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Full Length Research Paper

Compatibility nature of Azoxystrobin 25 SC with Pseudomonas fluorescens and Bacillus subtilis on chilli Plants

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An experiment was conducted to know the compatibility of Azoxystrobin 25 SC with biocontrol agents by poisoned food technique and turbidity method and with insecticides by emulsion stability test. The biological compatibility was done under glass house condition to find the percent injury. The compatibility showed that *Pseudomonas fluorescens* and *B. subtilis* were compatible with Azoxystrobin 25 SC at 5, 10, 50, 100 and 250 ppm, concentration. The physical compatibility studies showed that Azoxystrobin 25 SC was highly compatible commonly used with insecticides *viz.*, Profenphos, Dichlorvos, Monocrotophos, Carbaryl, Dimethoate, Triazophos and Quinalphos. The results from the biological compatibility revealed that insecticides, Dichlorvos and Profenphos were found to be less compatible with Azoxystrobin 25 SC when compared to other insecticides.

Key words: Chilli. Pseudomonas fluorescens, Bacillus subtilis, Azoxystrobin 25 SC, Insecticides.

INTRODUCTION

Chilli (Capsicum annum) is the fourth most important vegetable crops in the world and first in Asia, with world production approximately 122.34 million tonnes of fresh chilli and 2.8 tones of dry chilli in 2010 (Indian Horticultural Database, 2011-2012). The most important producers and exporters of chilli include China, India, Mexico, Morocco, Pakistan, Thailand and Turkey. Demand for chilli in the world is increasing every year. Chilli is a very remunerative spice crop of the Indian subcontinent (Sharma et al., 2005) and occupies an area of about 0.81 million ha (Suthin and Christopher, 2009) which accounts for 25% of the world production (Chandra Nayaka et al., 2009). In Tamil Nadu, chilli is cultivated on

49.0 thousand hectares with 31.8 thousand tonnes of production. Chilli not only meets domestic consumption but also helps in earning foreign exchange. Unlike other chilli-producing countries, about 90% of the production (estimated over 10 lakh tonnes of chilli) in India is absorbed by the huge domestic market. India exports only about 1.5 lakh tonnes of chilli out of the total production of 7.5 lakh tonnes (Anon, 2008).

Chilli is attacked by several fungal, bacterial and viral diseases among them; anthracnose and powdery mildew are found to be the major diseases incurring heavy losses, if not cared. Anthracnose (fruit rot and die back) caused by *Colletotrichumcapsici* (Syd. Butler and Bisby) is prevalent throughout the chilli growing areas of India (Jeyalakshmi, 1996).

Azoxystrobin was produced by the Basidiomycetes fungus, *Strobilurus tenacellus* having novel mode of action

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(Hewit, 1998). Its fungicidal activity results from the inhibiting mitochondrial respiration of higher fungi, which is achieved by the prevention of Electron transfer between cytochrome b and cytochrome c (Becker et al., 1981). Because of its novel mode of action, azoxystrobin is effective against pathogens which have developed reduced sensitivity to other fungicides. Azoxystrobin the exhibits no cross-resistance to ergosterol biosynthesis inhibitors, phenylamides, dicarboximades and benzimidazoles group of fungicides. Azoxystrobin shows a unique spectrum of disease control. It is active against the fungi belong to oomycetes, ascomycetes, basidiomycetes and deuteromycetes (Sauter et al., 1995).

Compatible means capable of existing together in harmony (that is) able to exist together with something also. Often it becomes necessary to mix two or three compatible pesticides (insecticide with fungicide) and biocontrol agents in a single preparation which saves time and expenses. These two chemicals and biocontrol agents when mixed in a single preparation due to reaction form a compound, which differs from the parent. Use of incompatible chemicals may result in the undesirable effects. The compatibility in such cases may be physical compatibility, biological compatibility and phytotoxicity. The compatibility on beneficial organisms such as nitrogen fixers, residential antagonists and mycorrhizal fungi are the other advantages of the application of fungicides (Rodriguez and Curl, 1980).

Since fungicides may have deleterious effects on the pathogen as well as antagonist, an understanding of the effect of fungicides on the pathogen and the antagonist, would provide information on the selection of selective fungicides and fungicide resistant antagonist. The idea of combining biocontrol agents with fungicides is for the development or establishment of desired microbes in the rhizosphere (Papvizas and Lewis, 1981). Considering all these points the present study was undertaken to test the compatibility of Azoxystrobin 25 SC with biocontrol agents and insecticides.

MATERIALS AND METHODS

Compatibility of bacterial antagonists with fungicide by poison food technique

Compatibility of biocontrol agents with Azoxystrobin were tested by poison food technique (Schmitz, 1930). Potato dextrose agar (PDA) was the basal medium to which the calculated quantity of Azoxystrobin 25 SC was mixed separately after sterilizing the medium to give required concentrations viz, 5, 10, 50, 100 and 250 ppm. In the sterilized Petri plates the poisoned medium was poured at 20 ml and allowed to solidify. Bacterial antagonists were streaked separately on the medium respectively and incubated at room temperature (28 + 2°C). The medium without addition of fungicide served as control.

Three replications were maintained for each treatment at the rate of 3 Petri dishes per replication. Bacterial growths of antagonists were recorded after 24 h and compared with control plates (Anand, 2005).

Compatibility of Azoxystrobin 25 SC with Pseudomonas fluorescens and Bacillus subtilis by turbidometric method

The compatibility of the Azoxystrobin 25 SC with the bacterial antagonists was tested using Turbidometric method (ISI, 1964). One ml of the bacterial culture was transferred to a 250 ml sidearm flask containing 50 ml of King's B broth amended with Azoxystrobin at five different concentrations *viz*, 5, 10,50, 100 and 250 ppm for *P. fluorescens and B. subtilis*. The control was maintained without inoculation of bacterial culture and Azoxystrobin technical standard in both the broth. The flasks were incubated at 28±2°C in a psycotherm shaker. The optical density values of the culture broth were determined in Spectro Photo colorimeter at 610 nm at regular intervals of 6 h.

Physical compatibility of Azoxystrobin 25 SC with insecticide by emulsion stability test

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 solution for all tests.

Physical compatibility

The test was carried out for Dichlorvos76% EC, Profenofos 50% EC, Monocrotophos, 36% EC, Carbaryl 50% WDP, Dimethoate 36% EC Triazophos 40% EC and Quinalphos 25% EC as prescribed by Indian standard specification for emulsion stability test. Azoxystrobinat different concentrations at 100, 125, 150 g a.i were mixed with different insecticides at 1 ml/L for Dichlorvos 76% EC, Profenophos 50% EC Triazophos 40% EC and Quinalphos 25% EC, Dimethoate 36% EC, at 1.4 ml/L for Monocrotophos 36% EC and 2 g/L for carbaryl 50% WDP. To 75ml of standard hard water before kept in a beaker at 30°C, the material was added by means of

Mohr's type pipette. The insecticide and fungicide mixture was added to the standard hard water at 25 ml/min with the material 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 4 revolutions per second during

Table 1. Compatibility of Azoxystrobin 25 SC with *P. fluorescens* (Strain Pf 1).

Concentration (ppm)	Absorbance at 610nm at different hours after inoculation*							
	12	18	24	30	36	42	48	
5	1.20	0.94 a	0.72 ^u	0.63	0.06	0.07	0.02	
10	1.62	0.72	0.96	0.84	0.82	0.09 a	0.03 a	
50	1.74	0.98	0.94	0.92	0.88 a	0.08 a	0.01	
100	1.87 ^a	1.32 a	1.26	1.14	0.06	0.06	0.01	
250	1.92	1.80 a	1.54	1.50 a	0.07 u	0.07 ^D	0.02	
Control	2.00 a	1.90 a	1.58	1.45	0.04	0.06	0.00 u	

Figures in the parentheses are arc sine transformed values. The data are mean of three replications In the column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 2. Compatibility of Azoxystrobin 25 SC with Bacillus subtilis (Strain Bs1).

Concentration	Absorbance at 610nm at different hours after inoculation*							
	12	18	24	30	36	42	48	
5	0.96	1.12 u	1.42 u	1.74	2.30 ab	2.12	1.62	
10	0.84 u	0.96 ^e	1.23 ^e	1.62 e	2.04 ^u	2.13 ^u	1.69 ^u	
50	1.30	1.40	1.60	1.72 u	2.10	2.20	1.63 ^u	
100	1.27	1.65	1.72	1.86	2.26 a	2.28	1.86	
250	1.60 a	1.72 b	1.96 a	1.90 a	2.24	2.36	1.96	
Control	1.60 a	1.86 a	1.97 a	1.94	2.26 a	2.84	2.02 a	

Figures in the parentheses are arc sine transformed values. The data are mean of three replications In the column, means followed by a common letter are not significantly different at 5% level by DMRT.

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 cylinders with its contents were kept at 30°C in thermostat for 30 min. The creaming matter at the top and sedimentation at the bottom were observed. For stable emulsion the creamy matter and/or the sediment if any should not exceed 2 ml should be considered for the compatibility.

Biological compatibility of Azoxystrobin 25 SC with insecticides under glass house condition

Azoxystrobin at different concentrations at 100,125,150 g a.i were mixed with different insecticides at 1 ml/L for Dichlorvos 76% EC, Profenophos 50% EC, Triazophos 40% EC, and Quinalphos 25% EC Dimethoate 36% EC, at 1.4 ml/L for Monocrotophos 36% EC and 2 g/L for Carbaryl 50% WDP and used for the study. The plants were sprayed with fungicides and insecticides mixture and incubated in glass house and observed for the phytotoxicity symptoms after 1 , 2 and 3 sprays. Five replications for each treatment.

RESULTS

Compatibility studies

Compatibility of Azoxystrobin 25 SC with bacterial antagonists by Poisoned food technique

The results conducted on the compatibility of Azoxystrobin 25 SC with Pf1 strain of *P. fluorescens* and Bs1 of *B. subtilis* indicated that Azoxystrobin 25 SC was highly compatible with *P. fluorescens* and *B. subtilis*. The fungicide Azoxystrobin 25 SC even at the highest concentration 250 ppm did not exhibit any inhibition to the growth of both the bacteria.

Compatibility of Azoxystrobin 25 SC with Pseudomonas fluorescens and Bacillus subtilis by turbidometric method

In order to study the compatibility of Azoxystrobin 25 SC with Pf1 strain of *P. fluorescens* and Bs1 of *B. subtilis* study was conducted using the different concentration of fungicides. The results from Tables 1 and 2 indicated that even at the highest concentration 250 ppm the bacterial

Table 3. Physical compatibility of Azoxystrobin 25 SC with commonly used insecticides in vitro based on emulsion stability.

	Physical condition of mixture					
Treatment (mg/ml)		opearance / uring cylinder	Sedimentation / 100 ml measuring cylinder			
	Below 2 ml	Above 2 ml	Below 2 ml	Above 2 ml		
I. Profenofos (Curacron 50% EC)						
Azoxystrobin100 g ai@ 0.8 ml I + Profenofos @ 1 ml I	+	-	+	-		
Azoxystrobin125 g ai @ 1 ml l + Profenofos @ 1 ml l -1	+	-	+	-		
Azoxystrobinin 150 g ai @ 1.2 ml l + Profenofos @ 1 ml l	+	-	+	-		
II. Dichlorvos (Nuvan 76% EC)						
Azoxystrobin100 g ai@ 0.8 ml I + Dichlorvos @1 ml I	+	-	+	-		
Azoxystrobin 125 g ai@ 1ml l ੍ + Dichlorvos @1 ml l	+	-	+	-		
Azoxystrobin150g ai@ 1.2 ml I + Dichlorvos @1 ml I	+	-	+	-		
III. Monocrotophos (Target 36% EC)						
Azoxystrobin100 g ai@ 0.8 ml l + Monocrotophos@ 1.4 ml l	+	-	+	-		
Azoxystrobin125 g ai @ 1 ml l + Monocrotophos @ 1.4 ml l	+	-	+	-		
Azoxystrobin150g ai@1.2 ml I + Monocrotophos @ 1.4 ml I	+	-	+	-		
IV.Carbaryl (Sevin 50% WDP)						
Azoxystrobin 100 g ai @0.8 ml I + Carbaryl@ 2 ml I	+	-	+	-		
Azoxystrobin125 g ai @1 ml l _+ Carbaryl@ 2 ml l	+	-	+	-		
Azoxystrobin150g ai@ 1.2 ml I + Carbaryl @ 2 ml I	+	-	+	-		
V. Dimethoate (Rogar 36% EC)						
Azoxystrobin100 g ai@0.8 ml I + Dimethoate @ 1 ml I	+	-	+	-		
Azoxystrobin125g ai@ 1 ml I + Dimethoate @ 1 ml I	+	-	+	-		
Azoxystrobin150 g ai @1.2 ml l + Dimethoate @ 1 ml l	+	-	+	-		
-VI Triazophos(Hostathion) 40% EC						
Azoxystrobin 100 g ai@ 0.8 ml I + Triazophos 40% EC@1ml	+	-	+	-		
Azoxystrobin 125 g ai @ 1 ml l + Triazophos 40% EC@1ml l	+	-	+	-		
Azoxystrobin 150 g ai@1.2 ml I + Trizophos 40% EC@1ml I	+	-	+	-		
VII. Quinalphos(Ekalaux) EC 25%						
Azoxystrobin 100 g ai @ 0.8 1ml ha + Quinalphos EC 25%1 ml l	+	-	+	-		
Azoxystrobin 125 g ai @1 ml l + Quinalphos EC 25% 1ml l	+	-	+	-		
Azoxystrobin 150 g ai@ 1.2 ml l + Quinalphos EC 25%1ml l Control	+	-	+	-		

⁺ indicates compatibility; - indicates incompatibility.

growths were not inhibited, as indicated by recording OD value of 1.92 as compared to 2.00 in control, this was followed by 100 ppm concentration. This result clearly indicated that Azoxystrobin 25 SC was compatible with *P. fluorescens*

Physical compatibility of Azoxystrobin 25 SC with insecticide by emulsion stability test

With the view to confirm the compatibility of Azoxystrobin

25 SC with insecticides the following were selected. Profenphos, Dichlorvos, Monocrotophos, Carbaryl, Dimethoate, Triazophos and Quinalphos. Three concentration of Azoxystrobin 25 SC of 100,125 and 150 g a.i at 0.08, 0.1 and 0.12 ml were used for the study. The results from Table 3 showed that all treatment concentrations showed creamy appearance and sedimentation data below 2 ml and above 2 ml respectively for the tests. Thus the results indicated the physical compatibility of these insecticides with Azoxystrobin 25 SC.

Table 4. Biological compatibility of Azoxystrobin 25 SC with insecticides under glasshouse condition.

	njury					
Treatment	Days after spraying*					
	First	Second	Third	Mean		
I. Dichlorvos (Nuvan 76% EC)	a	b	b			
Azoxystrobin 100 g ai @0.8 ml l + Dichlorvos @ 1 ml l	50.42 (45.23)	62.24 (52.65)	68.80 (56.04)	63.56		
Azoxystrobin 125 g ai @1 ml l + Dichlorvos @ 1 ml l	79.46 (63.01)	80.76 (63.95)	89.34 (70.91)	83.18		
Azoxystrobin 150 g ai1. @2 ml l + Dichlorvos @ 1 ml l	90.00 ^d (71.57)	94.46 (76.31)	94.68 ^d (76.56)	93.13		
Ⅱ.Monocrotophos (Target ຶ 36% SL)	а	а	а			
Azoxystrobin100g ai @0.8 ml l + Monocrotophos @ 1.4 ml l	0.00 a (0.50)	0.00 a (0.50)	0.00 a (0.50)	0.00		
Azoxystrobin 125g ai @1 ml l + Monocrotophos @ 1.4 ml l	0.00 (0.50)	0.00 ^a (0.50)	2.31 (11.71)	2.31		
Azoxystrobin 150 g ai @1.2 ml l ⁻¹ + Monocrotophos@ 1.4 ml l ⁻¹	3.24 (8.53)	5.32 ^b (13.31)	7.71 (16.11)	5.42		
III. Carbaryl (Sevin 50% WDP)						
Azoxystrobin 100 g ai @0.8 ml l + Carbaryl @ 2 ml l	0.00 (0.50)	0.00 a (0.50)	0.00 (0.50)	-		
Azoxystrobin 125 g ai @ 1 ml I + Carbaryl @ 2 ml I	6.67 (14.89)	15.42 (23.11)	16.72 (24.12)	12.93		
Azoxystrobin 150 g ai @1.2 ml I + Carbaryl @ 2 ml I	24.34 ^C (29.53)	36.62 ^C (37.23)	34.72 ^C (36.09)	31.90		
IV. Dimethoate (Rogar 36% EC)	а	2	а			
Azoxystrobin 100 g ai @0.8 ml l + Dimethoate@ 2 ml l	0.00 (0.50)	0.00 a (0.50)	0.00 (0.50)	-		
Azoxystrobin 125 g ai @1 ml l + Dimethoate@ 2 ml l	9.42 (17.85)	14.16 (22.06)	16.72 (24.12)	13.43		
Azoxystrobin 150 g ai @1.2 ml l + Dimethoate@ 2 ml l	24.36 ^C (29.53)	23.82 ^C (29.2)	34.82 ^C (35.79)	27.66		
V. Profenophos (curacron) 50% EC	а	а	а			
Azoxystrobin 100 g ai @0.8 ml l + Profenophos 50% EC @ 1ml l	0.00 a (0.50)	0.00 (0.50)	0.00 (0.50)	0.00		
Azoxystrobin 125 g ai@1 ml I + Profenophos 50% EC @ 1ml I	44.22 ^U (41.67) C	53.43 (46.95) C	60.12 (50.83)	52.59		
Azoxystrobin 150 g ai @1.2 ml I + Profenophos 50% EC @ 1ml I	60.42 (51.1)	74.36 (59.54)	80.12 (63.51)	71.36		
VI. Triazophos (Hostathion) 40% EC	a	0.00 (0.50)	a			
Azoxystrobin 100 g ai @0.8 ml l + Triazophos 40% EC@1ml l	0.00 a (0.50)		0.00 a (0.50)	0.00		
Azoxystrobin 125 g ai @1 ml l _+ Triazophos 40% EC@1ml l	0.00 (0.50) h	0.00 a (0.50)	0.00 (0.50) b	0.00		
Azoxystrobin 150 g ai@1.2 ml l + Trizophos 40% EC@1ml l	5.28 (13.18)	9.27 (17.66)	3.42 (10.62)	5.46		
VII. Quinalphos (Ekalaux) EC 2 <u>5</u> %	a	a a (2 = 2)	a a			
Azoxystrobin 100 g ai @0.8 ml l + QuinalphosEC 25% 1ml	0.00 (0.50)	0.00 (0.50)	0.00 (0.50)	0.00		
I Azoxystrobin 150 g ai @1.2 ml I + QuinalphosEC	25.12 (30.07)	28.18 (32.01)	36.76 (37.29)	26.74		
25%1ml I _1 Azoxystrobin 125 g ai @1 ml I + QuinalphosEC	10.16 (18.53) a	18.64 (25.55) a	17.67 (24.8) a	14.48		
25% 1ml Control	0.00 (0.50)	0.00 (0.50)	0.00 (0.50)	<u> </u>		

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

Biological compatibility with insecticides

The observations on the biological compatibility of Azoxystrobin 25 SC with all the above insecticides showed that insecticides, Dichlorovos and Profenphos were found to be less compatible as recorded higher injury when compared to other insecticides. Triazophos and quinalphos were found to show less percent leaf injury even at 125 g a.i of Azoxystrobin 25 SC. The study also indicated that there were no wilting, vein clearing, necrosis, epinasty, hyponasty and fruit injury (Table 4).

DISCUSSION

Compatibility by poisoned food technique

The compatibility of Azoxystrobin 25 SC with Pf1 strain of *P. fluorescens* and Bs1 of *B.subtilis* indicated that Azoxystrobin 25 SC was highly compatible with *P. fluorescens* and *B.subtilis* at 5 to 250 ppm concentration. The fungicide Azoxystrobin 25 SC combines with its breadth of spectrum at high levels of intrinsic activity and compatibility. The compatibility of fungicide with two

bacterial biocontrol agents has been reported by earlier workers (Mathiyazhagan, 2007; Sendilvel, 2003).

Turbidometric method

The results obtained from the physical compatibility by turbidometry indicated that Azoxystrobin 25 SC was highly compatible with P. fluorescens and B. subtilis. The bacterial growth was not affected by azoxystrobin 25 SC even at the increasing concentration there was no difference in optical density (OD) value in the bacterial suspension and turbidity. The findings are in agreement with results of a few workers. The bacterial growth was not suppressed by tetraconazole even at the highest concentration of 1000 ppm (Anand et al., 2007). also reported that the compatibility of P. fluorescens (Pf1) with azoxystrobin at different concentrations viz., 100, 150, 200, 250 and 300 ppm revealed that it was compatible with all the concentrations of azoxystrobin tested and the growth of the bacterium turbidity was unaffected even at the maximum concentration of 300 ppm. Archana (2009) reported that the compatibility of *P. fluorescens* (Pf1) with azoxystrobin 23 SC at different concentrations viz., 100, 150, 200, 250 and 300 ppm revealed that it was compatible with all the concentrations of Azoxystrobin 25 SC tested and the growth of the bacterium growth was unaffected even at the maximum concentration. Sendilvel et al. (2004) reported that turbidometric assay showed that the bacterial biocontrol agents (P. fluorescens and B. subtilis) growth in azoxystrobin-amended broth was not affected and is perfectly compatible with bacterial biocontrol agents.

Emulsion stability test

The results on the investigation carried out to give the physical compatibleness in terns of emulsion stability indicated that the chemicals when mixed change their physical form to one that is unstable. The three concentrations such as azoxystrobin @ 100, 125, 150 g a.i did not produce any creamy matter or sediment more than 2 ml at the top or bottom of the 100 ml of measuring cylinder. The present study is agreement with findings of Anand et al. (2007) and Sendhilvel (2003).

Biological compatibility

The indiscriminative use of potentially hazardous fungicides poses a serious threat to environment. In the present study of biological compatibleness indicates triazophos and quinalphos were found to show less percent chilli leaf injury even at 125 g a.i of Azoxystrobin 25 SC. Azoxystrobin 25 SC with all the other remaining insecticides viz., Dichlorovos and Profenphos were found

to be less compatible as recorded higher injury when compared to other insecticides. The reason for Dichlorovos and profenophos incompatibility could be because of formation of byproducts and toxic metabolites giving higher leaf injury and phytotoxic symptoms are in line with finding of Bagwan (2010).

Katria et al. (2002) tested *P. fluorescens* as seed treatment against *R. solani* in bean with the combination of Azoxystrobin, fluidioxanil (0.2%), pencyuron and tebuconazole (0.2%) found to be compatible which did not produce phytotoxic symptoms like hyponasty and epinasty. Sendhilvel (2004) inferred that azoxystrobin with dichlorvos and Profenofos caused leaf injury in grapevine. Anand (2005) reported that Azoxystrobin at

31.25, 62.50 and 125 g ai ha was biologically compatible with methyl demeton and dicofol in chilli, with methyl demeton in cucumber and also monocrotophos in tomato. Mareeswari (2002) reported that combination of hexaconazole and monocrotophos was biologically compatible and effective in reducing the powdery mildew incidence in okra and chilli. The fungicide hexaconazole and epiconazole and their combination with insecticides were significantly effective in reducing the sheath blight incidence in rice and combination suggested that it can be used to check the disease in rice (Reddy and Krishnaiah, 2003). Hence, the triazophos and quinalphos can be effectively combined with Azoxystrobin 25 SC on chilli plants which do not cause any phytotoxicity.

Conclusion

Considering these aspects present study would be useful to test the compatibility of Azoxystrobin 25 SC with beneficial biocontrol agents and also insecticides which are commonly applied to chilli plants without causing any phytotoxic symptoms.

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