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Research Article

Evaluation of fungicides for the management of Lasiodiplodia crown rot of strawberry (*Fragaria* × *ananassa* Duch.) in Kerala

P. Amrutha* and Reshmy Vijayaraghavan

Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala

Abstract

Strawberries are one of the most valued sweet fruit all over the world. During April and July 2016, strawberries grown under polyhouse conditions at Agricultural Research Station, Anakkayam in Malappuram district showed symptoms like complete wilting and withering of 82 and 75 per cent of plants. The infected crowns when cut open showed an internal reddish discoloration. Leaf petiole near the crown region also had a slight rotting. The fungus was then isolated from infected crown sections. Fungus produced initially white to smoky grey, dense mat like mycelium turning greyish black to deep black. Dark black pycnidia developed in twenty days which aggregated to form tough and round structures producing thick walled, brown, ellipsoid conidia with tapered apex and longitudinal striations. Single septa divided the conidia into two with both ends round with 23.54 - 32.17 x 12.75 -16.05 µm size. The morphology of the fungus was confirmed as *Lasiodiplodia theobromae* using ITS sequencing. *In vitro* fungicide testing with carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide) and carbendazim 50WP (Bavistin) recorded cent per cent inhibition at all three concentrations. Dual culture evaluation of *Trichoderma asperellum* inhibited the pathogen by 74.10 per cent overgrowth mechanism. Pot culture experiments revealed that carbendazim 12% + mancozeb 63% could control the disease by 87.5 per cent.

Keywords: crown rot, fungicides, *L. theobromae*, management, strawberry

***Correspondence**
Author: P. Amrutha
Email: amruthapvijayan8@gmail.com

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) known for its sweet and refreshing flavour is grown throughout India. United States followed by China and Spain are the leading strawberry producers in the world. Development of tropical varieties leads to its cultivation in high ranges like Munnar and Wayanad as well as plains of Kerala. This has lifted the cultivation all throughout the seasons. But, various fungal diseases are observed in the crop that has lead to serious yield loss. Complete crop loss occurs when the crown portion of the crop is affected. *L. theobromae* causing crown rot or die back is one among them. 11 per cent of the total strawberry in Turkey and Korea were infected with severe wilting and discoloration [1] and [2]. Works carried out internationally reveals the importance of the dreadful disease and no such work carried out in Kerala envisages the significance in characterising the pathogen and finding suitable management measures.

Materials and Methods

Assessment of disease incidence and its relation with weather parameters

Two rowing sample surveys were conducted in March-April and July-August at Agricultural research Station, Anakkayam, Malappuram. Plants showing definite symptoms were collected for isolation and the correlation with climatic parameters were calculated. Total number of plants infected was noted by randomly choosing 100 plants and Per cent Disease incidence (PDI) was calculated on the basis of formula given below [3].

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

Symptomatology and pathogenicity testing

Root and crown portion showing necrotic lesions were examined and symptoms were thoroughly noticed. After washing the affected tissues in running water 1cm bits were cut using sterile blade and disinfected with Sodium

hypochlorite solution (1%). Root bits were then placed onto Potato Dextrose Agar (PDA) for culturing using standard protocol. Obtained fungus was pure cultured and maintained at 4°C. Pathogenicity was carried out by preparing 2 X 10^6 spores mL^{-1} of the mycelial suspension and pouring it onto 3 week old strawberry plants [4]. Injury was made before the application of suspension.

Characterisation of the pathogen

Cultural and morphological characteristics of the isolated pathogen were recorded based on the colony colour, growth pattern and conidial colour, size and presence of septation. Conidial size was calculated by slide culture technique [5]. Genus level identification was done by sending the culture to National Centre for Fungal Taxonomy (NCFT) and molecular characterisation was carried out by ITS sequencing. Sequence analysis and nucleotide homology of pathogen were analysed through the BLASTn programme of NCBI (http://ncbi.nlm.nhm.gov/blast).

In vitro evaluation of fungicides and bio-control agents

Nine different fungicides like copper hydroxide 77WP (Kocide), copper oxychloride 50 WP (Fytolan), propineb 70WP (Antracol), difenoconazole 25 EC (Score), carbendazim 50WP (Bavistin) and Potassium phosphonate (Akomin 40), Bordeaux mixture and two combination fungicides *viz.,* cymoxanil 8% + mancozeb 64% (Curzate M8) and carbendazim 12% + mancozeb 63% (Saaf), at three different concentrations was evaluated by poison food technique (Zentmeyer, 1955) against the fungus. Similarly two bio-control agents *viz*., *T. asperellum* and *Pseudomonas fluorescens* selected from Kerala Agricultural University (KAU) were tested by dual culture method [6]. The per cent inhibition of mycelial growth was calculated using the formula given below [7].

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

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

Pot culture management of L. theobromae

A *in vivo* experiment was laid out to evaluate the efficacy of selected fungicides and bio-agents from *in vitro* studies against the pathogen. The pots planted with strawberry variety Winter dawn was grown at rain shelter conditions during December 2016 - January 2017 at College of Horticulture, Vellanikkara. The pathogen was inoculated in the root surface [4].

Results and Discussion

Disease assessment and correlation with weather parameters

Correlation studies revealed a positive influence of temperature on disease development. However, a negative correlation was noticed with RH and rainfall which indicated that the disease upsurge, when RH and rainfall decrease. This was evident from the incidence of the disease which decreased from 85 to 72 per cent when rainfall increased from 4.6 mm to 504 mm and RH from 48.58 per cent to 66.96 per cent. The influence of weather parameters on occurrence of *Botryodiplodia theobromae* in guava observed a negative correlation with relative humidity, maximum and minimum temperature and total rainfall [8].

Symptomatology and pathogenicity testing

Pathogen responsible for crown and root rot in strawberry was *L. theobromae*, in which the plants exhibited severe wilting and die back of runners with black rot on root and crown region (**Figure 1a** and **b**). Similar black necrotic discolouration on roots and crown of strawberry apart from the infection on leaf petiole was noticed [1] and [2]. Pathogenicity of the isolate was carried out by pouring the spore suspension to the root zone. Symptoms initiated as wilting and drooping of plants, 13 days after inoculation where the roots became black and crown tissue appeared brown in colour which is identical to that observed under natural conditions (**Figure 2**).

Characterisation of the pathogen

Crown and root rot pathogen isolated from Malappuram district produced white colonies that turned greyish black. These observations were in conformity with the observations of [1] and [9] where hyphae was septate with brown to

black colour *and* conidia single celled hyaline and brown when young, ellipsoid or obovate and thick walled, when mature (**Figure 3**). Dimension of conidia ranged from 23.54 - 32.17 x 12.75 -16.05 µm size (**Figure 4**). The above descriptions were also pointed out by [10] and [2] in mulberry plants infected by *L. theobromae.*

Figure 1 a and b Natural symptom

Figure 2 Symptom on artificial inoculation

Figure 3 Culture of *L. theobromae*

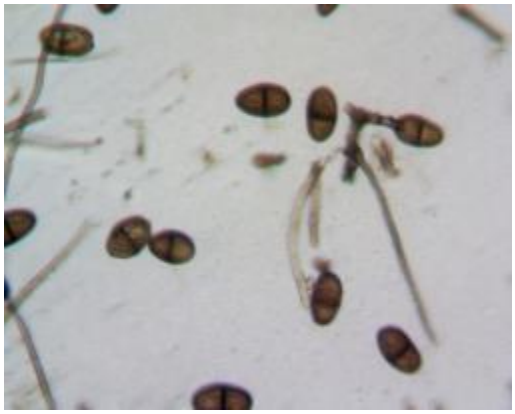


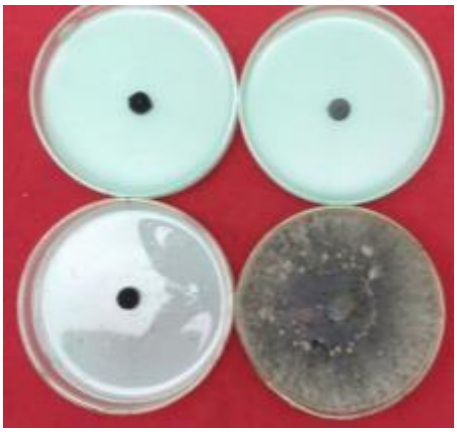
Figure 4 Conidia of *L. theobromae*

Sequence of *Lasiodiplodia* when compared with database retrieved from NCBI revealed that all of the sequences were homologous with *Lasiodiplodia theobromae* showing 100 per cent identity and 99 per cent query coverage. The strains of *Lasiodiplodia theobromae* were CMW25212, CF/UENF439, CF/UENF437, CF/UENF435, CF/UENF432, CF/UENF431 and CF/UENF430. Hence, the isolate was confirmed with the results of molecular characterisation as *Lasiodiplodia theobromae*.

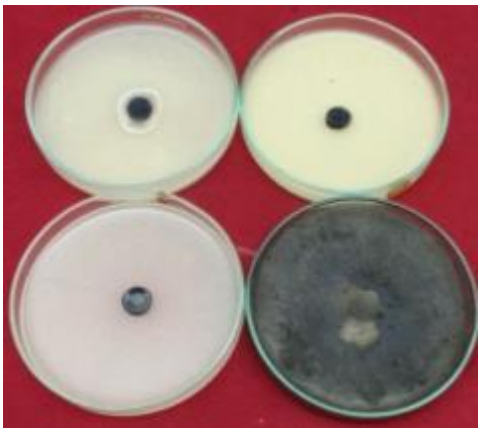
Evaluation of fungicides and bio-control agents

In the present investigation, *L. theobromae* was evaluated against several chemicals and cent per cent inhibition was observed with carbendazim 12% + mancozeb 63% (Saaf) and copper hydroxide 77 WP (Kocide) (**Figure 5a-f**).

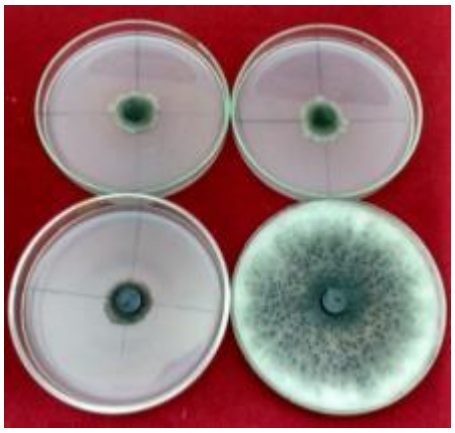
Cymoxanil 8% + mancozeb 64% (Curzate M8) at 0.2 per cent exhibited cent per cent inhibition and upto 96 per cent with the lower two concentrations (Figure 5b). On the other hand, Bordeaux mixture (Figure 5d) recorded only 69 to 81 per cent efficacy at all concentrations tested. The inhibitory action of difenoconazole 25 EC (Score), propineb (Figure 5c-e), and Saaf was in accordance with that of [11], who noticed 72 to 100 per cent inhibition with the three fungicides tested against *B. theobromae* in Jatropa. Cent per cent inhibition of the pathogen in case of bottle gourd incited by *B. theobromae* with carbendazim and Alliete was recorded which was contradictory to the present results [12]. Cent per cent sensitivity of *B. theobromae* infecting pineapple with Saaf and 94.25 per cent with Carbendazim was recorded [13]. Copper oxychloride 77 WP (Fytolan) was found the least effective with *Lasiodiplodia theobromae* which was in line with the findings of [14], [15] and [16] in mango (**Table 1**). No fungicide evaluation has been carried out with this pathogen isolated from strawberry.



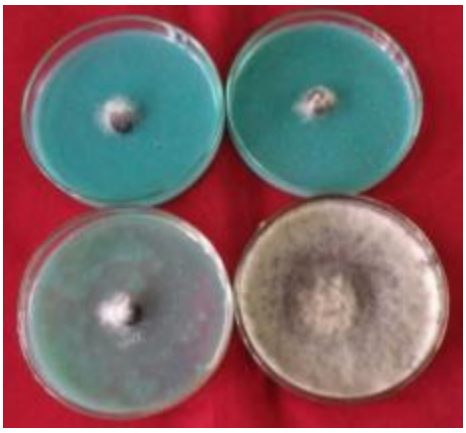
(a) Carbendazim 12% + mancozeb 63%



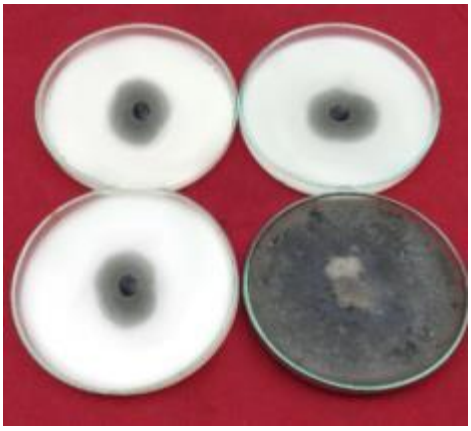
(b) Cymoxanil 8%+mancozeb 64%



(c) Difenoconazole 25EC



(d) Bordeaux mixture



(e) Propineb 70WP

(f) Copper hydroxide 77WP

Figure 5 *in vitro evaluations* of fungicides against *Lasiodiplodia theobromae*

Table 1 *In vitro* evaluation of fungicides against *Lasiodiplodia theobromae*

Sl No.	Fungicide	Conc (%)	Per cent inhibition over control *L. theobromae* (CRM-2)
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	93.2(9.76)[b]
		0.20	96.3(9.77)[b]
		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	0
		0.25	0
		0.30	0
5.	Propineb 70WP (Antracol)	0.25	55.55(7.52)[j]
		0.30	64.11(8.04)[h]
		0.35	69.44(8.37)[g]
6.	Carbendazim 50WP (Bavistin)	0.05	23.33(4.97)[m]
		0.10	31.67(5.68)[l]
		0.15	33.23(5.84)[k]
7.	Difenoconazole 25EC (score)	0.05	62.22(7.95)[i]
		0.10	75(8.68)[f]
		0.15	76.67(8.79)[e]
8.	Potassium phosphonate 40% (Akomin 40)	0.25	0
		0.30	0
		0.35	0
9.	Bordeaux Mixture	0.50	69.44(8.4)[g]
		1.0	81.11(8.99)[d]
		1.50	83.55(9.09)[c]
	CD (0.05)		0.038

In reviewing the effect of antagonists against *Lasiodiplodia theobromae, Trichoderma asperellum* recorded a maximum of 74.10 per cent efficacy against the pathogen (**Figure 6a**). However, *P. fluorescens* was found zero per cent effective (Figure 6b). Nonetheless, [17] noted 25.38 per cent control only with *T. viride* in cashew infected with *B. theobromae*.

(a) *T. asperellum* (b) *P. fluoresces*

Figure 6 *In vitro* evaluation of bio-control agents against *Lasiodiplodia theobromae*

Pot culture management of Lasiodiplodia theobromae

An attempt was made to study the effect of fungicides and biocontrol agents against *Lasiodiplodia theobromae* in strawberry. Spore suspension was poured into the root surface for challenge inoculation. About 75 to 100 per cent infection was noticed in various treatments with the maximum infection in T_1 (Control) and T_2 (cymoxanil 8% + mancozeb 63%) (0.2%) and lowest in T_6 (*Trichoderma asperellum*) (2%). Observations taken 10 days after first drenching recorded upto 37.5 per cent control with treatments T_3 (carbendazim 12 % + mancozeb 64%) (0.2%) and T_6 (*Trichoderma asperellum*) (2%), 25 and 12.5 per cent with T_4 (copper hydroxide 77WP) (0.2%) and T_5 (propineb 70WP) (0.3%). However, the treatment T_2 (cymoxanil 8% + mancozeb 63%) (0.2%) was found to be least effective

(**Table 2**). It is interesting to note that, application of *Trichoderma* was carried out as a prophylactic measure 10 days before the application of fungicides which is in congruence with the study of [18] where they pointed out that *Trichoderma* are early colonizers off substrates and reduce the activity of other fungi simply by substrate occupation and depletion when applied prophylactically. Second treatment application recorded 50 per cent reduction in disease with T_3 (carbendazim 12 % + mancozeb 64%) (0.2%) and T_4 (Copper hydroxide 77WP) (0.2%) and least control of only 25 per cent with T_5 (Propineb 70WP) (0.3%) and T_2 (cymoxanil 8% + mancozeb 63%) (0. 2%). In consonance with the above results, [19] and [16] reported the efficacy of carbendazim and [20] reported the 40 per cent field efficacy with mancozeb against *B. theobromae* in mango. [21] recorded the efficacy of carbendazim recording 63.10 per cent disease reduction and 33.4 per cent in case of *Trichoderma viride* treated along with FYM and neem cake in fields of Jatropha.

Table 2 Effect of treatments on per cent incidence of *Lasiodiplodia theobromae*

Treatment No.	Treatments (foliar spray)	Conc (%)	16 days after inoculation	10 days after first drench		10 days after second drench	
			*PDI	*PDI	Per cent disease reduction over control	*PDI	Per cent disease reduction over control
T_1	Control	-	100	100	-	100	-
T_2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	100	100	0	75	25
T_3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	87.5	62.5	37.5	50	50
T_4	Copper hydroxide 77WP (Kocide)	0.2	87.5	75	25	50	50
T_5	Propineb 70 WP(Antracol)	0.3	87.5	87.5	12.5	75	25
T_6	*Trichoderma asperellum*	2	75	62.5	37.5	62.5	37.5

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