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In-vitro Evaluation of Bioagents, Botanicals and Fungicides against Leaf Spot of Clove Caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc

S Aravindasamy and R Kannan*

Department of Plant Pathology,

Agricultural College and Research Institute (TNAU), Killikulam, Vallanad - 628 252, Tamil Nadu, India

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A B S T R A C T

Clove (*Syzygium aromaticum* L.) being an important spice and medicinal crop. It is being affected by several fungal diseases among which leaf spot caused by *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. is important pathogen. Among them, leaf spot disease causes considerable loss both in quality and quantity. An investigation to in vitro evaluates the different effective botanicals, fungicides and biocontrol agents were conducted to manage the fungal disease leaf spot of clove. Among the bioagents, *Trichoderma harzianum* was found effective with 72.22% growth inhibition. Among the botanicals, garlic extract at 5% and 10% concentration levels was found to be effective with 56.66 and 62.66% growth inhibition respectively followed by ginger extract 48.88 and 49.66% growth inhibition. Among chemicals, propiconazole (0.1% and 0.2%) was found to be the most effective fungicide in inhibiting 100% growth of *C. gloeosporioides* followed by hexaconazole with 87.44 and 88.55% growth inhibition at 0.1% and 0.2% respectively.

Key words: *Colletotrichum gloeosporioides*, Fungicides, Botanicals, Bioagents

The clove of commerce is the aromatic, dry, fully grown, but un-opened flower buds of the clove tree (*Syzygium aromaticum*) (Family: Myrtaceae). Clove grows well in rich loamy soils of the humid tropics and can be grown successfully in the red soils of the midlands of Kerala as well as in the hilly terrain of Western Ghats at higher elevations in Tamil Nadu. Leaf spot disease incidence in clove seedlings and grown up trees is a serious concern. Black to brown water soaked lesions appeared on the leaves with circular yellow halo margin. In advanced stage, these spots enlarged, coalesced and resulted in bigger patches. Severely affected leaves wither, droop down and dry up. In nursery seedlings, die back symptoms were observed. Twigs were infected as the symptoms extended from the leaves through petioles. The affected branches stand without leaves or only with young leaves at tips. The objective of the present study was to screen potential biocontrol agents, botanicals and fungicides for the inhibition of the leaf spot causing pathogen under *in vitro* condition.

MATERIALS AND METHODS

Effect of the growth of C. gloeosporioides– Dual culture technique (Fleming *et al*. 1975)

The antagonistic effect of *Pseudomonas* sp. and *Bacillus* sp. was tested against the *C. gloeosporioides.* Nine mm PDA culture disc of the pathogen was cut individually from seven-day old culture. This was placed at one side on the sterilized PDA previously plated in sterilized Petri dish. The pathogen was allowed to grow for three days. Actively growing *Pseudomonas* sp. and *Bacillus* sp. cultures were separately streaked on the opposite side of the pathogen after three days. Culture disc of *Trichoderma* spp. were placed on the opposite side of the plates. Three replications for each treatment and suitable controls were maintained. The plates were incubated at room temperature (28 ± 2°C) for seven day. The mean diameter of the mycelial growth was measured and the results were expressed in terms of per cent inhibition of the mycelium over control (Vincent 1947).

***Corresponding author:** Dr. R. Kannan, Professor and Head, Department of Plant Pathology, Agricultural College and Research Institute (TNAU), Killikulam, Vallanad - 628 252, Thoothukudi District, Tamil Nadu

e-mail: aravind3695@gmail.com | **Contact:** +91- 9489730880

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

Where, I = Per cent inhibition over control
C= Growth in control
T= Growth in treatment

Effect of botanicals on the growth of C. gloeosporioides – poisoned food technique

The botanical extract solutions were mixed with PDA medium to obtain 5 and 10 per cent concentrations. Nine mm actively growing PDA culture disc of *C. gloeosporioides* was cut by means of a sterilized cork borer and placed at the centre of the medium. The plates were incubated at room temperature (28 ± 2°C). PDA without botanical extract served as control. Three replications were maintained for individual treatment. The radial growth of the mycelium was measured in treatments on 10th day after inoculation when the fungus was fully grown (9 cm) in the control plate. The mean diameter of the mycelial growth of the pathogens was recorded and the results were expressed in terms of per cent inhibition of mycelium over control.

In-vitro efficacy of fungicides against the pathogens – Poisoned food technique

Under *in vitro* condition, different fungicides viz. Hexaconazole 5% EC(Contaf), Propiconozole 25% EC (Tilt), Metalaxyl 4% + Mancozeb 64% WG (Ridomil), Mancozeb 75% WP (Indo Fil M-45), Tebuconazole 50% + trifloxystrobin 25% (Nativo), Azoxystrobin 23% EC (Amistar), Carbendazim 12% + Mancozeb 63% WP (SAAF) and Carbendazim 75% WP (Bavistin) were tested against the *C. gloeosporioides.* 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 Petri plates and repeated thrice for each treatment. Potato dextrose agar without fungicides served as control. Each plate was inoculated with a 9 mm mycelial disc of the pathogen taken from 7-day old culture. The inoculated plates were incubated at room temperature. The colony diameter was recorded and per cent inhibition in each treatment over control was calculated using the formula (Vincent 1947) as described above.

RESULTS AND DISCUSSION

In-vitro evaluation of biocontrol agents against C. gloeosporioides - (Dual culture technique)

There were significant differences among the two fungal bioagents. *T. harzianum* recorded the maximum fungal growth inhibition (72.22%) followed by *T. viride* (67.44%). Among the bacterial bioagents, *Bacillus* sp. (55.22%) was found superior followed by *P. fluorescens1* (40.77%). *Bacillus* sp, was found to be more effective in mycelial growth inhibition than *P. fluorescens 1*. Fungal bioagents *T. viride, T. harzianum* isolates were found superior than bacterial biocontrol agents (Table 1). The biocontrol agent, *T. harzianum* isolate was best in inhibiting the mycelial growth of *C. gloeosporioides* followed by *T. viride* whereas *P. fluorescens* exhibited the lowest inhibition against *C. gloeosporioides* as far as the present study is concerned. In this present study *T. viride* and *T. harzianum* isolates were found to be effective in inhibiting the mycelial growth of *C. gloeosporioides* while *T. asperellum* showed low inhibition followed by *Bacillus cereus* (52.52.4%). Significant myco-parasitism between *T. viride, T. harzianum* and *T. asperellum* and anthracnose fungus leading to lysis of pathogen hyphae was also observed *in vitro*. This is agreement with the findings of Mandhare *et al*. (1996), Prashanth *et al*. (2008), Devamma *et al*. (2012), Pandey *et al*. (2012). Saju *et al*. (2012) reported that *Pseudomonas* sp was the most effective antagonist to inhibit mycelial growth of *C. gloeosporioides*.

Table 1 *In vitro* evaluation of biocontrol agents against *C. gloeosporioides* - (Dual culture technique)

Bioagents	Mycelial growth of pathogen (cm)*	Percent inhibition over control (%)
T_1: *Pseudomonas fluorescens 1*	5.33[b]	40.77
T_2: *Bacillus sp*	4.03[c]	55.22
T_3: *Trichoderma viride*	2.93[d]	67.44
T_4: *Trichoderma harzianum*	2.50[e]	72.22
T_5: Control	9.00[a]	0.00
CD(P=0.05)	0.57	

*Mean of three replications
The treatment means are compared using Duncan Multiple Range Test (DMRT)
In a column, means followed by a common letter (s) are not significantly different (P=0.05)

Efficacy of botanicals on the growth of C. gloeosporioides in vitro at 5% and 10% Concentrations

Among the ten plant extracts tested at 5 per cent concentration, maximum percent inhibition of mycelial growth (56.66%) was recorded in garlic bulb extract which was significantly superior to all other treatments, followed by ginger rhizome extract (48.88%). The neem leaf extract recorded minimal mycelial growth inhibition (7.11%) of *C. gloeosporioides*. At 10 per cent concentration of plant extracts, maximum inhibition (62.66%) of mycelial growth was recorded in garlic bulb extract followed by ginger rhizome extract (49.66%). The least mycelial inhibition (12.66%) was found in neem leaf extract (Table 2). In this present study, bulb extract of garlic was found effective against *C. gloeosporioides* followed by rhizome extract of ginger. Three plant extracts namely Nagadhale, Simarouba and Lantana leaf extract showed more than 60 per cent inhibition of mycelial growth at 20% concentration.

Effectiveness of Neem, Tulsi, Lantana and Pongamia leaf extract against *C. gloeosporioides* was also studied by many authors viz. Jadhav *et al*. (2008), Vinod *et al*. (2009), Watve *et al*. (2009), Mukherjee *et al*. (2011), Ademe *et al.* (2013).

Table 2 Efficacy of botanicals on the growth of *C. gloeosporioides in vitro* 5% and 10% Concentration

Treatment	Common name	Parts used	Mycelial growth (cm)*	Per cent inhibition over control (%)	Mycelial growth (cm)*	Per cent inhibition over control (%)
			5 % concentration		10 % concentration	
T_1	Prosphis	Leaf	6.16[d]	31.55	6.03[d]	33.00
T_2	Pungam	Leaf	5.26[e]	41.55	5.03[e]	44.11
T_3	Garlic	Bulb	3.90[g]	56.66	3.36[g]	62.66
T_4	Ginger	Rhizome	4.66[f]	48.88	4.53[f]	49.66
T_5	Bougainvillea	Leaf	7.13[c]	20.77	6.73[c]	25.22
T_6	Neem	Leaf	6.03[d]	33.00	5.80[d]	35.55
T_7	Onion	Bulb	8.36[b]	7.11	7.86[b]	12.66
T_8	Tulsi	Leaf	8.06[b]	10.44	7.60[b]	14.44
T_9	*Coleus forshikohli*	Leaf	7.16[c]	20.44	6.90[c]	23.33
T_{10}	*Coleus forshikohli*	Rhizome	6.13[d]	31.88	5.83[d]	35.22
T_{11}	Control		9.00[a]	0.00	9.00[a]	
	CD(P=0.05)		0.60		0.47	

*Mean of three replications

The treatment means are compared using Duncan Multiple Range Test (DMRT)

In a column, means followed by a common letter (s) are not significantly different (P=0.05)

In vitro evaluation of fungicides against C. gloeosporioides at 0.1% and 0.2% concentrations (Poison food technique)

Totally eight fungicides were tested at 0.1 and 0.2 per cent concentrations against the mycelial growth of *C. gloeosporioides* under *in vitro.* Maximum inhibition of pathogen growth was observed in plates incorporated with propiconazole (100%) and hexaconazole (87.4% and 88.5%) at 0.1and 0.2 per cent concentrations and were significantly superior to other fungicides and followed by Carbendazim 12% + Mancozeb 63% WP (86.6% and 87.4%). The least per cent inhibition of fungus was recorded in Carbendazim 50% WP (4.11% and 4.44%) (Table 3). In this present study, hexaconazole and propiconazole even at 0.1% concentration were found effective against *C. gloeosporioides.* Most of the fungicides viz. Hexaconazole, Propiconazole, Penconazole, Tebuconazole, Carbendazim, Azoxystrobin, Difenoconazole, Thifluzamide, Trifluoxystrobin inhibited maximum mycelial growth at 0.2% but decreased with reduced concentration such as 0.05 and 0.1 per cent. These observations are in accordance to the findings of Patel (2009), Vinod *et al*. (2009), Devamma *et al*. (2012), Pandey *et al*. (2012), Saju *et al*. (2012).

Table 3 *In vitro* evaluation of fungicides against *C. gloeosporioides* at 0.1% and 0.2% concentration (Poison food technique)

Treatment	Fungicide	Mycelial growth (cm)*	Per cent inhibition over control (%)	Mycelial growth (cm)*	Per cent inhibition over control (%)
		0.1% concentration		0.2% concentration	
T_1	Hexaconazole 5 % EC	1.13[g]	87.44	1.03[e]	88.55
T_2	Propiconazole 25 % EC	0.00[h]	100	0.00[f]	100
T_3	Metalaxyl 4% + Mancozeb 64% WG	7.63[c]	15.22	7.23[c]	19.66
T_4	Azoxystrobin 23% EC	7.33[d]	18.55	7.03[c]	21.88
T_5	Tebuconazole 50% + Trifloxystrobin 25% WG	1.40[f]	84.44	1.20[e]	86.66
T_6	Carbendazim 50% WP	8.63[b]	4.11	8.60[b]	4.44
T_7	Mancozeb 75% WP	3.23[e]	64.11	2.90[d]	67.77
T_8	Carbendazim 12% + Mancozeb 63% WP	1.20[g]	86.66	1.10[e]	87.44
T_9	Control	9.00[a]	0.00	9.00[a]	0.00
	CD(P=0.05)	0.46		0.41	

*Mean of three replications

The treatment means are compared using Duncan Multiple Range Test (DMRT)

In a column, means followed by a common letter (s) are not significantly different (P=0.05)

The experiments conducted to know the efficacy of bioagents under *In vitro* condition revealed that *Trichoderma harzianum* recorded the maximum growth inhibition (72.22%) of *Colletotrichum gloeosporioides* followed by *Trichoderma viride* (67.44%). Significant mycoparassitism between the *T. viride* and *T. harzianum*

fungi leading to the lysis of pathogen hyphae was also observed. Among the bacterial bioagents, *Bacillus* sp. was found more effective in inhibiting the mycelial growth of the pathogen as compared with *Pseudomonas fluorescens* 1. This is in contrast with Saju *et al.* (2012) who reported that *Pseudomonas* sp. was most effective against *Colletotrichum gloeosporioides*. In this present study, bulb extract of garlic (10%) was found effective against the pathogen by recording 62.66 per cent inhibition followed by rhizome extract of ginger (49.66%).

As far as fungicides are concerned, Propiconazole 25% EC was found superior by recording 100 per cent inhibition at 0.1 per cent concentration. In order to reduce the usage of chemicals in the field, the bioagents may be preferred in the plantations though they are next to fungicides in inhibiting the pathogen.

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