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Management of fruit rot of mango with local *Trichoderma* spp.

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Abstract
Anthracnose and die-back diseases of mango were noticed as endemic to the konkan region of Maharashtra. The causal organisms were isolated from leaf and twig samples showing typical symptoms of anthracnose and die-back diseases. The pathogens were identified as *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* on the basis of morphological characteristics. Out of the seven media tested to study cultural characteristics of the test pathogens, potato dextrose agar and oat meal agar were found to be the best media which recorded maximum (90 mm) mycelial growth of *C. gloeosporioides* and *L. theobromae* respectively. Under *in vitro* studies *Trichoderma harzianum* recorded maximum growth inhibition (73.11% and 72.00%) of *C. gloeosporioides* and *L. theobromae* respectively, followed by *T. longibrachiatum* (50.44% and 49.33%) and *T. koningii* (41.78% and 44.22%). When three *Trichoderma* species were evaluated under field conditions, *T. harzianum* was found to be effective with maximum (63.84%) per cent disease control of *C. gloeosporioides*. It was followed by *T. longibrachiatum* (56.92%) and *T. koningii* (39.23%). Similarly in case of *L. theobromae*, *T. harzianum* recorded maximum (57.88%) disease control followed by *T. longibrachiatum* (42.12%) and *T. koningii* (30.54%). The spray of three *Trichoderma* spp. under field conditions resulted in reduced postharvest rot of mango fruits. It was observed that the trees sprayed with *T. harzianum* displayed maximum (61.25%) per cent disease control followed by *T. longibrachiatum* (51.25%) and *T. koningii* (36.88%). Fruit dipped treatment of *T. harzianum* 10% for 30min was found the best among all the treatments and gave maximum (79.31%) disease control. While *T. longibrachiatum* at the same concentration and time period displayed 68.96% PDC. However, PDC exhibited by *T. koningii* at aforesaid conditions was 58.62%.

Keywords: *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Trichoderma* spp. Antagonism, field evaluation, fruit dip treatment

Introduction
Mango plays a decisive role in agricultural economy of Konkan region of Maharashtra. It succumbs to various diseases among which anthracnose and die- back are the most destructive. The hot and humid climatic conditions of Konkan region favour the perpetuation, dissemination and severity of these diseases. The infections of both these diseases occur on vegetative flush but due to the quiescent mode of infection of the pathogens it culminates in post harvest fruit rot. Till date, the disease management strategies mainly rely on chemical control.

Anthracnose disease caused by *C. gloeosporioides* is the major postharvest disease of mango in all mango producing areas of the world (Dodd *et al*., 1997) [5]. *Colletotrichum gloeosporioides* (Penz.) Penz.&Sacc., is an ubiquitous fungus. Anthracnose symptoms comprise of leaf spot, blossom blight, wither tip, twigs blight and fruit rot. Small, sunken, black spots develop on the leaves, twigs and fruits.

Lasiodiplodia theobromae (Pat.) Griffon &Moube [synonym: *Botryodiplodia theobromae*] causing die-back of mango is another serious threat to the mango growing belts of India. Die back sums up in drying and withering of twigs from tip downwards, followed by discolouration, drying and eventual dropping of leaves. Dried branches remain clinched to the canopy, lower healthy portion of the infected twigs show gummosis; sometimes infected branches show longitudinal cracks and the fruit infection results in rot (Khanzada *et al*., 2004) [8]. Use of fungicides is a routine method of disease management adopted by nearly all the cultivators. However, the aftermath is disgusting due to its detrimental effects on the ecology and human health. Also the recurrent use of fungicides resulted in fungicide resistance in pathogen (Kumar *et al*., 2007) [9].

On this background, eco-friendly strategies comprising, use of bio-agents, botanicals stand to be the potential alternatives to chemicals in management of diseases (Rungjindamai, 2016) [14]. Fungal plant pathogens were reported to be controlled successfully by using biological control agents (Zhang *et al*., 2013) [23]. Among various bio-control agents, the potential species in the fungal genus *Trichoderma* are more efficient and promising. *Trichoderma* spp. are present in nearly all agricultural soils and in some other environments. Use of *Trichoderma* species against soil borne plant pathogens has been assessed and proved effective (Jin *et al*., 1992) [7] but their usefulness against aerial plant pathogens will be worthwhile for management of some dreadful ones.

Material and Methods

Isolation of fungicausing anthracnose and dieback disease of mango

Naturally infected mango leaves/ twigs, showing typical anthracnose and die-back symptoms were collected from the mango orchard. Both the causal organisms were isolated on potato dextrose agar (PDA) medium in Petri plates by standard tissue isolation method. The plates were incubated at room temperature (27 ± 2^0C) for seven days and monitored for growth of the causal organism. The PDA slants with pure fungal growth were stored in refrigerator at 4^0C.

Cultural characterization of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* on different solid media

Eight media including potato dextrose agar (PDA), oat meal agar (OMA), Richard's Agar (RA), Czapek Dox agar (CDA), Asthana and Hawker's Agar (AHA), host leaf extract agar (HLEA), host leaf and twig extract agar (HLTEA), and water agar (WA) were selected to study cultural characteristics of the pathogens. Host leaf extract agar was used for *C. gloeosporioides* and twig extract agar was used for *L. theobromae*. The inoculated plates were incubated at 27±2°C. Each treatment was replicated thrice. The colony diameter, colony appearance, colony colour were recorded when the mycelial growth in control plates fully covered the surface of medium.

In vitro efficacy of local *Trichoderma* species against *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*.

Three local species of *Trichoderma viz*. *T. harzianum*, *T. longibrachiatum* and *T. koningii* which were isolated from Konkan soils (Barde, 2022) [1] and identified on the basis of molecular characters, were evaluated against the test pathogens by dual culture technique (Dennis and Webster, 1971) [3].

Per cent mycelial growth inhibition of the pathogensas compared to untreated control was calculated by using the formula given by Vincent (1947) [21].

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

Where,

I = Per cent inhibition.

C = Radial growth of test pathogen (mm) in control.

T = Radial growth of test pathogen (mm) in treatment.

Field evaluation of *Trichoderma* species against *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*

Mass multiplication of *Trichoderma* spp.

All the three *Trichoderma* spp. *viz*. *T. harzianum*, *T. longibrachiatum* and *T.koningii* were grown on potato dextrose broth (PDB) medium. The growth of *Trichoderma* species on broth medium was inoculated on rice grains for mass multiplication.A quantity of 1000 g of rice grains was washed 3-4 times to remove dirt and other material from grains. Then it was allowed to air dry up to 70-75% moisture level. These grains were filled in 1000 ml of conical flask (each flask containing 200g rice grains). Then all the flasks were autoclaved. Mycelial mat of respective *Trichoderma* species from PDB medium was transferred to the flasks containing rice grains under aseptic conditions and plugged with non-absorbent cotton. Then those flasks were maintained at room temperature. After completion of growth of *Trichoderma* spp. on rice grains, it was used for preparation of *Trichoderma* solution. In order to prepare spray solution, 200ml of distilled sterile water was added to the flask containing fully grown mycelium on rice grains, after shaking well, the spore suspension of *Trichoderma* was obtained which was passed through muslin cloth to get grain free spore suspension. This suspension was used as a stock solution.

Evaluation of *Trichoderma* species under field conditions

The above mentioned local *Trichoderma* species were further evaluated under field conditions on randomly selected mango plants of cultivar ‘Alphonso’.

Anthracnose

Mature scions of mango trees were randomly selected and 5 trees per treatment with 10 mature scions on each tree were maintained.

Die-back

Selected twigs in the canopy were tagged in such a way that there were 10 twigs per replication (per plant) and 5 trees per treatment.

Experiment was laid out in randomized block design (RBD) with five replications for each treatment. The spore suspension of each *Trichoderma* species was sprayed at a concentration of 10% (5 L of *Trichoderma* suspension in 50 L of water) thrice starting with the initiation of symptoms of both the diseases; followed by two more sprays at an interval of 10 days. The water-sprayed trees served as control. The observations on per cent disease incidence on leaves, twigs and fruits were recorded by adopting standard disease scales proposed by the authors mentioned below. Disease index for anthracnose and die-back disease on leaves was recorded a day before first spray and 10 days after each spray. Disease incidence on fruits harvested from *Trichoderma* solution sprayed trees was recorded 12 days after harvesting.

Disease rating scales for field evaluation

Disease rating scale for anthracnose of mango on leaves:

The observations of per cent disease index on leaves was recorded by adopting 0–5 disease rating scale given by Sundravadana *et al*. (2007) [19].

Table 1: Disease rating scale for anthracnose of mango on leaves

Disease Grade	Disease intensity (%)	Description
0	0	No spot
1	1-20	1-5 spots
2	21-40	6-10 spots
3	41-60	11-15 spots
4	60-80	16-25 spots
5	> 80	>25 spots

Disease rating scale for die-back of mango

The observations of per cent disease index was recorded by adopting 1-5 disease rating scale given by Saeed *et al.* (2011) [15]

Table 2: Disease rating scale for die-back of mango

Disease Rating	Disease intensity (%)	Description
1	0	No disease symptoms.
2	1-25	An early stage of infection characterized by browning of leaf petioles and mid-veins and presence of distal or marginal leaf blade necrosis in one or two branches.
3	25-50	The presence of dead leaves, which may remain attached to branch, in the tips of several branches, vascular browning, and evidence of pathogen invasion of vascular tissues.
4	51-75	Dead leaves and progressive defoliation extending too many larger branches.
5	76-100	Sever decline or dieback that extended to major portions of the plant.

Disease severity on fruits

Disease severity on fruits was recorded by using disease rating scale for postharvest fruit rot of mango given by Sudha *et al*. (2021) [17].

Table 3: Disease rating scale for postharvest rot of mango

Disease Grade	Description
0	0 per cent fruit area infected
1	1-10 per cent fruit area infected
2	11-25 per cent fruit area infected
3	26-50 per cent fruit area infected
4	51-75 per cent fruit area infected
5	> 75 per cent fruit area infected

The per cent disease index was recorded with the following formula as given by, Mckinney (1923) [11].

$$PDI = \frac{\text{Sum of individual disease ratings}}{\text{Total number of leaves assessed x Maximum disease grade}} X\ 100$$

Further, per cent Disease Control (PDC) was calculated by following formula:

$$\%\ \text{Disease control PDC} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} X\ 100$$

The per cent disease incidence was calculated by the following formula:

$$A = \frac{Y}{X} \times 100$$

Where,

A = Per cent fruit rot

X = Total number of fruits observed

Y = Total number of disease fruits

Fruit dip treatment

In order to study the effect of postharvest fruit dip treatment of spore suspension on development postharvest fruit rot, mature, green, apparently healthy, unsprayed and non-inoculated 'Alphonso' fruits were harvested. The experiment was laid in randomized block design with three replications and nine treatments. The fruits were washed in water and air-dried. Spore suspension of each *Trichoderma* species was prepared at two concentrations - 5% and 10%. Then a set of 18 fruits was dipped individually in the two concentrations for 10min, 15min, 20min and 30min respectively. Such fruits were air dried and allowed to ripen at ambient temperature. The observations on postharvest rot were recorded on 5th, 10th and 12th day after treatment. Per cent fruit rot was recorded as per the scale mentioned above and the disease incidence was calculated by the formula given by Mckinney (1923) [11].

Results and Discussion

Cultural characterization of *C. gloeosporioides* and *L. theobromae*on different solid media

The cultural characters were studied on seven different solid media. The radial growth of *C. gloeosporioides*and *L. theobromae* was measured after seven days of incubation at room temperature. The observations on various cultural characters were recorded.

Table 4: Cultural characterization of *C. gloeosporioides* and *L. theobromae* on different solid media

Sr. No	Treatment	*C. gloeosporioides*		*L. theobromae*	
		Colony colour	Mean colony diameter*	Colony colour	Mean colony diameter*
1	Host leaf extract agar/ Host leaf and twig extract agar	Off-white to grey	83.13	Milky white	82.07
2	Asthana and Hawker's agar	Light grey	75.20	Cottony white	78.17
3	Czapek dox agar	Light grey	81.10	Light grey	80.10
4	Richard's agar	Light grey	78.07	Creamy white	75.13
5	Oat meal agar	White to grey	87.17	Creamy white	90.00
6	Water agar	Creamy white	41.03	White to light grey	44.00
7	Potato dextrose agar	Creamy white to grey	90.00	Cottony white	88.03
	SE(m)±		0.11		0.06
	CD @ 1%		0.44		0.27

* Mean of three replications

The data presented in table 4, photo plate 1 and fig. 1 revealed that *C. gloeosporioides* exhibited high degree of variation on different media. All the treatments were statistically significant. Among the solid media evaluated, maximum growth was observed on PDA (90 mm) which was significantly superior to rest of the treatments. It was followed by OMA (87.17 mm), HLEA (83.13 mm), CDA (81.10 mm), RA (78.07 mm), AHA (75.20 mm) and WA (41.03 mm). Fungus produced creamy white to greyish, profuse mycelial growth on different solid media. Later the fungal colony turned black in colour. The pathogen *C. gloeosporioides* initially infects the tender leaves in the new vegetative flush and later on the inoculum switches over to mature leaves. Therefore, the growth of this pathogen was better on HLEA medium.

In case of *L. theobromae* (table 1, plate 2 and fig. 2) all the treatments were statistically significant with the maximum growth (90 mm) in OMA closely followed by PDA (88.03 mm). HLTEA also favoured better growth (82.07 mm) followed by CDA (80.10 mm), AHA (78.17mm), and RA (75.13 mm). Least growth (44.00 mm) was observed in WA. Here also HLTEA was the third effective medium. *L. theobromae* mainly infects the twigs and branches which contain higher amount of lignin. The pathogen therefore grew well in the HLTEA medium. WA is deficient in nutrients and therefore there was least growth of both the pathogens in this medium.

Similar results were reported by Jayalakshmi *et al*. (2013) [6] who reported that highest radial growth and sporulation of *C. gloeosporioides* was observed on PDA (90.00 mm), which was significantly superior to all the other media. Whereas OMA (84.90 mm), Sabouraud dextrose agar (82.00 mm), Richard's agar (81.00 mm), host leaf extract agar (81.00 mm), and malt extract agar (81.00 mm) were next in line. Czapek dox agar showed the least amount of radial growth (72.70 mm). The results of Dharbale *et al*. (2019) [4] are in concurrence with the results of present study in respect of PDA as they reported it as the best medium (90 mm) for the growth of *C. gloeosporioides*. However, their findings about least growth inhost leaf extract Agar (49.67 mm) are contradictory as the pathogen grew profusely in HLEA (83.13 mm). The results of both the experiments about growth in OMA are at similitude.

These findings match partially with the results obtained by Chaudhari *et al.* (2017) [2] as they reported better growth of *L. theobromae* on OMA and CDA. Further they have mentioned that the fungus produced very thin, uniform, thread-like mycelium in all the media. These findings are in agreement with the findings of present study. Also the results obtained in present investigation are in close confirmation with results reported by Suresh *et al*. (2017) [20]. They reported Potato Sucrose medium as the best (8.89 cm) followed by Potato Dextrose Agar (8.46 cm). But the whitish grey to blackish grey colony colour reported by them is contradictory to present results.

In vitro efficacy of *Trichoderma* species against *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*

In vitro efficacy of three *Trichoderma* species against two test pathogens *viz*. *C. gloeosporioides* and *L. theobromae* was carried out using standard dual culture technique with five replications in completely randomized design. All the three bio-agents studied were significantly effective in inhibiting the mycelial growth of both pathogens.

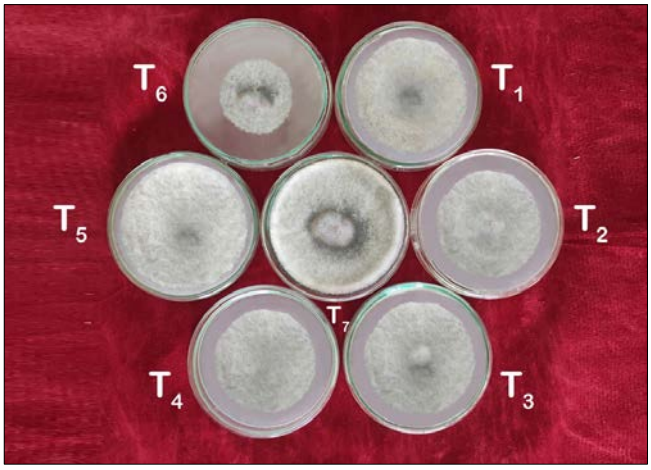

Plate I: Effect of different solid media on the growth of *C. gloeosporioides*

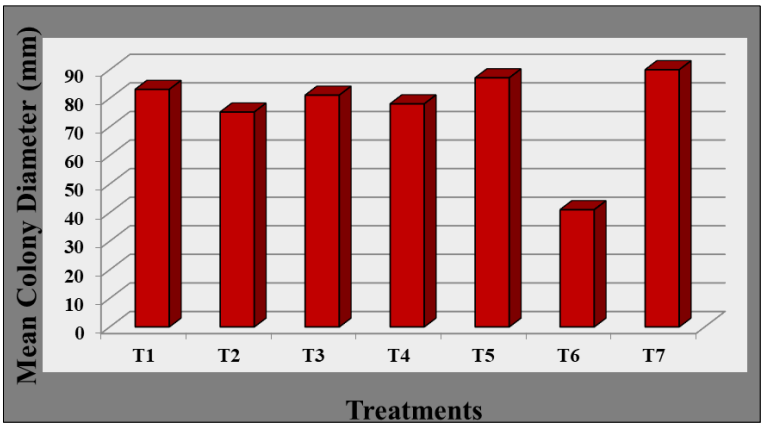

Fig 1: Effect of different solid media on the growth of *C. gloeosporioides*

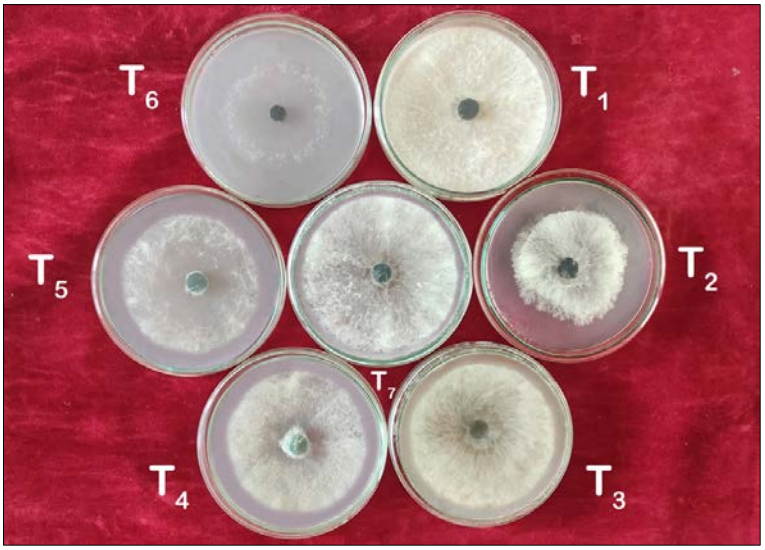

Plate 2: Effect of different solid media on the growth of *L. theobromae*

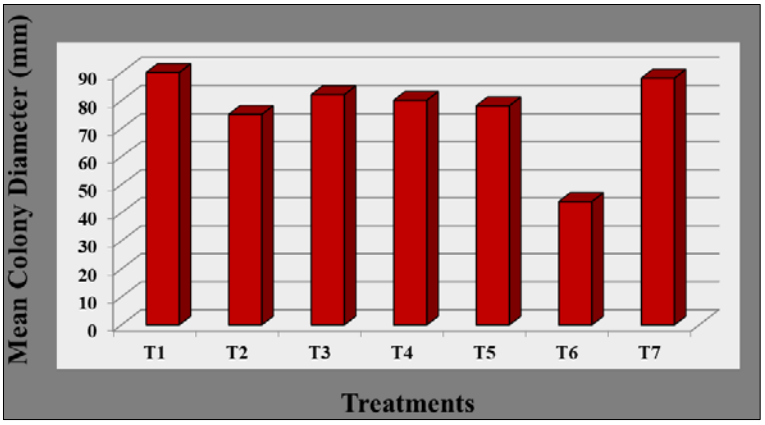

Fig 2: Effect of different solid media on the growth of *L. theobromae*

Table 5: *In vitro* efficacy of Trichoderma spp. against C. gloeosporioides and L. theobromae

Tr. No.	Treatment details	*C. gloeosporioides*		*L. theobromae*	
		Colony dia. (mm)*	Per cent inhibition	Colony dia. (mm)*	Per cent inhibition
T_1	*T. koningii*	52.40	41.78	50.20	44.22
T_2	*T. longibrachiatum*	44.60	50.44	45.60	49.33
T_3	*T. harzianum*	24.20	73.11	25.20	72.00
T_4	Control	90.00	-	90.00	-
SE(m) ±		0.20		0.19	
CD@ 1%		0.83		0.77	

(* Mean colony diameter)

Results presented in table 5, plate3 and fig. 3, showed that all the three bio-agents studied were significantly effective in inhibiting the mycelial growth of both pathogens. *T. harzianum* gave the highest per cent inhibition (73.11%) of *C. gloeosporioides* followed by *T. longibrachiatum* (50.44%). *T. koningii* was the least effective (41.78%) against *C. gloeosporioides*.

In case of *L. theobromae*, data represented in table 5, plate 3 and fig. 4 showed that, *T. harzianum* was the best with significantly superior inhibition (72.00%). Rest two antagonists *T. longibrachiatum* (49.33%) and *T. koningii* (44.22%) recorded less than 50 per cent inhibition.

The three *Trichoderma* species used in this study were isolated by Barde (2022) [1] from the rhizosphere of different plant species *viz*. *T. harzianum*- mango rhizosphere; *T. longibrachiatum*-cabbage rhizosphere and *T. koningii*- from rice. Therefore, the reason for the best performance of *T. harzianum* can attributed to the fact that the isolate was from mango rhizosphere.

The results obtained from present investigation are in congruence with results reported by Prabakar *et al.* (2008) [13]. According to them, *T. harzianum* was the best (inhibition 41.66%) and *T. koningii* (16.7%), and *T. longibrachiatum* (11.1%) were poor performers as they recorded below 20 per cent inhibition of *C. gloeosporioides*. The conclusions of Bhadra and Khair (2014) differ with the present results. According to their results, *T. koningii* was the best with 75% per cent inhibition while *T. harzianum* gave 60% inhibition. The findings of Sharma *et al*. (2021) [16] illustrated that the among all the *Trichoderma* species tested, *T. harzianum* isolate 1 was the most effective against *C. gloeosporioides* with 89.26% mycelial growth inhibition. These results are in conformity with present findings. Similar results were obtained by Wajinku *et al.* (2021) who stated that, among the 4 *Trichoderma* species- *T. atroviride*, *T.virens*, *T. asperellum*, and *T. harzianum* tested against *L. theobromae*, *T. harzianum* was a better performer with 54.57% growth inhibition and it was followed by *T. atroviride* (36.28%).

Field evaluation of *Trichoderma* spp. against *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*

Trichoderma spp. against *C. gloeosporioides*

Trichoderma spp. against *L. theobromae*

Plate 3: *In vitro* Evaluation of *Trichoderma* spp. against *C. gloeosporioides* and *L. theobromae*

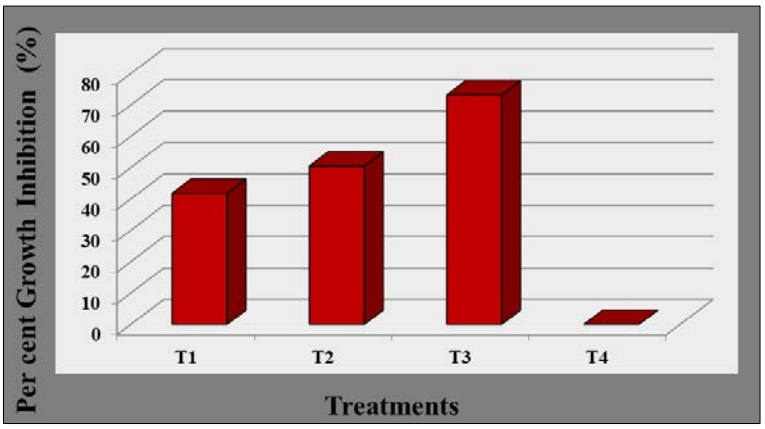

Fig 3: *In vitro* efficacy of *Trichoderma* species against *C. gloeosporioides*

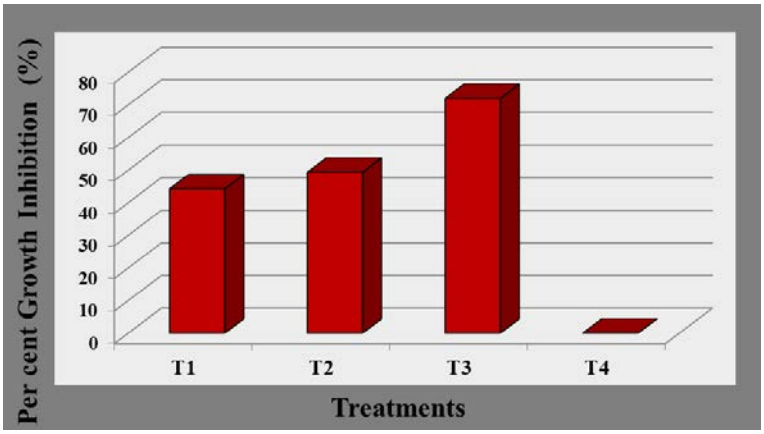

Fig 4: *In vitro* efficacy of *Trichoderma* species against *L. theobromae*

Table 6: Field evaluation of *Trichoderma* spp. against *C. gloeosporioides*

	Treatments	Per cent Disease Index (%)*				PDC
		Before spraying	10days after 1st spray	10days after 2nd spray	10days after 3rd spray	
T_1	*T. harzianum*	11.60(19.91)	13.80(21.81)	16.40(23.89)	18.80(25.69)	63.84
T_2	*T. longibrachiatum*	10.40(18.81)	16.00(23.57)	20.40(26.85)	22.40(28.23)	56.92
T_3	*T. koningii*	12.40(20.61)	24.60(29.73)	30.20(33.34)	31.60(34.20)	39.23
T_4	Control	12.60(20.78)	32.20(34.57)	44.20(41.67)	52.00(46.15)	_
	S.E(m)±	0.27	0.18	0.17	0.44	
	CD@ 5%	0.84	0.55	0.54	1.34	

* Mean of five replications, PDI (Per cent disease index), PDC (Per cent disease control)
Figures in parenthesis are arcsine values.

Data from table 6 and fig. 5 revealed that, among the three bio-agents, *T. harzianum* was found to be highly effective for the control of *C. gloeosporioides*, with highest per cent disease control (63.84%). It was followed by *T. longibrachiatum* (56.92%) and *T. koningii* (39.23%).

Table 7: Field evaluation of *Trichoderma*species against *L. theobromae*

Tr. No.	Treatments	Per cent Disease Index (%)*				PDC (%)
		Before spraying	10days after 1st spray	10days after 2nd spray	10days after 3rd spray	
T_1	*T. harzianum*	18.60(25.55)	20.80(27.13)	25.60(30.39)	29.20(32.70)	57.88
T_2	*T. longibrachiatum*	17.60(24.80)	28.20(32.07)	31.60(34.20)	36.00(36.86)	42.12
T_3	*T. koningii*	19.60(26.28)	31.00(33.83)	35.80(36.75)	43.20(41.09)	30.54
T_4	Control	19.20(25.99)	41.20(39.93)	51.60(45.92)	65.20(53.85)	_
	SE (m)±	0.19	0.18	0.14	0.62	
	CD@ 5%	0.58	0.55	0.44	1.92	

* Mean of five replications, PDI (Per cent disease index), PDC (Per cent disease control) Figures in parenthesis arcsine transformed values.

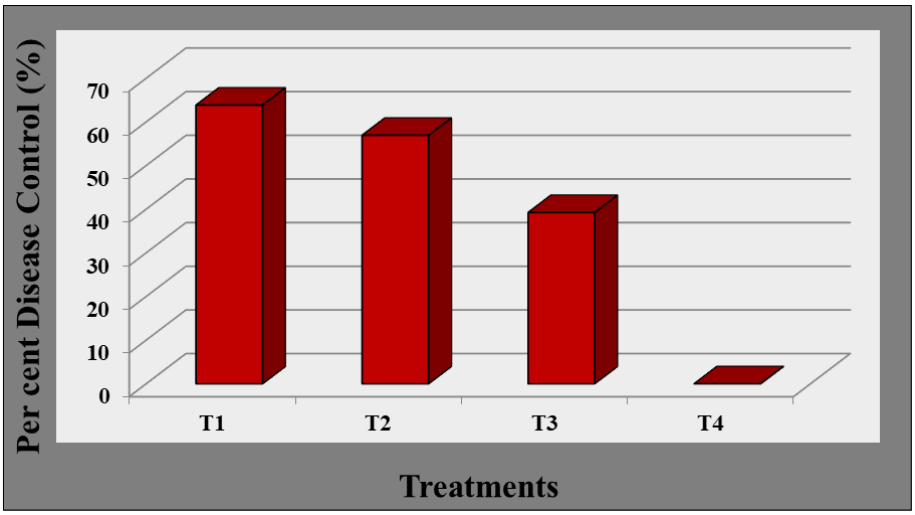

Fig 5: Field evaluation of *Trichoderma* species against *C. gloeosporioides*

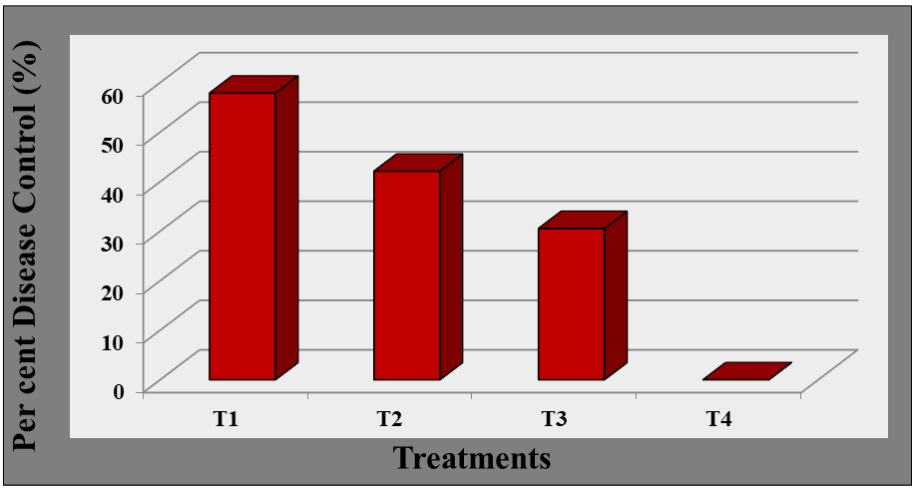

Fig 6: Field evaluation of *Trichoderma* species against *L. theobromae*

In case of *L. theobromae* the data presented in table 7 and fig. 6 revealed that, the maximum disease control (PDC-57.88%) was achieved by *T. harzianum* followed by *T. longibrachiatum* (PDC-42.12%) and *T. koningii* (PDC - 30.54%).

T. harzianum 10% conc.

T. longibrachiatum 10% conc.

T. koningii 10% conc.

Control

Table 8: Field evaluation of *Trichoderma* spp. against postharvest rot of mango fruits

Tr. No.	Treatments	Postharvest fruit rot	
		Per cent disease index (%)*	Per cent disease control
T_1	*T. harzianum*	24.80(29.86)	61.25
T_2	*T. longibrachiatum*	31.20(33.95)	51.25
T_3	*T. koningii*	40.40(39.46)	36.88
T_4	Control	64.00(53.13)	_
	SE(m)±	0.40	
	CD@ 5%	1.23	

* Mean of five replications, PDI (Per cent disease incidence), PDC (Per cent disease control) Figures in parenthesis arcsine transformed values

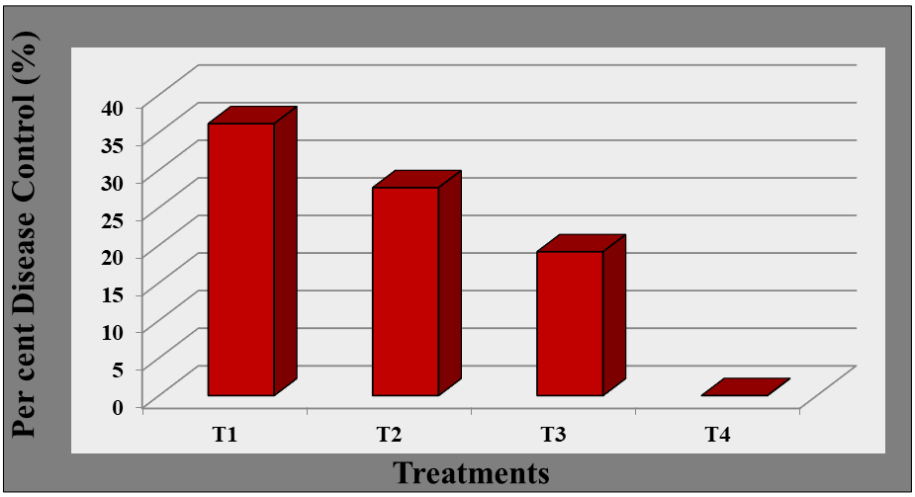

Fig 7: Field evaluation of *Trichoderma* spp. against postharvest rot of mango

It is evident from the data presented in table 8, fig. 7 that spraying of three different *Trichoderma* spp. under field conditions resulted in reduced postharvest rot of mango fruits. After 12 days of harvesting, it was observed that the trees sprayed with *T. harzianum* showed the highest disease control to the tune of 61.25% after which *T. longibrachiatum* recorded 51.25% disease control. *T. koningii* was the least effective with only 36.88% disease control.

Similar results were reported by Noiaium and soytong (2000) [12], who observed that spore suspension of *T. harzianum*and *T. hamatum* at 404x10^{10} spore/ml concentration reduced mango anthracnose incidence by 79.88% and 55.93% respectively. Also present results are in close conformity with Manasa *et al*. (2018) [10] who evaluated effectiveness of pre-harvest sprays of bio-agents like *Trichoderma viride* and *Trichoderma harzianum*, each at a concentration of 0.5 per cent, at various growth stages of mango fruit. When compared to control fruits (21. 33% index), it was found that *T. harzianum* and *T. viride* dramatically reduced the disease index of mango fruits to 17.00 per cent and 15.67 per cent, respectively.

Also the present results are comparable with results obtained by Sharma *et al*. in (2021) [16] who conducted a study where the usefulness of *T. harzianum* for management of mango anthracnose was investigated. Three consecutive sprays of *T. harzianum* at a 5% concentration were administered in the field. The end result revealed 59.91% reduction in disease.

Table 9: Efficacy of fruit dip treatment with spore suspension of *Trichoderma* spp. against postharvest rot of mango

Sr. No.	Treatment Concentration of spore suspension and time of fruit dip	*T. harzianum*		*T. longibrachiatum*		*T. koningii*	
		PDI	PDC	PDI	PDC	PDI	PDC
1	5% for 10min	35.37(36.48)	34.14	38.89(38.46)	27.59	46.29(42.87)	13.81
2	5% for 15min	27.78(31.81)	48.27	37.03(37.44)	31.06	40.74(39.61)	24.14
3	5% for 20min	18.52(25.13)	65.51	29.63(32.84)	44.83	31.48(34.07)	41.39
4	5% for 30min	16.67(23.90)	68.97	22.22(28.01)	58.62	27.78(31.50)	48.28
5	10% for 10min	29.63(32.84)	44.83	35.18(36.29)	34.50	40.74(39.66)	24.14
6	10% for 15min	24.07(28.73)	55.18	33.33(35.22)	37.94	33.33(35.06)	37.94
7	10% for 20min	18.52(25.13)	65.51	22.22(28.01)	58.62	29.63(32.96)	44.83
8	10% for 30min	11.11(19.07)	79.31	16.67(23.90)	68.96	22.22(28.01)	58.62
9	Control	53.71(47.13)	_	53.71(47.13)	_	53.71(47.13)	_
	SE(m) ±	3.17		2.63		2.38	
	CD@ 5%	9.50		7.89		7.14	

* Mean of three replications, PDI (Per cent disease incidence), PDC (Per cent disease control) Figures in parenthesis arcsine transformed values

It is apparent from the data presented in table 9, fig. 8 that when fruits were treated with *T. harzianum* at different concentrations and different time period, treatment comprising fruit dip at 10% concentration for 30min (T_8) was par excellent and significantly superior (79.31) to rest of the treatments. It was followed by T_4 (68.97%) which was at par with T_3 (65.51%) and T_7 (65.51%) which were numerically at par with each other. Further, T_6 (55.18%) was at par with T_2 (48.27%). The treatment T_2 was at par with T_5 which recorded the disease control up to 44.83%. The treatment T_1 recorded the least disease control (34.14%).

In terms of *T. longibrachiatum* (table 9, fig. 9) the treatment (T_8) was found to be the best with PDC of 68.96 per cent and was statistically significant over rest of treatments. It was followed by T_4 (58.62%) which was numerically par with T_7. Further, the treatment T_3 (44.83%) was found at par with T_6 (37.94%) which was at par with T_5 (34.50%) and T_2 (31.06%). The treatments T_5, T_2 and T_1 were statistically at par.

With respect to *T. koningii* also (table 9, fig. 10), (T_8) was the best (58.62%) and was statistically significant to rest of the treatments. It was followed by T_4 (48.28%) which was at par with T_7 (44.83%) and T_7 was at par with T_3 (41.39%) and T_6 (37.94%). These treatments were followed by T_5 (24.14%) and T_2 which were at numerically at par with each other. The least disease control was observed in case of treatment T_1 (13.81%).

These results indicate that dipping the fruits in spore suspension of all the three *Trichoderma* species at maximum

concentration (10%) for maximum length of time (30 min) was effective in reducing postharvest fruit rot. But *T. harzianum* was the most effective. The *T. harzianum* isolate used in this experiment was obtained from mango rhizosphere and that could be the reason for its excellent performance. Further, fruit dip for 20 minute and /or 30 min facilitated spore deposition followed by spore germination due to available free water which subsequently increased the colonization of the antagonists on fruit surface.

These results are in close consonance with the results reported by Prabakar *et al*. (2008) [13] who used inoculated as well as non-inoculated fruits for fruit dip treatment. They also concluded that *T. harzianum* spore suspension (10^6spores/ml) for 5 minutes demonstrated 41.67% and 40.00% disease index in non-inoculated and inoculated fruits respectively. Similarly Suhanna *et al*. (2013) [18] reported that treatment with *Trichoderma* spp. at $1x10^6$ conidia/ml concentration, considerably reduced the per cent disease lesion to31.7%, followed by 35% at $1x10^8$conidia/ml. The conclusions of this study confirms the findings of Sharma *et al*. (2021) [16] who assessed *T. harzianum* (1) and *T. viride* for their comparative effectiveness against postharvest anthracnose of mango at a concentration of $1.2x10^4$cfu/ml and reported that, *T. harzianum* isolate 1 was found effective with 81.67% reduced disease incidence.

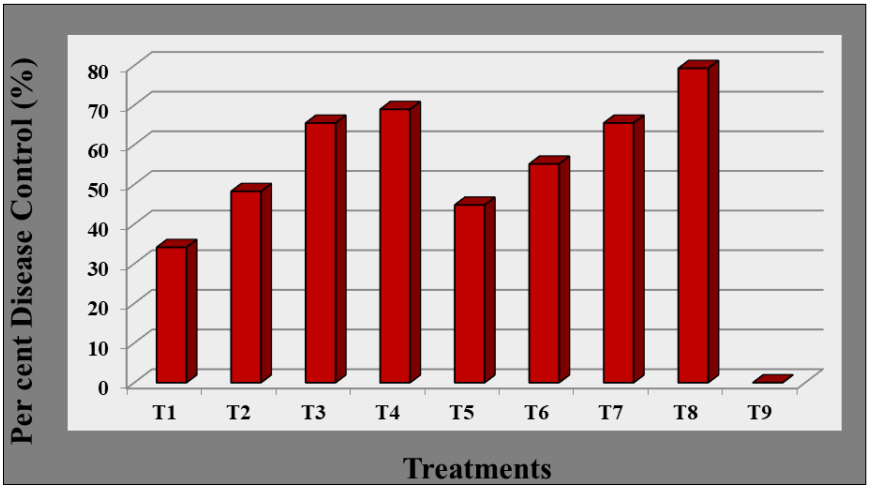

Fig 8: Efficacy of *T. harzianum* fruit dip treatment against postharvest rot of mango

T. harzianum 10% for 30 min

T. longibrachiatum 10% for 30min

T. koningii 10% for 30 min

Control

Plate V: Effective fruit dip treatments

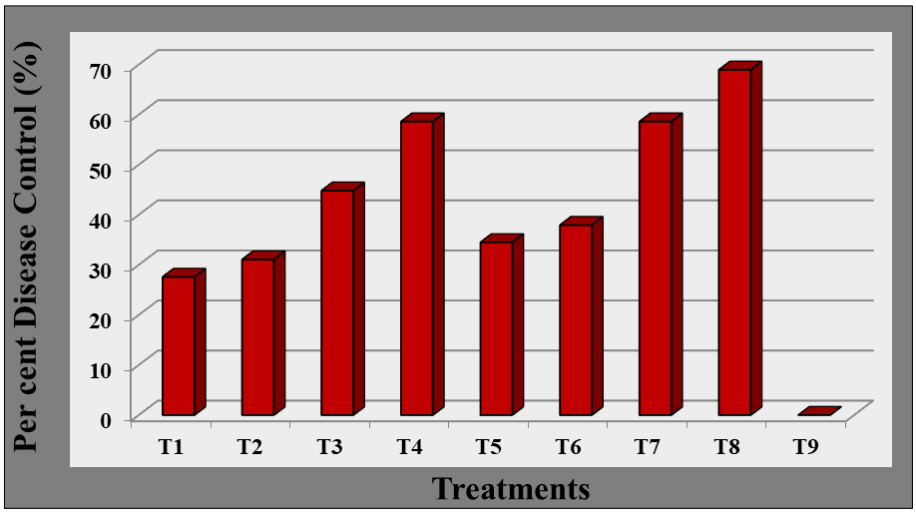

Fig 9: Efficacy of *T. longibrachiatum* fruit dip treatment against postharvest rot of mango

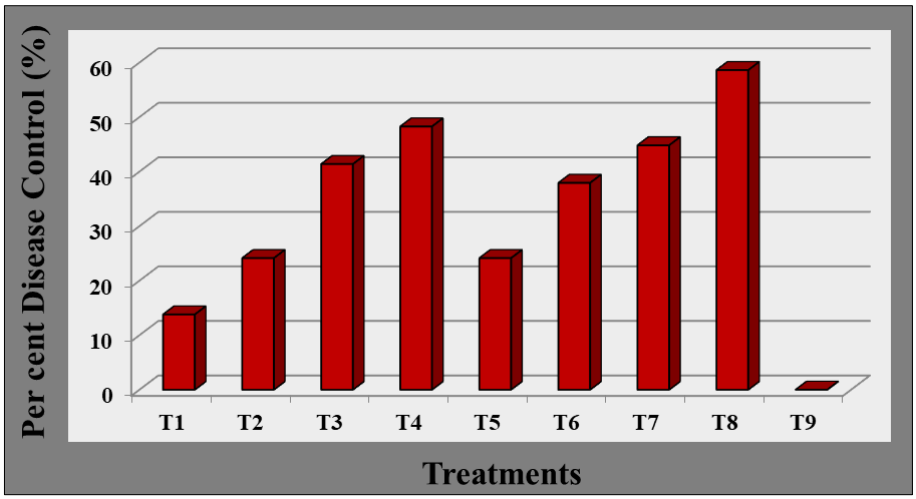

Fig 10: Efficacy of *T. koningii* fruit dip treatment against postharvest rot of mango

Conclusion

Trichoderma spp. was found to be the effective bio-agent for the control of notorious diseases like anthracnose and die-back of mango. Among all the species used, *Trichoderma harzianum* was observed to be the best under *in vitro*, field conditions as well as for fruit dip treatment.

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References

1. Barde PS. Investigation on potential *Trichoderma* spp. in konkan region. Ph.D. (Agri.) Thesis, Dr. B.S.K.K.V. Dapoli; c2022.
2. Chaudhuri T, Panja B, Saha J. Cultural and morphological characteristics of *Lasiodiplodia theobromae* of Dianella in various carbon and nitrogen containing media. J Pharmaco. and Phytochem. 2017;6(6):160-164.
3. Dennis C, Webster J. Antagonistic properties of species-groups of *Trichoderma* hyphal interaction. Trans. Br. Mycol. Soc. 1971;57(3):363-369.
4. Dharbale BB, Hingole DG, Bhalerao JB, Kardile PB. Studies on cultural and morphological characteristics of *Colletotrichum gloeosporioides* (Penz.) Sacc. in sweet orange at Marathwada region of Maharashtra. Journal of Pharmacognosy and Phytochemistry. 2019;8(2):1154-1158
5. Dodd JC, Prusky D, Jeffries P. Fruit diseases in: Litz, R.E. (Ed.), The Mango: Botany, Production and Uses. CAB International, UK. Hort. 1012, ISHS; c1997. p. 257-280.
6. Jayalakshmi K, Nargund VB, Raju J, Benagi VI. Cultural characterization of *Colletotrichum gloeosporioides* (Penz. And Sacc.) causing anthracnose of pomegranate. Bioinfolet. 2013;10(2A):498-501.
7. Jin X, Hayes CK, Harman GE. Principles in the development of biological control systems employing *Trichoderma* species against soil-borne plant pathogenic fungi. Frontiers in Industrial Mycology; c1992. p. 174-175.
8. Khanzada MA, Lodhi AM, Shahzad S. Mango dieback and gummosis in sindh, Pakistan Caused by *Lasiodiplodia theobromae*. Plant Health Prog. 2004;5(1):13.
9. Kumar AS, Reddy NPE, Reddy KH, Devi MC. Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in agri export zone of Andhra Pradesh, India. Plant Pathol. Bulletin. 2007;16(3):157-160.

10. Manasa B, Jagadeesh SL, Thammaiah N, Sandhyarani N, Gangadharappa PM, Jagadeesha RC, *et al*. Evaluation of fungicides, bioagents and botanicals on postharvest disease, shelf life and physico-chemical properties of 'Alphonso' mango. J Pharmacogn. Phytochem. 2018;7(4):1883-1888.
11. Mckinney HH. Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. J Agric. Res. 1923;26:195-217.
12. Noiaium S, Soytong K. Integrated biological control of mango var. Choke Anan. Acta. Hort; c2000. p. 509.
13. Prabakar K, Raguchander T, Saravanakumar D, Muthulakshmi P, Parthiban VK, Prakasam V. Management of postharvest disease of mango anthracnose incited by *Colletotrichum gloeosporioides*. Arch. Phytopathol. Pflanzenschutz. 2008;41(5)333-339.
14. Rungjindamai N. Isolation and evaluation of biocontrol agents in controlling anthracnose disease of mango in Thailand. J Plant Prot. Res; c2016. p. 56. 10.1515/jppr-2016-0034.
15. Saeed S, Khan M, Masood A. Symptom development after artificial inoculation of *Botryodiplodia theobromae*, a possible causal organism to quick decline in mango trees. Pak. J Agri. Sci. 2011;48(4):289-294.
16. Sharma A, Sharma IM, Sharma M, Sharma K, Sharma A. Effectiveness of fungal, bacterial and yeast antagonists for management of mango anthracnose (*Colletotrichum gloeosporioides*). Egypt. J Biol. Pest Control. 2021;31:135.
17. Sudha S, Narendrappa T, Sivakumar G. Management of post-harvest anthracnose disease in mango using promising biocontrol agents. J Pharm. Innov. 2021;10(3):210-214.
18. Suhanna A, Nor HAY, Shazalwardi S. *Trichoderma* spp. as a Biological Control Agent in the Postharvest Treatment of Mango Stem-End Rot. Acta; c2013.
19. Sundravadana S, Alice D, Kuttalam S, Samiyappan R. Efficacy of azoxystrobin on *Colletotrichum gloeosporioides* penz. growth and on controlling mango anthracnose; c2007. https://www.researchgate.net/publication/237779979
20. Suresh V, Vidya Sagar B, Sumalatha N, Rajendraprasad M. Effect of culture media and different temperatures on mycelial growth and pycnidial production of *Lasiodiplodia theobromae* causal agent of mango gummosis. J Mycopathol, Res. 2017;54(4):531-534.
21. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature; c1947. p. 159-180.
22. Wanjiku EK, Waceke JW, Mbaka JN. Suppression of Stem-End Rot on Avocado Fruit Using *Trichoderma* spp. in the Central Highlands of Kenya Adv Agric. 2021;8867858:6 pages. https://doi.org/10.1155/2021/8867858
23. Zhang F, Yuan J, Yang X, Cui Y, Chen L, Ran W, *et al*. Putative *Trichoderma harzianum* mutant promotes cucumber growth by enhanced production of indole acetic acid and plant colonization. Plant Soil. 2013;368(1-2):433-444.