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In vitro evaluation of non-systemic fungicides against *Colletotrichum gloeosporioides* causing fruit rot in banana

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Abstract
Banana (*Musa paradisiaca* L.) crop suffers from several fungal diseases, among which fruit rot caused by *Colletotrichum gloeosporioides* is one of the serious disease observed regularly in banana growing areas. Therefore, efforts were made to evaluate the different non-systemic fungicides *in vitro* condition against *Colletotrichum gloeosporioides*. Among the seven non-systemic fungicides evaluated *in vitro* against *C. gloeosporioides*, the pathogen was completely restricted by four non-systemic fungicides namely mancozeb 75% WP, copper oxychloride 50% WP, copper hydroxide 77% WP and zineb 75% WP at all three test concentrations (@ 1500, 2000 and 2500 ppm), whereas, propineb 70% WP (@ 2000 and 2500 ppm) and chlorothalonil 75% WP (@ 2500 ppm), also shows complete mycelial inhibition. These were followed by captan 50% WP, of the test pathogen.

Keywords: Banana, fruit rot, *Colletotrichum gloeosporioides*, contact fungicides, poisoned food technique and potato dextrose agar

Introduction
Banana (*Musa paradisiaca* L.) is a popular fruit crop grown widely in tropical countries and has a high consumer demand worldwide due to its flavour, texture, nutritional value and eating convenience (Robinson, 1996) [7]. It can be grown round the year and it is widely adopted in India. Major banana producing countries are India, China, Philippines, Brazil, Ecuador, Indonesia, Costa Rica, Mexico, Thailand and Colombia. In India, the area under banana is 8,57,000 hectare with 30,201,000 metric tonnes and productivity of 34.00 metric tonnes per hectare during 2017-18. The largest area under banana cultivation in India is in Tamil Nadu followed by Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Madhya Pradesh and Bihar. Maharashtra is the second highest banana producer state in India with 30,72,490 metric tonnes production with an area 74,680 hectare and productivity 44.00 metric tonnes per hectare during 2016-17 (Horticultural statistics- at a glance, 2017) [4]. Banana is a rich source of easily digestible carbohydrate and hence, useful as a food supplement. It provides vitamin B and C and minerals such as calcium and magnesium. Major economic part of the banana plant is the fruit, suffers from many post harvest diseases. These diseases has considerable influence on different aspects of cultivation, nutritive value, harvesting, transit and transhipment, storage of fruits. It is estimated that 20 to 25 per cent of harvested fruits are decayed by pathogens during post harvest handling even in developed countries (Zhu and Ma, 2007) [9]. In India, banana crop is affected by several diseases induced by fungi, bacteria, viruses and nematodes. Amongst them fruit rot of banana caused by *Colletotrichum gloeosporioides* is economically important. Post harvest decay of fruits causes tremendous losses. It is estimated that 20 to 25% of harvested fruits are decayed by pathogens during post harvest handling (Droby and Zhu, 2006) [3]. Considering these issues, present study was planned and conducted with the aim to evaluate the different non-systemic fungicides *in vitro* condition against *Colletotrichum gloeosporioides* causing post harvest fruit rot in banana.

Material and Methods
Seven non-systemic fungicides (each @ 1500, 2000 and 2500 ppm) were evaluated *in vitro* against *C. gloeosporioides*, using Potato dextrose agar as basal culture medium and applying Poisoned food technique (Nene and Thapliyal, 1993) [5]. The experiment was conducted in Completely Randomized Design (CRD) with three replication of each treatment. Seven non systemic fungicides namely: captan 50% WP, mancozeb 75% WP, chlorothalonil 75% WP, copper oxychloride 50% WP, propineb 70% WP, copper hydroxide 77% WP and zineb 75% WP were tested each at three concentrations (each @ 1500, 2000 and 2500 ppm). Based on active ingredient, requisite quantity of the test fungicides was calculated, dispensed separately

and mixed thoroughly with autoclaved and cooled (400C) 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. gloeosporioides* test isolate and incubated in an inverted position at 28±20C. Petri-plates filled with plain PDA (without any fungicide) and inoculated with pure culture disc of the test isolate was maintained as untreated control. Observations on radial mycelial growth / colony diameter of the test pathogen was recorded at 24 hrs interval and continued till growth of the test pathogen in untreated control plate was fully covered. Per cent inhibition of the test pathogen was calculated by applying following formula (Vincent, 1927) [8].

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

Where,
C= growth of the test fungus in untreated control plates T= growth of the test fungus in treated plates

Result and Discussion

In vitro evaluation of non-systemic fungicides against *Colletotrichum gloeosporioides* was done as described in material and methods by following Poisoned food technique. The non-systemic fungicides were tested at 1500, 2000 and 2500 ppm concentrations each and the observations on colony diameter and per cent inhibition of colony growth over control are presented in Table 1.

Radial mycelial growth

Results (Table 1, Fig 1 and Plate I) revealed that all the non-systemic fungicides tested exhibited a wide range of radial mycelial growth of *Colletotrichum gloeosporioides*, which was found to be decreased drastically with increase in their concentration. Radial mycelial growth / colony diameter of test isolate of *Colletotrichum gloeosporioides* was completely restricted by four non-systemic fungicides mancozeb 75% WP, copper oxychloride 50% WP, copper hydroxide 77% WP and zineb 75% WP at all three test concentrations (@ 1500, 2000 and 2500 ppm), propineb 70% WP (@ 2000 and 2500 ppm) and chlorothalonil 75% WP (@ 2500 ppm). Next to these fungicides, significantly least (8.00 mm) mycelial growth exhibited with propineb 70% WP (@ 1500 ppm) followed by captan 50% WP (36.33, 33.41 and 29.00 mm) and chorothalonil 75% WP (50.41 and 44.08 mm) at two concentrations (@ 1500 and 2000 ppm), respectively against maximum mycelial growth (90.00 mm) in untreated control.

Mycelial growth inhibition

Result (Table 1, Fig 1 and Plate I) revealed that all the non-systemic fungicides tested each @ 1500, 2000 and 2500 ppm significantly inhibited per cent mycelial growth of *Colletotrichum gloeosporioides*, over untreated control and it was directly proportional to concentrations of the fungicides tested. Among non-systemic fungicides tested per cent mycelial growth inhibition was (100.0%) over untreated control with non-systemic fungicides mancozeb 75% WP, copper oxychloride 50% WP, copper hydroxide 77% WP and zineb 75% WP at all three test concentrations (@ 1500, 2000 and 2500 ppm), propineb 70% WP (@ 2000 and 2500 ppm) and chlorothalonil 75% WP (@ 2500 ppm). These were followed by propineb 70% WP (@ 1500 ppm) with per cent mycelial growth inhibition (91.11%), captan 50% WP (59.63, 62.87 and 67.77%) at all three test concentrations (@ 1500, 2000 and 2500 ppm) and chlorothalonil 75% WP (43.98 and 51.02%) at two concentrations (@ 1500 and 2000 ppm), respectively against untreated control (0.00%).

Average per cent radial mycelial growth inhibition recorded with the non- systemic fungicides tested was ranged from 63.42 to 100.00%. However, it was significantly highest per cent (100%) mycelial growth inhibition with fungicides mancozeb 75% WP, copper oxychloride 50% WP, copper hydroxide 77% WP and zineb 75% WP followed by propineb 70% WP (97.03%), chlorothalonil 75% WP (65.00%) and captan 50% WP (63.42%), respectively.

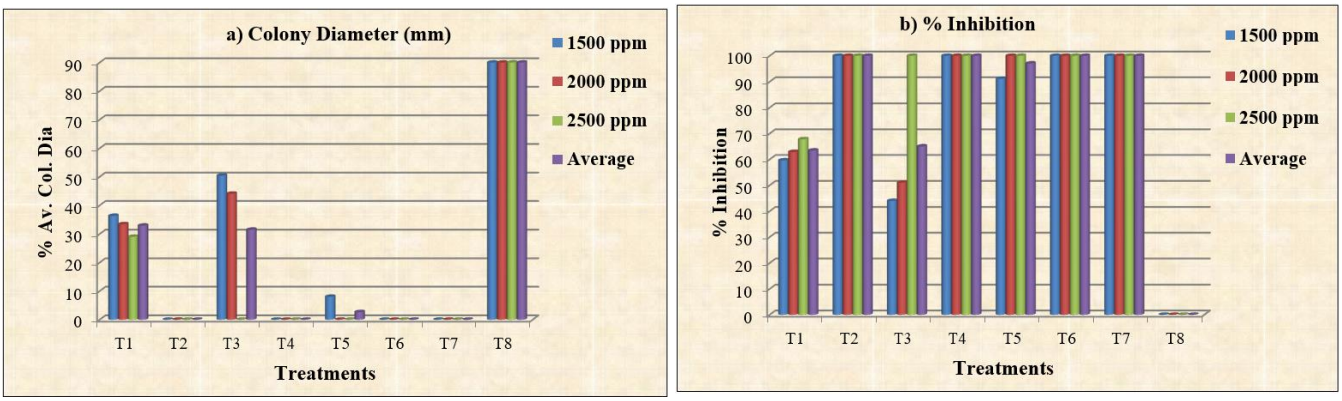
In test isolate, there was complete mycelial growth inhibition with fungicides mancozeb 75% WP, copper oxychloride 50% WP, copper hydroxide 77% WP and zineb 75% WP tested each @ 1500, 2000 and 2500 ppm, propineb 70% WP (@ 2000 and 2500 ppm) and chlorothalonil 75% WP (@ 2500 ppm) followed by propineb 70% WP (@ 1500 ppm), captan 50% WP (@ 1500, 2000 and 2500 ppm) and chlorothalonil 75% WP (@ 1500 and 2000 ppm). Fungicides mancozeb 75% WP, copper oxychloride 50% WP, copper hydroxide 77% WP, zineb 75% WP and propineb 70% WP were reported to cause maximum mycelial growth inhibition in many *Colletotrichum gloeosporioides*. These results were in conformity to the finding of several earlier workers. Similar results were reported by Bhat, 1991 [2]; Ashoka, 2005 [1] and Ramani *et al.*, 2015 [6]

Table 1: *In vitro* efficacy of non-systemic fungicides against *Colletotrichum gloeosporioides*

Tr. No.	Treatments	Col. Dia.* (mm) at ppm			Av (mm)	% Inhibition* at ppm			Av. Inhib. (%)
		1500	2000	2500		1500	2000	2500	
T1	Captan 50% WP	36.33	33.41	29.00	32.91	59.63 (50.55)	62.87 (52.45)	67.77 (55.40)	63.42 (52.78)
T2	Mancozeb 75% WP	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T3	Chlorothalo nil 75% WP	50.41	44.08	0.00	31.49	43.98 (41.54)	51.02 (45.58)	100.00 (90.00)	65.00 (53.72)
T4	Copper oxychloride 50% WP	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Propineb 70% WP	8.00	0.00	0.00	2.66	91.11 (72.65)	100.00 (90.00)	100.00 (90.00)	97.03 (80.07)
T6	Copper hydroxide 77% WP	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00

						(90.00)	(90.00)	(90.00)	(90.00)
T7	Zineb 75% WP	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T8	Control (untreated)	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE ±	0.25	0.51	0.14	-	0.29	0.34	0.26	-
	CD (P=0.01)	0.73	1.50	0.42	-	0.85	1.01	0.77	-

*- Mean of three replications, Figures in parentheses are arcsine transformed values



Tr. No.	Treatments	Tr. No.	Treatments
T1	Captan 50% WP	T5	Propineb 70% WP
T2	Mancozeb 75% WP	T6	Copper hydroxide 77% WP
T3	Chlorothalonil 75% WP	T7	Zineb 75% WP
T4	Copper oxychloride 50% WP	T8	Control (untreated)

Fig 1: *In vitro* efficacy of non-systemic fungicides against *Colletotrichum gloeosporioides*.

Plate 1: *In vitro* efficacy of non-systemic fungicides at various concentrations against *Colletotrichum gloeosporioides*

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