Full Length Research Article

© 2025, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 16; Issue 01 (Jan–Feb 2025); pp 29–33

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Evaluation of Fungicides and Biocontrol Agents against *Lasiodiplodia theobromae* (Pat.) Griffon and *Maubl.* Inciting Dieback Disease of Mango in Kerala

Hima Mariya Dixon*[1], Deepa James[2], Reshmy Vijayaraghavan[1], Gleena Mary C. F.[1] and Aswini A[2]

[1] Department of Plant Pathology, College of Agriculture, Vellanikkara - 680 656, Kerala Agricultural University, Kerala, India
[2] Cocoa Research Centre, College of Agriculture, Vellanikkara - 680 656, Kerala Agricultural University, Kerala, India

Received: 07 Dec 2024; Revised accepted: 05 Jan 2025

Abstract

Mango dieback caused by *Lasiodiplodia theobromae* is a grave disease affecting the fruit quality and quantity. Numerous disease management options are available nowadays, with chemical control being the most efficient option while biopesticides present the most environment-friendly alternative in controlling various crop diseases. With this objective, chemical and biological control of *Lasiodiplodia theobromae* was assessed in the present study using the three most virulent isolates of the pathogen collected from three different mango varieties and three different districts of Kerala. The *in vitro* evaluation of fungicides revealed that the fungicides viz., hexaconazole, azoxystrobin, Bordeaux mixture and carbendazim + mancozeb showed 100 per cent inhibition of the virulent pathogen isolates, while difenoconazole had inhibition in the range of 96.10-100 per cent, copper hydroxide in the range 68.14-88.88 per cent and propineb in the range 33.88-79.25 per cent. Among the biocontrol agents, *Trichoderma asperellum* and plant growth promoting microorganisms caused inhibition in the range 41.88-51.33 per cent and 14.82-48.66 per cent respectively against three virulent isolates of the pathogen while *Pseudomonas fluorescens* had zero per cent inhibition.

***Key words*:** Fungicides, Chemical control, Biocontrol, *Lasiodiplodia theobromae*, Biological control, Efficacy

India takes the top position in production of mango (2,62,99,000 T) but not in productivity (9,726 kg/ha) [1]. This might be due to major biotic and abiotic production constraints affecting the mango ecosystem. Major abiotic constraints affecting mango production include unseasonal rains during flowering, heat stress, poor soil drainage, deficiency of nutrients, drought, salinity issues, natural disasters etc. Major biotic constraints include pests like mango hopper, fruit flies, mealy bugs, stem borers etc.; diseases like dieback, powdery mildew, anthracnose, malformation, bacterial canker etc.; weeds and nematodes like root-knot nematode. Addressing these constraints is essential for ensuring optimal mango yield in mango orchards worldwide. Fungicide intervention could be used as a viable option for mitigating different diseases affecting mango.

Among the diseases affecting mango, dieback is the most serious threat in all mango producing regions. Mango dieback arises from the infection of the fungus *Lasiodiplodia theobromae*. The fungus *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. is placed within the Ascomycetes, in the order Botryosphaeriales and family Botryosphaeriaceae. Organisms of this family may either attack a wide variety of host plants or thrive as saprophytes or endophytes in seeds and other living cells [2]. It is regarded as plurivorous, cosmopolitan, polyphagous and opportunistic pathogen infesting about 500 hosts [3]. Mango dieback can be distinguished by the presence of necrotic leaves, dark discolouration on stem, vascular discolouration, gummosis, bark splitting etc. During initial stages of disease development necrotic leaves can be seen, failure to manage the disease in the initial stages could spread the disease to other parts of the tree; potentially causing death of the whole tree. The disease is known to occur in various regions globally, often in severe form. Such significant losses are caused by mango dieback which can be curbed only by the judicious use of fungicides.

The use of fungicides for disease control dates back to late 1807s and fungicides assumes the primary means of disease control in every crop ecosystem. The fungicides offered great control of the diseases, which leads to an uncontrollable dependency of farmers on fungicides. For some phytopathogens for which host resistance is not known till now such as polycyclic oomycetes, fungicides remain as the exclusive strategy for disease control. Fungicides remain to be a common preference among peasants nowadays due to their faster action. The use of fungicides has its own shortcomings. While looking into different aspects of resistance development, environmental consequences, health and biodiversity impact etc., an alternative for chemical fungicides is the need of the decade. In this context, biopesticides emerge as an effective option in mitigating phytopathogens. Different biocontrol agents have been used by farmers in controlling a wide variety of crop diseases worldwide. Thus, this research was formulated

***Correspondence to:** Hima Mariya Dixon, E-mail: hima-2022-11-091@student.kau.in; Tel: +91 8078279287

Citation: Dixon HM, James D, Vijayaraghavan R, Gleena Mary CF, Aswini A. 2025. Evaluation of fungicides and biocontrol agents against *Lasiodiplodia theobromae* (Pat.) Griffon and *Maubl.* inciting dieback disease of mango in Kerala. *Res. Jr. Agril. Sci.* 16(1): 29-33.

in assessing the efficiency of different fungicides and biocontrol agents against the virulent isolates of *Lasiodiplodia theobromae* responsible for dieback in mango.

MATERIALS AND METHODS

Three isolates of *Lasiodiplodia theobromae* having higher virulence were chosen from a total of 37 *Lasiodiplodia theobromae* isolates causing dieback in mango collected from Kerala. These three isolates were collected from three different mango cultivars and three different districts of Kerala. The three virulent isolates selected were designated as I_3, I_4 and I_5. Isolate I_3 was collected from Mallika mango cultivar from the location Mankomb of Wayanad district; I_4 was collected from Banganapally mango variety from Padanakkad of Kasargod district while I_5 from Bennet Alphonso variety from Kumarakom of Kottayam district.

Evaluation of fungicides against virulent isolates of Lasiodiplodia theobromae

Poisoned food technique [4] was used for studying efficacy of different fungicides on the virulent isolates of *Lasiodiplodia theobromae*. Into a conical flask containing 100 mL of molten PDA (Potato Dextrose Agar), requisite amount of fungicides was added, mixed well, and poured to the Petri plate. A 5 mm mycelial disc of 10-day old culture of the isolate was placed at the centre of the Petri plate. A control was also maintained. The experiment was done in Completely Randomized block Design (CRD) having eight treatments and three replications. Different fungicides evaluated *in vitro* are represented in (Table 1).

Per cent inhibition (PI) was calculated as per the formula given by Vincent [5].

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

Where; C denotes radial growth of control plates, while T denotes radial growth of treated plates

Table 1 Fungicides used for *in vitro* evaluation along with their doses

Treatment	Fungicides	Dosage
T_1	Copper hydroxide (77 WP)	0.2%
T_2	Hexaconazole (5 SC)	0.2%
T_3	Propineb (70 WP)	0.2%
T_4	Difenoconazole (25 EC)	0.1%
T_5	Carbendazim (12 WP) + Mancozeb (64 WP)	0.2%
T_6	Azoxystrobin (23 SC)	0.1%
T_7	Bordeaux mixture	1%
T_8	Control	

Evaluation of biocontrol agents against virulent isolates of Lasiodiplodia theobromae

Different biopesticides evaluated against the three virulent isolates of *Lasiodiplodia theobromae* were *Trichoderma asperellum* (KAU reference culture), *Pseudomonas fluorescens* (KAU reference culture PN026) and PGPM (Kerala Agricultural University). PGPM (Plant growth promoting microorganisms) is a consortium of *Trichoderma viridae*, *Trichoderma harzianum*, *Bacillus megaterium* and *Pseudomonas fluorescens*. Dual culture technique [6] was used for *in vitro* evaluation of *Trichoderma asperellum* and *Pseudomonas fluorescens* against the virulent isolates of the pathogen while poisoned food technique [4] was used for *in vitro* evaluation of PGPM. Completely Randomized block Design (CRD) was followed with three treatments and five replications.

Inhibitory effect of *Trchoderma asperellum* was assessed by placing 7 mm sized mycelial disc of test isolate 2 cm away from the edges of Petri dish containing PDA medium. A 7 mm sized mycelial bit of *Trichoderma asperellum* was placed at the opposite end, 2 cm away from the edges of the Petri dish. The efficacy of *Pseudomonas fluorescens* was evaluated by placing 7 mm mycelial disc of isolate on centre of Petri plate containing PDA media. The bacterial antagonist was streaked 2 cm away from both edges of Petri plate. The sensitivity of different isolates of the fungus to PGPM was assessed by mixing 2 g of PGPM with 100 mL of molten PDA. This was poured into Petri plate, allowed to solidify and a 5 mm mycelial disc was kept on centre of Petri dish. Five replications and a control were kept for all the three biocontrol agents. Per cent inhibition was calculated for all three antagonists.

RESULTS AND DISCUSSION

Efficacy of different fungicides against different isolates of the pathogen

Among the different fungicides tested, hexaconazole, azoxystrobin, difenoconazole, Bordeaux mixture and carbendazim + mancozeb showed 100% inhibition against isolates, I_3 and I_5. Considering the efficacy against isolate I_4, hexaconazole, azoxystrobin, Bordeaux mixture, and carbendazim + mancozeb showed cent per cent inhibition of mycelial growth over the control. The fungicide difenoconazole showed 96.10 per cent inhibition as compared to untreated control to the isolate I_4. Results of efficacy of different fungicides have been represented in (Table 2, Fig 1-3). Rao *et al.* [7] conducted *in vitro* screening of different fungicides effective against *Lasiodiplodia theobromae* infecting coconut and observed that the fungicide hexaconazole completely arrested the growth of the fungal pathogen. Talaviya *et al.* [8] also reported that mango dieback caused by *Lasiodiplodia theobromae* was successfully suppressed by hexaconazole at 100-1000 ppm *in vitro*. Bordeaux mixture at one per cent showed 100 per cent mycelial inhibition of *Lasiodiplodia theobromae* infecting anthurium [9] and of *Botryodiplodia theobromae* infecting mango [10]. Bhure *et al.* [11] and Nath *et al.* [12] also reported cent per cent inhibition of radial growth of *Lasiodiplodia theobromae* by the combi product carbendazim + mancozeb in jackfruit and banana respectively. Rafi [9] also noted that difenoconazole at 0.05-0.15 per cent had complete inhibitory action on *Lasiodiplodia theobromae* infecting anthurium.

Mycelial inhibition of 33.88, 79.25 and 74.62 per cent was shown by propineb against the isolates I_3, I_4 and I_5 respectively whereas copper hydroxide showed 81.94, 68.14 and 88.88 per cent mycelial inhibition respectively. Hossain *et al.* [13] in their studies involving *Lasiodiplodia theobromae* causing gummosis and sudden decline in mango noted that propineb (66.08%) was least effective among eight fungicides tested and copper hydroxide showed only 86.36 per cent inhibition against the pathogen. Amrutha and Vijayaraghavan [14] in their studies on crown rot of strawberry caused by *Lasiodiplodia theobromae* reported that propineb at different concentrations recorded per cent inhibition in the range 55.55-69.44. Similarly, Baloch *et al.* [15] while studying on *Botryodiplodia theobromae* infecting guava found that antracol was moderately effective against the pathogen. Chinnuswamy and Saralemma [16] noted that copper hydroxide at 0.05-0.25% showed inhibition in the range 63-72.6 per cent against *Botryodiplodia theobromae* infecting mango. The treatments hexaconazole, difenoconazole, carbendazim + mancozeb, azoxystrobin and Bordeaux mixture were found on par for

isolate I_3 and I_5. These five treatments were found significantly different from copper hydroxide, propineb and control for isolate I_3 and I_5. The treatments hexaconazole, difenoconazole, carbendazim + mancozeb, azoxystrobin and Bordeaux mixture were found on par for isolate I_4. Copper hydroxide and propineb were also found on par for isolate I_4. The treatments hexaconazole, difenoconazole, carbendazim + mancozeb, azoxystrobin and Bordeaux mixture; copper hydroxide and propineb, and control were found significantly different for isolate I_4.

In the present study, systemic fungicides were found to be more effective than contact fungicides in suppressing the pathogen. Shahbaz *et al.* [17] while assessing effectiveness of different fungicides noted that systemic fungicides (thiophanate methyl, carbendazim) produced cent per cent sensitivity, while contact fungicides (captan, copper oxychloride) produced inhibition of less than 35 per cent only. Honger *et al.* [18] studied efficacy of different fungicides against *Lasiodiplodia theobromae* causing stem end rot in mango and noticed that all systemic fungicides produced 100 per cent inhibition; one contact fungicide 100 per cent; two contact fungicides (Funguran and Kocide) zero per cent and remaining one contact fungicide produced inhibition less than 12 per cent on the 3rd day.

Table 2 Efficacy of different fungicides against different isolates of the pathogen *Lasiodiplodia theobromae*

Treatments	Concentration (%)	Per cent inhibition (%)		
		I_3	I_4	I_5
T_1: Copper hydroxide	0.2	81.94 (65.03)^b	68.14(55.64)^b	88.88(70.61)^b
T_2: Hexaconazole	0.2	100.00(89.04)^a	100.00(89.04)^a	100.00(89.04)^a
T_3: Propineb	0.2	33.88(35.58)^c	79.25(63.25)^b	74.62(60.01)^c
T_4: Difenoconazole	0.1	100.00(89.04)^a	96.10(82.69)^a	100.00(89.04)^a
T_5: Carbendazim 12% + Mancozeb 63%	0.2	100.00(89.04)^a	100.00(89.04)^a	100.00(89.04)^a
T_6: Azoxystrobin	0.2	100.00(89.04)^a	100.00(89.04)^a	100.00(89.04)^a
T_7: Bordeaux mixture	1.0	100.00(89.04)^a	100.00(89.04)^a	100.00(89.04)^a
T_8: Control		0.00 (0.95)^d	0.00(0.95)^c	0.00 (0.95)^d
CV %		2.32	6.55	3.40
CD (0.05%)		2.74	7.87	4.24

Mean of three replications. In each column figure followed by same letter do not differ significantly according to DMRT.
Values in parenthesis are angular transformed values

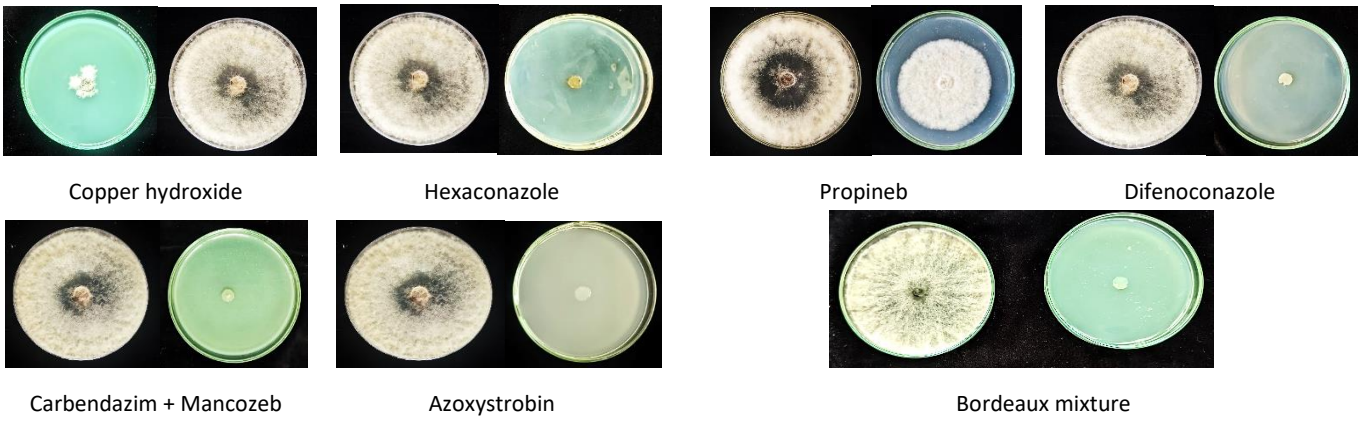

Fig 1 Efficacy of different fungicides to isolate I_3

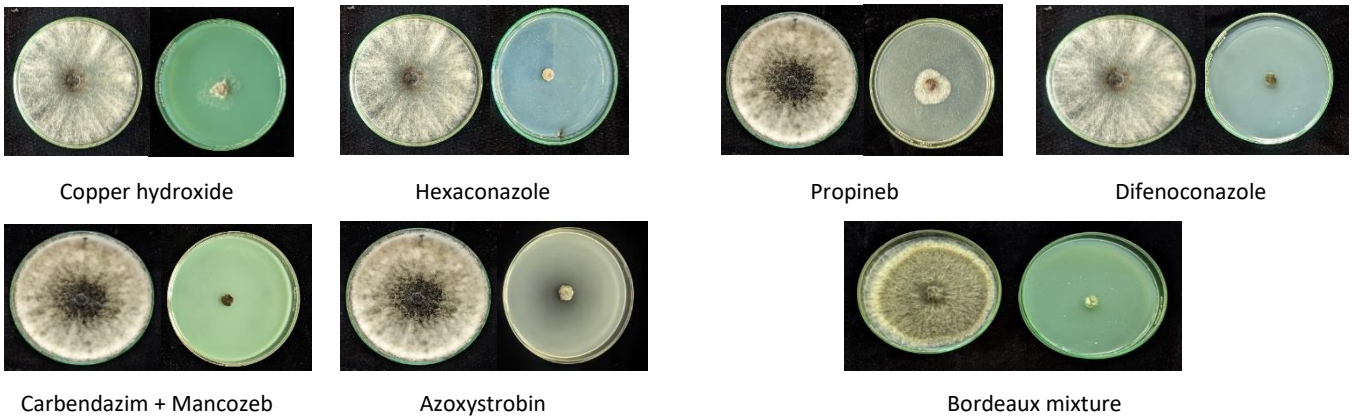

Fig 2 Efficacy of different fungicides to isolate I_4

Efficacy of different biocontrol agents against different isolates of the pathogen

Per cent inhibition of 45.24, 51.33 and 41.88 was shown by the biocontrol agent *Trichoderma asperellum* against different isolates of *Lasiodiplodia theobromae* viz. I_3, I_4 and I_5 respectively. Plant growth promoting microorganisms showed 28.77, 14.82 and 48.66 inhibition against I_3, I_4 and I_5 respectively while *Pseudomonas fluorescens* had no inhibitory effect on three isolates of the pathogen. Results of efficacy of different fungicides have been represented in (Table 3, Fig 4-6). These three different biocontrol agents were found significantly different from each other for each isolate. Boat *et al.* [19] reported that different strains of *Trichoderma asperellum* caused mycelial inhibition of *Lasiodiplodia*

theobromae causing papaya stem end rot in the range 49.3-75.4 per cent. Thangavelu *et al*. [20] observed that different species of *Trichoderma* viz., *T. viride*, *T. pseudokoningii*, *T. hamatum*, *T. koningii*, *T. reesei*, *T. virens*, *T. harzianum* caused mycelial inhibition of *Lasiodiplodia theobromae* causing crown rot in banana. Chandran [21] noted that the bacterial antagonist, *Pseudomonas fluorescens* had no antagonistic activity on *Lasiodiplodia theobromae* causing crown rot of banana.

Fig 3 Efficacy of different fungicides to isolate I_5

Fig 4 Efficacy of different biocontrol agents to isolate I_3

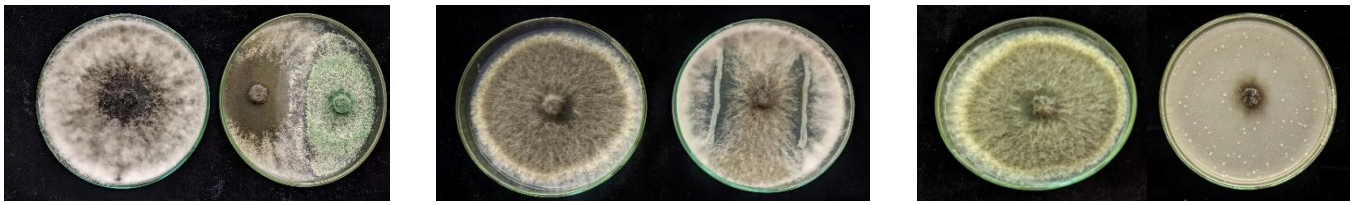

Fig 5 Efficacy of different biocontrol agents to isolate I_4

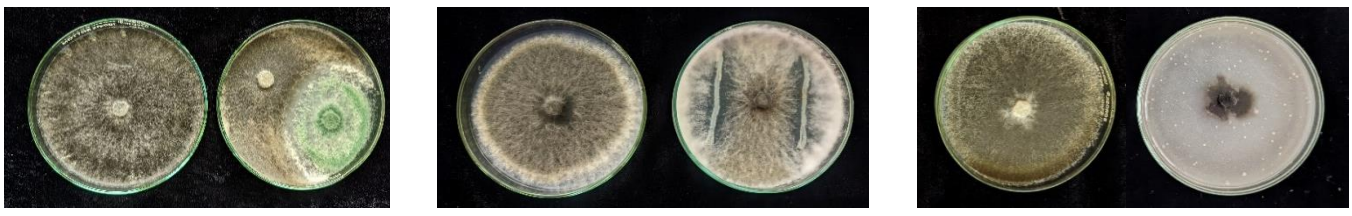

Fig 5 Efficacy of different biocontrol agents to isolate I_6

Table 3 Efficacy of different biocontrol agents against most virulent isolates of the pathogen *Lasiodiplodia theobromae*

S. No	Treatments	Per cent inhibition (%)		
		I_3	I_4	I_5
1.	*Trichoderma asperellum*	45.24 (42.26)[a]	51.33 (22.62)[b]	41.88 (40.22)[a]
2.	*Pseudomonas fluorescens*	0.00 (0.95)[c]	0.00 (0.95)[c]	0.00 (0.95)[b]
3.	Plant growth promoting microorganisms	28.77 (32.20)[b]	14.82 (45.76)[a]	48.66 (44.23)[a]
4.	Control	0.00 (0.95)[c]	0.00 (0.95)[c]	0.00 (0.95)[b]
	CV %	15.94	5.15	15.08
	CD (0.05%)	4.08	1.30	4.36

Mean of five replications. In each column figure followed by same letter do not differ significantly according to DMRT.
Values in parenthesis are angular transformed values

CONCLUSION

The assessment study of different fungicides and biocontrol agents against the pathogen, *Lasiodiplodia theobromae* revealed that the synthetic fungicides were most effective compared to biological antagonists in deterring the mycelial growth of the pathogen. Among the fungicides, hexaconazole, azoxystrobin, Bordeaux mixture, and the combination fungicide carbendazim + mancozeb were found most effective. Among the biological antagonists, *Trichoderma asperellum* was found highly effective against the pathogen.

LITERATURE CITED

1. FAOSTAT (Food and Agricultural Organization). FAOSTAT homepage(online). Available: https://www.fao.org/faostat/en/#home. (04 Dec. 2024).
2. Crous PW, Slippers B,Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Stud. Mycology* 55: 235-253.
3. Punithalingam E. 1980. Plant diseases attributed to *Botryodiplodia theobromae*. *Vaduz: Pat. Journal Cramer* pp 123.
4. Zentmeyer GA. 1955. A laboratory method for testing soil fungicides with *Phytophthora cinnamomia* as test organism. *Phytopathology* 45: 398-404.
5. Vincent JM. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nat*. 159: 850-853.
6. Morton DT, Stroube NH. 1955. Antagonistic and stimulatory effects of microorganisms upon *Sclerotium rolfsii*. *Phytopathology* 45(8): 419-420.
7. Rao VG, Rao NC, Sreenivas C, Neeraja B, Kireeti A, Koteswarao G, Bhagavan B, Srinivasulu B. 2023. Abiotic stress as a potential contributing factor to onset and severity of infection caused by *Lasiodiplodia theobrome* an opportunistic fungal pathogen in coconut. *Pollut. Research* 42(2): 262-269.
8. Talaviya J, Lathiya S, Dhingani J, Gevariya S, Savaliya V. 2020. Evaluation of fungicides against *Lasiodiplodia theobromae* causing dieback of mango. *Int. Res. Jr. Chemistry* 32: 1-5.
9. Rafi N. 2019. Etiology and characterization of diseases of anthurium (*Anthurium andraeanum* L.) in Kerala. *M. Sc. Thesis*, Kerala Agricultural University, Thrissur. pp 181.
10. Mengal A, Hussain S, Ali M, Abro MA, Maari AKSA, Jatoi GH, Nisa T, Rafiq M, Iqbal S. 2015. Evaluation of different botanical extracts on the linear colony growth of the fungus *Fusarium wilt* of mango nursery and its in-vitro control. *European Journal of Biotechnology and Bioscience* 3(11): 7-14.
11. Bhure SS, Patil PD, Pawar HD, Kadam JJ, Joshi MS, Mahadik SG, Thorat SB. 2023. Evaluation of fungicides, phytoextracts and germplasm screening against *Lasiodiplodia theobromae* causing fruit rot of jackfruit. *Jr. Plant Science* 18(1): 26-30.
12. Nath K, Solanky KU, Bala M. 2015. Management of banana (*Musa paradisiaca* L) fruit rot diseases using fungicides. *Jr. Plant Pathol. Microbiology* 6(8): 1-7.
13. Hossain MM. 2020. Study on gummosis and sudden decline disease of mango plants in Rajshahi region of Bangladesh. *Ph. D. Thesis*, University of Rajshahi, Rajshahi. pp 171.
14. Amrutha P, Vijayaraghavan R. 2020. Evaluation of fungicides for the management of *Lasiodiplodia* crown rot of strawberry (Fragaria× ananassa Duch.) in Kerala. *Chinese Chem. Letters* 9: 425-431.
15. Baloch A, Abro MA, Jatoi GH, Abro NA, Dad S, Rafique M, Ali R, Memon S, Mangi N. 2017. *In-vitro* evaluation of different fungicides and the bio-control agent *Trichoderma harzianum* against *Botryodiplodia theobromae*, the cause of guava decline. *Pakistan Journal of Biotechnology* 14(3): 491-496.
16. Chinnusamy S, Saralemma B. 2017. Fungicidal studies on foliar fungi associated with die back disease of mango grafts in nursery. *Int. Jr. Chem. Studies* 5(5): 2287-2291.
17. Shahbaz M, Iqbal Z, Saleem A, Anjum AM. 2009. Association of *Lasiodiplodia theobromae* with different decline disorders in mango. *Pakistan Jr. Plant Pathology* 41: 359-368.
18. Honger JO, William CE, Owusu-Ansah D, Siaw-Adane K, Odamtten GT. 2015. Pathogenicity and fungicide sensitivity of the causal agent of postharvest stem end rot disease of mango in Ghana. *Ghana Jr. Agric. Science* 49(1): 37-52.
19. Bedine, MAB, Taïeb N, Agriopoulou S, Miché L, Moussango D, Sameza ML, Dupuy N, Roussos S, Fekam Boyom F. 2022. Identification of native soil-derived *Trichoderma* spp. isolates and analysis of their antagonist traits against *Lasiodiplodia theobromae* causing stem-end rot in papaya. *Arch. Phytopathol. Plant Protection* 55(15):1766-1794.
20. Thangavelu R, Sangeetha G, Mustaffa MM. 2007. Cross-infection potential of crown rot pathogen (*Lasiodiplodia theobromae*) isolates and their management using potential native bioagents in banana. *Australasian Plant Pathology* 36: 595-605.
21. Chandran DR. 2017. Management of postharvest crown rot of banana using biocontrol agents and essential oils. *M. Sc. (Agriculture) Thesis*, Kerala Agricultural University, Thrissur. pp 168.