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In vitro management of ginger rhizome rot causing pathogen by fungicides

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Abstract

Ginger, a basic Indian spice widely used in Indian culinary and alternative medicines as well. An experiment on screening of fungicides against the major test pathogen *Fusarium oxysporum* f. sp. *zingiberi* was performed *in vitro* by poisoned food technique using contact and systemic fungicides. The fungicides tested at 24, 48, 72 and 96 h. Amid fungicides tested, carbendazim + mancozeb @ 0.15, 0.2 and 0.25%, carbendazim @ 0.1 and 0.15% and copper oxychloride @ 0.25% were found best in inhibiting the pathogen growth by 89.97 and 85.14 per cent respectively and recorded highest inhibition (%) over other treatments at 24h, carbendazim+mancozeb@ 0.25% noticed a significant inhibition of 89.97 per cent after 48h of incubation, similarly, carbendazim+mancozeb (0.25%) proved its inhibition ability and found with highest inhibition of 85.42, 79.42 per cent at 72h and 96h respectively over control.

Keywords: Ginger, SAAF, fungicide, poisoned food technique, *Fusarium* spp.

Introduction

Ginger being a prominent crop grown commercially for its aromatic rhizomes. India shares a major part in the world's production and from North East region (NER) of India farmers producing 184'000 MT of fresh ginger in 174'000 Ha (NHB, 2018) [17]. This spice used to treat several health problems and improves the appetite (Hanumant *et al.*, 2020) [8]. In India, it is growing widely in Himachal Pradesh, Meghalaya, Sikkim, West Bengal, Karnataka, Andhra Pradesh, Orissa, Tamil Nadu, and Kerala. Meghalaya is one among the NER contributes the country's total productivity in major (Archana *et al.*, 2020) [3]. The cultivation of this crop is majorly targeted by diseases like rhizome rot or yellows, soft rot, storage rot (Meenu and Kaushal, 2017) [16] caused by pathogens *Fusarium* spp., *Ralstonia solanacearum*. *Fusarium oxysporum*, is one such ubiquitous soil-borne fungus responsible for rot, vascular wilt and damping off diseases in plants (Archana *et al.*, 2010) [2]. Though many management strategies are being practised to control the disease, use of fungicides is a quick and efficient method in disease management (Tarafder *et al.*, 2019) [24]. The aim of the study is to analyse various contact and systemic fungicides against *Fusarium oxysporum* f.sp. *zingiberi* in order to understand judicial dose of the fungicide for control this soil-borne pathogenic fungus.

Material and Methods

Isolation of pathogen

Isolation of the causal agent of rhizome rot, *Fusarium oxysporum* f.sp. *zingiberi* was done from the diseased plant. Small portions of the sliced infected portions were surface sterilised with NaOCl (1%) solution for 30 seconds followed by three subsequent washings with sterilized distilled water and dried for 10 min on tissue paper and then transferred to potato dextrose agar in Petri dishes. The seeded plates were then incubated at 27±1°C for ten days and purified the culture by hyphal tip cut method. The culture was then used for the assay.

Evaluation of fungicides against rhizome rot pathogens

Efficacy of seven fungicides against *Fusarium oxysporum* f.sp. *zingiberi* was performed *in vitro* by poisoned food technique (Magar *et al.*, 2020) [11] using contact and systemic fungicides, which include mancozeb (0.15, 0.2 and 0.25%); captan (0.15, 0.2 and 0.25%); copper oxychloride (0.15, 0.2 and 0.25%); propiconazole (0.05, 0.1 and 0.15%); carbendazim (0.05, 0.1 and 0.15%); tebuconazole (0.05, 0.1 and 0.15%); carbendazim + mancozeb (0.15, 0.2 and 0.25%).

The amount of each fungicide to make the desired concentration was mixed aseptically with melted potato dextrose agar media (PDA) and poured aseptically into the sterilized Petri plates and allowed them to solidify. After the solidification each Petri plate was inoculated with 5 mm mycelial disc cut from an actively growing seven day old culture. A check without fungicide inoculated with fungal disc at the centre of the plate served as a control. The Petri plates were then incubated at $27 \pm 1^\circ\text{C}$. Radial mycelial growth (cm) of the test agent was measured at 24, 48, 72 and 96 h after inoculation. Per cent growth inhibition over control was calculated using the formula of Kumari *et al.*, (2017). The inhibition (%) of the respective treatment over control was calculated by considering by the radial mycelial growth of pathogen in control plate at the stated time intervals.

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

Where

I = Per cent inhibition

C = Mycelial growth in control

T = Mycelial growth in treatment

Results and Discussion

Evaluation of the fungicides (Table 1) against the rhizome rot pathogen revealed that, carbendazim + mancozeb (0.15, 0.2 and 0.25%), carbendazim (0.1 and 0.15%), propiconazole (0.15%), tebuconazole (0.15%), copper oxychloride (0.25%) and copper oxychloride (0.2%) could inhibit 89.96, 85.14 and 84.80 per cent on growth and recorded highest inhibition (%) over other treatments. Propiconazole and tebuconazole each at 0.1 per cent evidenced an inhibition per cent of 74.81 and were significantly on par with propiconazole (72.57%) at 0.05 per cent concentration. Captan (68.26%) showed its maximum inhibition at 0.25 per cent concentration and carbendazim (0.05%), copper oxychloride (0.15%), tebuconazole (0.05%) also evidenced a similar inhibition of 66.47 per cent against the test pathogen. Whereas, least mycelial inhibition was recorded with mancozeb (0.15%) by 46.02 per cent over control after 24h of observation in the assay.

At 48h of incubation in the test, carbendazim+mancozeb@ 0.25% showed a significant inhibition of 89.96 against the pathogen followed by carbendazim (0.15%), tebuconazole (0.15%) and carbendazim+mancozeb (0.2%) by 86.33 and 86.19 respectively and were significantly on par with each other. Treatments, propiconazole (0.15%) with an inhibition per cent of 75.66 followed ahead to carbendazim+mancozeb at 0.15 per cent (74.06%). Propiconazole at 0.1 per cent noticed a significantly on par mycelial growth inhibition result of 72.81% on the pathogen followed by carbendazim (0.1%) and tebuconazole (0.1%) by 71.74 and 71.56 per cent. Fungicide, propiconazole (0.05%) and tebuconazole (0.05%) recorded a per cent inhibition of 68.25, 67.17 next to tebuconazole (0.1%) and the treatments carbendazim (66.20%), copper oxychloride (66.20%), captan (64.37%) noticed an on par relation with the respective treatments at 0.05, 0.25 and 0.25 per cent concentrations respectively. Fungicide, copper oxychloride (0.2%) significantly on lead with a per cent inhibition of 58.40 over other treatments, captan (52.90%) at 0.2, copper oxychloride (51.50%) at 0.15 and mancozeb (50.04%) at 0.25 per cent respectively. Lowest inhibition (%) was recorded from mancozeb (0.2%), captan (0.15%) and mancozeb (0.15%) by 43.50, 42.03 and 39.96 per

cent and was noticed not much variation among the treatments when compared with control.

At 72h incubation, carbendazim+mancozeb (0.25%) showed its maximum inhibition of 85.42 per cent followed by tebuconazole (73.14%), carbendazim+mancozeb (72.12%) at 0.15 and 0.2 per cent and were on par with propiconazole and carbendazim each of 0.15% by 71.20 and 70.42 per cent respectively. Treatment, tebuconazole at 0.1 per cent (68.67%) was on par with propiconazole (67.87%) at 0.1 per cent, carbendazim+mancozeb (67.13%) and carbendazim (67.13%) each at 0.15 and 0.1 per cent, tebuconazole (65.67), carbendazim (63.53) at 0.05 per cent and copper oxychloride (62.32) at 0.25 per cent. Whereas, captan 0.25% (61.00), propiconazole 0.05% (60.30) were significantly differs in per cent growth inhibition with captan (50.42), copper oxychloride (45.57) at 0.2 per cent. Treatments, copper oxychloride (0.15%), mancozeb (0.25%) recorded an inhibition per cent of 40.66 and significantly on par with captan @ (0.15), mancozeb @ (0.2), mancozeb @ (0.15) by 33.81, 33.17 and 32.09 (%) respectively over control.

At 96h, carbendazim+mancozeb (79.42%) at 0.25 per cent showed highest per cent inhibition (Fig. 1) and was significantly different from the treatment tebuconazole (0.15%) by 68.60 per cent. Tebuconazole 0.1% (66.77) recorded next and was on par with treatments, carbendazim 0.15% (65.63), carbendazim+mancozeb 0.2% (65.60), propiconazole 0.15% (63.95), carbendazim+mancozeb 0.15% (62.89). Carbendazim (0.1%) recorded a mycelial growth inhibition of 62.89 per cent followed by tebuconazole 0.05% (61.86), carbendazim 0.05% (60.36%), captan 0.25% (58.93%) and copper oxychloride 0.15% (58.93%). Treatment propiconazole (0.1%) evidenced with a per cent inhibition of 56.57 followed by captan 0.2% (51.21%), propiconazole 0.05% (50.75) and significantly on par with copper oxychloride at (0.25%), mancozeb (0.25%), copper oxychloride (0.2%) by 38.77, 35.24 and 35.22 respectively. The fungicides mancozeb (0.15%), captan (0.15%) and mancozeb (0.2%) were least in inhibition efficiency and were recorded with 29.55, 26.46 and 24.90 per cent respectively. The findings of the present investigation are supported by Ghante *et al.*, 2019^[7]; Manju *et al.*, 2020^[14], Sanapo *et al.*, 2020, who reported that carbendazim + mancozeb inhibited *Fusarium oxysporum* at 200 and 2500 ppm concentration. Similar results are in conformity with Rao *et al.*, 2020^[20] stated that propiconazole @0.05% influences the growth of *Fusarium* spp. and best inhibitory action of carbendazim on *Fusarium oxysporum* reported (Poddar *et al.*, 2004)^[19]. Fungistatic effects of non-systemic and combi fungicides against *F. oxysporum* were also reported earlier by several workers (Boyacioglu *et al.*, 1992; Amini and Sidovich, 2010; Maitlo *et al.*, 2014; Bashir *et al.*, 2018)^[6, 1, 12, 4]. Fungicides propiconazole, hexaconazole, mancozeb and captan also proved its efficiency in inhibiting the mycelial growth of *Fusarium* sp. (Padvi *et al.*, 2018)^[18]. Propiconazole acts by inhibiting the demethylation step in the biosynthesis of sterol, which is needed in fungal cell walls, they most likely bind to cytochrome P-450 involved in sterol demethylation in *Fusarium* spp. (Manasa *et al.*, 2017)^[13]. Carbendazim may be because of its mitosis inhibiting nature in fungi effectively controlling the test pathogen *in vitro*. Carbendazim+mancozeb exhibits best in inhibiting the spindle microtubules assembly and restricting the mitotic and cell division of the fungi showed its best fungistatic activity over pathogen (yang *et al.*, 2011)^[25]. Fungicides captan, mancozeb with its multisite activity on pathogen restricted the

pathogenic growth *in vitro*. Carbamates such as mancozeb targets β -tubulin engaged in mitosis (Louis *et al.*, 2014) [10]. The fungicide copper oxychloride can be attributed its action of *Fusarium* spp. by complex-forming reactions of the copper(II) ions that penetrate the cell with the thiol or amino groups that may result in nonspecific inhibition of enzymes and denaturation of proteins of pathogen *Fusarium*

oxysporum (Matolcsy *et al.*, 1988) [15]. Savita and Raj (2019) [22] also supported the statement by reporting the efficacy of fungicides carbendazim+mancozeb and carbendazim over *Fusarium oxysporum* f.sp. *dianthi*. Effectiveness of carbendazim against *Fusarium* sp. has been reported by several other workers also (Bhat and Srivastava, 2003; Singh *et al.*, 2010) [5, 23].

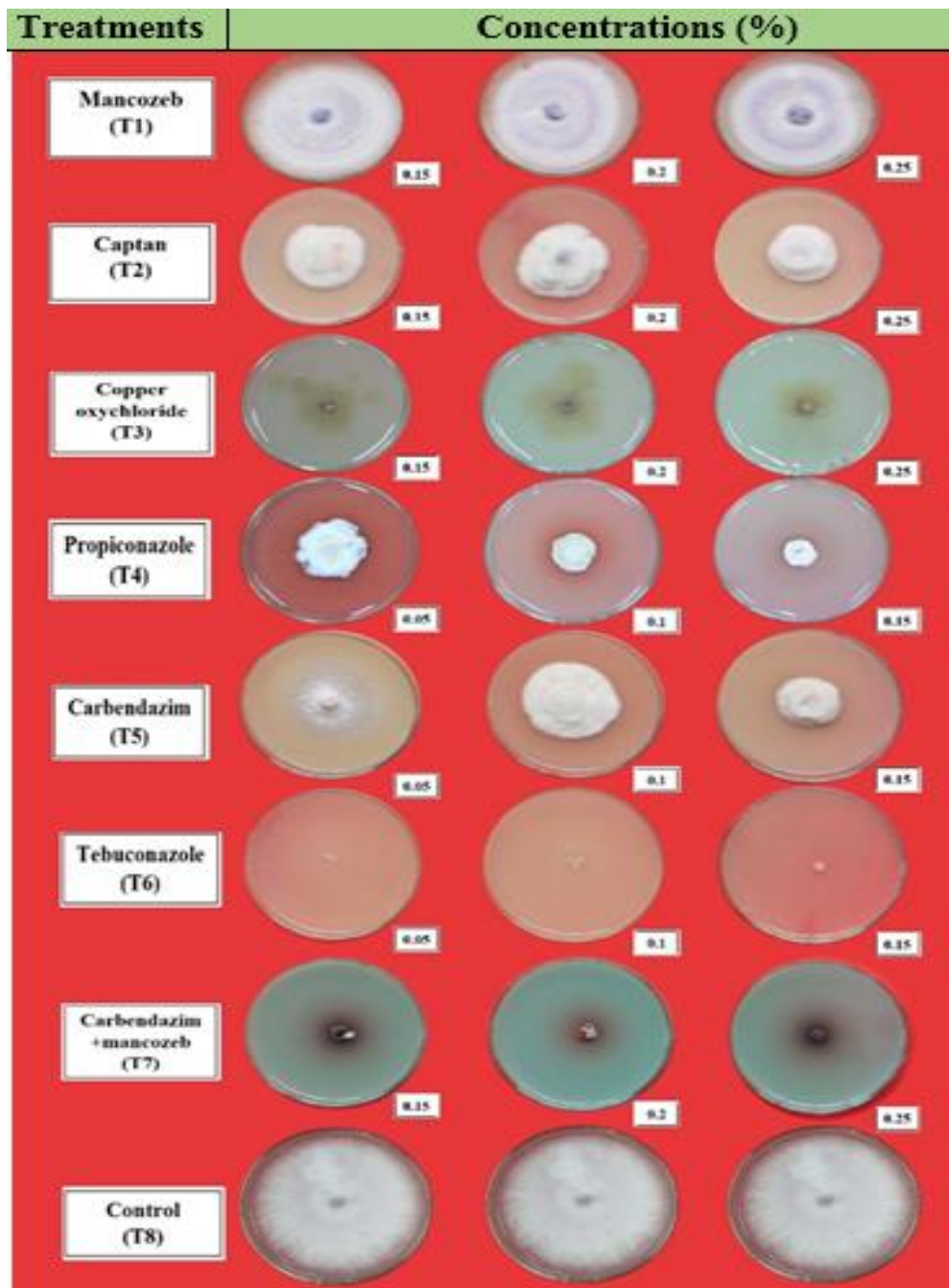


Fig 1: Efficacy of fungicides against *Fusarium oxysporium* f.sp. *zingiberi* at 96h

Table 1: Effect of different concentrations of fungicides on rhizome rot pathogen over control

Treatment (s)	Conc. (%)	% Growth Inhibition			
		24h	48h	72h	96h
Mancozeb 75% WP (DITHANE M-45)	0.15	51.79±5.7 (46.03) ^h	41.30±2.4 (39.96) ^h	28.27±1.2 (32.10) ^l	24.44±2.6 (29.56) ^j
	0.2	67.86±3.6 (55.51) ^{fg}	47.44±2.6 (43.51) ^h	30.28±5.4 (33.17) ^l	17.78±1.3 (24.90) ^j
	0.25	77.08±2.9 (61.46) ^{ef}	58.79±1.4 (50.05) ^g	42.48±0.8 (40.66) ^k	33.33±1.3 (35.24) ⁱ
Captan 50% WP (CAPTAN)	0.15	63.39±3.4 (52.79) ^g	44.87±2.6 (42.03) ^h	31.05±2.3 (33.82) ^l	20.00±2.6 (26.46) ^j
	0.2	77.08±2.9 (61.46) ^{ef}	63.55±4.1 (52.91) ^g	59.42±2.4 (50.43) ⁱ	60.74±3.2 (51.21) ^h
	0.25	86.31±0.6 (68.27) ^{cd}	81.23±2.3 (64.37) ^e	76.47±2.2 (61.00) ^h	73.33±2.6 (58.94) ^{fg}
Copper oxychloride 50% WP (BLITOX)	0.15	83.93±2.7 (66.48) ^{de}	61.26±2.2 (51.50) ^g	42.48±0.8 (40.66) ^k	73.33±2.6 (58.94) ^{fg}
	0.2	97.62±2.4 (84.80) ^a	72.53±2.3 (58.41) ^f	51.03±3.4 (45.58) ^j	33.33±2.6 (35.22) ⁱ

	0.25	97.92±2.1 (85.14) ^a	83.70±1.5 (66.20) ^e	78.38±2.1 (62.32) ^{gh}	39.26±2.0 (38.77) ⁱ
Propiconazole 25% EC (TILT)	0.05	90.77±2.5 (72.58) ^{bc}	86.26±1.1 (68.25) ^{cde}	75.49±0.5 (60.30) ^h	60.00±1.3 (50.75) ^h
	0.1	93.15±0.3 (74.81) ^b	91.21±1.4 (72.82) ^{bc}	85.84±0.3 (67.87) ^{cdef}	69.63±2.7 (56.58) ^g
	0.15	100.00±0.0 (89.97) ^a	93.77±1.2 (75.67) ^b	89.60±1.1 (71.20) ^{bcd}	80.74±0.7 (63.95) ^{bcde}
Carbendazim 50% WP (BAVISTIN)	0.05	83.93±2.7 (66.48) ^{de}	83.70±1.5 (66.20) ^e	80.17±0.4 (63.54) ^{fgh}	75.56±1.3 (60.36) ^{efg}
	0.1	100.00±0.0 (89.97) ^a	89.93±2.7 (71.74) ^{bcd}	84.91±0.8 (67.13) ^{def}	79.26±0.7 (62.89) ^{cdef}
	0.15	100.00±0.0 (89.97) ^a	98.81±1.2 (86.34) ^a	88.73±1.5 (70.43) ^{bcd}	82.96±1.5 (65.63) ^{bcd}
Tebuconazole 250 EC (FOLICUR)	0.05	83.93±2.7 (66.48) ^{de}	84.98±0.4 (67.18) ^{de}	83.01±1.6 (65.68) ^{efg}	77.78±1.3 (61.87) ^{def}
	0.1	93.15±0.3 (74.81) ^b	89.93±1.5 (71.57) ^{bcd}	86.76±1.1 (68.67) ^{bcde}	84.44±1.3 (66.78) ^{bc}
	0.15	100.00±0.0 (89.97) ^a	98.81±1.2 (86.34) ^a	91.45±1.8 (73.15) ^b	86.67±1.3 (68.60) ^b
Carbendazim 12%+ Mancozeb 64% WP (SAAF)	0.15	100.00±0.0 (89.97) ^a	92.49±0.2 (74.07) ^b	84.91±0.8 (67.13) ^{def}	79.26±0.7 (62.89) ^{cdef}
	0.2	100.00±0.0 (89.97) ^a	98.72±1.3 (86.20) ^a	90.58±0.9 (72.13) ^{bc}	82.96±0.7 (65.61) ^{bcd}
	0.25	100.00±0.0 (89.97) ^a	100.00±0.0 (89.97) ^a	98.15±1.9 (85.42) ^a	94.81±3.2 (79.42) ^a
SEm(±)		2.10	1.86	1.61	1.69
CD (p=0.05)		5.98	5.30	4.57	4.80

Note: Figures in parentheses are arc sine transformed values

Data followed by same letters in the same column are not statistically significant

Conclusion

Fungicides provides a quick response and action over the soil-borne pathogens, among various fungicides tested carbendazim+mancozeb influenced less mycelial growth and found best in controlling the rhizome rot pathogen of ginger.

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