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## Compatability of Strobilurin fungicides with *Bacillus subtilis* and their efficacy against chilli *Colletotrichum truncatum*

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### Abstract

Chilli is considered an important tropical and subtropical crop. Fruit rot disease in chilli is widely distributed worldwide causing considerable losses in yield and quality. Five different fungicides viz., Azoxystrobin (Amistar 23%SC), Azoxystrobin+ Difenconazole (Amistar Top 30% W/W) Pyraclostrobin + Metiram (Carbrio Top 60%WG), Tebuconazole + Trifloxystrobin (Nativo 75WG) and Fenamidone + Mancozeb (Sectin 60 WG) were assayed for their compatibility with *Bacillus subtilis*. Testing the compatibility of Strobilurin fungicides with biocontrol agents for phytotoxic symptoms in chilli plants. The compatible fungicides were assayed for their efficacy against *Colletotrichum truncatum* by Poison food technique at concentration of 250ppm, 500ppm, 750ppm, 1000ppm and 2000ppm. Amistar Top with per cent inhibition of 94.43% found to be best in inhibiting mycelia growth of pathogen and found to be superior over other chemical fungicides. There are distinct fungicides available in the market for control of fruit rot disease. However some fungicides are highly effective against the pathogen. Therefore this investigation will helpful to the farmer for selection of suitable fungicides used for management of fruit rot disease, an integrated way to save their crop from the disease and obtain better yield.

**Keywords:** Chilli, Fruit rot, *Colletotrichum truncatum*, fungicides, poison food technique, compatibility

### Introduction

Chilli (*Capsicum annum* L.) is considered an important tropical and subtropical crop on the basis of high consumption, nutritional and cash values to the farmers and consumers both in developing and developed countries. It 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. Fruit rot caused by *Colletotrichum truncatum* is prevalent throughout the chilli growing areas of India. Strobilurin fungicides were produced by the Basidiomycetes fungus, *Strobilurus tenacellus* having novel mode of action (Hewit, 1998) [1]. Amistar Top (azoxystrobin and difenoconazole) have a mode of action that inhibits mitochondrial respiration, preventing spore germination, reducing mycelial growth and are active against many plant pathogenic fungi and shown to induce growth stimulation, hormonal changes and delayed senescence that contribute to increased yields (Mahoney, 2015) [3]. Compatible means capable of existing together in harmony (that is) able to exist together with something also. Compatible fungicides and biocontrol agents in a single preparation which saves time and expenses. Use of incompatible chemicals may result in the undesirable effects like phytotoxicity. Liu *et al.* (2018) [2] reported Synergistic effects of the combined application of *Bacillus subtilis* H158 and strobilurins for rice sheath blight control. 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) [4]. Considering all these points the present study was undertaken to test the compatibility of Strobilurin fungicides with biocontrol agents. Zhang *et al.* (2020) [8] reported that seed immersion with Amistar top suspension at 650ppm control 99.2% fungal infection i.e. *A. alternata*. Although, earlier several studies have been conducted for management of chilli anthracnose in which different fungicides have used against *Colletotrichum truncatum* and few are found highly effective Keeping in view the present investigation was undertaken for effect of different strobilurin fungicides against

*Colletotrichum truncatum* caused chilli fruit rot disease.

**Materials and Methods**

**Compatibility of bacterial antagonists with fungicide by poison food technique**

Compatibility of biocontrol agents with Strobilurin fungicides were tested by poison food technique (Schmitz, 1930) [5]. Five different fungicides viz., Azoxystrobin (Amistar 23%SC), Azoxystrobin+ Difenconazole (Amistar Top 30%W/W) Pyraclostrobin + Metiram (Carbrio Top 60%WG), Tebuconazole + Trifloxystrobin (Nativo 75WG) and Fenamidone + Mancozeb (Sectin 60 WG) (Table1) at five concentrations viz. 250 ppm, 500ppm, 750ppm, 1000ppm and 2000ppm were evaluated with *Bacillus* sp. Potato dextrose agar (PDA) was the basal medium to which the calculated quantity of Strobilurin fungicides were mixed separately after sterilizing the medium to give required concentrations viz, 250, 500, 750, 1000and 2000 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.

**Efficacy of Strobilurin fungicides against *Colletotrichum truncatum***

Three compatible strobilurin fungicides viz., Azoxystrobin (Amistar 23%SC), Azoxystrobin+ Difenconazole (Amistar Top 30%W/W), Tebuconazole + Trifloxystrobin (Nativo 75WG)at five concentrations viz. 250 ppm, 500ppm, 750ppm,1000ppm and 2000ppm were evaluated against *Colletotrichum truncatum* by employing poisoned food technique (Schmitz, 1930) [5]. The desired concentrations were obtained by adding appropriate amount of stock solution of fungicides to potato dextrose agar taken in conical flask and then transferred to petriplates and repeated thrice for each treatment. Potato dextrose agar without fungicides served as control. Each plate was inoculated with a 5 mm mycelial disc of the pathogen taken from 7 days old culture of

*Colletotrichum truncatum*. The inoculated plates were incubated in BOD incubator at 27°C for mycelia growth of pathogen. The colony diameter was recorded and per cent inhibition in each treatment over control was calculated by using the formula (Vincent, 1947) [7]. Radial growth of the fungus was measured with metric scale (mm).

$$I = C - T / C \times 100$$

Where,

I = per cent inhibition over control

C = mean maximum radial growth of colony in control

T = mean maximum radial growth of colony in treatment

**Results**

**Compatibility of Strobilurin fungicides with bacterial antagonists by Poisoned food technique**

The results conducted on the compatibility of Strobilurin fungicides with Bs-PLM and Bc-ADP of *B. subtilis* and *B. cerus* (Table. 2) indicated that three strobilurin fungicides viz., Azoxystrobin (Amistar 23% SC), Azoxystrobin+ Difenconazole (Amistar Top 30%W/W), Tebuconazole + Trifloxystrobin (Nativo 75WG) was highly compatible with *Bacillus* sp. These fungicides even at the highest concentration 2000 ppm did not exhibit any inhibition to the growth of both the bacteria.

**Efficacy of Strobilurin fungicides against *Colletotrichum truncatum***

The results conducted on the efficacy of compatible Strobilurin fungicides against *Colletotrichum truncatum* (Table. 3) indicated that Azoxystrobin+ Difenconazole (Amistar Top 30%W/W) was found highly efficient in controlling the growth of *Colletotrichum truncatum* at concentration viz., 2000 ppm and inhibition percentage was reported 94.43% respectively followed by Azoxystrobin+ Difenconazole (Amistar Top 30%W/W) and Tebuconazole + Trifloxystrobin (Nativo 75WG). Fig.1

**Table 1:** List of fungicides used for *in vitro* evaluation against *Colletotrichum truncatum*

S. No	Fungicide	Trade name
1	Amistar	Azoxystrobin 23%SC
2	Amistar Top	Azoxystrobin 18% + Difenconazole 12% W/W
3	Carbrio Top	Pyraclostrobin 5% + Metiram 55%WG
4	Nativo	Tebuconazole 50% + Trifloxystrobin 25% WG
5	Sectin	Fenamidone 10% + Mancozeb 50% WG

**Table 2:** Comptability of different strobilurin fungicides with *Bacillus* sp

Fungicides	<i>Bacillus subtilis</i>					<i>Bacillus cerus</i>				
	250 ppm	500 ppm	750 ppm	1000 ppm	2000 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	2000 ppm
Azoxystrobin (Amistar 23%SC)	+	+	+	+	+	+	+	+	+	+
Azoxystrobin+ Difenconazole (Amistar Top 30%W/W)	+	+	+	+	+	+	+	+	+	+
Pyraclostrobin + Metiram (Carbrio Top 60%WG)	-	-	-	-	-	-	-	-	-	-
Tebuconazole + Trifloxystrobin (Nativo 75WG)	+	+	+	+	+	+	+	+	+	+
Fenamidone + Mancozeb (Sectin 60 WG)	-	-	-	-	-	-	-	-	-	-

-Sensitive; + Compatible

**Table 3:** Effect of different strobilurin fungicides against *Colletotrichum truncatum*

Fungicides	Radial growth (cm)					Percent inhibition (%)				
	250 ppm	500 ppm	750 ppm	1000 ppm	2000 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	2000 ppm
Azoxystrobin (Amistar 23%SC)	2.52	2.26	2.15	1.82	1.20	72.00	74.87	76.11	79.76	85.87

Azoxystrobin+ Difenconazole (Amistar Top 30%W/W)	2.31	1.65	1.3	0.9	0.5	74.33	81.65	85.55	90.00	94.43
Tebuconazole + Trifloxystrobin (Nativo 75WG)	4.62	4.29	3.9	3.11	3.14	48.66	52.32	56.65	65.43	65.11
Control	9.0									
CD (0.05)	0.21	0.19	0.06	0.19	0.26					

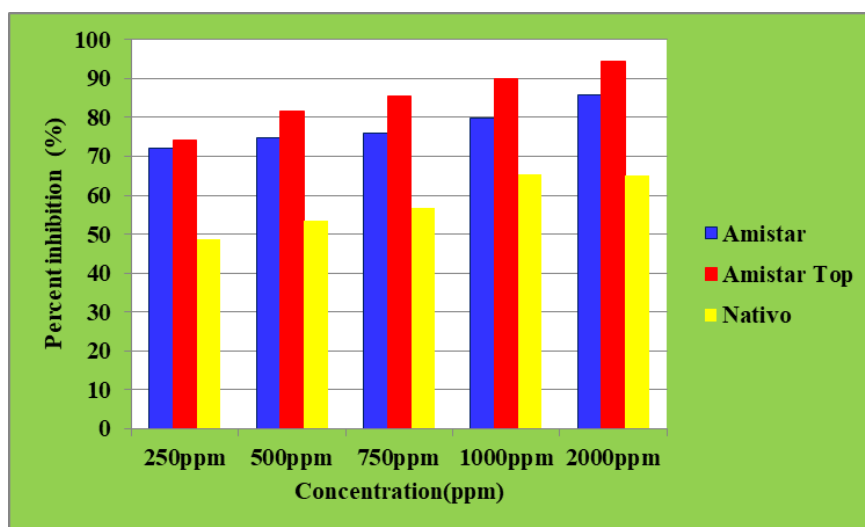


Fig 1: Percent Inhibition Rate (%) of strobilurin fungicides against *Colletotrichum truncatum*.

## Discussion

### Compatibility by poisoned food technique

The compatibility of Strobilurin fungicides with Bs-PLM and Bc-ADP of *B. subtilis* and *B. cerus* indicated that Azoxystrobin (Amistar 23%SC), Azoxystrobin+ Difenconazole (Amistar Top 30%W/W), Tebuconazole + Trifloxystrobin (Nativo 75WG) were highly compatible with *Bacillus* sp at 250 to 2000ppm concentration. The strobilurin fungicides combines with its breadth of spectrum at high levels of intrinsic activity and compatibility. Amistar Top 32.5% EC (Azoxystrobin+ Difenconazole) along with biocontrol agents showed highest efficacy for decreasing downy mildew in onion (Shahin, 2017) [6].

### Efficacy by poisoned food technique

Efficacy of Strobilurin fungicides against *Colletotrichum truncatum* indicated that Azoxystrobin+ Difenconazole (Amistar Top 30%W/W) was effective at highest concentration of 2000ppm. Amistar Top showed considerable potential to control *Sclerotinia sclerotiorum* in pea at 1.0% concentration (Rabiul, 2021). It also showed 99.2% control at 650ppm in carrot *Alternaria alternata* (Zhang, 2020) [8].

## Conclusion

Chilli (*Capsicum annum* L.) is considered an important tropical and subtropical crop on the basis of high consumption, nutritional and cash values to the farmers and consumers both in developing and developed countries. The disease caused by *Colletotrichum truncatum* is one of the most worst plant pathogens worldwide causing the economically important disease anthracnose in a wide range of horticultural crops. Testing the compatibility of Strobilurin fungicides with beneficial biocontrol agents which are commonly applied to chilli plants without causing any phytotoxic symptoms. Presently many farmers are using fungicides and chemicals for control of chilli fruit rot. However farmers are unable for select suitable fungicides for control of this disease because different fungicides and chemicals available in the market for control of chilli

anthracnose. There are some fungicides highly effective against pathogen and disease and some fungicides are not effectively control the anthracnose disease of chilli. Therefore this investigation will helpful to the farmer for selection of suitable fungicides used as a control of fruit rot disease, an integrated way to save their crop from the disease and obtain better yield.

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