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Compatibility of Azoxystrobin 23 SC with Biocontrol Agents and Insecticides

S. Archana*, Manjunath Hubballi, T. Prema Ranjitham, K. Prabakar and T. Raguchander
Department of Plant Pathology, Centre for Plant Protection Studies,
Tamil Nadu Agricultural University, Coimbatore - 641 003

An experiment was conducted to study the compatibility of azoxystrobin 23 SC with bacterial and fungal biocontrol agents and insecticides under *in vitro* and glass house conditions, respectively. Bacterial biocontrol agents *viz., Pseudomonas fluorescens* and *Bacillus subtilis* were compatible with azoxystrobin 23 SC even at a high concentration of 300 ppm whereas fungal biocontrol agent *Trichoderma viride* was inhibited by azoxystrobin 23 SC at a concentration above 15 ppm. Among the four insecticides tested for compatibility, all insecticides were physically compatible with azoxystrobin 23 SC at 125, 250 and 500 g ai ha^{-1} whereas dichlorvos was biologically incompatible even at the lowest concentration tested.

Key words: Azoxystrobin, *Pseudomonas fluorescens, Bacillus subtilis, Trichoderma viride* and insecticides.

Azoxystrobin is a potent strobilurin compound produced by the Basidiomycete *Strobilurus tenacellus* having novel biochemical mode of action (Hewitt, 1998). Its fungicidal activity results from the inhibition of mitochondrial respiration in fungi and this is achieved by the prevention of electron transfer between cytochrome b and cytochrome c. It is a systemic compound that is translocated in the transpiration stream from roots to the stem and into the leaves. Taken up by leaves, roots and seeds, it is claimed to have protectant and eradicant properties. Compared with the major classes of systemic fungicides, azoxystrobin has a high level of intrinsic activity and the broadest spectrum; therefore, it is active at very low doses against a wide range of fungal pathogens. Indeed, one of the key reasons for the outstanding commercial success of azoxystrobin is that it gives control of fungi from all four classes of plant pathogens, namely the Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes. Therefore, azoxystrobin gives control of combinations of pathogens which was previously only possible through the mixture of two or more fungicides, e.g. downy and powdery mildew of grapevines.

Since fungicides may have deleterious effects on the pathogen as well as the antagonist, an understanding of the effect of fungicides on the pathogen and the antagonist, would provide an information on the selection of selective fungicides and fungicide resistant antagonists. The idea of combining biocontrol agents (BCA) with fungicides is for the development or establishment of desired microbes in the rhizosphere (Papavizas and Lewis, 1981). Further, the antagonism of BCA was influenced by the addition of fungicides (Kay and Stewart, 1994; Naar and Kecskes, 1999). Many authors reported the compatibility of fungicides with biocontrol agents in various crops (Utkhede and Koch, 2002; Senthilvel *et al.,* 2004; Anand *et al*., 2007).

In intensive agriculture, crop production is challenged by an array of insects and pathogen leading to wide variety of damage. In order to combat these maladies, often it becomes necessary to mix two or three compatible pesticides (insecticide with fungicide) in a single preparation to save time and also expenses. Under such circumstances, it is desirable to generate information regarding the compatibility of insecticide and fungicide based on their efficacy against insect pest and diseases. The compatibility of insecticides with fungicides has been reported earlier in many cases (Varalakshmi *et al*., 2000; Sendhilvel, 2003). Considering all these points the present study was undertaken to test the compatibility of azoxystrobin 23 SC with biocontrol agents and also insecticides.

Materials and Methods

The bacterial biocontrol agent, *Pseudomonas fluorescens* Migula (Pf 1) and *Bacillus subtilis* (SVPR4) and fungal biocontrol agents *Trichoderma viride* (TV1) were obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. Insecticides and azoxystrobin 23 SC were purchased from pesticides market at Coimbatore.

Compatibility of azoxystrobin 23 SC with biocontrol agents

Turbidometric method

One ml of the each bacterial culture *viz*., *P. fluorescens* and *B. subtilis* was transferred to a 250 ml sidearm flask containing 50 ml of King's B and

*Corresponding author email: archanas_agri@yahoo.co.in

Nutrient Agar broth, respectively and amended with azoxystrobin 23 SC technical standard at five different concentrations *viz.,* 100, 150, 200, 250 and 300 ppm. The control was maintained without inoculation of bacterial culture and azoxystrobin technical standard in both. The flasks were incubated at 28 ± 1 °C in a psychotherm shaker. The optical density values of the culture broth were determined in Spectrophoto colorimeter at 610 nm at regular intervals of 6 h.

Poisoned food technique

The compatibility of *Trichoderma viride* with azoxystrobin was tested using lower doses unlike in bacterial biocontrol agents as the *T. viride* is highly sensitive to fungicide and in addition to this it is mainly delivered as soil application. Potato dextrose agar (PDA) was used as the basal medium to which calculated quantities of azoxystrobin 23 SC was separately mixed aseptically after sterilizing the medium to give required concentrations *viz*., 5, 10, 15, 20 and 25 ppm. For each concentration, azoxystrobin 23 SC was taken into a 100 ml Erlenmeyer flask containing 100 ml of the sterilized and molten medium, mixed thoroughly by gently swirling the flask, poured 15 ml in each sterile Petri dish and allowed to solidify. A nine mm actively growing PDA culture disc of test fungus was placed at the centre of the plate and the plates were incubated in inverted position at room temperature (28 ± 2°C). The PDA medium without azoxystrobin 23 SC and inoculated with *T. viride* served as control. Three replications were maintained for each concentration. The radial growth of mycelium was measured periodically at 1, 2, 3, 4 and 5 days after inoculation.

Compatibility of azoxystrobin 23 SC with insecticides

In vitro *condition*

Preparation of standard hard water

Standard hard water is defined as water, which provides a hardness of 342 ppm calculated as calcium carbonate. For getting this hardness, 304 mg of anhydrous calcium chloride and 139 mg of magnesium chloride were dissolved in distilled water.

This solution was used to prepare insecticide solutions for all tests.

Emulsion stability test for physical compatiblity

The test was carried out for dichlorvos, monocrotophos, carbaryl and dimethoate as prescribed by Indian Standard Specification for emulsion stability test (ISI, 1964). To 75 to 80 ml of standard hard water kept in a beaker at 30 °C, the insecticide and fungicide was added by means of Mohr's type pipette. The insecticide and fungicide mixture was added to the standard hard water @ 25 to 30 ml pouring directly into the beaker and not along the sides of the beaker. The contents of the beaker were stirred with a glass rod at four revolutions per second during the addition.

The diluted emulsion was made up to 100 ml mark with water which was transferred immediately to a clean dry graduated cylinder. Then the cylinder with its contents was kept at 30°C in a thermostat for 30 min. The creaming matter at the top and sedimentation at the bottom were observed. The creamy/sedimentation above two ml was considered as unstable.

Biological compatibility under glass house condition

Grapevines cv. Muscat was raised in pots under glass house conditions. Three month old vines were sprayed with mixture containing insecticides *viz.,* monocrotophos (1.4 ml l^{-1}), dichlorvos (1 ml l^{-1}), carbaryl (2 ml l^{-1}) and dimethoate (2 ml l^{-1}) with the three concentrations of azoxystrobin 23 SC *viz*., 125, 250 and 500 g ai ha^{-1} to test the compatibility. The trial was conducted with three replications and three pots constituted one replication. Observations on leaf injury were taken after first, second and third days after spray.

Statistical analysis

The data generated from various experiments of this study were statistically analyzed by DMRT with IRRISTAT software. The data with per cent values were subjected to arc sine transformation.

Results and Discusssion

Bacterial biocontrol agents

Azoxystrobin 23 SC at different concentrations was evaluated *invitro* for its compatibility with the bacterial biocontrol agents, *P. fluorescens* – Pf 1 and *B. subtilis* (SVPR4). The growth of bacteria in azoxystrobin 23 SC amended broth (100, 150, 200, 250 and 300 ppm) was assessed by turbidomertic method and the results are presented in Table 1 and 2. The bacterial growth was not affected by

Table 1. Growth of *P. fluorescens* - Pf 1 in medium amended with azoxystrobin 23 SC

Concentration (ppm)	Absorbance at 610 nm at different hours after inoculation*						
	12	18	24	30	36	42	48
100	1.52 [a]	1.80 [a]	1.91 [a]	1.95 [a]	2.22 [a]	2.32 [a]	1.95 [a]
150	1.55 [a]	1.84 [a]	1.94 [a]	1.94 [a]	2.21 [a]	2.28 [a]	1.99 [a]
200	1.33 [a]	1.82 [a]	1.96 [a]	1.97 [a]	2.15 [a]	2.22 [a]	1.93 [a]
250	1.49 [a]	1.83 [a]	1.94 [a]	1.95 [a]	2.09 [a]	2.17 [a]	1.93 [a]
300	1.49 [a]	1.92 [a]	1.94 [a]	1.94 [a]	2.06 [a]	2.14 [a]	1.97 [a]
Control	1.50 [a]	1.88 [a]	1.94 [a]	1.99 [a]	2.27 [a]	2.35 [a]	2.01 [a]

*****Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

azoxystrobin 23 SC even at the highest concentration of 300 ppm. There was no significant difference in OD value of the bacterial growth tested at all the concentrations under the study. The turbidity increased with increase in incubation time as in control. The compatibility of azoxystrobin with bacterial biocontrol agents could be attributed for

Table 2. Growth of *B. subtilis*– SVPR4 in medium amended with azoxystrobin 23 SC

Concentration (ppm)	Absorbance at 610 nm at different hours after inoculation*						
	12	18	24	30	36	42	48
100	1.08 [a]	1.72 [a]	1.87 [a]	1.90 [a]	1.98 [a]	2.19 [a]	2.19 [a]
150	1.11 [b]	1.81 [a]	1.82 [a]	1.83 [a]	1.90 [a]	2.18 [a]	2.18 [a]
200	1.81 [a]	1.84 [a]	1.85 [a]	1.92 [a]	1.98 [a]	2.18 [a]	2.18 [a]
250	1.17 [a]	1.77 [a]	1.88 [a]	1.94 [a]	1.97 [a]	2.18 [a]	2.19 [a]
300	1.15 [a]	1.75 [a]	1.84 [a]	1.85 [a]	1.95 [a]	2.18 [a]	2.19 [a]
Control	1.18 [a]	1.77 [a]	1.87 [a]	1.98 [a]	2.02 [a]	2.18 [a]	2.20 [a]

*Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

the reason that it acts on mitochondria as an inhibitor of electron transfer and mitochondria are not present in bacteria. These findings are in agreement with

Table 3. Mycelial growth of *T. viride* – TV1 in medium amended with azoxystrobin 23 SC

Concentration	Diameter of mycelial growth (mm) at different days after inoculation* (ppm)				
	1	2	3	4	5
5	13[b]	35[b]	53[b]	81[ab]	90[a]
10	12[b]	31[b]	48[c]	73[b]	90[a]
15	13[b]	38[b]	47[c]	72[b]	90[a]
20	11[c]	22[c]	41[cd]	49[c]	57[c]
25	12[b]	18[c]	33[d]	50[c]	52[b]
Control	22[a]	52[a]	65[a]	86[a]	90[a]

*Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

the results of a few workers. Kataria *et al*. (2002) reported that lower rates of azoxystrobin applied as seed treatment in combination with *P. fluorescens*

Table 4. Physical compatibility of azoxystrobin 23 SC with insecticides *in vitro* based on emulsion stability test

Treatment	Physical condition of mixture			
	Creamy appearance /100 ml measuring cylinder		Sedimentation /100 ml measuring cylinder	
	Below 2ml	Above 2ml	Below 2ml	Above 2ml
I. Dichlorvos (76EC)				
Azoxystrobin 125 g ai ha^{-1} + Dichlorvos @1 ml l^{-1}	+	-	+	-
Azoxystrobin 250 g ai ha^{-1} + Dichlorvos @1 ml l^{-1}	+	-	+	-
Azoxystrobin 500 g ai ha^{-1} + Dichlorvos @1 ml l^{-1}	+	-	+	-
II. Monocrotophos (36EC)				
Azoxystrobin 125 g ai ha^{-1} + Monocrotophos @ 1.4 ml l^{-1}	+	-	+	-
Azoxystrobin 250 g ai ha^{-1} + Monocrotophos @ 1.4 ml l^{-1}	+	-	+	-
Azoxystrobin 500 g ai ha^{-1} + Monocrotophos@ 1.4 ml l^{-1}	+	-	+	-
III.Carbaryl (50WDP)				
Azoxystrobin 125 g ai ha^{-1} + Carbaryl @ 2 ml l^{-1}	+	-	+	-
Azoxystrobin 250 g ai ha^{-1} + Carbaryl@ 2 ml l^{-1}	+	-	+	-
Azoxystrobin 500 g ai ha^{-1} + Carbaryl@ 2 ml l^{-1}	+	-	+	-
IV. Dimethoate (30EC)				
Azoxystrobin 125 g ai ha^{-1} + Dimethoate @ 1 ml l^{-1}	+	-	+	-
Azoxystrobin 250 g ai ha^{-1} + Dimethoate @ 1 ml l^{-1}	+	-	+	-
Azoxystrobin 500 g ai ha^{-1} + Dimethoate @ 1 ml l^{-1}	+	-	+	-

+ indicates compatibility; - indicates incompatibility

strain W36 showed better antagonist interaction against *Rhizoctonia solani* Kuhn. in bean and cucumber. Sendhilvel *et al*. (2004) found that *P. fluorescens* and *B. subtilis* (Ehrenberg) Cohn. growth was not affected by azoxystrobin at different concentrations of 100, 150, 200, 250 and 300 ppm. Similarly, Anand *et al.* (2007) also reported the compatibility of *P. fluorescens* (Pf1) and *B. subtilis* with azoxystrobin.

Fungal biocontrol agent

The compatibility of *T. viride* isolate TV1 with five different concentrations of azoxystrobin 23 SC was tested and results are presented in Table 3. From the data it was inferred that among the five concentrations tested, three concentrations namely 5, 10 and 15 was found to be compatible whereas the other two concentrations inhibited the growth of *T. viride*. The least mycelial growth of 52 mm was observed in case of 25 ppm as against 90 mm in case of control at five days after inoculation. Over all, from the results it was concluded that up to 15 ppm concentration, the test fungicide was compatible with *T. viride*. The compatibility of fungicide with *T. viride* has been reported by earlier workers (Anand *et al.*, 2007; Sendhilvel *et al*., 2004).

Compatibility of azoxystrobin 23 SC with commonly used insecticides

Physical compatibility of azoxystrobin 23 SC with insecticides

The results on the investigations carried out to study the physical compatibility in terms of emulsion stability revealed that recommended dose of insecticides *viz.,* monocrotophos (1.4 ml l^{-1}), dichlorvos (1 ml l^{-1}), carbaryl (2 ml l^{-1}) and dimethoate (2 ml l^{-1}) with recommended dose of azoxystrobin 23 SC at three different concentrations *viz.,* 125, 250 and 500 g ai ha^{-1} did not produce any creamy matter (or) sediment more than 2.0 ml at the top or bottom of the 100 ml measuring cylinder. The results confirmed the physical compatibility of the above insecticides (dichlorvos, cabaryl, monocrotophos and dimethoate) with azoxystrobin 23 SC (Table 4). The results of present study are in agreement with findings of Anand *et al*. (2007) and Sendhilvel (2003) who reported physical compatibility insecticides with azoxystrobin.

Biological compatibility of azoxystrobin 23 SC with commonly used insecticides under glass house conditions

The biological compatibility of azoxystrobin 23 SC at three concentrations *viz.,* 125, 250 and 500 g ai ha^{-1} with the commonly used insecticides *viz*., dichlorvos, cabaryl, monocrotophos and dimethoate was studied under glass house conditions.

The results of the experiment are presented in Table 5. Among the four insecticides used monocrotophos exhibited good compatibility with azoxystrobin 23 SC with the concentration of 125,

Table 5. Biological compatibility of azoxystrobin 23 SC with insecticides under glass house condition

Treatment	Per cent leaf injury / days after spraying*			
	First	Second	Third	Four
I. Dichlorvos (76 EC)				
Azoxystrobin 125 g ai ha^{-1} + Dichlorvos @ 1 ml l^{-1}	55.32 [b]	60.25 [b]	66.01 [b]	60.52
Azoxystrobin 250 g ai ha^{-1} + Dichlorvos @ 1 ml l^{-1}	80.40 [c]	85.73 [c]	88.36 [c]	84.83
Azoxystrobin 500 g ai ha^{-1} + Dichlorvos @ 1 ml l^{-1}	95.33 [d]	96.67 [d]	95.68 [d]	95.89
II. Monocrotophos (36 SL)				
Azoxystrobin 125 g ai ha^{-1} + Monocrotophos @ 1.4 ml l^{-1}	0.00 [a]	0.00 [a]	0.00 [a]	-
Azoxystrobin 250 g ai ha^{-1} + Monocrotophos @ 1.4 ml l^{-1}	0.00 [a]	0.00 [a]	2.31 [b]	0.77
Azoxystrobin 500 g ai ha^{-1} + Monocrotophos @ 1.4 ml l^{-1}	3.12 [b]	5.31 [b]	8.71 [c]	11.33
III. Carbaryl (50 WDP)				
Azoxystrobin 125 g ai ha^{-1} + Carbaryl @ 2 ml l^{-1}	0.00[a]	0.00 [a]	0.00 [a]	-
Azoxystrobin 250 g ai ha^{-1} + Carbaryl @ 2 ml l^{-1}	7.79 [b]	16.48 [b]	16.70 [b]	13.66
Azoxystrobin 500 g ai ha^{-1} + Carbaryl @ 2 ml l^{-1}	26.32 [c]	32.65 [c]	35.79 [c]	31.58
IV. Dimethoate (30 EC)				
Azoxystrobin 125 g ai ha^{-1} + Dimethoate@ 2 ml l^{-1}	0.00 [a]	0.00 [a]	0.00 [a]	-
Azoxystrobin 250 g ai ha^{-1} + Dimethoate@ 2 ml l^{-1}	10.76 [b]	15.12 [b]	17.76 [b]	14.54
Azoxystrobin 500 g ai ha^{-1} + Dimethoate@ 2 ml l^{-1}	25.75 [c]	27.79 [c]	35.76 [c]	29.77
Control	0.00 [a]	0.00 [a]	0.00 [a]	-

*Mean of three replications
Values in parenthesis are arcsine-transformed values
In a column, means followed by common letter are not significantly different at the 5% level by DMRT

250 and 500 g ai ha^{-1}. Dichlorvos produced severe phytotoxic injury with all the concentrations of azoxystrobin 23 SC (125, 250 and 500 g ai ha^{-1}) under glass house conditions revealing its incompatibility. Carbaryl and dimethoate produced no phytotoxic symptoms with azoxystrobin 23 SC at the concentration of 125 g ai ha^{-1}, but both these insecticides produced mild phytotoxic symptoms *viz.,* leaf injury with azoxystrobin 23 SC at 250 and 500 g ai ha^{-1} concentrations under glass house conditions indicating their incompatibility with azoxystrobin above 125 g ai ha^{-1}. The possible reason for incompatibility of dichlorovos could be because of formation more toxic compound through chemical reaction with test fungicide. The results of the experiment are in line with findings of Bagwan (2010) who reported the compatibility of insecticides with fungicides. Hence, monocrotophos can be combined with azoxystrobin 23 SC during application.

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