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Studies on compatibility of biocontrol agents with chemical fungicides for integrated management of *Alternaria* leaf spot of cabbage

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Abstract
The compatibility of fungal biocontrol agents (*Trichoderma viride* and *T. harzianum*) and bacterial biocontrol agents (*Bacillus subtilis*) was assessed with chemical fungicides viz., propiconazole, hexaconazole, tebuconazole and copper oxychloride. Studies on the compatibility of these effective fungicides and bioagents revealed that the triazole fungicides viz., propiconazole, hexaconazole, tebuconazole completely inhibited the growth of two bioagents viz., *T. viride* and *T. harzianum* at all the three concentrations but compatible with *B. subtilis* at all the three concentrations. The percentage of inhibition of *T. viride* with copper oxychloride was 61.4, 74.4 and 80 % and with *T. harzianum* was 65.5, 80.0 and 85.5% at 0.1%, 0.2% and 0.4% concentrations respectively. Copperoxy chloride found to be incompatible with *B. subtilis* as inhibition zone increased with increase in the concentration of fungicide.

Keywords: Fungal antagonists, bacterial antagonists, poisoned food technique, disc diffusion method.

Introduction
Alternaria leaf spot of cabbage is the most destructive disease and cause tremendous yield losses worldwide. Most of the plant diseases were controlled by utilization of either by utilization of chemical fungicides or by fungal and bacterial antagonists. In several disease management strategies, the addition of fungicide at reduced rate in combination with biocontrol agents has significantly enhanced disease control compared to treatments with biocontrol agents alone (Buck, 2004) [4]. Hence the present study was undertaken to test the compatibility of biocontrol agents viz., *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* with commonly used fungicides at different concentrations under *in vitro* conditions for the control of *Alternaria brassicicola* causing *Alternaria* leaf spot of cabbage.

Mclean *et al.* (2001) stated that sporulation of *T. harzianum* (C52) was completely inhibited by the tebuconazole (0.05%) and mancozeb (0.1%). Pandey *et al*. (2006) [12] reported that both hexaconazole and tebuconazole fungicides showed 100% inhibition of mycelial growth of both *T. viride* and *T. harzianum* under *in vitro* conditions at 500 ppm concentration. Bagwan (2010) [1] conducted an *in vitro* experiment and observed the compatibility of fungicides with *T. viride* and *T. harzianum* and reported that propiconazole, tebuconazole, hexaconazole and chlorothalonil were incompatible with both bioagents as they shown 100% inhibition at 0.2% concentration. Percentage inhibition with copper oxychloride was 34.8% and 32.6% with *T. viride* and *T. harzianum* respectively at 0.2% concentration.

Bhai and Thomas (2010) [3] stated that *T. harzianum* was not inhibited by copper oxychloride at 0.25% concentration. Gaur and Sharma (2010) [6] studied the compatibility of copper oxychloride with *T. viride* and *T. harzianum* and reported that the percentage of inhibition was 26.27% and 36.37% with *T. viride* and 38.07% and 48.03% with *T. harzianum* at 500 ppm and 1000 ppm respectively. Madhusudhan *et al*. (2010) [9] reported that two Trichoderma isolates T2 and T4 were completely incompatible with propiconazole and hexaconazole as they showed 100% inhibition at 500 and 1000 ppm concentrations. Sarkar *et al*. (2010) [14] tested the compatibility of propiconazole, hexaconazole and tebuconazole with *T. harzianum* at 5, 10, 25, 50, 100, 200, 300 ppm concentrations and reported that all the three fungicides completely inhibited the growth of bioagent at 200 and 300 ppm concentration and at the remaining concentrations hexaconazole found to be more toxic compared to propiconazole and tebuconazole. In addition to that, compatibility was also tested with copper oxychloride and found to be moderately sensitive as the percentage of inhibition was 60% and 50% at 300 ppm and 200 ppm concentrations.

Madhavi *et al*. (2011) [8] tested the compatibility of different fungicides with *T. viride* and the percentage of inhibition reported with highly sensitive fungicides propiconazole (0.1%),

Hexaconazole (0.2%), tebuconazole (0.15%) and moderately sensitive copper oxychloride (0.3%) was 93%, 94.4%, 94.4% and 62.9% respectively. Pandya *et al*. (2012) [13] studied the compatibility of propiconazole (0.05%) with *T. harzianum* and reported that as an incompatible combination as mycelial growth was completely inhibited. Zalte *et al*. (2013) [18] reported that *B. subtilis* was compatible with propiconazole at 0.2% concentration. Singh *et al*. (2015) [16] reported that *T. viride* was compatible with copper oxychloride at 500 ppm but mycelial growth was least inhibited at 1000 and 1500 ppm. At 2000 ppm concentration of copper oxychloride the growth of the bioagent was completely inhibited.

Dhanya *et al.* (2016) [5] observed the compatibility of *T. viride* with hexaconazole under *in vitro* conditions and reported that mycelial growth was completely inhibited at 0.1% concentration. Sharma *et al*. (2016) [15] conducted an experiment under *in vitro* conditions and determined the compatibility of *T. harzianum* with propiconazole and observed that the interaction was incompatible as the growth of *Trichoderma* spp. was completely prevented at 31.2, 62.5 and 125 ppm concentrations of the fungicide.

Gayatri *et al*. (2016) [7] reported that *T. viride* and *T. harzianum* showed 21.01% and 13.37% inhibition with copper oxychloride and found to be least sensitive but the sporulation was prevented. In contrast to this *B. subtilis* was reported to be most sensitive to copper oxychloride as it showed 89.36% inhibition. Mareeswaran and Asir (2016) [10] reported that *T. viride* was incompatible with propiconazole and hexaconazole as it shown 100% and 89.06% inhibition over control at 10 ppm concentration but compatible with copper oxychloride at 10 ppm concentration as both bioagents grown completely without any inhibition and also reported that *B. subtilis* was compatible with hexaconazole and propiconazole compared to copper oxychloride. Barooah (2016) [2] reported that propiconazole and hexaconazole were fully incompatible with *T. viride* and *T. harzianum* at 0.1% concentration but compatible with *B. subtilis* at 0.1% concentration.

Materials and methods

Four fungicides propiconazole, hexaconazole, tebuconazole at 0.05%, 0.1% and 0.2% concentrations and copper oxychloride at 0.1%, 0.2% and 0.4% concentrations were selected to test the compatibility with biocontrol agents.

a) *In vitro* evaluation of compatibility of fungicides with fungal antagonists

The compatibility of fungicides with fungal biocontrol agents was tested using poisoned food technique. In order to study this, 50 ml of double strength PDA medium and 50 ml of distilled water were taken in two separate conical flasks and sterilized in an autoclave. Under aseptic conditions in laminar airflow chamber required concentration of chemical is added to the sterile distilled water and stir well. Thereafter the fungicide suspension added to the 50 ml melted and cooled double strength PDA medium and stirred well. Then 15 ml of the poisoned medium was poured into the petriplate and biocontrol agent was inoculated at the centre of the petriplate. For each treatment three replications were maintained and incubated at room temperature. Biocontrol agent placed at the centre of unamended media plate served as control.

Per cent inhibition of the bioagent over control was determined as described by Vincent (1927) [17].

$$I = \frac{C - T}{C} \times 100$$

Where

I = Per cent growth inhibition

C = Growth of bioagent in control plate

T = Growth of bioagent in treatment plate

b) *In vitro* evaluation of compatibility of fungicides with Bacterial antagonists

The compatibility of fungicides with bacterial biocontrol agents was tested using Disc diffusion method. In order to study this, 15 ml of the nutrient agar was poured in the petriplate and allowed to solidify it under aseptic conditions in laminar airflow chamber. Overnight culture of B. subtilis (0.1 ml) was spread over the nutrient agar with the help of spreader. Fungicide solutions of required concentrations (propiconazole, hexaconazole, tebuconazole at 0.05%, 0.1% and 0.2% concentrations and copper oxychloride at 0.1%, 0.2% and 0.4% concentrations) were prepared in separate test tube and 5mm diameter filter paper discs were prepared. There after these discs were dipped in fungicide solutions at required concentrations and placed on the nutrient agar and properly labelled it. Discs dipped in normal water and placed on the nutrient agar which served as control. For each treatment three replications were maintained and incubated at room temperature. Diameter of the inhibition zone was recorded and compared it with control treatment.

Results

a) *In vitro* evaluation of compatibility of fungicides with fungal antagonists

The compatibility of *T. viride* and *T. harzianum* were tested with the fungicides propiconazole, hexaconazole, and tebuconazole at 0.05%, 0.1% and 0.2% concentrations and copper oxychloride at 0.1%, 0.2% and 0.4% concentrations using poisoned food technique. The results showed that the triazole fungicides viz., propiconazole, hexaconazole, tebuconazole completely inhibited the growth of two bioagents viz., *T. viride* and *T. harzianum* at all the three concentrations. The percentage of inhibition of *T. viride* with copper oxychloride was 61.4, 74.4 and 80 % and with *T. harzianum* was 65.5, 80.0 and 85.5% at 0.1%, 0.2% and 0.4% concentrations respectively (Table 1, Plate 1-2).

An *in vitro* experiment was conducted to test the compatibility of four fungicides viz., propiconazole, hexaconazole, and tebuconazole, copper oxychloride with three bioagents viz., *T. viride*, *T. harzianum* and *B. subtilis* which were effective against *A. brassicicola*. The results showed that triazole fungicides viz., propiconazole, hexaconazole and tebuconazole at 0.05%, 0.1% and 0.2% concentrations were incompatible with *T. viride* and *T. harzianum* as it showed cent per cent inhibition (Table 24). Compatibility of both *T. viride* and *T. harzianum* was tested with hexaconazole (0.05%) and tebuconazole (0.05%) fungicides and reported that both combinations were incompatible by Pandey *et al.* (2006) [12] as they showed cent percentage inhibition. The results of the present study were also in accordance with Bagwan (2010) [1]. Sarkar *et al.* (2010) [14] revealed that *T. harzianum* was incompatible with propiconazole, hexaconazole and tebuconazole at 300 ppm concentration.

In the present study, the fungicide copper oxychloride were incomapatible with two fungal bioagents viz., *T. viride* and *T. harzianum* as they recorded more than 50% inhibition at three concentrations 0.1%, 0.2% and 0.4%. Madhavi *et al.* (2011) [8] reported that growth of *T. harzianum* was highly sensitive with copper oxychloride as it showed 62.9% inhibition at 0.3

per cent concentration. Singh *et al*. (2015) [16] stated that copper oxychloride was incompatible with *T. viride* at 0.1, 0.15 and 0.2% concentrations. In *in vitro* study, copper oxychloride was incompatible with *B. subtilis* as the zone of inhibition increased with increase in the concentration of fungicide which is similar with the results of Mareeswaran and Asir (2016) [10].

Table 1: Effect of fungicides on the growth of *T. viride* and *T. harzianum*

Fungicides	Percentage inhibition *					
	0.05%		0.1%		0.2%	
	T. viride	T. harzianum	T. viride	T. harzianum	T. viride	T. harzianum
Propiconazole	100	100	100	100	100	100
Tebuconazole	100	100	100	100	100	100
Hexaconazole	100	100	100	100	100	100
	0.1%		0.2%		0.4%	
Copper oxychloride	61.4	65.5	74.4	80	80	85.5

*Mean of four replications

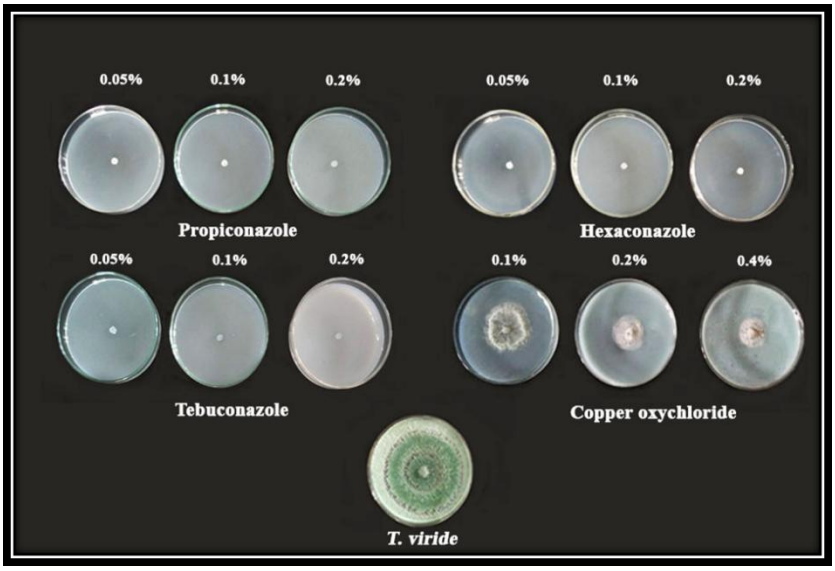

Plate 1: Compatibility of *T. viride* with effective fungicides

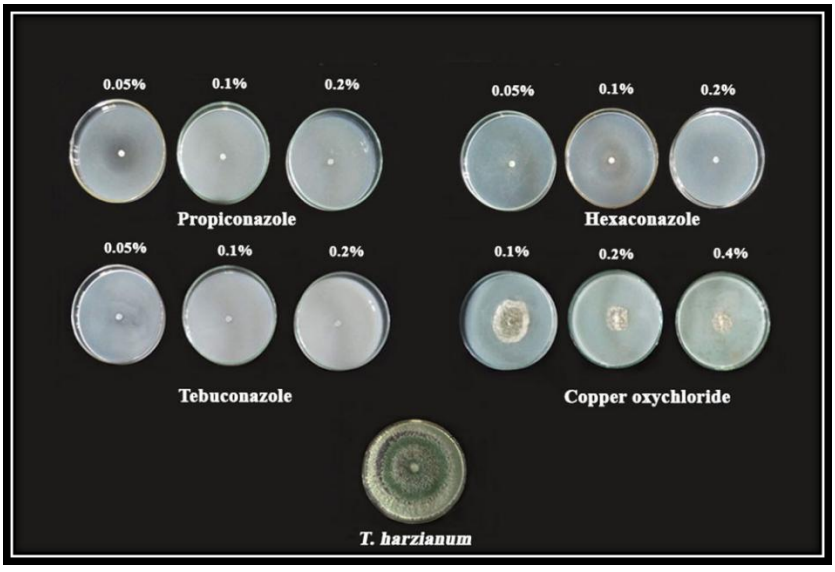

Plate 2: Compatibility of *T. harzianum* with effective fungicides

b) *In vitro* evaluation of compatibility of fungicides with Bacterial antagonists

The compatibility of *B. subtilis* was tested with the fungicides propiconazole, hexaconazole, Tebuconazole at 0.05%, 0.1% and 0.2% concentrations and copper oxychloride at 0.1%, 0.2% and 0.4% concentrations by disc diffusion method. The results were expressed in inhibition zone in diameter and 5 mm indicates 0% inhibition as it was the diameter of the disc placed in the petriplate. The inhibition zone of triazole fungicides i.e., propiconazole, hexaconazole and tebuconazole was 5 mm at all the three concentrations. The diameter of inhibition zone observed in copper oxychloride treatment was 7, 8 and 11 mm at 0.1, 0.2 and 0.4% concentration (Table 2, Plate 3).

The compatibility of *B. subtilis* was also tested under *in vitro* and found to be compatible with three triazole fungicides viz.,

propiconazole, hexaconazole and tebuconazole at 0.05, 0.1 And 0.2% concentrations and these results were in accordance with Mareeswaran and Asir (2016) [10].

Table 2: Compatibility of effective fungicides with *B. subtilis*

Fungicides	Inhibition Zone (mm) *		
	0.05%	0.1%	0.2%
Propiconazole	5	5	5
Tebuconazole	5	5	5
Hexaconazole	5	5	5
	0.1%	0.2%	0.4%
Copper oxychloride	7	8	11

*Mean of four replications

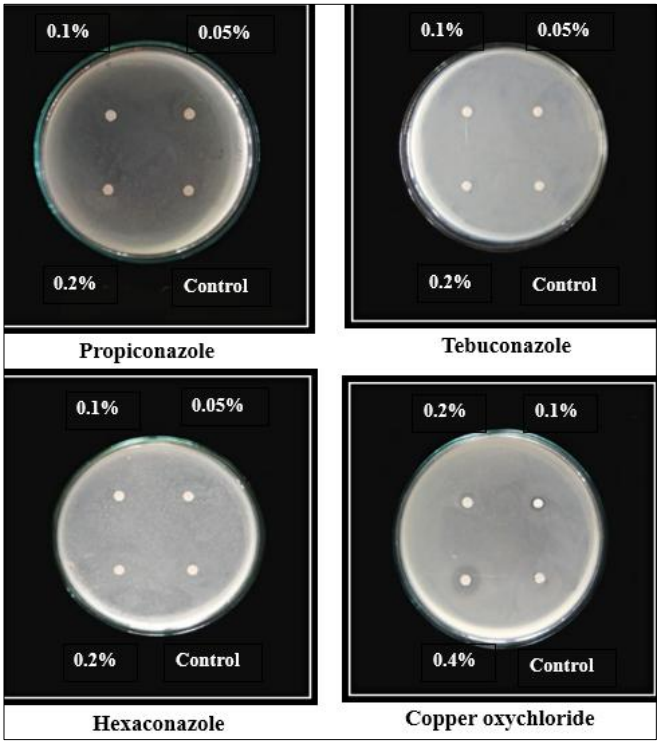

Plate 3: Compatibility of *B. subtilis* with selective fungicides

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