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In vitro evaluation of fungicides against pathogen *Fusarium oxysporum f. sp. zingiberi* causing rhizome rot of ginger in Maharashtra state

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Abstract
A total of thirteen fungicides were evaluated *in vitro* against *Fusarium oxysporum f.sp. zingiberi*, applying Poisoned Food Technique and using Potato Dextrose Agar as basal medium. Effect of these fungicides on radial mycelial growth and inhibition of test pathogen were recorded. All the treatments were replicated three times and a suitable untreated control (without fungicides) was also maintained. Among the different fungicides tested the maximum of 100 per cent inhibition was found in Carbendazim, Carbendazim + Mancozeb and Benomyl and the least inhibition was recorded with the (Metalaxyl + Copper oxychloride) with 21.55 per cent**.** The pot with Rhizome dip treatment 30 min and soil drenching after one month of sowing were treated with Carbendazim (0.1%) + Mancozeb (0.2%) showed most effective in managing the disease with per cent disease incidence of 8.33 per cent and the next best treatment was Carbendazim (0.1%) alone with PDI of 13.88 per cent, followed by Benomyl (36.11%), the least PDI was recorded with control (77.77%).

Keywords: *Fungicides, fusarium oxysporum f.sp. zingiberi*, carbendazim, mancozeb

Introduction
Ginger (*Zingiber officinale* Rosc.) belonging to the family Zingiberaceae is an important commercial crop grown for its aromatic rhizomes which are used as a spice and a medicine (Sharma and Pandey, 2014). It is obtained from the underground stems or rhizome of *Zingiber officinale* Rosc. Ginger originated in South- East Asia, probably in India (Burkill, 1966, Purseglove *et al*., 1981) [1, 4]. It is usually grown as an annual. The whole plant is refreshingly aromatic, but it is the underground rhizome (raw or processed) which is valued as spice. Its medicinal value is increasingly being recognized now days the name itself supports this view.
India is considered as 'The land of spices' and enjoys from time immemorial a unique position in the production and export of ginger. This crop is cultivated for its underground rhizomes, which are used in many ways. Ginger is used as flavoring agent, a preservative, used in pickle and ginger oil in soft drinks, Ginger is commonly used to treat various types of "stomach problems," including motion sickness, morning sickness, colic, upset stomach, gas, diarrhoea, nausea caused by cancer treatment, nausea and vomiting after surgery, as well as loss of appetite. Among the major constraints for growing these crop is the rhizome rot. Even though, important foliar diseases do exist in these crops, rhizome rot is very important in view of severe crop losses.
A survey was conducted during *Kharif* 2003-2005 in major ginger growing areas of Karnataka. Rhizome rot incidence was noticed in all the locations surveyed with a range from 5.50 to 45.60 per cent (Sagar *et al.*, 2008) [5]. It occurs in several parts of India wherever this crop is grown. The term rhizome rot is loosely used for all the diseases affecting the rhizome, irrespective of pathogens involved, since the ultimate result is the partial or total loss of rhizome caused by *Fusarium oxysporum f.sp. zingiberi*. The pathogens involved, decide the nature of damage and also symptom expression. The major diseases identified are the soft rot resulting in wet rot, yellows and bacterial wilt.
Purpose of this experiment was, to study the effect of different fungicides on rhizome. rot of ginger under controlled condition in Department of Plant Pathology and Agril.

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Material and Methods

Fungicides

The total Thirteen fungicides (systemic and contact) *viz.*, Carbendazim + Mancozeb, Carbendazim 50WP, Metalaxyl 35 WS, Captan 50WP, Hexaconazole + Mancozeb, Propaconazole 25 EC, Copper oxychloride 50 WP, Hexaconazole 5 EC, Mancozeb 75 WP, Bordeaux mixture, Metalaxyl + Copper oxychloride, Benomyl 50 WP and Hexaconazole + Captan were used for *in vitro* and *in vivo* (Pot culture) experiments conducted during present studies.

Potato dextrose agar medium

For all the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for culturing *F. oxysporum.*

Table 1: The composition of PDA used is given below

Materials	Quantity
Peeled and sliced potato	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	1000 ml (to make up the volume)

Two hundred grams of peeled potatoes were cut into small pieces. These pieces were boiled in water and the extract was collected by filtering through muslin cloth. Each of 20 g of dextrose and agar-agar were dissolved in potato extract and the final volume was made up to 1000 ml by adding distilled water. A known quantity of such medium was dispensed into number of conical flasks and plugged with non-absorbent cotton and finally wrapped with paper. The flasks containing dispensed medium were sterilized at 1.1 kg cm^{-2} pressure for 20 min.

Methodology

In vitro* evaluation of fungicides against *Fusarium oxysporum f.sp. zingiberi

Poisoned food technique Efficacy of Thirteen fungicides was evaluated *in vitro* at concentrations @ against *Fusarium oxysporum f.sp. zingiberi* (FOZ-8 isolate), applying Poisoned Food Technique (Nene and Thapliyal, 1993) and using Potato dextrose agar (PDA) as basal culture medium. Based on active ingredient, requisite quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (40^0C) PDA medium separately in conical flasks (250 ml cap.) to obtain desired concentrations of the test fungicides. Fungicide amended PDA medium was then poured (20 ml/plate) aseptically in glass Petri plates (90.33 mm dia) and allowed to solidify at room temperature. For each of the test fungicide and its test concentrations, three plates/treatment/replication were maintained and each test fungicide with various concentrations was replicated thrice. After solidification of the medium, all the plates were inoculated aseptically with a 7 mm culture disc obtained from a week old actively growing pure culture of *Fusarium oxysporum f.sp. zingiberi.* The culture disc was placed on PDA in inverted position in the centre of the Petri plate and plates were incubated at 28 + 2^0C. Petri plates filled with PDA (without any fungicide) and inoculated with the culture disc of *Fusarium oxysporum f.sp. zingiberi* were maintained as untreated control.

Experimental details
Design: C.R.D.
Replications: Three
Treatments: Thirteen

Table 2: List of fungicides tested against the *Fusarium oxysporum f.sp. zingiberi*

Tr. No.	Fungicides Name	Trade name	Concentrations (%)
T_1	Hexaconazole	Contaf 5% EC	0.1
T_2	Copper oxychloride	Blitox 70% WP	0.2
T_3	Carbendazim	Bavistin 50% WP	0.1
T_4	Mancozeb	Indofil M-45 75% WP	0.2
T_5	Metalaxyl	Togron 35 WS	0.2
T_6	Captan	Captaf 50 WP	0.2
T_7	Propaconazole	Tilt 25% EC	0.1
T_8	Bordeaux mixture	Kwicbordo	0.1
T_9	Benomyl	Benlate 50 WP	0.1
T_{10}	Control		

Observations on radial mycelial growth were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Per cent inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying following formula (Vincent, 1927).

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

Where,
C = Growth of the test fungus in untreated control plate
T = Growth of the test fungus in treated plate

Result and Discussion

In vitro evaluation of fungicides

A total of thirteen fungicides *viz.*, Hexaconazole 5 EC Copperoxychloride 50 WP, Carbendazim 50 WP, Metalaxyl 35 WS, Carbendazim + Mancozeb, Metalaxyl + Copper oxychloride, Mancozeb, Hexaconazole + Captan, Propaconazole 25 EC, Bordeaux mixture, Benomyl 50 WP, Hexaconazole + Mancozeb and Captan 50 WP were evaluated *in vitro* against *F. oxysporum*, applying Poisoned Food Technique and using Potato Dextrose Agar as basal medium. Effect of these fungicides on radial mycelial growth and inhibition of test pathogen were recorded. All the treatments were replicated three times and a suitable untreated control (without fungicides) was also maintained.

Radial mycelial growth

Results revealed that all the fungicides tested at recorded a wide range of radial mycelial growth (colony diameter) of the test pathogen. The mycelia growths were ranged from 0.00 to 39.16 mm mean colony diameter (Plate 1).

It was observed from the data given in (Table 2) that the Carbendazim (0.1%), Carbendazim (0.1%) + Mancozeb (0.20%) and Benomyl (0.1%), completely inhibited the growth of the pathogen *Fusarium oxysporum* on potato dextrose agar medium. This showed that, the fungicides Carbendazim, Carbendazim + Mancozeb and Benomyl, in the given concentration were 100 per cent effective against *F. oxysporum.*

Fungicides Carbendazim, Carbendazim + Mancozeb and Benomyl was found most effective and recorded least mean

growth of 0.00 mm. This was followed by Hexaconazole + Mancozeb (10.10 mm), Hexaconazole + Captan (12.40 mm), Propaconazole (18.30 mm) and Captan (20.16 mm).
Significantly least mean radial mycelial growth was recorded with Carbendazim (0.00mm), which was followed by Carbendazim + Mancozeb and Benomyl (0.00 mm), compared to highest mean radial growth (90.00 mm) in untreated control plates.

Mycelial inhibition
Results (Table 2) revealed that all the fungicides tested significantly inhibited mycelial growth of the test fungus over untreated control (00.00%). The per centage mycelial growth inhibition was ranged from 21.55 per cent (Metalaxyl + Copper oxychloride) to 100 per cent (Carbendazim, Carbendazim + Mancozeb and Benomyl). However, highest per centage of mycelial growth inhibition was recorded with Carbendazim, Carbendazim + Mancozeb and Benomyl (100%). This was followed by the fungicides, Hexaconazole + Mancozeb (88.77%), Hexaconazole + Captan (86.22%), Propaconazole (79.66%) and Captan (77.60%). Least mycelial growth inhibition was recorded with Bordeaux mixture (73.15%) which was followed by Mancozeb (57.54%), Hexaconazole (57.52%), Copper oxychloride (56.48%) and Metalaxyl (55.11%). Fungicides *viz.*, Hexaconazole, Copperoxychloride, Carbendazim, Metalaxyl, Carbendazim + Mancozeb, Metalaxyl + Copper oxychloride Mancozeb, Hexaconazole + Captan, Propaconazole, Bordeaux mixture, Benomyl, Hexaconazole + Mancozeb and Captan were reported to cause significant inhibition of mycelial growth of *Fusarium oxysporum*.
Barnoczkine-Stoilova (1988) reported that carbendazim has been reported to be very effective for *Fusarium oxysporum f.sp. cepae* under field condition.
Meena and Mathur (2003) [3] observed that fungicidal mixture of Ridomil MZ and Bavistin was effective in treating seed rhizomes and soil individually and in combination for the suppression of rhizome rot of ginger.
Dohroo (2006) [2] concluded that rhizome treatment with fungicide Mancozeb + Carbendazim (0.3 + 0.1%) to be effective against rhizome rot of ginger.

Table 3: *In vitro* evaluations of fungicides at different concentration on radial mycelial growth and inhibition of *Fusarium oxysporum f. sp. zingiberi*

Tr. No.	Fungicides	Concentration (%) used	Mean colony diameter (mm)* (after 7) days of inoculation	Percent inhibition of growth
1	Hexaconazole	0.15	38.23 (38.19)	57.52
2	Copper oxychloride	0.30	39.16 (38.74)	56.48
3	Carbendazim	0.1	0.00 (0.00)	100.00
4	Metalaxyl	0.2	40.40 (39.46)	55.11
5	Carbendazim + Mancozeb	0.1+0.2	0.00 (0.00)	100.00
6	Metalaxyl + Copper oxychloride	0.2 +0.10	70.60 (57.16)	21.55
7	Mancozeb	0.25	38.03 (38.07)	57.74
8	Hexaconazole + Captan	0.15+0.20	12.40 (20.61)	86.22
9	Propaconazole	0.15	18.30 (25.32)	79.66
10	Bordeaux mixture	0.1	24.16 (29.44)	73.15
11	Benomyl	0.1	0.00 (0.00)	100.00
12	Hexaconazole+ Mancozeb	0.15+0.20	10.10 (18.53)	88.77
13	Captan	0.20	20.16 (26.68)	77.60
14	Control		90.00 (75.82)	-
	CV		0.34	00.00
	S.E. +		0.058	
	CD at 1%		0.167	

*=Average of three replications
Figures in parenthesis are angular transformed values

Plate 1: *In vitro* evaluation of fungicides at different concentration on radial mycelial growth and inhibition of *Fusarium oxysporum f.sp. zingiberi*

Table 4: Show the Fungicides and Fungicides different

Tr. No.	Fungicides	Tr. No.	Fungicides
1	Hexaconazole	8	Hexaconazole + Captan
2	Copper oxychloride	9	Propaconazole

3	Carbendazim	10	Bordeaux mixture
4	Metalaxyl	11	Benomyl
5	Carbendazim + Mancozeb	12	Hexaconazole+ Mancozeb
6	Metalaxyl + Copper oxychloride	13	Captan
7	Mancozeb	14	Control

Conclusion

Among the different fungicides tested the maximum of 100 per cent inhibition was found in Carbendazim, Carbendazim + Mancozeb and Benomyl and the least inhibition was recorded with the (Metalaxyl + Copper oxychloride) with 21.55 per cent**.**

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