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Isolation and *in vitro* evaluation of fungicides against *Pestalotiopsis mangiferae* causing grey blight of mango

Alle Rakesh, Jai Prakash Mishra, Rajendra Prasad, J Chandra Sekhar, Dharmesh Gupta, Vedukola Pulla Reddy and Sunil Kumar

Abstract
The present investigation entitled "Isolation and *in vitro* evaluation of fungicides against *Pestalotiopsis mangiferae* causing grey blight of mango" was under taken to evaluate for various management studies of the given test fungus. The diseased samples were collected from different localities of Uttarkhandi.e Dehradun, Haridwar and Rishikesh during the 2019-20. The pathogen was isolated from the diseased samples and the pathogen was maintained in potato dextrose agar. The potential species of *Pestalotiopsis mangiferae* was identified and isolated. *In vitro* evaluation of fungicides were tested. The data regarding the growth inhibition by three fungicides in which (*viz.*, two systemic and one non-systemic) are as follows, among Systemic fungicides were found more inhibitory as compared to non-systemic fungicides even at equal levels of concentration against the pathogen. The data regarding the growth inhibition by two systemic and one non-systemic are as follows, among the systemic fungicide, Carbendazim and Mancozeb shows high efficacious, copper oxychloride has poor inhibiting capacity.

Keywords: Modified wakimoto's Agar (MWA), rifampicin

Introduction
Mango ***(****Mangiferaindica L.*) is the most important commercially grown fruit crop of the country. Mango is low in calories yet high in nutrients particularly vitamin C, which aids immunity, iron absorption and growth. Mango is a good source of several B vitamins, as well as vitamins A, C, K and E which all helps to boost immunity. In some parts of the world, mango is called "king of fruits". It's a drupe or stone fruit, which means that it has a large seed in the middle. India ranks first among the world's mango producing countries include China, Thailand, Mexico, Pakistan etc. India's share of production in all over the world is 52%. There are hundreds of types of mango, each with a unique taste, shape, size and colour.
India with 24,71,000 acres of mangos (70%of its fruit-growing area) produces 65% of the world's mango crop 99,20,700 tons. In 1985, mango growers around Hyderabad sought government protection against terrorists who cut down mango orchards unless the owners paid ransom (50,000 rupees in one case). India far outranks all other countries as an exporter of processed mangos, shipping 2/3 of the total 22,046 tons. India in volume of exports are Thailand, 7,74,365 tons, Pakistan and Bangladesh followed by brazil. Mexico ranks 5th with about 1,00,800 acres and an annual yield of approximately 6,40,000 tons. The Philippines have risen to 6th place. Tanzania is 7th, the Dominican Republic 8th and Colombia 9th place.
Egypt produces 1,10,230 tons of mangos annually and exports moderate amounts to 20 countries in the near East and Europe. Mango culture in the Sudan occupies about 24,710 acres producing a total of 66,138 tons per year.

Materials and Methods
Isolation of *Pestalotiopsis mangiferae* causing grey blight of mango
In the present investigation the diseases samples were collected from the field. Small tissues from infected stem or roots (5mm) along with the healthy tissue were cut with sterile scalpel. The tissues were surface sterilized with 0.1 % mercury chloride for 30 seconds. The tissues were subsequently washed in three changes of sterile distilled water to eliminate mercury ions. The surface sterilized tissues were transferred on to the PDA and incubated at 25 ± 2°C in BOD incubator and growth was observed periodically.

In-Vitro evaluation of fungicides against pestalotiopsis mangiferae causing grey blight of mango
The relative efficacy of three fungicides including systemic and non-systemic fungicides were evaluated under *in vitro* conditions at three different concentration levels i.e.

200, 400 and 600ppm by using Poisoned Food Technique (Nene and Thapliyal, 1979). From the stock double strength potato dextrose agar medium, 50ml double strength potato dextrose medium in a conical flask (150ml) were sterilized at 15 psi (1.05 kg/cm^2) pressure at 121.6ºC for 20 minutes. Simultaneously, concentrations of different fungicides were also prepared in equal amount (50 ml) of sterilized distilled water so as to get the desired concentration of fungicides after mixing the fungicide solutions in the double strength media. Fungicides solution were added separately to equal quantities of double strength PDA medium aseptically before pouring in petri plates. The culture discs (6 mm) cut from the margin of 7 days old vigorously growing culture of the test pathogen were placed in the center of each prepared Petri plate. A control was also maintained in which only plain sterilized water was added to the double strength medium. Each treatment was replicated four times and the inoculated plates were incubated at 25 ± 2°C in BOD incubator. The colony diameter of the test pathogen was recorded until the control plates were full of mycelial growth of the test pathogen. The per cent inhibition of mycelial growth at different test concentrations in relation to control was calculated by using the formula (Vincent, 1947) given as below:

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

Where,
I% = (%) Mycelial growth inhibition
C = Mycelial dial growth (mm) in control plates
T = Mycelial dial growth (mm) in treatment plates

Table 1: The following fungicides were used for *in vitro* evaluation are given below

S. No.	Common name	Test concentration (ppm)
1.	Carbendazim	200,400,600
2.	Copper oxychloride	200,400,600
3.	Mancozeb	200,400,600

Results and Discussion

In-vitro evaluation of fungicides against *Pestalotiopsis mangiferae* causing grey blight of mango

Three fungicides among which there are systemic and non-systemic fungicides were tested against *Pestalotiopsis mangiferae* under *in vitro* conditions through Poison Food Technique. The data regarding the growth inhibition by different fungicides at different concentration levels 200, 400 and 600 ppm. Variable inhibition reactions of different fungicides against *Pestalotiopsis mangiferae* were described in the report. Systemic fungicides were found more inhibitory as compared to non-systemic fungicides even at equal levels of concentration against the pathogen. Irrespective of different concentration levels of fungicide tested against mycelial growth of *Pestalotiopsis mangiferae*, Carbendazim proved most efficacious providing growth inhibition at 400 and 600 ppm and mancozeb showed less efficacious against mycelial growth of *Pestalotiopsis mangiferae*.

Table 2: *In-vitro* evaluation of Mancozeb against *Pestalotiopsis mangiferae* causing grey leaf blight of mango.

Fungicide concentration	T1- Mancozeb						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
200ppm	0.85	1.58	1.85	2.18	2.48	2.75	3.10
400ppm	0.78	1.18	1.48	1.75	2.18	2.48	2.78
600ppm	0.65	0.88	0.90	1.18	1.30	1.50	1.68
Control	1.18	2.58	3.68	4.48	5.28	6.08	7.00
C.D	0.12	0.15	0.13	0.14	0.14	0.13	0.15
SE(m)	0.04	0.05	0.04	0.04	0.05	0.04	0.05

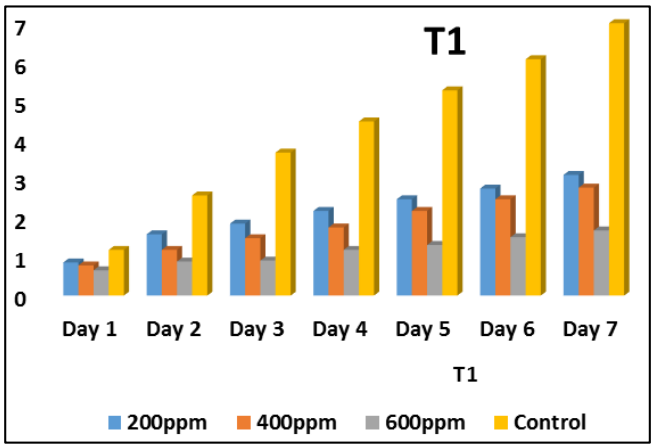

Fig 1: *In-vitro* evaluation of Mancozeb against Pestalotiopsis mangiferae causing grey leaf blight of mango.

Table 3: *In-vitro* evaluation of Carbendazim against *Pestalotiopsis mangiferae* causing grey leaf blight of mango

Fungicide concentration	T2- Carbendazim						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
200ppm	0.80	1.30	1.70	1.90	2.20	2.40	2.60
400ppm	0.65	1.10	1.40	1.60	1.85	2.00	2.20
600ppm	0.65	0.70	0.98	1.08	1.15	1.40	1.60
Control	1.48	2.68	3.48	4.78	5.38	6.28	7.00
C.D	0.12	0.13	0.14	0.14	0.17	0.13	0.11
SE(m)	0.04	0.04	0.04	0.04	0.06	0.04	0.04

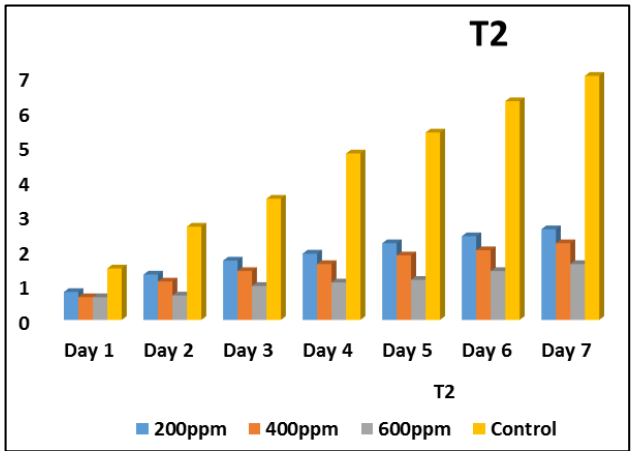

Fig 2: *In-vitro* evaluation of Carbendazim against Pestalotiopsismangiferae causing grey leaf blight of mango.

Table 4: *In-vitro* evaluation of Copper oxychloride against *Pestalotiopsis mangiferae* causing grey leaf blight of mango.

Fungicide concentration	T3 - Copper oxychloride						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
200ppm	0.75	1.60	1.75	2.10	2.53	2.83	3.28
400ppm	0.65	1.00	1.35	1.43	1.88	2.08	2.25
600ppm	0.65	1.05	1.05	1.20	1.55	1.80	2.00
Control	1.38	2.68	3.78	4.98	6.18	6.68	7.00
C.D	0.11	0.36	0.19	0.14	0.16	0.14	0.14
SE(m)	0.04	0.12	0.06	0.04	0.05	0.05	0.05

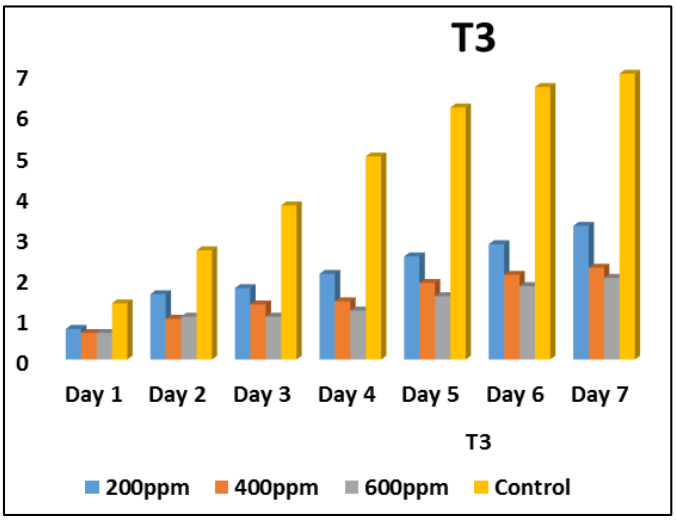

Fig 3: *In-vitro* evaluation of Copper oxychloride against Pestalotiopsis mangiferae causing grey leaf blight of mango.

High efficacies of Carbendazim have also been recorded against *Pestalotiopsis mangiferae* by P. Amrutha and Reshmy Vijayaraghavan (2018). While evaluating different fungicides Manjit Kumar Ray, Piyush Kumar Mishra (2016) reported that Carbendazim were highly effective fungicides for controlling growth and Copper oxychloride showed less effective. According to the S. Rahman, SK Adikary (2013) [1] Carbendazim showed very effective where as Mancozeb is less effective. Katherine E Hopkins (1996) also showed that Chlorothalonil is also effective in reducing the severity of *Pestalotiopsis mangiferae.* Chandra Sekhar J, *et al*, 2020 [3] who also used same fungicides at concentration levels against the pathogen and similar results were observed.

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