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Compatibility of Copper hydroxide (Kocide 3000) with Biocontrol Agents

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***Abstract*:** *An experiment was conducted to study the compatibility of copper hydroxide (Kocide 3000) with bacterial and fungal biocontrol agents under in vitro conditions. Bacterial biocontrol agents viz., Pseudomonas fluorescens and Bacillus subtilis were compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm. Fungal biocontrol agent, Trichoderma viride was inhibited by copper hydroxide at a concentration above 2500 ppm. The fungal biocontrol agent was highly compatible with the fungicide than the bacterial biocontrol agents.*

Key words: *Copper hydroxide (Kocide 3000), Pseudomonas fluorescens, Bacillus subtilis, Trichoderma viride*

I. Introduction

Kocide 3000 manufactured by DuPont, a copper based fungicide/bactericide was reported to inhibit wide range of fungal and bacterial pathogens. The active ingredient is copper hydroxide (35 per cent) with 30 per cent metallic copper equivalent. It was used for the management of wide range of fungal and bacterial diseases in various crops viz., in citrus against *Phytophthora* foot rot and canker; in almond and apricot against *Pseudomonas*; in apple against scab and anthracnose; in banana against sigatoka leaf spot; in grapes against downy mildew and black rot; in coffee against bacterial blight caused by *Pseudomonas syringae*; in peach against bacterial canker and bacterial spot (caused by *Xanthomonas* spp), in tomato against bacterial speck. (http://www.dupont.com/ProductionAgriculture/en_US/products_services/fungicides/Kocide3000_fungicide.html - 25k) [1]

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 [2]). Further, the antagonism of BCA was influenced by the addition of fungicides (Kay and Stewart, 1994 [3]; Naar and Kecskes, 1999 [4]). Many authors reported the compatibility of fungicides with biocontrol agents in various crops (Utkhede and Koch, 2002 [5]; Senthilvel *et al.,* 2004 [6]; Anand *et al*., 2007 [7]).

The indiscriminative use of potentially hazardous fungicides poses a serious threat to environment. The compatibility on beneficial organisms such as nitrogen fixers, residential antagonists and mycorrhizal fungi are the other advantages of the application of fungicides (Rodriguez- kabana and Curl, 1980 [8]). Singh *et al*., 1995 [9] reported that selected isolates of *T. harzianum, T. viride, T. reesei and T. koningi* were tested with Captaf® 500 ppm, Dithane M- 45® @ 500 ppm and Thiram @ 200ppm. The fungicides were highly inhibitory to *T. reesei* and they were compatible to *T. koningi*. Thiram at 200 ppm inhibited *T. viride* while the rest of the two fungicides were compatible with *T. viride*. Dubey, H. C., 2000 [10] recommended the combined use of fungicides and bio control agents for the management of web blight caused by *R. solani* in groundnut. The combination of biological control agents with fungicides would provide similar disease supression as achieved with higher fungicide use. With this literature as background, studies were carried out to find the compatibility of copper hydroxide with bacterial and fungal biocontrol agents.

II. 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. Copper hydroxide (Kocide 3000) was purchased from the Du Pont regional manager.

1.1. Compatibility of Copper hydroxide (Kocide 3000) with biocontrol agents

1.1.1. 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 Nutrient Agar broth, respectively and amended with Copper

hydroxide (Kocide 3000) technical standard at five different concentrations viz., 100, 200, 300, 400 and 500 ppm. The control was maintained without inoculation of bacterial culture and copper hydroxide (Kocide 3000) 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 Spectrophotocolorimeter at 610 nm at regular intervals of 6 h.

1.1.2. Poisoned food technique

The compatibility of *Trichoderma viride* with copper hydroxide (Kocide 3000) was tested. Potato dextrose agar (PDA) was used as the basal medium to which calculated quantities of copper hydroxide (Kocide 3000) was separately mixed aseptically after sterilizing the medium to give required concentrations viz., 1000, 1500, 2000, 2500 and 3000 ppm. For each concentration, copper hydroxide (Kocide 3000) 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 Petridish 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 copper hydroxide (Kocide 3000) 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.

1.1.3. 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.

III. Results

1.2. Bacterial biocontrol agents

The growth of bacteria (*P. fluorescens* and *B. subtilis*) in Copper hydroxide (Kocide 3000) amended broth was assessed by turbidomertic method and the results are presented in Tables 1 and 2. The bacterial growth was not affected by Copper hydroxide (Kocide 3000) even at the highest concentration of 500 ppm.

Table 1. Compatibility of Copper hydroxide (Kocide 3000) with *Pseudomonas fluorescens*

Time (hrs) after inoculation	Concentration (ppm) of Kocide/ OD value at 610 nm*						
	100	200	300	400	500	Control	Bacterial control
12	1.49 ^{d}	1.42 ^{e}	1.32 ^{e}	1.40 ^{e}	0.87 ^{d}	0.07 ^{a}	1.82 ^{e}
18	1.87 ^{c}	1.60 ^{d}	1.78 ^{d}	1.62 ^{d}	1.45 ^{c}	0.07 ^{a}	2.00 ^{d}
24	2.09 ^{b}	1.92 ^{c}	2.00 ^{bc}	1.95 ^{c}	1.62 ^{c}	0.07 ^{a}	2.14 ^{cd}
30	2.15 ^{ab}	2.10 ^{ab}	2.13 ^{ab}	2.00 ^{bc}	1.87 ^{b}	0.07 ^{a}	2.25 ^{bc}
36	2.28 ^{a}	2.02 ^{abc}	2.23 ^{a}	2.13 ^{ab}	2.00 ^{ab}	0.07 ^{a}	2.40 ^{b}
42	2.32 ^{a}	2.17 ^{a}	2.29 ^{a}	2.21 ^{a}	2.12 ^{a}	0.06 ^{a}	2.62 ^{a}
48	2.00 ^{bc}	1.93 ^{bc}	1.92 ^{cd}	1.88 ^{c}	1.87 ^{a}	0.06 ^{a}	2.30 ^{bc}

*Values are mean of three replications

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

In *P. fluorescens* inoculated broth the turbidity increased with increase in incubation time (0.87 to 2.12) in Copper hydroxide treated concentration of 500 ppm while in control the turbidity did not increase (0.07). When the incubation period was increased beyond 42 hours the turbidity was found to be decrease at all the concentrations.

In case of *B. subtilis*, the turbidity increased with increase in incubation time (0.62 to 1.25) in Copper hydroxide treated concentration of 500 ppm while in control the turbidity did not increase (0.06). But the turbidity was found to decrease with incubation period of more than 42 hours at all the concentrations (Table 2.)

Table 2. Compatibility of Copper hydroxide (Kocide 3000) with *Bacillus subtilis*

Time (hrs) after inoculation	Concentration (ppm) of Kocide/ OD value at 610 nm*						
	100	200	300	400	500	Control	Bacterial control
12	1.03 ^{e}	0.90 ^{d}	0.82 ^{c}	0.70 ^{d}	0.62 ^{e}	0.06 ^{a}	1.95 ^{e}
18	1.43 ^{d}	0.98 ^{d}	0.95 ^{c}	0.84 ^{cd}	0.72 ^{de}	0.06 ^{a}	2.16 ^{d}
24	1.67 ^{c}	1.05 cd	0.98 ^{c}	0.97 ^{c}	0.85 ^{cd}	0.06 ^{a}	2.32 ^{d}
30	1.77 ^{bc}	1.32 ^{c}	0.98 ^{c}	0.97 ^{c}	0.94 ^{bc}	0.06 ^{a}	2.53 ^{c}
36	1.95 ^{a}	1.72 ^{b}	1.32 ^{b}	1.22 ^{b}	1.18 ^{ab}	0.06 ^{a}	2.89 ^{b}
42	2.02 ^{a}	1.96 ^{a}	1.51 ^{a}	1.42 ^{a}	1.25 ^{a}	0.06 ^{a}	3.12 ^{ab}
48	1.90 ^{ab}	1.80 ^{ab}	1.40 ^{a}	1.30 ^{ab}	1.10 ^{ab}	0.06 ^{a}	3.10 ^{a}

*Values are mean of three replications

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

1.3. Fungal biocontrol agent

Copper hydroxide (Kocide 3000) at 100 through 500 ppm concentration did not increase the growth of the mycelium of *Trichoderma viride* under *in-vitro* condition, hence a higher dosage of 1000 to 3000 ppm was used to test compatibility with *T. viride*. The concentration of 1000 and 1500 ppm was not inhibitory with 7.50 and 6.83 cm of mycelial growth in 5 days as against 9.00 cm in control. A significant difference in mycelial growth of *T. viride* was observed in concentration from 2000 ppm to 3000 ppm when compared to control [Plate 1; Table 3].

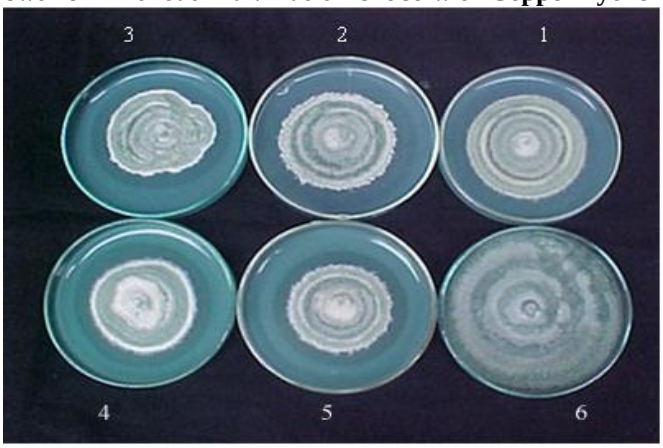
Table 3. Mycelial growth of *Trichoderma viride* amended with Copper hydroxide (Kocide 3000)

Days after inoculation	Concentration (ppm) of Kocide/ diameter of mycelial growth (cm) *					
	1000	1500	2000	2500	3000	Control
1	1.57 [e]	1.20 [e]	0.60 [e]	0.50 [e]	0.40 [e]	2.30 [d]
2	3.20 [d]	2.50 [d]	1.70 [d]	1.40 [d]	1.00 [d]	3.70 [c]
3	5.60 [c]	4.10 [c]	3.50 [c]	2.60 [c]	2.10 [c]	7.80 [b]
4	7.20 [b]	5.60 [b]	3.90 [b]	3.80 [b]	2.80 [b]	9.00 [a]
5	7.50 [a]	6.83 [a]	4.30 [a]	4.20 [a]	3.00 [a]	9.00 [a]

*Values are mean of three replications

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

Fig. 1 Mycelial growth of *Trichoderma viride* amended with Copper hydroxide (Kocide 3000)



1. 1000 ppm	2. 2500 ppm
2. 1500 ppm	3. 3000 ppm
3. 2000 ppm	4. Control

IV. Discussion

Fungicides that are active against a narrow spectrum of plant pathogens but not against biocontrol agents afford an opportunity for the integration of chemical with biological agents. Knowledge of compatibility of biocontrol agents with other components of the production system is needed to develop feasible management strategies. There are very few reports about the compatibility of copper fungicides with the biocontrol agents.

Our findings are in similar with the studies by using Copper oxychloride, mancozeb, fosetyl-Al and cymoxanil 8% + mancozeb 64% mixture fungicides showed moderate to good compatibility with *T. viride1* by exhibiting tolerance limits (ED_{50}) of 848, 710, 578 and 448 µg/ml respectively (Gaur and Sharma, 2010) [11].

Many reports showed compatibility of biocontrol agents with systemic fungicides like azoxystrobin. Kataria *et al.* (2002) [12] reported that lower rates of azoxystrobin applied as seed treatment in combination with *P. fluorescens*. strain W36 showed better antagonist interaction against *Rhizoctonia solani* Kuhn. in bean and cucumber. Sendhilvel *et al.* (2004) [6] 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) [7] also reported the compatibility of *P. fluorescens* (Pf1) and *B. subtilis* with azoxystrobin. Copper oxychloride was found as highly compatible with *T. harzanium* (Susheela and Thomas, 2010) [13].

From the above study, it was concluded that apart from effective management of bacterial and fungal diseases by copper hydroxide (Kocide 3000), the compatibility with bacterial and fungal bio control agents enhances wider opportunity in the agro ecosystem with minimal residual effect.

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