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In vitro Evaluation of Certain Fungicides, Botanicals and Bio control Agents against *Lasiodiplodia theobromae*

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ABSTRACT

Mango (*Mangifera indica* L.) being an important tropical and subtropical fruit crop, is being affected by several fungal diseases among which gummosis caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Moube [synonym: Botryodiplodia theobromae] is becoming a serious problem in India on many popular varieties of mango particularly during monsoon and post-monsoon periods. An investigation to in vitro evaluates the different effective fungicides, botanicals and bio control agents were conducted to manage the gummosis disease of mango. Among the 10 fungicides tested at different two concentrations (250 and 500 ppm) carbendazim, carbendazim + mancozeb and propiconazole, completely inhibited the growth of *L. theobromae* concentrations whereas pyraclostrobin was least effective. Among the different botanicals, extract of Garlic bulb at 10 per cent found to be superior (35.93%) followed by Neem at 10 per cent (8.15%). Among Trichoderma isolates T_9, T_6, T_3 and T_2 were found effective against *L. theobromae.*

Key words: *Lasiodiplodia theobromae*, Fungicides, Botanicals, *Trichoderma* isolates

Mango (*Mangifera indica* L.) is one of the most important fruit of India and it is grown on an area of 25 lakh ha with an annual production of 1800.4 MT. In Andhra Pradesh, it is grown in an area of 4.89 lakh ha with a production of 4,404 thousand MT. The mango production is reduced by important diseases like anthracnose, powdery mildew and die-back diseases. Since late nineties, mango gummosis has become one of the most serious problem in Andhra Pradesh (Narasimhudu and Reddy 1992). In most cases, the disease has been characterized by the exudation of gum, wilting, die back, vascular discolouration and death of whole tree (Narasimhudu and Reddy 1992, Khanzada *et al*. 2004). Previous studies have established *L. theobromae* (Syn. *Botryodiplodia theobromae*) as the causative fungus of this disease (Al-Adawi *et al*. 2006). The present study was undertaken to evaluate different fungicides, botanicals and bioagents against *L. theobromaein vitro.*

MATERIALS AND METHODS

To test the in vitro efficacy of fungicides

The fungicides viz carbendazim, mancozeb, carbendazim + mancozeb, propiconazole, azoxystrobin, propineb, pyraclostrobin + metiram, pyraclostrobin and tebuconazole + trifloxystrobinwere tested at 250 and 500 ppm concentrations were added to the autoclaved PDA medium. Agar discs obtained with the help of sterilized cork borer (6 mm) from three days old culture of *L. theorbromae* were placed in the center of each dish. Petri-dishes without fungicides served as control and incubated at 18 ± 2°C till the control treatments were fully covered with the fungus mycelium. The experiment was conducted in completely randomized design (CRD) and there were three replications for each treatment. Data on the mycelial growth of *L. theobromae* were recorded and per cent inhibition of mycelial growth was calculated by using formula:
Per cent inhibition of mycelial growth was calculated using the formula (Vincent 1927).

$$I = (C - T/C) \times 100$$

Where,
I = Per cent inhibition of mycelia
C = Colony diameter in control
T = Colony diameter in treatment (cm)

The present investigation was carried out by eight botanicals to evaluate different plant species for their fungitoxicant properties against *L. theobromae.*

In vitro evaluation of plant extracts

The present studies were planned to find out effective plant extracts against *L. theobromae*. Sensitivity *of L. theobromae* was studied by using poison food technique against eight plant extracts viz Neem (*Azadirachta indica*), Wild tulasi (*Ocimum sanctum*), Glory flower, (*Clerodendron infortunatum*), Datura (*Datura metal*), Karanj (*Pongamiapinnata*), Bitter oleander (*Holarrhenapubescen*), Duranta (*Duranta erecta*), Garlic (*Allium sativum*). In which only garlic plant extract obtained from bulb, other plant extracts obtained from leaf.

For the preparation of aqueous extract, 100gm fresh sample of each plant were macerated in100 ml of sterilized water with the help of pestle and mortar. The macerated plant extract was first passed through four layered sterilized muslin cloth and then filtered through Whatman's filter paper No. 14. The extract obtained was considered standard solution. Ten ml and five ml of stock solution was mixed with 90 and 95 ml of sterilized molten PDA medium respectively, so as to get 10 and 5 percent concentration. The medium was shaken thoroughly for uniform, mixing of test extract.

The sterilized PDA (potato dextrose agar) medium was poured into the sterilized Petri plates of 9 cm diameter. These Petri plates were allowed to solidify and divide them in two equal parts by marking. Wells of 1 cm diameter were made in the center of each portion of a Petri plate with the help of sterilized cork borer, and plant extracts 5ml or 10 ml were poured into these wells with the help of sterilized pipette. Than inoculation of actively growing culture of *B. theobromae* were done in other well on each Petri plate with the help of sterilized inoculating needle. All these Petri plates were then transferred to incubator at 18 ± 2°C and The radial growth of the colony was recorded when the maximum growth was observed in control and per cent XZinhibition of mycelial growth over control was calculated. Per cent inhibition of mycelial growth was calculated using the formula (Vincent 1927).

$$I = (C – T/C) \times 100$$

Where,
I = Per cent inhibition of mycelial growth
C = Colony diameter in control (cm)
T = Colony diameter in treatment (cm)

In vitro evaluation of bio agents against L. theobromae

Eleven Trichoderma isolates were obtained from department of plant pathology, College of Agriculture, Rajendranagar, Hyderabad. The *Trichoderma* isolates were screened for their antagonism against the *Lasiodiplodia theobromae* by using dual culture technique (Dennis and Webster 1971). About 20 ml PDA was poured into sterile Petriplates and allowed to solidify, from previously grown young cultures of antagonists and test pathogen, a 0.5 cm fungal disc of test fungus and respective bio agent were transferred aseptically to Petriplates simultaneously by leaving sufficient space in between two discs. Three replications were maintained for each treatment. The Petriplates were incubated at 18 ± 2°C till the growth of culture in control covered entire Petriplates. Colony diameter of both the test fungus and bio agent were measured and per cent inhibition was calculated and the

$$I = (C – T/C) \times 100$$

Where
I = Per cent inhibition of mycelial growth
C = Colony diameter in control (cm)
T = Colony diameter in treatment (cm)

The effective antagonistic bio agent screened by dual culture method against *L. theobromae* were tested and further used for the management of the disease.

RESULTS AND DISCUSSION

In vitro evaluation of fungicides against *L. theobromae*

The effectiveness of the test fungicides in reducing the mycelial growth of *L. theobromae* varied greatly (Table 1). Complete inhibition in growth of pathogen was recorded by carbendazim, carbendazim + mancozeb and propiconazole in both 500 ppm and 250 ppm concentrations.The fungicides mancozeb and propineb recorded 66.30 and 72.59 per cent inhibition respectively at 250 ppm but these exhibited maximum (100) inhibition at 500 ppm. While least inhibition of the pathogen was recorded in pyraclostrobin (26.11), (20.00) in both concentrations 500 and 250 ppm respectively (Table 1). Carbendazim either alone or in combination completely inhibited the growth of *L. theobromae*. Among systemic fungicides carbendazim was successful in completely (100%) inhibiting the growth of *L. theobromae* reported by Rawal and Ullasa, (1988) and Renganathan (2008). Meijiao *et al*. (2009) reported that propiconazole, carbendazim and mancozeb effective against control the pathogen. The effectiveness of carbendazim + mancozeb and propiconazolewas also reported by Bhatt and Jadeja (2010). In the present investigation the fungicides viz carbendazim, carbendazim + mancozeb, propiconazole were also found effective. The results in agreement with the Attah and Ahiasti (2010), Meijiao *et al.* (2009), Sahi *et al*. (2012). Carbendazim being a Benzimidazole group fungicide, they interfere with energy production and cell wall synthesis of fungi (Nene and Thapliyal 1982). Further, they also reported the effectiveness of triazole, which inhibit sterol biosynthesis pathway in fungi.

In vitro evaluation of botanicals against L. theobromae

In the present investigation eight plant extracts were evaluated under *in vitro* condition at 5% and 10% concentrations against *L. theobromae* to know the fungitoxic nature of their extracts. Though complete inhibition of the pathogen was not observed in any of the plant extracts tested but considerable amount of inhibition was noticed in some of them, the results are presented in (Table 2). Among the 8 plant extracts tested against *L. theobromae*, garlic at both concentrations 5% (25.56) and 10% (35.93) were significantly superior over all other plant extracts next followed by neem (8.15), Glory flower (7.41) and karanj (5.93) at 10% concentration however all other treatments are ineffective. Sahi *et al.* (2012) reported garlic and neem shows inhibitory effect on *L. theobromae.* The results of the present study are in agreement with the Okigbo *et al*. (2009)

reported that *A. sativum* extracts effective inhibition (25.2-86.9%) on mycelial growth of the *L. theobromae*. The most toxic effect of the extracts was observed with *A. sativum* at 10%, with significant (P<0.01) inhibition on the fungi. However in the present investigation neem was recorded 8.15 per cent inhibition by pathogen. This may be due to variation in isolate or due to different environmental conditions effecting the growth of the pathogen.

Table 1 *In vitro* evaluation of fungicides on radial growth of *Lasiodiplodia theobromae*

Fungicides	250 ppm *Per cent inhibition	500 ppm *Per cent inhibition
Carbendazim	100 (87.10)**	100 (87.10)
Mancozeb	66.30 (54.52)	100 (87.10)
Carbendazim+ Mancozeb	100.00 (87.10)	100 (87.10)
Propiconazole	100.00 (87.10)	100 (87.10)
Pyraclostrobin	20.00 (26.55)	26.11 (30.71)
Pyraclostrobin + Metiram	83.70 (66.18)	86.11 (68.12)
Azoxystrobin	35.37 (36.48)	46.85 (43.18)
Propineb	72.59 (58.58)	100.00 (87.10)
Trifloxystrobin+ Tebuconazole	75.00 (59.98)	79.81 (63.29)
Control	0.00 (2.87)	0.00m (2.87)
CD at 5%	3.33	1.19
SEm±	1.12	0.40

Table 2 *In vitro* evaluation of botanicals on radial growth of *L. theobromae*

Botanicals	*Percent inhibition at 5% concentration	*Percent inhibition at 10% concentration
Neem	4.81 (12.65)**	8.15 (16.57)
Wild Tulasi	2.22 (8.37)	2.59 (9.21)
Glory flower	4.07 (11.45)	7.41(15.78)
Datura	2.59 (9.21)	3.52 (10.70)
Karanj	2.04 (8.18)	5.93 (14.08)
Bitter oleander	2.22 (8.37)	2.22 (8.37)
Duranta	1.85 (7.73)	2.22 (8.37)
Garlic	25.56 (30.35)	35.93 (36.81)
Control	0.00 (2.87)	0.00 (2.87)
CD at 5%	2.64	2.15
SEm±	0.88	0.72

Table 3 Antagonistic activity of *Trichoderma* isolates against *Lasiodiplodia theobromae*

Isolate	*Percent inhibition
T_1: *Trichoderma* isolate 1	71.11 (57.47)**
T_2: *Trichoderma* isolate 2	80.74 (64.50)
T_3: *Trichoderma* isolate 3	80.37 (63.69)
T_4: *Trichoderma* isolate 4	61.11 (51.40)
T_5: *Trichoderma* isolate 5	69.63 (56.77)
T_6: *Trichoderma* isolate 6	81.48 (64.53)
T_7: *Trichoderma* isolate 7	70.00 (57.47)
T_8: *Trichoderma* isolate 8	65.19 (53.82)
T_9: *Trichoderma* isolate 9	81.85 (64.77)
T_{10}: *Trichoderma* isolate 10	74.44 (59.61)
T_{11}: *Trichoderma* isolate 11	70.00 (56.77)
Control	0.00 (2.87)
CD at 5%	1.460
SEm±	0.497

In vitro evaluation of *Trichoderma* isolates against *Lasiodiplodia theobromae*

The antagonistic effect of eleven *Trichoderma* isolates was assayed by dual culture method as mentioned in materials and methods. *Trichoderma* isolates showed significant difference in inhibiting the mycelial growth of *L. theobromae* (Table 3). Differences among the treatments were observed with per cent inhibition in mycelial growth ranging from 61.1 (T_4) to 81.85 (T_9). Among the treatments tested, the *Trichoderma* isolates 9, 6, 3 and 2 recorded maximum inhibition against *L. theobromae* and are statistically on par with each other and superior over the treatment, T_{10}. The *Trichoderma* isolates, T_1, T_7, T_{11}, T_5, T_8 and T_4 recorded 71.11, 70, 70, 69.63, 65.19 and 61.11 per cent inhibition against *L. theobromae*, respectively.

The results of the present investigation revealed that *Trichoderma* isolates effectively inhibited the growth of *L. theobromae*. Suhannaa *et al*. (2013) also reported the potential use of *Trichoderma* sp. against *B. theobromae,* the causal agent of mango stem end rot. *T. virens* and *T. hamatum* were also found effective in inhibiting the growth of *L. theobromae* by producing volatile metabolites. Similarly, *T. pseudokoningii* was also effective against the

pathogen through non-volatile metabolite production (Priya and Nagaveni 2009, Sangeetha *et al.* 2009). Moreover, the variation in the growth inhibition of *L. theobromae* by the same species of *Trichoderma* was also reported.

The antagonism of *Trichoderma* spp. against many fungi is mainly due to production at acetaldehyde compound (Dennis and Webster 1971). This may also be the reason for its antagonistic effect on *L. theobromae*.

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