Fungicides and bioagents: evaluation of pathogen eradication under laboratory conditions

Abstract

In an *in-vitro* study evaluating eight different fungicides against *C. musae*, carbendazim, carbendazim 12% + mancozeb 63%, and azoxystrobin 11% + tebuconazole 18.3% exhibited 100% inhibition of fungal growth at concentrations of 0.1%, 0.25%, and 0.1%, respectively. Propiconazole (0.05%) was also the most effective fungicide, showing 83.03% mycelial inhibition, followed by carboxin 37.5% + thiram 37.5% (73.51%) and chlorothalonil (76.84%) at concentrations of 0.25% and 0.2%, respectively. Mancozeb exhibited the minimum mycelial inhibition (66.44%) at a concentration of 0.25%, while copper oxychloride showed the least inhibition (48.96%) at 0.25% concentration. In addition, five biocontrol agents were screened for linear growth inhibition of *C. musae* through the dual culture method. Among the *Trichoderma* isolates, *T. harzianum* demonstrated the highest growth inhibition (84.38%), followed by *Pseudomonas fluorescens* (80.16%).

Keywords: *C. musae*, bioagents, efficacy, inhibition, potential biocontrol agents

1. INTRODUCTION

Banana (*Musa paradisica* L.) is an important fruit crop in tropical and sub-tropical regions. The term banana was introduced from the Guinea Coast of West Africa by the Portuguese while; the term ‘Plantain’ (for cooking bananas) was derived from ‘Plantano’ of the Spaniards. The banana, which is a member of the *Musaceae* family, is India's and the world's most significant fruit crop. It is renowned for its age and has a long history that dates back to the beginning of civilization in India. Due to its socioeconomic importance and variety of uses, the banana crop is known as "Kalpataru" (Plant of Heaven) and defines the socioeconomic standing of the farmers. It is known as "Poor man's apple" and is inexpensive and nutrient-rich. Unripe fruit is consumed as a starchy food. Banana is cultivated in nearly 120 countries in the world. Banana is the fourth largest fruit crop of the world. Major banana producing countries are India, China, Indonesia, Brazil, Ecuador, Philippines, Guatemala, Angola, United Republic of Tanzania, Colombia, Costa Rica. In world, 5.6 million ha of land are dedicated to banana with production of 119.8 million metric tons [1]. India being the largest producer at 30.5 million metric tons on 866,000 ha. China comes second with 12 million metric tons per year on 358,924 ha. with 7.2 million metric tons of production per year, Indonesia is the third largest producer of banana [1].

In terms of production among fruits, bananas top the list in India. After mango and citrus, it is the third largest in terms of area. India produces 324.54 lacs MT of bananas annually from an area of 8.84 lac ha. Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu, Karnataka, Uttar Pradesh, Bihar, Madhya Pradesh, West Bengal, Assam, Chhattisgarh are the top banana producing states of India. The production of Andhra Pradesh is 58.38 lakh MT and share is 17.99% Maharashtra ranks second with production of 46.28 lakh MT and share of 14.26%. Gujarat being third on position with production of 39.07 lakh MT and share of 12.04 % of India's total production [13]. In addition to being a great

source of potassium, vitamin A, vitamin C, vitamin B-6, and fiber, bananas are also low in fat, cholesterol-free, and salt. Certain blood pressure drugs reduce the body potassium reserves. The potassium balance is restored by consuming one banana each day. At least five servings of fruits or vegetables per day are advised in order to maintain a healthy diet and reduce the risk of cancer. According to a recent study, consuming nine or ten servings of fruits and vegetables per day, together with three portions of low-fat dairy products, can significantly lower blood pressure. The ripe banana fruits are edible, delicious and very nutritious. Banana is a rich source of vitamin A and fair source of vitamin C, B and B1. The fruits are also rich in carbohydrates, magnesium, sodium, potassium and phosphorus. It contains 17 mg calcium, 36 mg phosphorus, 27 g carbohydrates and 1 g protein in 100 g fruit. From the nutritional point of view banana has a calorific value of 116 calories per 100 g fruit. The food value is about three times that of wheat. It makes healthy and salt free balanced diet than many of the fruits [9]. The lack of proper storage and transportation infrastructure makes post-harvest losses more common in underdeveloped nations [18]. For many years, mycologists have focused their attention on the microbes responsible for fruit rotting after harvest. Fruit deterioration after harvest results in enormous losses. According to [8, 10]. infections cause 20 to 25 percent of harvested fruits to rot during post-harvest handling. Banana fruits are extremely prone to spoilage. At room temperature, bananas have a limited shelf life as climacteric fruit. Without any restrictions on the use of ripening agents, banana fruits are professionally ripened and kept by fruit merchants. Due to inappropriate handling, inadequate storage, and post-harvest infections, there is a 25 to 30 % post-harvest loss of bananas [8, 10]. Fruits like bananas suffer significant post-harvest losses in tropical nations like India. The cultivated banana is prone to a wide range of diseases, mostly fungi that affect the plant's numerous parts from the root to the fruit. During storage, banana fruits deteriorate through the activity of microorganisms and their activity is favored by the changing physiological state of the fruit. Banana fruit suffers from many serious diseases such as anthracnose, crown rot, finger rot, white rot, cigar end rot etc. Due to all these diseases storage of banana is difficult. The two primary postharvest rots of banana fruits are anthracnose and crown rot. The fungus *Colletotrichum musae* can cause both crown rot and anthracnose; in addition, crown rot diseases may also be caused by fungal pathogens in the genera *Fusarium*, *Acremonium*, *Verticillium* and *Curvularia* [25, 26]. The fungus *Colletotrichum* has been the most notorious fungal pathogen, which causes severe rots which rapidly deteriorating fruit quality and rendering the fruit completely to a rotten with sticky mass tickling from the infected pulpy banana [27]. One of the most significant postharvest diseases of bananas is anthracnose, which is typically brought on by the fungus *Colletotrichum musae* (Berk. & Curt.), which affects both mature and damaged green fruits [27]. Additionally, banana tip rot, crown rot, and blossom end rot have all been caused by *C. musae* [28, 29, 30, 311, 32, 33, 34, 35]. The disease typically develops during extended periods of storage and transit that are characterized by low temperatures and high humidity. Banana anthracnose is characterized by brown patches that develop into depressed lesions with acervuli that are orange or pink in color. The disease won't be seen until the fruit ripens. The following objectives guided the planning and execution of the current investigations on post-harvest fruit rot of bananas in light of the disease's significance [27].

2. MATERIAL AND METHODOLOGY

2.1 Source of pathogen

The pathogen *C. musae* was isolated from naturally infected banana fruit which is brought from Dhule fruit market showing typical symptoms circular to angular / irregular ring, light to dark brown spots with a dark red or blackish margin were used to isolate the pathogen by tissue segment method [16] on potato dextrose agar (PDA) medium. Further, the seven days old culture of *C. musae* were used for the *in vitro* efficacy of bioagents and fungicides test.

2.1 *In-vitro* evaluation of different fungicides

Eight different fungicides were evaluated *in vitro* against *C. musae*, using Potato dextrose agar as basal culture medium and applying Poisoned food technique (Nene and Thapliyal, 1993). Based on active ingredient, requisite quantity of the test fungicides was calculated, dispensed separately and mixed thoroughly with autoclaved and cooled (40 ^{0}C) PDA medium in glass conical flasks (250 ml capacity) to obtain desired concentrations. This PDA medium amended separately with the test fungicides was then poured (20 ml / plate) aseptically in sterile glass Petri-plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its test concentration, three plates / treatment / replication were maintained. After solidification of the PDA medium, all these plates were inoculated aseptically by placing in the centre a 5 mm culture disc obtained from actively growing 7 days old pure culture of *C. musae* test isolate and incubated in an inverted position at 25±2 ^{0}C. Petri-plates filled with plain PDA (without any fungicide) and inoculated with pure culture disc of the test isolate were maintained as untreated control. The experimental details were as follows: Design: Completely Randomized Design (CRD), Replications: Three, Treatments: Nine

2.2 *In-vitro* evaluation of bio-agents

The bioagents were evaluated *in vitro* against *C. musae* test pathogens, applying dual culture technique [6]. Seven days old cultures of the test bioagents and test isolates of the pathogens grown on respective culture media were used for the study. Two 5 mm culture discs, one each of the test isolates and the test bioagents were cut out with sterilized Cork-borer, placed at equidistance, exactly opposite to each other on autoclaved and solidified PDA medium in Petri-plates and incubated at 25 ± 2 ^{0}C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogens were also incubated as untreated control. The results were expressed as per cent growth inhibition over control. After incubation the growth of antagonist and test fungus was measured by linear measurement and average value were calculated. Index of antagonism was determined in each treatment by following standard formula given by Vincent [24].

$$AI = \frac{C - T}{C} \times 100$$

Where, AI= Percent antagonism index, C= Area of test fungus in control (mm), T= Area of test fungus in respective treatment (mm). The experimental details were as given below: Design: Completely Randomized Design (CRD), Replications: Four, Treatments: Six

3. RESULT AND DISCUSSION

3.1 *In-vitro* evaluation of fungicides

Eight different fungicides belonging to different groups were tested *in vitro* for their efficacy against *C. musae*, by employing poisoned food technique and using PDA as basal medium. The obtained data on the effect of different fungicides on the inhibition and radial mycelial growth of the test pathogen was recorded and results obtained are presented in Table 1 and Fig-1.

Table 1: *In-vitro* effect of fungicides on mycelial growth and inhibition of *C. musae.*

Treat.	Fungicides	Concentration Used (%)	Radial mycelial growth (mm)	Percent inhibition at concentration (%)
T_1	Propiconazole	0.05	15.26[#]	83.03
			(3.91)[**]	(65.68)[*]
T_2	Carbendazim	0.1	00.00	100.00
			(0.00)	(0.00)
T_3	Mancozeb	0.25	32.20	66.44
			(5.67)	(54.60)
T_4	Chlorothalonil	0.2	20.83	76.84
			(4.57)	(61.24)
T_5	Copper oxychloride	0.25	45.93	48.96
			(6.77)	(44.40)
T_6	Carbendazim 12% + Mancozeb 63%	0.25	00.00	100.00
			(0.00)	(0.00)
T_7	Carboxin 37.5% + Thiram 37.5%	0.25	23.83	73.51
			(5.49)	(59.02)
T_8	Azoxystrobin 11% +Tebuconazole 18.3%	0.1	00.00	100.00
			(0.00)	(0.00)
T_9	Control (Untreated)	--	90.00	0.00
			(9.49)	(0.00)
	S.E.± (m)	--	0.14	0.16
	C.D. at 1%	--	0.42	0.47

Average of three replications, *Figures in parenthesis are arc sine transformation values.
**Figures in parenthesis are square root transformation ($\sqrt{n+1}$) values.

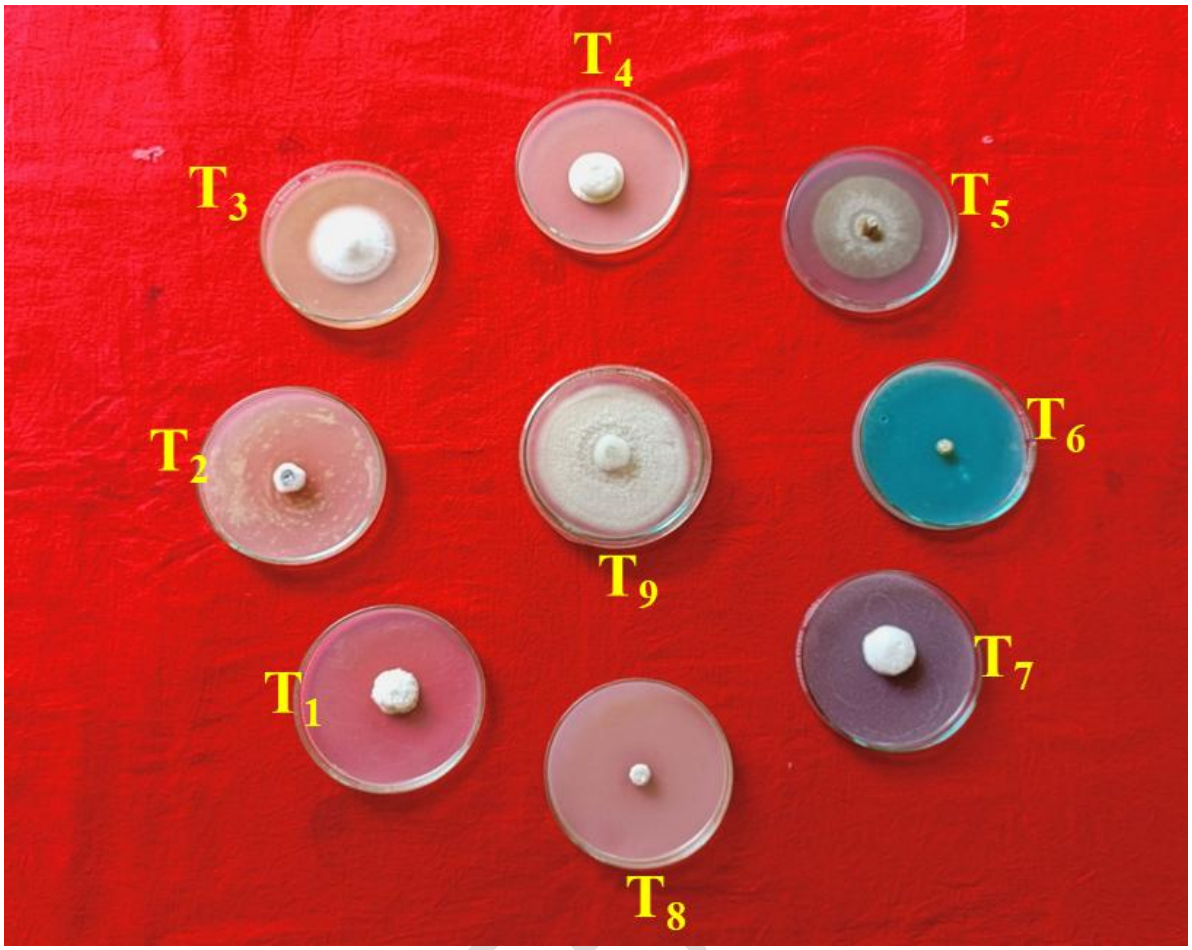

Fig 1: In vitro evaluation of fungicides on mycelial growth and inhibition of *C. musae*

Result (Fig 1) revealed that treatment of carbendazim, carbendazim 12% + mancozeb 63% and azoxystrobin 11% + tebuconazole 18.3%, were found 100 % mycelial inhibition at 0.1 %, 0.25 % and 0.1 % concentration of *C. musae* which were statistically, at par with each other and significant with rest of the treatments including untreated control. The treatment of propiconazole 0.05 % concentration was found next best fungicide and showed 83.03 % mycelial inhibition, followed by chlorothalonil (76.84 %) and carboxin 37.5 %+ thiram 37.5 % (73.51%) mycelial growth inhibition at 0.2 % and 0.25% respectively. Mancozeb was found minimum mycelial growth inhibition (66.44%) at 0.25% concentration, followed by copper oxychloride (48.96%) mycelial growth inhibition at 0.25%. All the fungicides were found effective against test fungus the observations of present investigation are in conformity with reports of Kolase [11] who also stated that, carbendazim at 0.1 per cent was shown to be effective in reducing the mycelial growth of *C. gloeosporioides*. Similarly, [19] who also reported that, carbendazim + mancozeb and propiconazole at 500 and 1000 ppm were found completely inhibited fungus growth and proved to be highly toxic to *C. gloeosporioides.* Somashekhara and Vani [23] noticed that propiconazole, difenconazole and tebuconazole were determined to be the most effective in inhibition of growth of fungus in laboratory condition.

3.2 *In-vitro* evaluation of different bioagents

The results obtained on mycelial growth and inhibition of *C. musae* with five fungal antagonists are presented in Table 2 revealed that all the bioagents evaluated exhibited antifungal activity against *C. musae* and significantly inhibited its growth over untreated control (Table 2, Fig 2). The antagonistic actions of five bio agents were evaluated against the *C. musae* fungus by dual culture technique. Based on observations of radial mycelial growth of antagonist and anthracnose fungus, per cent inhibition was calculated. The results are expressed in Table 2, Fig 2.

Table 2: *In-vitro* evaluation of bioagents on mycelial growth and inhibition of *C. musae.*

Tr. No.	Bioagents	Radial mycelial growth (mm)	Percent inhibition (%)
T_1	*Trichoderma viride*	24.15[#] (4.92)[**]	73.16 (58.80)[*]
T_2	*T. hamatum*	20.07 (4.47)	77.69 (61.82)
T_3	*T. harzianum*	14.05 (3.77)	84.38 (66.72)
T_4	*Bacillus substilis*	30.10 (5.51)	66.55 (54.67)
T_5	*Pseudomonas fluorescens*	17.85 (4.20)	80.16 (63.55)
T_6	Control (Untreated)	90.00 (9.49)	0.00 (0.00)
	S.E.±(m)	0.24	0.27
	C.D at 1%	0.72	0.80

#Average of four replications. *Figures in parenthesis are arc sine transformation values.

**Figures in parenthesis are square root transformation ($\sqrt{n+1}$) values.

Fig 2: *In-vitro* evaluation of bioagents on mycelial growth and inhibition of *C. musae*

The result revealed that all antagonists were capable to inhibiting the mycelial growth of *C. musae*. All the biocontrol agents significantly inhibited the mycelia growth of *C. musae* as compared to lower inhibiting bacterial biocontrol agent *B. subtilis*. Among all the bioagents significantly maximum inhibition of test pathogen (84.38 %) was observed in the presence of *T. harzianum* which was significantly superior as compared to other tested biocontrol agents. *P. fluorescens* (80.16 %) remained better effective biocontrol agent. The *T. hamatum* found moderately effective with (77.69%) per cent growth inhibition. The least inhibition of pathogen (73.16%) was observed in the presence of *T. viride*. Similarly, [7, 22] observed the antagonistic effects of *T. harzianum* over the *Colletotrichum* spp. [3, 4, 20, 21] also reported reduction in mycelium growth of *Colletotrichum* spp. due to presence of *Trichoderma* spp. in culture media. Costa and Subasinghe [5, 15, 16] observed the effect of *P. fluorescens* on the *C. musae*. The results regarding the effect of bio agents are also agreements with the many plant pathologists, [14] who also reported that *T. harzianum* and *T. viride* were shown to be the most effective *in vitro* condition against *C. gloeosporioides*. Above mentioned findings are also tallying with the [12] who tested the effect of bioagents *in vitro* and stated that, *T. viride* with 74.22 percent *P.s fluorescens* 57.56 per cent inhibition against *C. gloeosporioides*.

CONCLUSION

The current research concluded that, among the eight different fungicides evaluated in vitro against *C. musae* the pathogen was completely restricted by three fungicides namely, carbendazim, carbendazim 12% + mancozeb 63% and azoxystrobin 11% + tebuconazole 18.3% at concentration 0.1%, 0.25% and 0.1% respectively. Where, among the five different bioagents tested in vitro against *C. musae*, the *Trichoderma harzianum* was found most effective and recorded highest per cent mycelial inhibition (84.38 %).

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