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Evaluation of Fungicides and Biocontrol Agents against *Neopestalotiopsis clavispora* Causing Leaf Blight of Strawberry (*Fragaria* x *ananassa* Duch.)

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A B S T R A C T

A leaf blight disease caused by *Neopestalotiopsis clavispora* is a major problem in various strawberry growing areas causing mild to severe damage to plants. The present investigation was carried out to find out an effective management strategy against the disease using fungicides and biocontrol agents. Among the nine fungicides tested *in vitro*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide), copper oxychloride 50WP (Fytolan), propineb 70WP (Antracol) and Bordeaux Mixture showed cent per cent inhibition of the fungus. Bioagents *viz*., *Trichoderma asperellum* and *Pseudomonas fluorescens* exhibited an inhibition of 66.67 and 56.67 per cent respectively. Under *in vivo* conditions, fungicides like propineb 70WP (Antracol), carbendazim 12% + mancozeb 63% (Saaf) and the bioagent, *T. asperellum* showed more than 74 per cent reduction in disease over control.

Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is one of the most important fruit crops grown all over the world and Kerala has made its immense contribution towards country's strawberry export basket. Though, strawberry represents a very profitable crop for the fresh market, the occurrence of diseases on flowers, fruit, leaves, crowns and roots play a significant role in reducing its production and productivity. Among the various fungal diseases reported, *Neopestalotiopsis* leaf blight is one of the serious disease inflicting strawberry plants that reduces its fruit quality and market value.

Materials and Methods

In vitro evaluation of fungicides and bioagents

The efficacy of nine fungicides including systemic, contact and combination products, and bioagents were tested against the pathogen under aseptic conditions by employing poisoned food technique (Zentmeyer, 1955). The experiment was carried out in a completely randomized design (CRD) with three replications for each of the three treatments. The per cent inhibition of mycelial growth in each treatment was calculated using the formula suggested by Vincent (1947).

$$\text{Per cent inhibition of growth} = \frac{C-T}{C} \times 100$$

Where,

C= Growth of fungus in control (mm)
T= Growth of fungus in treatment (mm)

KAU isolates *viz*., *T. asperellum* and *Pseudomonas fluorescens* was tested by following the dual culture method suggested by Skidmore and Dickinson (1976). In case of fungal biocontrol agent, a mycelial plug of 8mm diameter of pathogen and antagonist grown on PDA was inoculated aseptically 2cm away from the periphery of the sterilized Petri plate on opposite sides. The bacterial antagonist, *Pseudomonas fluorescens*, was evaluated against each fungal pathogen by simultaneous antagonism by following the method of Utkhede and Rahe (1983). For this, mycelial disc of 8mm diameter from the centre of the pathogen was placed in the centre of the Petri plate, plated with PDA medium and a loopful of the bacterial culture was streaked at both ends of Petri plate, 2cm away from the periphery of the plate. Plates inoculated with pathogen alone served as control in both cases.

In vivo evaluation of fungicides and biocontrol agents

An experiment was laid out in CRD at College of Horticulture, Vellanikkara and the fungicides and bioagents which were found promising under *in vitro* were chosen for the experiment so as to find out whether these showed similar results under *in vivo* conditions. Thus, four fungicides and a biocontrol agent (*T.asperellum*) were selected and the variety used for the study was Winter Dawn. The plants were challenge inoculated with the pathogen by spraying with the spore suspension of 2 x 10^6 spores ml^{-1} concentration using a hand sprayer. The inoculated plants were kept in humid chamber for 24-48 h in the net house and high relative humidity was maintained throughout the experiment by frequent irrigation. Formulation of *Trichoderma asperellum* was applied as a prophylactic treatment 10 days prior to challenge inoculation of the pathogen and fungicidal treatments were given as foliar spray on symptom appearance and then at 10 days interval after each treatment. After the disease development and at ten days intervals of treatment applications, observations were taken and PDI as well as PDS were calculated.

Results and Discussion

In vitro evaluation of fungicides and bioagents

Findings of the study showed that *in vitro* evaluation of six fungicides *viz*., carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide77WP (Kocide), copper oxychloride 50 WP (Fytolan), propineb70WP (Antracol) at all concentrations were cent per cent effective against *Neopestalotiopsis clavispora*. Contradictory to the above results, Kumhar *et al.,* (2016) recorded only more than 69 per cent inhibition with Saaf, copper hydroxide and copper oxychloride against *Pestalotiopsis theae* causing grey blight of tea. However, Rahman *et al.,* (2013) showed cent per cent efficacy with combination of difenoconazole + propiconazole against *Pestalotia palmarum* in coconut which was in accordance with the present study. Difenoconazole 25 EC (Score) at 0.1 and 0.15 per cent showed cent per cent inhibition. According to Islam *et al.,* (2004), Barman *et al.,* (2015) and Ray *et al.,* (2016), carbendazim was cent per cent effective against the pathogen which was in opposition to the present study that recorded only 57 to 75 per cent inhibition against *Pestalotiopsis* sp. isolated from betel nut, tea and Somtree.

Moreover, Saju *et al.*, (2012) noticed 88.6 per cent restriction of *Pestalotiopsis* sp. with carbendazim in cardamom and Ray *et al.*, (2016) also reported 88.44 per cent with copper oxychloride against *Pestalotiopsis disseminata* in Somtree. Carrie-Missio *et al.*, (2010) reported that spraying of azoxystrobin and mancozeb has a potent role in reducing the symptoms of *Pestalotia* leaf blight on strawberry leaves.

Fungal antagonist when tested against *Neopestalotiopsis clavispora*, it recorded an inhibition of 66.67 per cent while, *Pseudomonas fluorescens* inhibited the pathogen by 56.67 per cent. Saju *et al.*, (2012) recorded 50.9 per cent control with *Trichoderma viride* and 41.3 per cent by *Pseudomonas fluorescens* in case of *Pestalotiopsis* sp. isolated from cardamom. Barman *et al.*, (2015) recorded a higher inhibition of 72.4 per cent by *T. viride* and 35.4 per cent by *Pseudomonas fluorescens* and Kumhar *et al.*, (2016) noticed 62.5 per cent control over pathogen with *T. asperellum* against *Pestalotiopsis theae* in tea (Table 1 and 2).

Table.1 *In vitro* evaluation of fungicides against *Neopestalotiopsis clavispora*

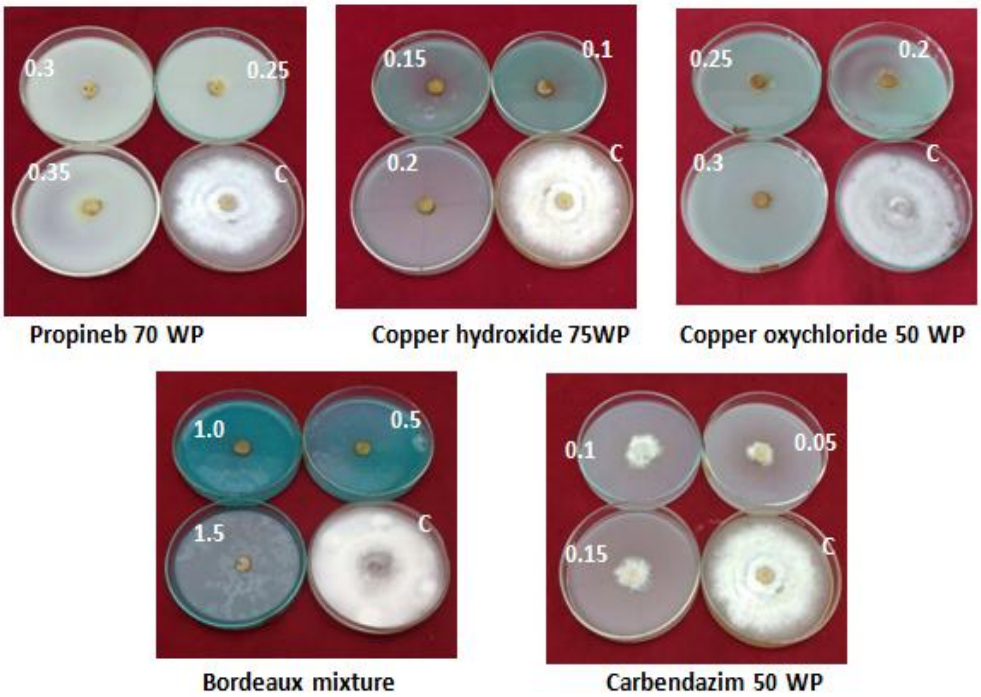
Sl No.	Fungicide	Conc (%)	Per cent Inhibition over control
			Neopestalotiopsis clavispora
1.	Carbendazim 12% + Mancozeb 63% (Saaf)	0.15	100(10) [a]
		0.20	100(10) [a]
		0.25	100(10) [a]
2.	Cymoxanil 8% + Mancozeb 64% (Curzate M8)	0.15	100(10) [a]
		0.20	100(10) [a]
		0.25	100(10) [a]
3.	Copper hydroxide 77WP (Kocide)	0.10	100(10) [a]
		0.15	100(10) [a]
		0.20	100(10) [a]
4.	Copper oxychloride 50WP (Fytolan)	0.20	100(10) [a]
		0.25	100(10) [a]
		0.30	100(10) [a]
5.	Propineb 70WP (Antracol)	0.25	100(10) [a]
		0.30	100(10) [a]
		0.35	100(10) [a]
6.	Carbendazim 50WP (Bavistin)	0.05	57.22(7.59)[g]
		0.10	66.11(8.14)[f]
		0.15	75(8.83)[e]
7.	Difenoconazole 25EC (score)	0.05	81.11(9.04)[d]
		0.10	83.55(9.12)[c]
		0.15	85.55(9.27)[b]
8.	Potassium phosphonate (Akomin 40)	0.25	27.77(5.27)[j]
		0.30	30.55(5.57)[i]
		0.35	36.67(6.08)[h]
9.	Bordeaux Mixture	0.50	100(10) [a]
		1.0	100(10) [a]
		1.50	100(10) [a]
	CD (0.05)		0.028

Table.2 Effect of treatments on per cent disease incidence and per cent disease severity of *Neopestalotiopsis clavispora*

Treatment No.	Treatments (foliar spray)	Conc (%)	7 days after inoculation		10 days after first spray		10 days after second spray	
			*PDI	*PDS	*PDS	Per cent disease reduction over control	*PDS	Per cent disease reduction over control
T_1	Control	-	55.98	37.17	39.79	-	43.43 (6.56)[a]	-
T_2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	43.39	31.80	23.27	39.20	13.20 (3.91)[b]	69.60
T_3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	51.63	28.1	19.43	51.16	11.12 (3.39)[bc]	74.39
T_4	Copper hydroxide 77WP (Kocide)	0.2	43.71	35.17	25.59	35.68	11.37 (3.52)[b]	73.81
T_5	Propineb 70 WP(Antracol)	0.3	47.33	33.58	23.76	40.28	9.54 (3.89)[b]	78.03
T_6	*Trichoderma asperellum*	2	35.45	23.6	18.53	53.43	10.52 (2.28)[c]	75.77
	CD (0.05)			NS	NS		1.139	
	CV			29.08	29.93		28.72	

*Mean of Replications
In each column figure followed by same letter do not differ significantly according to DMRT.
√x+0.5 transformed values are given in parentheses
PDS- Per cent disease severity, PDI- Per cent diseases incidence

Plate.1 *In vitro* evaluation of fungicides and biocontrol agents



Trichoderma asperellum

Plate.2 Challenge inoculation of pathogen

Plate.3 Symptom appearance

In vivo evaluation of fungicides and bioagents

Challenge inoculation of the leaf blight pathogen, *Neopestalotiopsis clavispora*, on two month old strawberry plants revealed that the severity of infection ranged from 35.45 to 55.98 per cent with maximum incidence in T_1 (Control). Treatments were applied twice at 10 days interval. Though there was no significant difference between the treatments seven days after challenge inoculation, the maximum disease reduction was recorded with T_6 (*Trichoderma asperellum*) (2%) (53.43%) and T_3 (carbendazim 12% + mancozeb 63%) (0.2%) (51.16%) followed by T_5 (propineb 70WP) (0.3%) (40.28%) and T_2 (cymoxanil 8% + mancozeb 64) (0.2%) (39.2%) and least per cent disease reduction was noticed with T_4 (copper hydroxide 77WP) (0.2%) (35.68%). However, after the second fungicidal application, a significant difference was recorded among the treatments with a per cent reduction of 78.03 with T_5 (propineb 70WP), followed by T_6 (*Trichoderma asperellum*) (75.77 per cent) and T_3 (carbendazim 12% + mancozeb 63%) showing 74.39 per cent reduction over control. Minimum disease severity was noticed with the treatment T_2 (cymoxanil 8% + mancozeb 64) (0.2%). Moustafa *et al.*, (2015) noticed that propineb and copper hydroxide could manage the disease caused by *Pestalotia psidii* only upto 60 per cent in guava. Antu (2013) conducted an experiment in guava infected with *Pestalotia* where he observed the effectiveness of Saaf and Curzate M8 showing a PDS of less than 18 per cent. According to Shin *et al.*, (2010) copper hydroxide and carbendazim are highly insensitive against *Pestalotiopsis longisetula* and *P. theae.* While, Sanjay *et al.*, (2008) observed only 22.6 per cent disease incidence when treated with the combination fungicide, Companion and 20.7 per cent each with mancozeb, carbendazim and copper oxychloride against *Pestalotiopsis theae*. Carre-Missio *et al.*, (2010) described the efficacy of mancozeb in reducing the infection of *Pestalotiopsis longisetula* in strawberry. The efficacy of the biocontrol agent, *Trichoderma viride* was also reported by Sanjay *et al.*, (2008) against *Pestalotiopsis theae* of tea.

Hence, the present study suggests that the contact fungicide Propineb 70 WP and the combination product of carbendazim 12% + mancozeb 63% exhibited highest per cent disease reduction of 78.03 and 74.39 respectively whereas the bioagent, *Trichoderma asperellum* could reduce the severity under *in vivo* conditions by 75.77 against *Neopestalotiopsis* leaf blight. This shows that the results of the *in vitro* study with various fungicides and biocontrol agents will not always be in consonance with the observations under *in vitro* conditions. However, the results of the research have depicted a clear picture on the management with different plant protection chemicals and bioagents on the leaf blight disease inflicting strawberry plants though multilocational trials should be conducted in various agroclimatic conditions in different strawberry growing tracts of Kerala so as to prove the efficacy of fungicides and biocontrol agents.

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