

EPIDEMIOLOGY AND MANAGEMENT OF *COLLETOTRICHUM GLOEOSPORIOIDES* CAUSING LEAF SPOT OF STRAWBERRY (*Fragaria x ananassa* Duch.) IN KERALA

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KEYWORDS

C. gloeosporioides
Trichoderma asperellum
Bordeaux mixture
carbendazim

Received on :
15.02.2020

Accepted on :
02.05.2020

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ABSTRACT

A fungal leaf spot caused by *Colletotrichum gloeosporioides* was found as a major problem in three strawberry growing tracts of Kerala viz., Malappuram, Idukki and Wayanad. Wayanad recorded highest Per Disease Index (PDI) and severity of 52.90 and 22.80 in December-January followed by 52.40 and 23.40 in March-April in Malappuram. Disease severity was negatively correlated with humidity (-0.868) and rainfall (-0.919) in Wayanad and positive correlation of 0.501 and 0.541 in Malappuram. Three isolates were obtained and brought into culture. *In vitro* management when carried out with nine fungicides, Saaf, Antracol, Bavistin and Curzate M8 recorded 100 per cent inhibition, 70.77 to 100 per cent in case of Score, 77.77 to 100 in case of Fytolan and Bordeaux mixture. Akomin 40 recorded least inhibition ranging from 11.11 to 43.88 per cent. Biocontrol agents *Trichoderma asperellum* and *Pseudomonas fluorescens* recorded 100 and 66-70 per cent control. A pot culture experiment was conducted with *C. gloeosporioides* isolate from Wayanad using strawberry variety Winter dawn. *T. asperellum* followed by carbendazim 12% + mancozeb 63% recorded 88.07 and 86.48 per cent control which can be followed for efficient management of the disease under field conditions. Thus, *T. asperellum* and fungicide carbendazim 12% + mancozeb 63% is effective in reducing the leaf spot under *in vivo* conditions.

INTRODUCTION

Strawberry is a small fruit crop of worldwide importance native to temperate regions. In India, the crop is widely cultivated in tropical as well as hilly regions. Presently there has been a huge increase in strawberry cultivation, but the cultivation is heavily fluctuating due to heavy pest and disease attack. There is little information regarding the fungal diseases of strawberry. Strawberry anthracnose by *Colletotrichum* spp. is a pathogen causing serious damage. Brooks (1931) first described a disease incited by *Colletotrichum fragariae* in strawberry runners from Florida and called it as anthracnose. Black to greyish circular leaf spots on leaves was observed by Howard *et al.* (1983) and Tanaka *et al.* (1996) along with anthracnose symptoms on stolons and petioles. Murthy and Pramanick (2012) noticed *Colletotrichum acutatum* infection from India. Several findings suggest that the disease is closely related to weather parameters. Smith and Black (1987) observed a high disease severity at high temperature and 100 per cent RH with a positive correlation. Similarly, Wilson *et al.* (1992) reported maximum disease incidence of *C. acutatum* during March, where the temperature ranged from 11.86 to 23.9°C, relative humidity of 61-94 per cent and rainfall of 62.9mm.

An integrated disease management approach with cultural, biological, organic, physical methods, use of resistant varieties and chemicals are commonly in practice accomplished for better disease management. Although many practices are present for the management, farmers mostly rely on use of chemicals and bio-control agents. Freeman *et al.* (1997)

observed good control over anthracnose by *C. acutatum* using prochloraz-Zn and prochloraz-Mn alone or in combination with folpet than captan, difenoconazole and propiconazole. Similarly, Munoz (2002) noticed the effectiveness of propiconazole, bitertanol, hexaconazole, imazalil, carbendazim and thiabendazole against *C. acutatum* CECT 20240 under *in vitro*. Karande *et al.* (2007) noticed least per cent inhibition of mancozeb and Bordeaux mixture and complete inhibition of Bavistin, copper oxychloride and Tilt over *C. gloeosporioides*. According to Filoda (2008), Sarfun 500 SC (Carbendazim) and Amistar 250 SC (Azoxystrobin) limited the growth and development of *C. gloeosporioides*. Although different works have been carried out internationally, little works are there nationally and no work has been carried out till now in Kerala. Studies on epidemiological parameters leading to disease spread and use of chemical fungicides and Biocontrol agents are of immense use in understanding the nature of the disease and its management. Therefore, the present work was envisaged to explore the common weather factors that can lead to disease development and to know the feasible fungicides for effective and efficient management of anthracnose in strawberry under Kerala conditions.

MATERIALS AND METHODS

Survey, assessment of disease incidence and correlation with weather parameters

Intensive surveys were carried out from 2015 to 2017 in December-January, March-April and July- August from in

different strawberry growing tracts of Wayanad, Idukki and Malappuram district. The isolates were designated along with the name of location, ie, LSW for leaf spot pathogen isolated from Wayanad, LSI from Idukki and LSM from Malappuram. The PDI was calculated using the formula given by Wheeler (1969).

$$\text{Per cent disease incidence(PDI)} = \frac{\text{Number of plant infected}}{\text{Total number of plant observed}} \times 100$$

The per cent disease severity (PDS) was assessed in case of all

Table 1: Score chart for assessing the disease severity

Grade	Description
0	No symptom
1	≤ 1 per cent leaf area infected
2	> 1-10 per cent leaf area infected
3	> 10-25 per cent leaf area infected
4	> 25-50 per cent leaf area infected
5	> 50 per cent leaf area infected

foliage diseases following a standard score chart of 0-5 scale given by Sharma and Kolthe (1994) as depicted in Table 1.

Per cent disease incidence was calculated using the formula:

Weather parameters like relative humidity (RH), temperature and rainfall was noted throughout the survey period (December-January, March-April and July- August) and correlation analysis was carried out to study the spread of disease.

In vitro evaluation of fungicides, organic preparations and Biocontrol agents

Nine selected fungicides at recommended, next higher and next lower concentrations was evaluated under in vitro against the fungal isolates by poison food technique suggested by Nene and Thapliyal, 1993. 100 ml Potato Dextrose Agar (PDA) was prepared aseptically in conical flasks and desired concentration was made by adding respective fungicides and organic preparations. Twenty five ml of the respective poisoned medium was plated onto sterile Petri dishes of 90 cm diameter and 8mm mycelial disc of test pathogen was placed at the centre under aseptic conditions and incubated. Efficacy of biocontrol agents *Trichoderma asperellum* and *Pseudomonas fluorescens* were tested by following dual culture technique (Dennis and Webster, 1971). Test fungus and *T. asperellum* was placed 2cm away from the periphery of Petri plate on opposite sides and the bacterial biocontrol agent was streaked on both sides 2cm away from the periphery and test pathogen was kept at the centre. The per cent inhibition of mycelial growth in each treatment was calculated using the formula suggested by Vincent (1947).

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

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

Pot culture evaluation of fungicides and biocontrol agents

The isolate with higher PDS (LSW) during survey was chosen for pot culture experiment. Two month old strawberry plants of variety Winter dawn was grown in pots at net house of Department of Plant Pathology. Four best fungicides and one bioagent were chosen from in vitro experiment and thus experiment was laid as CRD with six treatments including control each replicated ten times. Plants were initially kept in humid chamber for 48hr prior to inoculation for maximum disease development. Spore suspensions of each pathogen was prepared by gently scraping the pure culture and mixed with 100ml sterile water, which was then filtered through a muslin cloth to get a final spore count of 2×10^6 spores ml⁻¹ (Denoyes and Baudry, 1995). Suspension was sprayed onto healthy leaves using a hand atomizer after making injuries using sterile needles and then incubated in a moist humid chamber. Control plants were maintained by treating with sterile water. Biocontrol agent was inoculated ten days before pathogen inoculation and fungicides were sprayed on 10 days interval after pathogen inoculation and PDS was calculated using the score chart to know the efficacy of the treatments.

RESULTS AND DISCUSSION

Survey, assessment of disease incidence and correlation with weather parameters

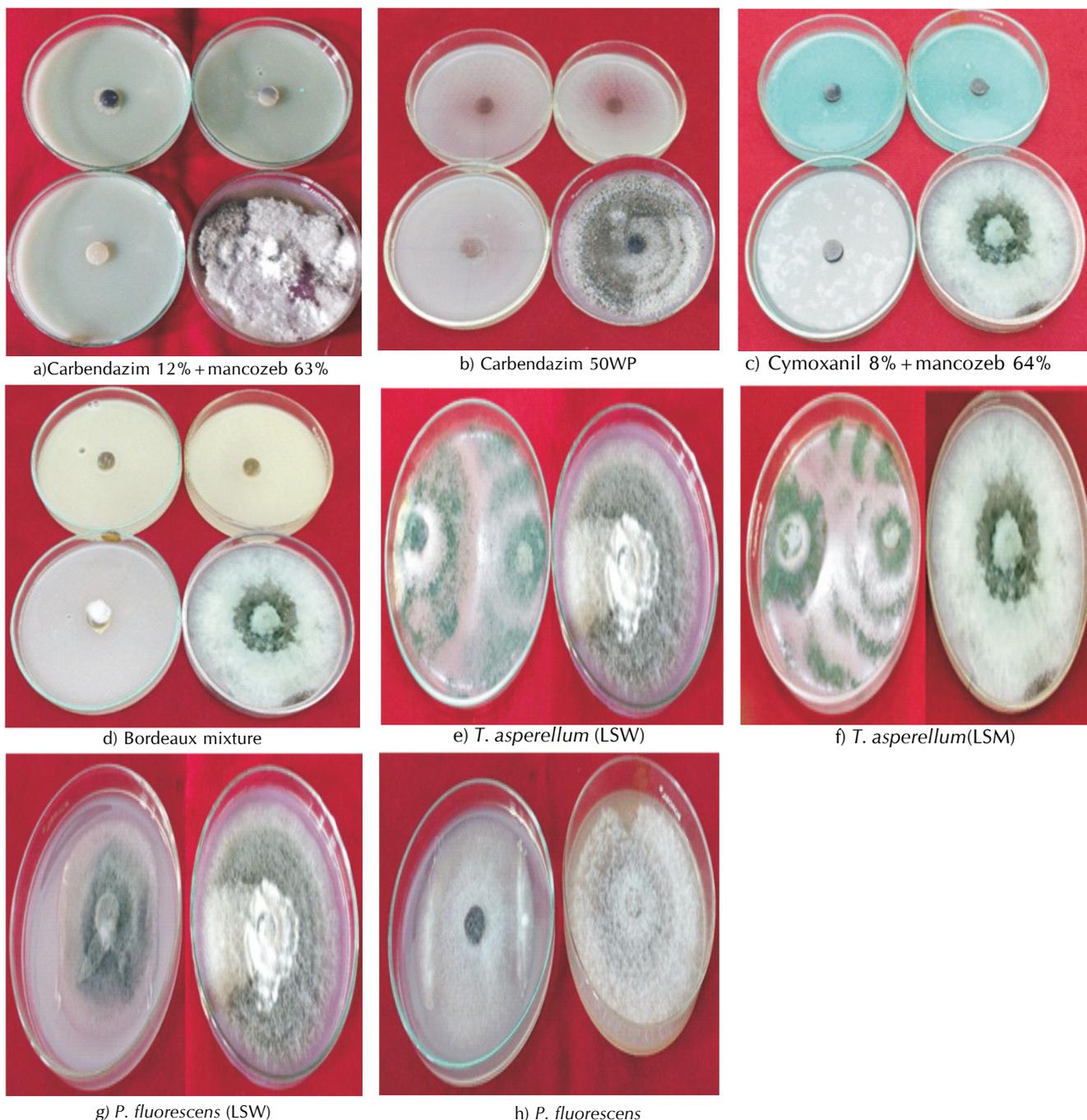
Leaf spot caused by *Colletotrichum gloeosporioides* was of common occurrence in all three districts viz., Wayanad, Idukki and Malappuram. Correlation studies of *C. gloeosporioides* with weather parameters depicted no relation with temperature at Wayanad and Malappuram. However, negative correlation existed with relative humidity (-0.868) and rainfall (-0.919) in Wayanad and positive correlation of 0.501 and 0.541 respectively in Malappuram. At Idukki temperature (0.872) and relative (0.501) humidity showed a positive correlation.

Ambalavayal recorded a higher incidence and severity of 52.9 and 22.8 per cent respectively during December-January, 43.30 and 21.78 per cent during March-April and 32.30 and 15.37 per cent during July-August and here, increase in RH and rainfall plays an important role in reducing the disease severity over the seasons as it is negatively correlated with the disease (Table 2). The results were in accordance with Ji *et al.* (2012), where he opined that the pathogen *C. fragariae* infecting strawberry spreads at a temperature of 25-30°C in rainy days of midsummer.

LSI in Idukki recorded a maximum incidence and severity of

Table 2: Per cent disease incidence and severity of leaf spot of strawberry

Sl. No	Location	Disease	Period					
			Dec- Jan		March-April		July- August	
			PDI	PDS	PDI	PDS	PDI	PDS
1	Wayanad	LSW	52.9	22.8	43.3	21.78	32.3	15.37
2	Idukki	LSI	29.6	10.4	40.2	19.9	23.6	11.4
3	Malappuram	LSM	32.3	21.4	52.4	23.4	49.6	20.9



40.20 and 19.9 per cent during March-April and a minimum severity of 10.4 per cent in December-January when temperature was 24.04°C and 19.74°C respectively depicting a positive correlation with temperature and rainfall. The relative humidity was observed as 95.48 during March-April. Smith and Black (1987) observed a high disease severity of *C. fragariae* in strawberry at a high temperature of 35°C and cent per cent relative humidity, showing a positive influence of temperature and RH on disease development. Similarly, Hang *et al.* (2007) observed that the optimum temperature required for *C. gloeosporioides* to cause infection in strawberry is 28-32°C along with a relative humidity of above 90 per cent

which was not comparable with the results of present study. Average rainfall recorded during the survey was 103.11 mm. LSM in Anakkayam recorded a positive correlation with RH and rainfall where maximum severity of 23.4 per cent was observed during March-April at a higher temperature of 29.2°C and lower RH and rainfall of 48.58 per cent and 4.6 mm. It was interesting to note that a relative humidity of 66.96 per cent and a heavy rainfall of 504 mm resulted in lower severity of 20.9 per cent during July-August. However, the above findings were closely supported by Wilson *et al.* (1992), where they reported maximum disease incidence of *Colletotrichum acutatum* during March when the temperature ranged from

Table 3: In vitro evaluation of fungicides against *Colletotrichum gloeosporioides*

Sl No.	Fungicide	Conc (%)	Per cent Inhibition over control		
			<i>Colletotrichum gloeosporioides</i> (LSW)	<i>Colletotrichum gloeosporioides</i> (LSI)	<i>Colletotrichum gloeosporioides</i> (LSM)
1	Carbendazim 12% + Mancozeb 63% (Saaf)	0.15	100(10) a	100(10) a	100(10) a
		0.2	100(10) a	100(10) a	100(10) a
		0.25	100(10) a	100(10) a	100(10) a
2	Cymoxanil 8% + Mancozeb 64% (Curzate M8)	0.15	97.67(9.86)b	93.44(9.66)d	100(10) a
		0.2	100(10) a	95.44(9.76)c	100(10) a
		0.25	100(10) a	97.67(9.89)b	100(10) a
3	Copper hydroxide 77WP (Kocide)	0.1	100(10) a	46.67(6.84)m	100(10) a
		0.15	100(10) a	47.22(6.88)m	100(10) a
		0.2	100(10) a	50(7.1)l	100(10) a
4	Copper oxychloride 50WP (Fytolan)	0.2	100(10) a	83.33(8.7)i	77.22(8.81)e
		0.25	100(10) a	86.67(8.9)f	80.33(8.98)c
		0.3	100(10) a	100(10)a	83.77(9.19)b
5	Propineb 70WP (Antracol)	0.25	100(10) a	93.33(9.68)d	84.44(9.22)b
		0.3	100(10) a	100(10) a	100(10) a
		0.35	100(10) a	100(10) a	100(10) a
6	Carbendazim 50WP (Bavistin)	0.05	100(10) a	100(10) a	100(10) a
		0.1	100(10) a	100(10) a	100(10) a
		0.15	100(10) a	100(10) a	100(10) a
7	Difenoconazole 25EC (score)	0.05	100(10) a	70.77(8.39)k	79.44(8.93)d
		0.1	100(10) a	72.77(8.54)j	100(10) a
		0.15	100(10) a	82.44(9.1)e	100(10) a
8	Potassium phosphonate (Akomin 40)	0.25	42.67(6.52)f	11.11(3.35)p	19.44(4.49)h
		0.3	43.33(6.61)e	12.44(3.51)o	26.67(5.1)g
		0.35	43.88(6.73)d	13.67(3.72)n	31.67(5.65)f
9	Bordeaux Mixture	0.5	88.88(9.38)c	77.77(8.8)h	100(10) a
		1	100(10) a	78.88(8.86)g	100(10) a
		1.5	100(10) a	79.44(8.93)f	100(10) a
	CD (0.05)		0.044	0.042	0.036

Table 4: Effect of treatments on disease incidence and severity of *Colletotrichum gloeosporioides*

Trea tment No.	Treatments (foliar spray)	Conc (%)	8 days after inoculation		10 days after first spray		10 days after second spray	
			PDI	PDS	PDS	*PDR over control	PDS	*PDR over control
T1	Control	-38	19.2 (4.35)a	23.5 (4.83)a	-	27.83	-	
T2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	18.13 (3.7)a	14.41 (3.12)a	10.21	56.55 (5.26)a	6.21	77.68
T3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	14.77 (3.02)ab	9.29 (2.47)bc	6.16	73.78 (1.99)bc	3.76	86.48
T4	Copper hydroxide 77WP (Kocide)	0.2	14.35 (3.19)ab	11.38 (2.76)bc	8.53	63.7 (2.19)bc	5.34	80.81
T5	Propineb 70 WP(Antracol)	0.3	25.28 (3.89)a	19.16 (3.34)b	13.54	42.38 (2.76)b	9.31	66.54
T6	Trichoderma asperellum	2	7.98	7.84 (1.92)ab	5.02 (1.77)c	78.63	3.32 (1.53)c	88.07
	CD (0.05)	--	-	1.49	1.31		1.089	
	CV			44.19	42.5		39.89	

PDR- Per cent Disease Reduction

11.86 to 23.9°C, relative humidity of 61-94 per cent and rainfall of 62.9mm. According to them the disease is considered as a typical temperature and high humidity disease. Nevertheless, Pan and Mishra (2010) noticed a non-significant positive correlation with maximum relative humidity, temperature and total rainfall and significant positive results with minimum relative humidity, temperature and number of rainy days in case of *C. gloeosporioides* infecting guava.

In vitro evaluation of fungicides

In vitro evaluation of LSW pathogen with six fungicides viz., carbendazim 12% + mancozeb 63% (Saaf), copper hydroxide 77WP (Kocide), copper oxychloride 50WP (Fytolan), propineb 70WP (Antracol), difenoconazole 25 EC (Score) and carbendazim 50WP (Bavistin) revealed 100 per cent inhibition at all concentrations (Plate 1a). From the Table 3, it was



Plate 2: Challenge inoculation of pathogen



Plate 3: Symptom appearance

observed that cymoxanil 8% + mancozeb 64% (Curzate M8) and Bordeaux mixture at its lower concentration of 0.15 and 0.5 per cent inhibited the mycelial growth by 97.67 and 88.88 per cent whereas cent per cent inhibition was noticed at two higher concentrations. However, among various fungicides tested, potassium phosphonate (Akomin 40) showed the least inhibition of 42.67, 43.33 and 43.88 per cent at 0.25, 0.3 and 0.35 per cent concentration respectively.

In case of LSI, fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf) and carbendazim 50WP (Bavistin) recorded cent per cent inhibition of the pathogen. Also, propineb 70 WP (Antracol) at higher two concentrations of 0.3 and 0.35 per cent inhibited the mycelial growth by cent per cent (Plate 1b). These observations were closely followed by cymoxanil 8% + mancozeb 64% (Curzate M8) where concentrations of 0.15, 0.2 and 0.25 per cent restricted the fungal growth by 93.44, 95.44 and 97.67 per cent respectively. It was also noticed that difenoconazole 25 EC (Score), copper oxychloride 50WP (Fytolan) and Bordeaux mixture exhibited 70 to 80 per cent inhibition in the growth of the pathogen. However, fungicides like copper hydroxide 77WP (Kocide) and potassium phosphonate (Akomin 40) were found comparatively less effective against the pathogen.

Studies conducted on LSM-1 pathogen revealed that fungicides *viz.*, carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide), carbendazim 50WP (Bavistin) and Bordeaux mixture at all the three concentrations and highest two concentrations of Propineb 70WP (Antracol) and difenoconazole 25 EC (Score) showed cent per cent inhibition against the pathogen (Plate 1c and d). However, copper oxychloride 50WP (Fytolan) at 0.2, 0.25 and 0.3 per cent showed an inhibition of 77.22, 80.33 and 83.77 per cent respectively. Potassium phosphonate (Akomin 40) showed the least inhibition on the pathogen at all its concentrations.

Singh *et al.* (2008) reported that carbendazim and difenoconazole completely inhibited the pathogen *C. gloeosporioides* at a concentration 100 µg/ml when tested in vitro in guava. According to Filoda (2008), Carbendazim limited the growth and development of *C. gloeosporioides*. Only potassium phosphonate showed the least inhibition of

40 per cent and above at all concentrations tested. Prashanth *et al.* (2008) made similar findings regarding the ability of copper oxychloride and difenoconazole to inhibit *C. gloeosporioides* infecting pomegranate. Copper hydroxide 77WP and potassium phosphonate were found comparatively less effective. Patil *et al.* (2009) noticed 96.26 per cent control with mancozeb + carbendazim (0.2%) followed by carbendazim (0.1%) which showed 68.34 per cent control and copper oxychloride (0.3%) 64.88 per cent in long pepper. Akhter *et al.* (2009) observed 100 per cent inhibition of radial growth and sporulation of *C. gloeosporioides* in strawberry at 500, 1000, 1500 and 2500 ppm concentrations.

Saju *et al.* (2013) evaluated the effect of carbendazim, copper oxychloride, and carbendazim + mancozeb at different recommended concentrations. Copper oxychloride was found more effective with 91.9% inhibition of the pathogen at 0.3% than other two fungicides tested. Carbendazim at 0.5%, 0.1%, 0.15%, reduced the mycelial spread by 89.3, 91.7 and 92 per cent. Carbendazim + mancozeb showed significantly higher inhibition of the pathogen at 0.2% (90.3%).

Carbendazim + Mancozeb 0.25% was evaluated against soybean leaf spot pathogen *C. gloeosporioides* in vitro which recorded 93.15 per cent control and reduction in growth (Ingle *et al.* 2014). Ramani *et al.* (2015) reported cent per cent efficacy of *C. gloeosporioides* with carbendazim, copper oxychloride and the combination fungicide, carbendazim (12%) + mancozeb (63%), while 56.68 per cent inhibition was reported with Curzate M8 in banana.

Deepthi *et al.* (2015) reported an average growth inhibition of 62.10 per cent over *C. truncatum* in soybean with fungicide Saff. Rathva *et al.* (2017) isolated *C. gloeosporioides* from pointed gourd and tested the efficacy of some fungicides in which, carbendazim + mancozeb at 500 and 1000ppm could reduce the mycelial growth by 100 per cent, however difenoconazole reduced the growth upto 93.30 per cent at 1000ppm, followed by cymoxanil + mancozeb (73.23 %). But copper hydroxide and propineb showed 87.36 and 66.91 per cent inhibition at 2500 ppm.

Combination fungicide carbendazim 50% WP+ mancozeb 64% WP at 500, 1000, 1500 and 2500 ppm recorded 100 per cent inhibition followed by carbendazim 50% WP showing

91.92-100 per cent in Aonla. Whereas, difenoconazole 25% EC recorded an average inhibition of 90.33 per cent, followed by Copper oxychloride 50 % WP (89.20), Copper hydroxide 77 % WP (68.97) and Propineb 70 % WP (59.07) (Asalkar et al., 2019).

In vitro evaluation of biocontrol agents

Two reference cultures from KAU were evaluated against the isolated pathogens. It was observed that *T. asperellum* when evaluated against three isolates of the pathogens, they exhibited overgrowth mechanism of antagonism with cent per cent inhibition (Plate 1e and 1f). However, *P. fluorescens* could inhibit the pathogen only by 67.7 to 70 per cent (Plate 1g and 1h). Patil et al. (2009) recorded 70.42 per cent and 20.72 per cent inhibition of *C. gloeosporioides* with *T. viride* and *P. fluorescens* in long pepper. Conversely, Tasiwal et al. (2009), Ramani et al. (2015) and Tapwal et al. (2015) pointed out only less than 61 per cent control with *Trichoderma viride* and 42.87 per cent using *P. fluorescens* with *C. gloeosporioides* infecting papaya and banana, In congruence with the present results, Dev et al. (2016) observed 100 per cent efficacy with several isolates of *T. viride* and 47.6 per cent with *P. fluorescens* in pomegranate infected with *C. gloeosporioides*.

Pot culture evaluation of fungicides and biocontrol agents

In the present study, *C. gloeosporioides* was challenge inoculated to the leaves by spraying spore suspension to two month old strawberry plants maintained under ambient conditions (Plate 2 and 3). It was observed that the per cent disease severity, eight days after inoculation ranged from 7.98 to 25.28 per cent with highest in T5 (propineb 70WP) (0.3%) and least in T6 (*T. asperellum*) (2%). However, after the first treatment application a significant difference was noticed among various treatments, where T6 (*T. asperellum*) (2%) showed the maximum per cent reduction of 78.63 per cent, closely followed by T3 (carbendazim 12% + mancozeb 63%) (0.2%) recording 73.78 per cent reduction over control. The least per cent reduction of 42.38 per cent was noticed with T5 (propineb 70WP) (0.3%) compared with other treatments.

After two rounds of fungicide application, the treatment T6 (*T. asperellum*) (2%) showed the maximum reduction of 88.07 per cent followed by T3 (carbendazim 12% + mancozeb 63%) (0.2%) (86.48 per cent) and T4 (copper hydroxide 77WP) (0.2%) (80.81 per cent) which were on par with each other. These treatments were followed by T2 (cymoxanil 8% + mancozeb 64) (0.2%) and T5 (propineb 70WP) (0.3%) which depicted comparatively lower efficiency of 77.68 and 66.54 per cent respectively (Table 4). The efficacy of *Trichoderma* in soil may be attributed to the presence of proper amount of organic matter that lead to the multiplication and activation of spores. Moreover, according to Baker and Cook, (1982), Weltzein, (1991) and Ma et al. (2001), antagonists compete with the pathogen for infection sites, leaving limited space for pathogens to proliferate or they may secrete secondary metabolites on the plant surface, and may parasitize pathogens directly when applied prophylactically.

A perusal of literature revealed that antagonistic potential of different species of *Trichoderma* against *Colletotrichum* spp. Sivakumar et al. (2000) and Soyong et al. (2005), proved the efficacy of *Trichoderma* in rambutan and grapes. Patil et al.

(2009) observed 28.36 per cent reduction of *C. gloeosporioides* with *Trichoderma viride* in infected pepper plants. Karande et al. (2007) conducted a field experiment and observed that spraying of mancozeb + carbendazim (0.2%) was found effective leading to 33.38 per cent disease control followed by copper oxychloride (0.3%) and *T. viride* (6×10^7 CFU/ml) 30.10 and 28.46 per cent disease control. Similarly, the efficacy of field application of *Trichoderma* against *Colletotrichum lindemuthianum* in beans was recorded by Padder et al. (2010). Likewise, Sawant et al. (2012) recorded a subsequent reduction in *C. gloeosporioides* infection of grapevine foliage with *Trichoderma* sp. Diedhiou et al. (2014) recorded 84 percent control with mancozeb. Contrary to the above observations, Ingle et al. (2014) and Subedi et al. (2015) observed 42.19 and 56.37 per cent disease control with Saaf against *C. gloeosporioides* of soybean and pepper. While, Solanki (2015) observed comparable results with Saaf and propineb against *C. gloeosporioides* in mango showing 79.03 and 69.01 per cent efficacy over control. No much pot culture works have been carried out in strawberry to manage the disease.

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