-10 min 40-5% A,10-16 min 5% 16-17 min 5-90% A and 17-22 min 90% A. Trifloxystrobin and tebuconazole were eluted at the retention time of 12.49 and 11.75 min., respectively. The analysis was performed in positive polarity mode by multiple reaction monitoring (MRM) with mass transition $[M+H]^+$ 308.1/70.1 [Declustering potential (DP) = 30 V, Collision energy (CE) = 39 V, Collision cell exit potential (CXP) = 4 V] and 308.0/ 125.0 (DP = 30 V, CE = 38V, CXP = 4 V) for tebuconazole; whereas, for trifloxystrobin, the MS parameters included $[M+H]^+$ 409.0/ 186.0 (DP = 14.3 V, CE = 23 V, CXP = 6.5 V) and 409.0/ 206.0 (DP = 14.3 V, CE = 23 V, CXP = 7 V) with dwell time of 70 ms.

Method Validation

The analytical methodology was validated as per the single laboratory validation approach [13]. Certified reference standards of trifloxystrobin (99.7% purity) and tebuconazole (98.8% purity) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Six point calibration standards (0.001 to 0.05 µg ml^{-1}) were prepared in methanol:water (50:50) and the calibration curves were obtained by plotting the peak area against the concentration. The limit of detection (LOD) and limits of quantification (LOQ) were determined by considering a signal-to-noise ratio of 3 and 10 in the matrix standard, respectively.The matrix effect (ME) was estimated by comparing the detector response of calibration standards at different levels in solvent versus matrix. Recovery experiments were carried out at three concentration levels viz. 0.01, 0.025 and 0.05 mg Kg^{-1} by fortifying the untreated cabbage samples in six replicates and the precision in terms of repeatability was determined by calculating the associated RSD.

Analysis of field samples and dissipation study

The samples collected during the residue trial were extracted and analyzed as described above.

The day wise residue dissipation data of the pesticides was analyzed using the curve fitting software Table curve 2D v5.01. The equation parameters, regression equation, half-lives and pre-harvest intervals (PHI) were calculated by the software by different models as listed below;

$$\text{First-order model: } [A]_t = [A]_1 \exp(-k_1 t)$$
$$\text{First + first-order model: } [A]_t = [A]_1 \exp^{(-k1t)} + [A]_2 \exp^{(-k2t)}$$

where $[A]_t$ is the concentration (mg Kg^{-1} of sample) of A at time t (days), $[A]_1$ and $[A]_2$ are the initial concentrations of A at time 0 degraded through first order processes 1 and 2 and k_1 and k_2 are the degradation rate constants for 1 and 2. The units of k depend on the model used. The half-life (DT_{50}), which is the time at which the concentration of initial deposits reaches the 50% level, was determined by the following equation:

$$\text{First-order model: } DT_{50} = \ln(2) \times k_1^{-1}$$

The PHI, i.e. the time period (in days) required for dissipation of the initial residue deposits to below the maximum residue limit (MRL) for first-order kinetics, was determined by the equation;

PHI = [log (intercept) – log (MRL)] / slope of first-order equation

Since the first + first order model cannot be described in a differential form, DT_{50} and PHI were calculated by an iterative procedure.

Results and discussion

In vitro evaluation of the test fungicide against Alternaria brassicae (Berk.) Sacc

All the treatments significantly reduced the radial growth of the pathogen, *Alternaria brassicae* (Berk.) Sacc, as compared to the untreated control (**Table 1**). The best inhibition of pathogen (80.83%) was exhibited by the combination fungicide trifloxystrobin 25% +tebuconazole 50%75WP at 300 mg/Kg, followed by its lower dose @250 mg Kg^{-1}. These two doses gave a significantly higher efficacy in controlling the pathogen over the respective solo doses of trifloxystrobin 50WG (40.65%) and tebuconazole 250 EC (40.08%), which gave an inhibition of 79.27%.The present study confirms to the reports where the test fungicide can cause a growth rate inhibition at different concentrations and even lead to the total cessation of the growth of *A. solani* [14]. The *in vitro* efficacy of strobilurins was also reported in *A. alternate* [15]. Recently, trifloxystrobin 25% + tebuconazole 50% 75WP were reported to inhibit the mycelial growth of *Alternaria brassicicola* to an extent of 83.55% at 500ppm [16].

Table 1 Effect of different doses of trifloxystrobin 25% + tebuconazole 50% on radial growth of *Alternaria brassicae* (Berk.) Sacc

S. No	Treatment	Dose (ppm)	Average radial growth (mm)	Inhibition* (%)
1	Trifloxystrobin 25%+Tebuconazole 50%	100	24.97	43.83 (41.45)
2	Trifloxystrobin 25% +Tebuconazole 50%	150	17.96	62.15 (52.04)
3	Trifloxystrobin 25% +Tebuconazole 50%	200	15.22	76.09 (60.78)
4	Trifloxystrobin 25% +Tebuconazole 50%	250	15.20	79.27 (62.92)
5	Trifloxystrobin 25% +Tebuconazole 50%	300	12.53	80.83 (64.04)
6	Trifloxystrobin 50WG	150	29.51	40.65 (39.61)
7	Tebuconazole 250 EC	600	25.15	40.08 (39.26)
8	Hexaconazole 5EC	500	22.70	41.85 (40.31)
9	Untreated control	-	54.3	0 (0)
	CD (0.05%)	-	4.41	2.79
	SEM±	-	1.47	0.93

* Data in the parenthesis are arcsine transformed value

On field efficacy of the test fungicide against leaf spot of cabbage

During both the seasons, the test fungicide at 250 g ha^{-1} and 300 g ha^{-1} gave the best control of the disease as compared to the untreated check. In this period, the individual components, that is trifloxystrobin 50 WG and tebuconazole 250 EC were able to control the disease, but the efficacy was significantly higher in their combination. The test fungicide trifloxystrobin 25% + tebuconazole 50% at 300 g ha^{-1} manifested 68.96% and 69.05% control with the corresponding PDI of 12.92 and 12.55, respectively in the two seasons (**Table 2**). It was also noted that all the three doses of trifloxystrobin 25% + tebuconazole 50% gave a better control of the disease as compared to standard check fungicide hexaconazole 5EC in both the seasons. The yield of head was also highest in the treatment of the test fungicide at 300 g ha^{-1}. The untreated control had PDI values of 41.62 and 40.55 in 2011–12 and 2012–13, respectively. On the other hand, the standard fungicide, hexaconazole 5EC at 500 g ha^{-1} manifested a control of 42.24% and 43.83% with a corresponding PDI of 23.88 and 22.77 in both the seasons. The yield of head of the test fungicide at 300 g ha^{-1} was 17.4% and 15.85% more as compared to the untreated control in two seasons, respectively.

The combination of trifloxystrobin and tebuconazole was effective against *Alternaria brassicae* [16] as well as *Alternaria* in tomato [17]. Moreover, among the strobilurins, trifloxystrobin is reported to be more effective against *A. solani* isolates which were resistant to azoxystrobin and pyraclostrobin [18]. The combination of trifloxystrobin and tebuconazole proved to be in synergy as the individual components have different modes of action. Tebuconazole, a triazole acts as demethylation inhibitor (DMI) in fungal sterol biosynthesis and is highly prone to cross resistance but it has an excellent broad spectrum activity to control the disease. The risk of cross resistance is regulated by using a component having a different mode of action. Trifloxystrobin, a Quinone outside Inhibitor (QoI) affects the mitochondrial respiration of the fungi [19]. As trifloxystrobin decreased the transpiration rate, it was endowed with

stress management properties as well [20]. Thus, trifloxystrobin 25% + tebuconazole 50% 75WG may be recommended against black spot of cabbage.

Table 2 Effect of different treatments of trifloxystrobin 25% + tebuconazole 50% on *Alternaria brassicae* of cabbage under field conditions

Sr. No.	Treatments	Dose (g/ha)	Per cent Disease Index[1]			Yield (Q/ha)[2]		
			2011-2012	2012-2013	Mean	2011-2012	2012-2013	Mean
1.	Untreated control	-	41.62 (-)	40.55 (-)	41.08 (-)	747.67 (-)	756.67 (-)	752.17 (-)
2.	Trifloxystrobin 25 % + Tebuconazole 50 % - 75 WG	200	15.88 (61.64)	14.89 (62.75)	15.38 (62.56)	807.33 (8.26)	809.67 (7.00)	808.50 (7.4)
3.	Trifloxystrobin 25 % + Tebuconazole 50 % - 75 WG	250	14.73 (64.64)	13.66 (66.31)	14.19 (65.45)	863.00 (15.73)	864.00 (14.18)	863.50 (14.80)
4.	Trifloxystrobin 25 % + Tebuconazole 50 % - 75 WG	300	12.92 (68.96)	12.55 (69.05)	12.73 (69.01)	875.00 (17.44)	876.67 (15.85)	875.83 (16.44)
5.	Trifloxystrobin 50 WG	150	25.22 (39.40)	23.89 (41.08)	24.55 (40.23)	793.00 (6.34)	798.33 (5.50)	795.66 (5.7)
6.	Tebuconazole250 EC	600	31.88 (23.40)	29.66 (26.85)	30.77 (25.09)	781.33 (4.78)	783.67 (3.56)	782.50 (4.03)
7.	Hexaconazole5 EC	500	23.88 (42.24)	22.77 (43.84)	23.32 (43.23)	813.67 (9.11)	817.33 (8.01)	815.50 (8.41)
8.	CD (0.05%)	-	8.28	7.99	-	5.85	32.37	-
9	SEm ±	-	3.80	3.66	-	17.94	14.85	-

[1]figure in parentheses denotes percent disease reduction over control
[2]figures in parentheses denote percent increase in yield over control
n.b.: the percent values were arcsine transformed prior to statistical analysis

Residue dissipation

Method Validation

The linearity of the calibration curves in solvent and matrix for both the chemicals was established with a correlation coefficient (R^2) of >0.995 in the range 0.001-0.05 µg ml^{-1}. The LOQs were set at 0.005 and 0.01 mg $Kg^{-1}$for trifloxystrobin and tebuconazole, respectively. The average recoveries (%) and corresponding repeatability (RSD) of trifloxystrobin and tebuconazole at 0.01, 0.025 and 0.05 mg Kg^{-1} levels of fortification were 70.2 (±4)%, 77.5 (±9)%, 75.3 (±7)% and 99.7(±11)%, 101.3 (±11)%, 97.5 (±8)%, respectively. Thus, mean recoveries were within the range 70-120% (RSD < 20%) fulfilling the required validation criteria. The matrix effect was prominent for both the pesticides with ionization suppression being 18 and 8% for trifloxystrobin and tebuconazole, respectively. In view of this, the matrix matched calibration was used for quantification of the residues for both the fungicides.

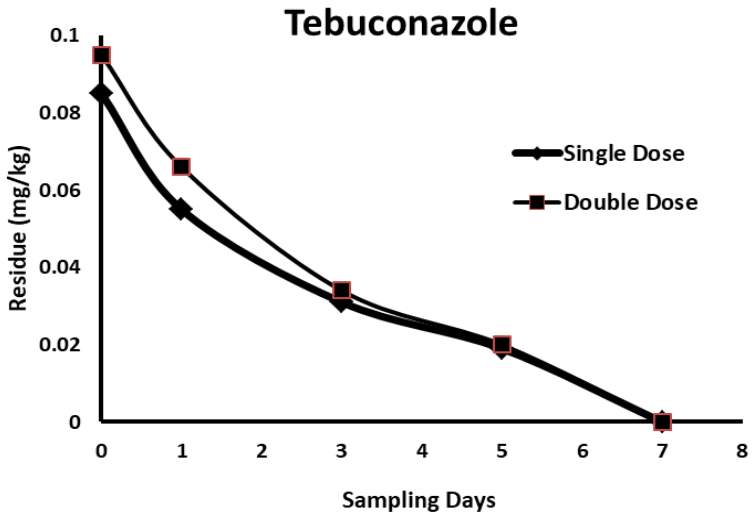

Figure 1 Dissipation of residues of tebuconazole in cabbage

Dissipation of residues in field experiments

The relative concentrations of the day wise residues of the test chemicals (7 days) are represented in the **Figure 1-2**. The comparative data of first (linear kinetics) and first + first order (non-linear) rate kinetics are presented in **Table 3**. The correlation coefficient (R^2) for the first + first order was comparatively superior indicating its better applicability vis-à-vis the first order model. The DT_{50} values obtained by the first + first order model were more realistic as manifested by the day wise residue data. The residues were below the prescribed MRL of trifloxystrobin (0.5 mg Kg^{-1}) and tebuconazole (0.7 mg Kg^{-1}) on all the sampling days (including the day of final application), no PHI is applicable. The uses of both the chemicals in cabbage are therefore quiet safe from the food safety point of view.

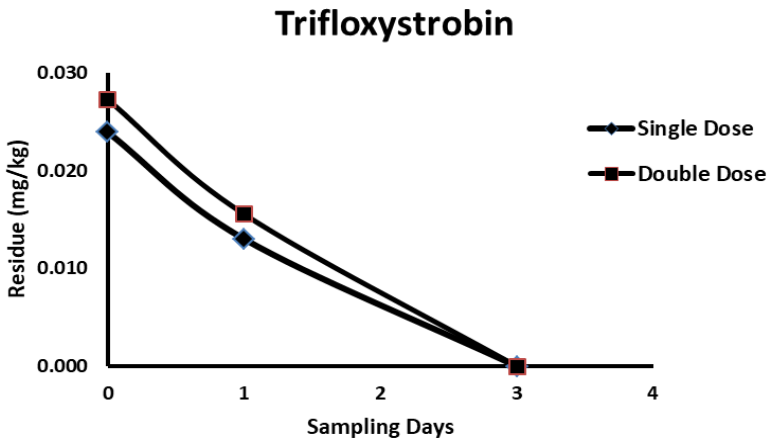

Figure 2 Dissipation of residues of trifloxystrobin in cabbage

Table 3 Day-wise residue data and residue dissipation parameters of Trifloxystrobin (25 %) + tebuconazole (50%) in Cabbage

Days after spray		Tebuconazole (mg/kg)		Trifloxystrobin (mg/kg)	
		Standard dose	Double dose	Standard dose	Double dose
0		0.085	0.095	0.024	0.027
1		0.055	0.066	0.013	0.016
3		0.031	0.034	BLQ	BLQ
5		0.019	0.020	BLQ	BLQ
7		BLQ	BLQ	BLQ	BLQ
10		BLQ	BLQ	BLQ	BLQ
1^{st} order	[*A*]1 (mg/kg)	0.083	0.095	0.02	0.095
	k1 (Day^{-1})	0.354	0.356	0.76	0.356
	[A]2 (mg/kg)	N/A	N/A	N/A	N/A
	k2 (Day^{-1})	N/A	N/A	N/A	N/A
	R^2	0.98	0.98	0.98	0.98
	DT_{50} (Day)	2	2	1	1
	PHI (Day)	Not applicable	Not applicable	Not applicable	Not applicable
1^{st} +1^{st} order	[*A*]1 (mg/kg)	0.007	0.031	-	-
	k1 (Day^{-1})	0.25	0.356	-	-
	[A]2 (mg/kg)	0.078	0.064	-	-
	k2 (Day^{-1})	0.326	0.356	-	-
	R^2	0.98	0.98	-	-
	DT_{50} (Day)	2	2	-	-
	PHI (Day)	Not applicable	Not applicable	-	-

Conclusion

Trifloxystrobin 25% + tebuconazole 50% 75WG @ 250-300 g ha^{-1} provides an excellent control against leaf spot disease of cabbage in which harvesting continues once the heads start maturing to the end of the harvest season. Safety evaluation through residue dynamics studies of trifloxystrobin 25% + tebuconazole 50-75WG proved advantageous to the farmers as well as the exporters in producing disease free MRL compliant produce. Not only in

cabbages, the use of the fungicide can be extended to other vegetable crops as well and will be an integral part for formulating the Good Agricultural Practice (GAP) for different crops. Effect of the fungicides on the morpho-physiological parameters of cabbage may also be looked into, along with its translocation, and distribution in the host tissues. Moreover, the combined bioefficacy, residue analysis and safety assessment of the fungicide study will help in its inclusion in the package of practices for recommendation to the farmers in managing the disease.

Acknowledgements

The authors are grateful to the Directors of ICAR-NRCG, Pune and ICAR- IIVR, Varanasi for their keen interest and ardent support during the duration of the study.

References

[1] Anon. (2015). Area and production estimates for horticultural crops. In Indian Horticulture Database. National Horticulture Board, Ministry of Agriculture, Government of India: New Delhi, India, 2015:139.
[2] Kahlon, T.S., Chiu, M.C., Chapman, M.H. (2008). Steam cooking significantly improves in vitro bile acid binding of collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage. Nut. Res., 28: 351-357.
[3] Surviliene, E., Sidlauskiene, A., Vasinauskiene, M. Zitikaite, M. (2004). Resistance to Brassica L. vegetables to phytopathogens. Hort. Veg. Growing., 23: 115-125.
[4] Kolte, S.J. (1985). Diseases of Annual Edible Oilseed Crops, Vol II. Rapeseed-Mustard and Sesame Diseases. CRC Press Inc. Boca Raton, Florida, USA. 135pp.
[5] Cherukuri, S.C., Plaha, P., Sharma, R. (2011). Evaluation of some cultivated Brassicas and their related alien species for disease resistance In: Cruciferae Newsletter., 30: 18-20.
[6] Nowicki, M., Nowakowska, M., Niezgoda, A., Kozik, E.U. (2012). Alternaria black spot of crucifers: Symptoms, importance of disease, and perspectives of resistance breeding. Veg. Crop Res. Bull. 76: 5-19.
[7] Survilienė, E. Valiuškaitė, A., Duchov-skienė, L., Kavaliauskaitė, D. (2010). Influence of fungicide treatment on grey mould of cabbage. Veg. Crop Res. Bull. 73: 133-142.
[8] EU pesticide database 2016. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN
[9] Zentmeyer, G.A. (1955). A laboratory method for testing soil fungicides with Phytophthora cinnamomii, a test organism. Phytopathol., 45: 398-404.
[10] Vincent, J.M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 159: 850.
[11] Kashyap, P.L., Dhiman, J.S. (2010). Eco-Friendly Strategies to Suppress the Development of Alternaria Blight and Black Rot of Cauliflower. Aca. J. Plant Sci., 3: 140-146.
[12] Wheeler, B.E.J. (1969). Pathometric calculations. In An introduction to plant diseases. John Wiley and Sons. Ltd.: London, pp 374.
[13] Thompson, M., Ellison, S.L., Wood, R. (2002). Harmonized guidelines for single laboratory validation of methods of analysis. IUPAC technical report. Pure and Appl. Chem. 74: 835-855.
[14] Nandi, S., Mondal, A., Chaudhuri, S., Dutta, S. (2012). Physiological changes in Alternaria solani treated with Nativo 75 WG. Aus.Mycolo. 30: 5-12.
[15] Badoc, A. (2005). Biologieetphysiologie des principauxagentsfongiquesde la pourriture des pommesen conservation etluttechimiqueparl'Azoxystrobine. Bull. Soc. Pharm. Bordeaux. 144: 47-62.
[16] Tu, D.V., Somasekhara, M.Y., Govindaraju, C. (2015). Evaluation of new molecules of fungicides against leaf spot (Alternaria brassicicola (schw) Wilt shire of cabbage (Brassica oleraceavar. capitatal.). Intl. J. Agric. Sci. Res. 5: 349-354.
[17] Saha, S., Ahammed, S., Purath, T., Jadhav, R.M., Loganathan, M., Banerjee, K., Rai, A.B. (2014). Bioefficacy, residue dynamics and safety assessment of the combination fungicide Trifloxystrobin 25% + tebuconazole 50%-75 WG in managing early blight of tomato (Lycopersicon esculentum Mill.). J. Env. Sci. Health Part B. 49: 134-141.
[18] Pasche, J.S., Wharam, C.M., Gudmestad, N.C. (2004). Shift in Sensitivity of Alternaria solani in response to QoI fungicides. Plant Dis. 88: 181-187.
[19] Ziegler, H., Benet-Buchholz, J., Etzel, W., Gayer, H. (2003). Trifloxystrobin–a new strobilurin fungicide with an outstanding biological activity. Pflanzenschutz-Nachrichten Bayer. 2: 213-230.
[20] Weber, R.W.S., Hahn, M. (2011). A rapid and simple method for determining fungicide resistance in Botrytis. J. Plant Dis. Protect. 118: 17-25.

Publication History
Received 07th Aug 2018
Revised 20th Sep 2018
Accepted 04th Oct 2018
Online 30th Oct 2018