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RESEARCH ARTICLE

Determination of median inhibitory concentrations of two fungicides against *Fusarium oxysporum* f.sp. *vanillae* associated with stem rot disease of vanillae

■ L.S.VANITHA*[1], J. JAYAPPA[2], MALLU GOVARDHANA[1], L. MANJUNATHA[1] AND S.C. CHANDRASHEKAR[1]

[1]Department of Plant Pathology, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA
[2]Department of Agricultural Entomology, K.R.C.College of Horticulture, (U.H.S.) Arabhavi, BELGAUM (KARNATAKA) INDIA

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*Corresponding author:
vanithalakshmanaih@gmail.com

ABSTRACT

Two fungicides were evaluated for their *in vitro* effect on the colony growth of *Fusarium oxysporum* f.sp. *vanillae,* the causal agent of stem rot of vanillae after 48 hrs and 144 hrs of inoculation in pre-amended Potato dextrose agar (PDA) medium. The fungicides showed variable response in inhibiting the growth of the pathogen according to their nature and specificity at different inhibitory concentrations of Tricyclazole (Beam 75% WP) and Matco (Metalaxyl 8 %WP Mancozeb 64% WP). The maximum per cent inhibition on growth of *Fusarium oxysporum* f.sp. *vanillae* by Tricyclazole was high at concentration of 800 ppm 79.99 per cent at 48 hrs, 90.99 per cent at 144 hrs of incubation. The Matco fungicide showed 79.87 per cent inhibition at 800 ppm concentration at 48 hrs and inhibition was 90.78 per cent at 144 hrs post treatment. Interestingly, the toxicity of Tricyclazole was found to increase with time intervals from 24 hrs (IC_{50} value=589.9 ppm) till 144 hrs (IC_{50} value=107.1 ppm). The toxicity of fungicide Matco was also found to increase over time from 24 hrs (IC_{50} value=575.2 ppm) to 144 hrs (IC_{50} value=33.00 ppm) post treatment against *Fusarium oxysporum* f.sp. *vanillae.*

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INTRODUCTION

Plant diseases are one of major constraints in food production. Among several diseases of crop plants, fungal diseases constitute the major group and some are often difficult to control. Vanilla is a crop of highly economic value for its pleasant flavour and the second expensive spice after saffron (Anonymous, 2004). The crop is grown in several parts of the world including India.

The substance responsible for the unique fragrance and flavour of the vanilla bean is *Vanillin* (C8H8O3). In Karnataka, vanilla is cultivated as intercrop as well as pure crop in the districts, like Chikmagalur, Udupi, Shimoga, Hassan, Mysore, Kodagu, Dakshina Kannada and Uttara Kannada. The exporting countries of vanilla are Indonesia, Comoros, Uganda and India. The crop has economic significance in the Indian economy and has significant demand in the international market.

Vanilla plant is affected by several plant pathogens. Among the diseases on vanilla, stem rot caused by *Fusarium oxysporum* f. sp. *vanillae* is one of the important constraints for the vanilla cultivation and responsible for the decreasing of vanilla production (Suprapta *et al.,* 2006; Jayasekhar *et al.,* 2008).

Fusarium wilt is considered as one of the important diseases of banana. The evaluation of newly available fungicide both *in vitro* and *in vivo* was carried out to test their efficacy against the pathogen. Among tested fungicides Prochloraz and Propiconazole significantly inhibited the mycelial growth at concentrations of 1 and 5 µg/ml (Nel *et al.,* 2007).

Currently the word avocado industry relies almost solely on phrosphorus acid to control *Phytophthora cinnamoni* root rot. Alternative chemical such as liquid potassium silicate was used to test its activity against several types of plant pathogenic fungi and *in vitro* dose reponse was determined for *Phytophthora cinnamoni, Sclerotinia sclerotiarum, Sclerotium rolfsii, Fusarium oxysporum, Alternaria solani* and *Colletotrichum coccodes*. The standard agar amended method was used for these tests. Inhibition of mycelial growth was dose dependent, with 100% inhibition at 80 ml and 40 ml soluble silicon (20.7% silicon dioxide) per litre of agar (Kaiser, *et al*., 2005).

MATERIALS AND METHODS

Collection of diseased specimens, isolation of phytopathogenic fungus and maintenance of culture :

Vanilla plant infected by rot pathogen *Fusarium oxysporum* f.sp. *vanillae* were collected from the field. Isolation of the fungus by standard tissue isolation techniques. The infected stem tissues were cut into small bits of 5-6 mm size and surface sterilized with 4:100 ratio sodium hypochlorite solution for one minute and washed repeatedly thrice in sterile water to remove traces of sodium hypochlorite before transferring them to sterile potato dextrose agar (PDA) plates under aseptic conditions. The plates were incubated at a temperature of 28±1^0 C and observed for fungal growth. The hyphal tips of the fungi radiating from the tissues were cut and transferred with a sterile loop to PDA slants to obtain pure culture of the fungus. The fungi were sub cultured on sterile PDA slants and allowed to grow at 28 ± 1^0 C for 7-8 days and such slants were preserved in refrigerator at 5^0 C. The cultures were used for further studies. The pathogen identity was established by proving Koch's postulates.

***In vitro* evaluation of fungicides against *Fusarium oxysporum* f.sp. *vanillae* :**

One systemic (Tricyclazole) and one combination of non systemic + systemic fungicide (Matco) were tested against *Fusarium oxysporum* f. sp. *vanillae* on Potato dextrose agar medium using poison food technique under *in vitro* condition. The fungicides were tried at 10, 25, 50, 100, 200, 400, 600 and 800 ppm concentrations. Poison food technique (Shravelle, 1961) was followed to evaluate the efficacy of fungicides inhibiting the mycelial growth of *Fusarium oxysporum* f. sp. *vanillae*. The fungus was grown on PDA medium for seven days prior to setting up of the experiment. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted medium to obtain required concentrations on quantitative basis of the chemical. 20 ml of poisoned medium was poured in to each of the sterilized Petri plates. Suitable check was maintained without adding any fungicides. 5 mm mycelial discs were taken from the periphery of eight days old colony and were placed at the centre of Petri plates and incubated at 28±1^{0}C for 10 days. The replicates were maintained for each treatment. The diameter of the colony was measured in two directions and average was calculated by using the formula of Vincent (1927).

The observation on growth of the fungus was recorded at 48 and 144 hrs interval. The per cent inhibition by different concentrations of each fungicide was calculated and the data were analyzed with suitable statistical tests. The results of the study are presented in this section.

RESULTS AND DISCUSSION

The per cent inhibition of *Fusarium oxysporum* f.sp. *vanillae* was significantly different between the eight concentrations of Tricyclazole ($F_{7,32}$ = 38.7; p<0.001) and interaction between concentrations and fungus was also

Table1: *In vitro* evaluation of different concentrations of Tricyclazole against *Fusarium oxysporum* f.sp. *vanillae* at 48 and 144 hrs post treatment

Concentrations (ppm)	Per cent inhibition (48 hrs)	Per cent inhibition (144 hrs)
10	20.90±11.33 (26.78±8.12)	30.70±0.46 (33.64±0.28)
25	34.85±1.34 (36.17±0.80)	36.82±4.84 (37.21±2.87)
50	36.02±30.87 (24.14±25.30)	40.43±4.28 (39.46±2.49)
100	37.38±17.58 (27.26±12.57)	46.27±3.10 (40.54±1.80)
200	40.69±25.15 (39.23±15.05)	60.38±6.53 (51.01±3.83)
400	54.34±6.08 (47.49±3.49)	67.86±2.91 (55.47±1.78)
600	79.88±0.48 (63.35±0.34)	87.34±1.46 (69.17±1.26)
800	79.88±0.48 (63.35±0.34)	90.99±0.12 (72.53±0.12)
Pooled mean	47.99±24.54 (40.97±17.49)c	57.59±26.58 49.88±14.40b

Figures in parentheses are arcsin transformed values

exhibited significant difference at 48 hrs post treatment.

The pooled mean of per cent inhibition of fungus was significantly different between the concentrations. However, higher per cent inhibition was recorded at 800 ppm (79.88±0.48) and this was followed by 400 ppm (54.34±6.08) and lowest per cent inhibition was observed at 10 ppm (20.90±11.33). The per cent inhibition of fungus was significantly different between the concentrations at 144 hrs post treatment. However, higher per cent inhibition was recorded at concentration 800 ppm (90.99±0.12) and this was followed by 600 ppm (87.34±1.46), 400 ppm (67.86±2.91) and lowest per cent inhibition was documented at concentration 10 ppm (30.70±0.46) (Table 1). The studies are in conformity with *Fusarium pallidoroseum* on castor. The result showed that the fungicides, Benomyl, Benomyl+Thiram and Tricyclazole, Metalaxyl + Thiomethozan + Difenconozole, Carbendazim and Mancozeb evaluated had inhibitory effect on mycelial growth of *Fusarium pallidoroseum in vitro* at all the three concentrations used especially high concentration.

The per cent inhibition of fungi was significantly different between the eight concentrations of Metalaxyl + Mancozeb ($F_{7,32}$= 84.44; P<0.001) and interaction between concentrations and fungus was also exhibited significant difference at 48 hrs post treatment. The highest per cent inhibition was recorded at 800 ppm (79.88±0.48) and this was followed by 600 ppm (79.44±0.55) and 400 ppm (79.12±0.33) and lowest per cent inhibition was observed at concentration 10 ppm (43.46±0.98) at 48 hrs of post treatment.

The per cent inhibition of the fungi were significantly different between the eight concentrations of Metalaxyl + Mancozeb ($F_{7,32}$=378.8; p<0.001) and interaction between concentrations and fungus was also exhibited significant difference at 96 hrs post treatment.

The pooled mean of per cent inhibition of fungi were significantly different between the concentrations. However, higher per cent inhibition was recorded at 800 ppm (90.99±0.12) and this was followed by 600 ppm (90.79±0.15) 400 ppm (90.65±0.05) and lowest per cent inhibition was observed at

Table 2: *In vitro* evaluation of different concentrations of Metalaxyl+Mancozeb *Fusarium oxysporum* f.sp. *vanillae* at 48 and 144 hrs. post treatment

Concentrations (ppm)	Per cent inhibition (48 hrs)	Per cent inhibition (144 hrs)
10	43.46±0.98 (41.23±0.56)	46.38±8.63 (35.81±5.22)
25	62.07±3.30 (51.99±1.94)	68.93±8.50 (44.38±4.88)
50	63.38±2.72 (52.76±1.62)	69.07±0.63 (41.82±0.36)
100	77.69±1.73 (61.82±1.18)	79.52±1.17 (51.66±0.68)
200	79.29±0.92 (62.93±0.65)	80.69±3.66 (54.76±2.22)
400	79.12±0.33 (62.80±0.23)	90.65±0.05 (72.19±0.04)
600	79.44±0.55 (63.03±0.38)	90.79±0.15 (72.33±0.14)
800	79.88±0.48 (63.35±0.34)	90.99±0.12 (72.53±0.12)
Pooled mean	70.57±13.22 (57.49±7.85)d	77.05±2.75 (55.68±14.57)d

Figures in parentheses are arcsin transformed values

Table 3 : Toxicity (median inhibitory concentration: IC_{50}) values of two fungicides against *Fusarium oxysporum* f.sp. *vanillae* at different hours post treatment

Sr. No.	Fungicides	Hours	IC_{50}
1.	Tricyclazole (Beam 75% WP)	24	589.9
		48	331.2
		72	282.5
		96	168.1
		120	123.9
		144	107.1
2.	Metalaxyl 8 %WP Mancozeb 64% WP (Matco)	24	575.2
		48	79.9
		72	53.8
		96	53.3
		120	41.62
		144	33.0

concentration 10 ppm (46.38±8.63) at 96 hrs post treatment (Table. 2). These results are supported by studies conducted by Naik *et al.* (2011) on stem rot disease of vanilla using bioagent, *Trichoderma harzianum* (2g/pl) and in combination with fungicides, Carbendazim (0.1%), Cymoxanil and Mancozeb (0.2%) with 77.77 per cent survival of plants was observed both in pot and field condition.

Toxicity of two fungicides (median inhibitory concentration (IC_{50}) against *Fusarium oxysporum* f.sp. *vanillae* :

Since the per cent inhibition of fungus was exhibited sigmoid relationship with the concentration of fungicides tested (dose-response=sigmoid curve). Therefore, an attempt was made to find the median inhibitory concentration (IC_{50}) for two fungicides at particular hour following Probit analysis (Finney, 1971). The outcome of the Probit analysis is presented in this section. The toxicity of Tricyclazole was found to increase with time intervals from 24 hrs (589.9 ppm) till 144 hrs (107.1 ppm) against *Fusarium oxysporum* f.sp. *vanillae*. The toxicity of fungicide, Metalaxyl+Mancozeb (Matco) was found to increase over time from 24 hrs (575.2 ppm) to 144 hrs (33.00 ppm) post treatment against *Fusarium oxysporum* f.sp. *vanillae* (Table 3). These results suggested that the response of *Fusarium oxysporum* f.sp. *vanillae* was variable with the type of fungicide used. These findings are in conformity with the results of Kuo (2001) reported the inhibitory effect of Prochloraz on *Colletotrichum gloeosporioides*.

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