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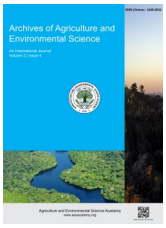

ORIGINAL RESEARCH ARTICLE

Efficacy of chemical fungicides against the fusarium rhizome rot of Ginger (*Zingiber officinale*)

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ABSTRACT

The present investigation was conducted to analyze efficacy of different commercially available fungicides against the Fusarium spp. causing rhizome rot of ginger adopting poison food technique. The experiment was carried out in a completely randomized design (CRD) with 8 treatment and 3 replications. The fungicides SAAF (Carbendazim 12% WP + Mancozeb WP 63%), Nativo (Tebuconazole 50% WP + Trifloxystrobin 25% WP), Caviet (Tebuconazole 25% WP), Kingsin M (Thiophanate –methyl 70%WP), Moximate (Cymoxil 8% WP + Mancozeb 64% WP), Custodia (Azoxystrobin 11% SC + Tebuconazole 18.3% SC), Melody duo (Iprovalicarp 5.5% WP + Probineb 61.5% WP) were used as a treatment for poison food technique. The results of this study indicated that there was a highly significant difference ($p \leq 0.001$) among the treatments in mycelial growth of the pathogen and inhibition of pathogen by different fungicides. The maximum mycelial growth of pathogen was observed on control plate (79.67mm) which was followed by melody duo and Moximate with the radial mycelial growth of 57.33mm and 55.83mm, respectively. Whereas the least mycelial growth of pathogen was recorded in SAAF (0.00mm) which was followed by Nativo, Custodia, Caviet and Kingsin M with the radial mycelial growth of 10.33mm, 14.83mm, 15.50, 21.83mm, respectively. Therefore, SAAF fully inhibited the growth of pathogen and found most effective which was followed by Nativo, Custodia, and Caviet with 87.04%, 81.40%, 80.55%, respectively.

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INTRODUCTION

Ginger (*Zingiber officinale* rocs.) is an important commercial spice crop in Nepal. It belongs to the family zingiberaceae, which includes 56 genera and 1300 plant species like, Turmeric, Cardamom, Javanese ginger and many more (Lakhan *et al.*, 2015). Ginger is herbaceous perennial plant with false steam of about a meter tall with narrow to broad leaf blades whose rhizome is used as a spice and medicine (Bharati *et al.*, 2019). It is believed that the ginger first appeared in southern region of ancient China. Then distributed to India, Maluku islands (Spice Island) and then rest of the Asia and West Africa (Langner *et al.*, 1998). In FY 2018/19, Nepal's total ginger production was 297,512 MT in 22,132 ha of land with the productivity of 13.44 MT/ha. Where province no.1 was the largest ginger producing region (111,604 MT) of Nepal (MoAD 2020). Ginger is very susceptible to several disease and pests which became the major problem on its production. Among the several diseases the rhizome rot of ginger is the most common and economically very important disease of ginger growing area because it causes the huge economic loss by reducing the yield by 50-92% (Meenu and Jebasingh, 2019a). The fungal pathogens (Pythium spp., and Fusarium spp.) and bacteria [Pseudomonas (Ralstonia) solaniserum] are the major pathogen responsible for the rhizome rot of ginger (Rai, 2006). The fusarium oxysporium f. sp. Zinziberis is mostly found the

rhizome rot (dry rot) complex of ginger, while the Pythium spp. are mostly found to be causing the soft rot of rhizome of ginger (Vivek *et al.*, 2013, Rosangkima *et al.*, 2018a).

The fusarium spp. is both soil born and seed born (Rosangkima *et al.*, 2018b) pathogen and produces different spores such as micro and macro-conidia, chlamydospores and zoospores which survives as a pathogen in dry weather condition and excess soil moisture favors to the disease development (Nelson, 1992, Booth, 1971a). The disease causes huge damage if the favorable condition met by the pathogen during the cropping season. The disease can affect all parts of the plants such as roots, sprouts, developing rhizomes and collar region of the pseudo stem. Initially the symptom appears as the older leaf starts to turn yellow and chlorosis proceeds downwards along the margins involving rest of the leave blade and, eventually the leaf sheath and ultimately resulting in withering and death of the leaves. As on older leaves, the similar symptoms progression appears on the younger leaves until the entire plant dies (Meenu and Jebasingh, 2019b). The foot of the plant and rhizomes turns into pale, watery and soft appearance on just above the ground level. The rhizome gradually turns into a mass of decaying of tissue and bad foul smell can be experienced which makes the rhizome inconsumable and yield reduction (Ayub *et al.*, 2009). The disease caused due to these soil and seed borne pathogenic fungi are very serious disease, which affects the several cultivated plants worldwide which results in poor production, poor quality and decreased the economic returns and income of the farmers (Punja and Rodriguez 2018, Booth, 1971b, Rosangkima *et al.*, 2018c).

Farmers in Sunsari Nepal are much conscious about enhancement of production, yield, and economic return but there is lack of effective fungicides recommendation. Thus, abundantly use of different classes of fungicide had also creating the problems from the economic aspects. So, the Plant protective and curative measures are progressively depending upon an application of effective fungicides. Similarly, successful management of rhizome rot disease is pivotal to ensure economic viability of ginger production whereas to inhibit incidence of this thermophilic soil borne and seed borne disease through chemicals is an integral part of the contemporary studies. In addition, identification of the systemic fungicide is the demand of the current era. Therefore, it is the need of time to introduce such fungicides which exhibit rapidity in action against the fusarium rhizome rot and cause less phytotoxicity. Thus, most effective fungicides against the rhizome rot disease were exploited to prevent plants from the disease.

MATERIALS AND METHODS

Sample collection and pathogen isolation

The soil and the disease sample for the isolation of pathogen were collected from diseased prone area of turmeric / ginger zone of Sunsari PMAMP. The isolation of pathogen was done in the plant protection lab of GPCAR, Morang. The diseased rhizome was collected from the ginger zone of Sunsari, PMAMP. The surface of the samples was sterilized with 2% sodium hypo-chloride for 5 minutes, and then inoculation was done in PDA tissue transplanting method with streptomycin to prevent the growth of bacteria. The pathogen was identified by the observation of their morphological and cultural characteristics. Figure 1 represent the pathogen isolated from rotted rhizome of ginger.

Experimental design

The lab experiment was laid out in CRD design consisting of eight treatments following three replications. There were eight treatments with seven types of fungicides and one leave as naturally control for the analysis of fungicides against the pathogen of fusarium rhizome rot of ginger as given in Table 1.

Preparation of poison bait, inoculation of pathogen and observation

Potato Dextrose Agar (PDA) was prepared with composition of 200gm of potato, 20 gm of dextrose and 20 gm of agar for 1000 ml of distilled water. The media was autoclaved at 121˚C and 15 psi pressure for 20 minutes and allowed to cool to bring around 40 ˚C in room temperature before pouring in sterilized petri plates under the laminar air flow chamber. Stock solution was prepared for each fungicide by diluting 1gm in 100 ml of distilled water and required amount was incorporated into the conical flask containing Potato Dextrose Agar to get 200ppm concentration and mixed thoroughly before autoclaving. The poisoned food technique was followed to evaluate the efficacy of fungicides at concentrations of 200ppm in inhibiting the mycelial growth of fusarium spp.

After autoclaving, 20 ml of the poisoned media was poured aseptically into the sterilized petri plates each of 9 cm diameter under laminar air flow chamber and allowed to solidify. As for control, only PDA media was used without addition of any fungicide. With the help of cork borer, 5mm radial disc of the pathogen from the 7 days old culture plate was placed on the center of the petri plate containing PDA media and incubated at 28±1 ^{0}C. Figure 2, 3 and 4 represent the 1st day of inoculation of the pathogen, 10th day of pathogen inoculation and 10th day of pathogen inoculation in T8, respectively.

Data collection

The diameter of the pathogen was taken from two directions after 2ndday of inoculation (DAI) and recorded up to the 10th-day. The percent inhibition of growth of pathogen was calculated by using the formula given by Vincent (1947).

i.e., I=C-T/C

Where, I= percent inhibition, C= growth in control, T= growth in treatment

Statistical analysis

Data was entered in MS-Excel (2007) and subjected to ANOVA with the help of R-studio (R-version 3.5.3 statistical data analysis package). Mean comparison among significant variables was carried out by Fisher-LSD test at 5% level of significance. MS-Excel (2007) was used for construction of graph and tables.

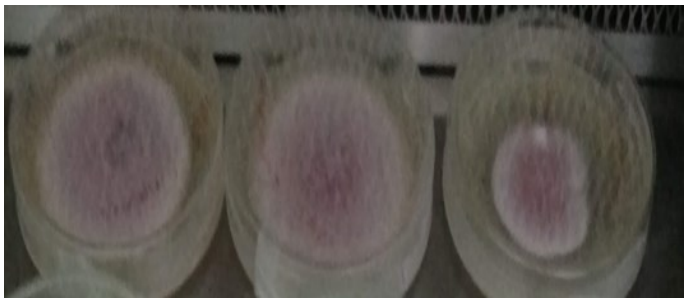

Figure 1. *Pathogen isolated from rotted rhizome of ginger.*

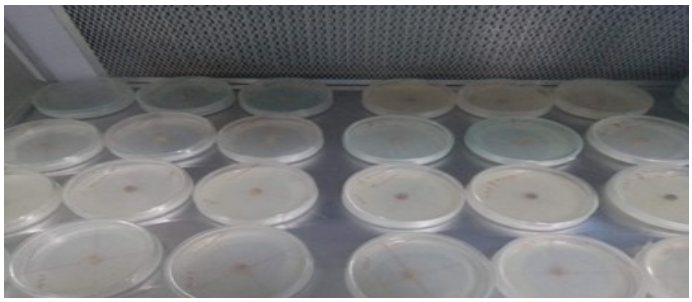

Figure 2. *1st day of inoculation of the pathogen.*

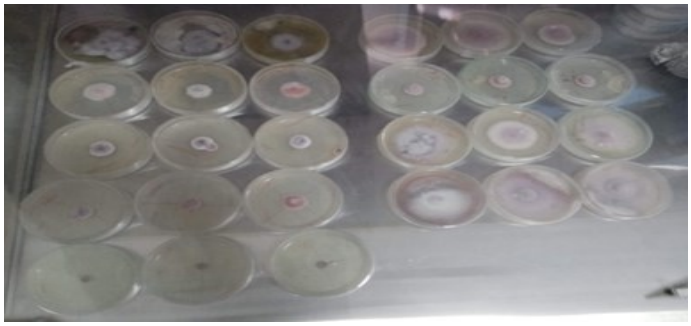

Figure 3. *10th day of pathogen inoculation.*

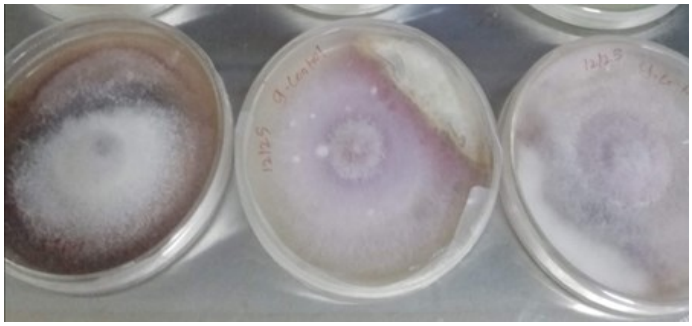

Figure 4. *10th day of pathogen inoculation in T8.*

Table 1. List of chemical fungicides used as a treatment used in the experiment of poison food technique method.

S.N.	Fungicides	Treatment number
1	SAAF (carbendazim 12%+mancozeb63%WP	1
2	NATIVO (tebuconazole 50%+trfloxystrobin 25%WP	2
3	CAVIET (tebuconazole 25%WP)	3
4	KINGSIN M (thiophanate-methyl 70% WP)	4
5	MOXIMATE (cymoxil 8%+mancozeb 64%WP)	5
6	CUSTODIA (azoxystrobin 11%+ tebuconazole 18.3% SC)	6
7	MELODY DUO (iprovalicarb 5.5%+probineb 61.25% WP)	7
8	CONTROL	8

RESULTS AND DISCUSSION

There was a highly significant difference ($p \leq 0.001$) among the treatments in mycelial growth of the pathogen and inhibition of pathogen by different fungicides (Table 2). The maximum mycelial growth of pathogen was observed on control plate (79.67mm) which was followed by melody duo and Moximate with the radial mycelial growth of 57.33mm and 55.83mm, respectively. Whereas the least mycelial growth of pathogen was recorded in SAAF (0.00mm) which was followed by Nativo, Custodia, Caviet and Kingsin M with the radial mycelial growth of 10.33mm, 14.83mm, 15.50, 21.83mm, respectively as shown in Table 2. Among seven different fungicides, SAAF was found to be the best for the 100% inhibition of mycelial growth of the pathogen followed by Nativo with 87.04%. Custodia and Caviet was also found to be most effective with the growth inhibition rate of 81.40% and 80.55%, respectively and satisfactory result to Kingsin M with the growth inhibition rate of 72.62%. While the Moximate and Melody duo showed low 17 inhibitions of the fungal mycelia 29.90% and 28.11% respectively, as compared to other fungicides and Moximate and Melody duo was found ineffective against the pathogen with least inhibition percentage. Figure 5 shows the pathogen at the concentration of 200ppm of different fungicides showed different growth pattern from 2nd to 10th day of inoculation (Figure 5). At 200ppm, no growth of pathogen was observed in SAAF treated plate. Very slow growth of pathogen was observed in Nativo treated plate during the early days but later the growth of pathogen was observed. The growth pattern of mycelia of the pathogen was observed similar in case of Caviet and Custodia. Heavily growth of pathogen was observed in Moximate, and Melody duo treated plates. The growth of pathogen in Moximate and Melody duo was quite similar up to the day 6 but the growth of pathogen in Melody dou was suddenly increased after day 6 whereas the growth of pathogen in Moximate treated plate was increasing uniformly. The growth of pathogen was increasing every day in control plates until the plate was fully covered. Hence the least growth of pathogen was found on SAAF treated plate which was followed by Nativo, Custodia, and Caviet. Moderate growth of pathogen was found on Kingsin M but among the fungicides the maximum growth of pathogen was recorded on Melody duo and Moximate. Thus, Melody duo and Moximate were not effective enough to inhibit the growth of pathogen.

Table 2. Effect of different fungicides on inhibition of mycelial growth of Fusarium spp. after 10th day of inoculation.

Treatments	Radial mycelial growth (mm)	Growth inhibition (%)
1	0.00 ^{f}	100.00^{a}
2	10.33^{e}	87.04^{b}
6	14.83^{d}	81.40^{c}
3	15.50^{d}	80.55^{c}
4	21.83^{c}	72.62^{d}
5	55.83^{b}	29.90^{e}
7	57.33^{b}	28.11^{e}
8	79.67^{a}	0.00^{f}
$LSD_{0.05}$	4.34	4.65
CV (%)	7.85	4.48
F-Test	***	***

CV: Coefficient of Variation; ***: Significant at 0.1% level of significance; LSD: Least Significant Difference; Values with same letters in a column are not significantly different at 5% level of significance by Fisher-LSD test.

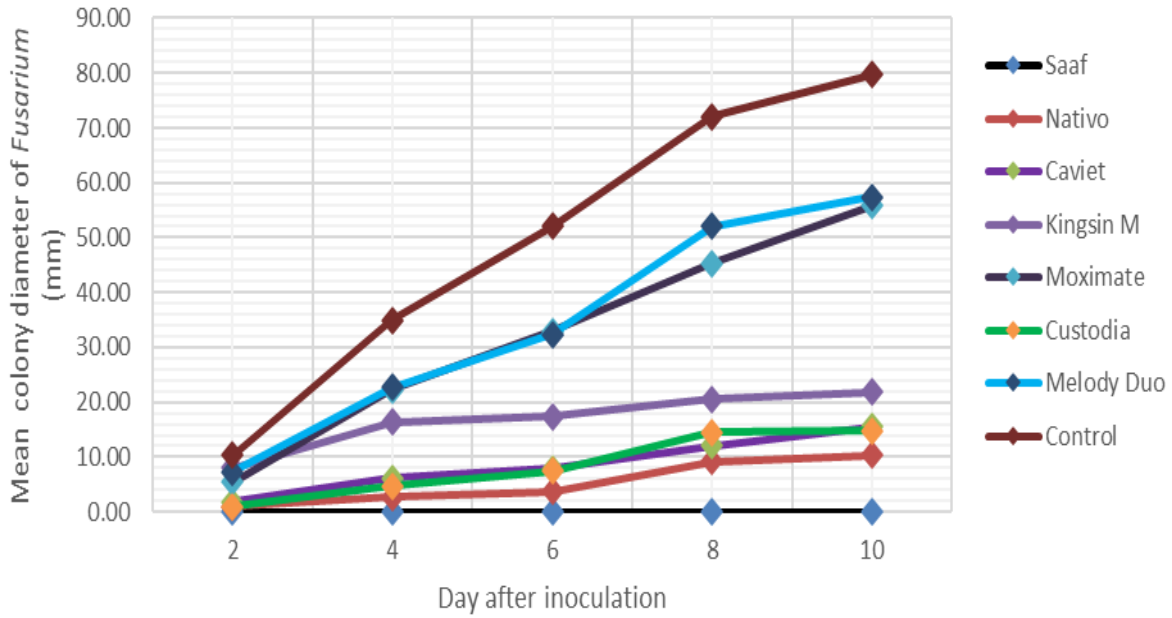

Figure 5. *Effect of different fungicides on mycelial growth of fusarium at different days after inoculation.*

SAAF has performed best on inhibition of the pathogen which was supported by Rajput *et al.* in 2006 on an experiment on efficacy of different fungicides against Fusarium wilt of cotton caused by Fusarium oxysporium f. sp. Vasinfectum and found Carbendazim as a most effective fungicides against the mycelial growth of the pathogen Fusarium spp. followed by Thiophanate methyl, and also the maximum shoot and root length was recorded and Carbendazim also reduced the colonization of Fusarium oxysporium f.sp. Vasinfectum. Dahal and Shrestha (2018) and Hegde *et al.* (2017) reported that the fungicides like Carbendazim and Mancozeb were found to be the effective against the *Fusarium* spp. In 2011, Madhavi *et al.* (2011) reported that combination of Carbendazim + Mancozeb was found effective in inhibiting mycelial growth (93.6%), followed by Carbendazim alone (92.4%). Kumar *et al.* (2021 on an experiment of efficacy of fungicides and bio-agents against Fusarium oxysporium f.sp. lentis causing vascular wilt of lentil (Lens culinaris Medik) in-vitro, observed that the 100 per cent inhibition of mycelial growth of pathogen was found in the treatment with Carbendazim 50 %WP, Cymoxil 8%+ Mancozeb 64%, Tebuconazole 25.9% EC and Propiconazole 25% EC at all three concentrations (50ppm, 75ppm and 100ppm). The least inhibition per cent of mycelial growth was recorded (50.00%, 54.17% and 60.42%) in Mancozeb 75 % WP at all three concentrations (50ppm, 75ppm and 100ppm) after 144 hrs. Suneeta *et al.* (2017) on the experiment of promissory action of Trichoderma spp. and fungicides in the management of Fusarium wilt of gerbera found that the Tebuconazole 50% + Trifloxystrobin 25%WP completely restricted the growth of Fusarium oxysporium f.sp. gerbera. In 2018, Nagar, U. S. conducted an experiment of in-vitro and glass house evaluation of fungicides against the pathogens associated with rhizome rot complex of ginger in Kumaon region of Uttarakhand with nine fungicides and found that carbendazim show complete inhibited of growth of Fusarium spp. in-vitro and Azoxystrobin, Tebuconazole, Carbendazim showed complete inhibition of Pythium aphanidermatum.

Conclusion

From the experiment it is concluded that the least growth of pathogen was found on SAAF treated plate which was followed by Nativo, Custodia, and Caviet. Moderate growth of pathogen was found on Kingsin M but among the fungicides the maximum growth of pathogen was recorded on Melody duo and Moximate. Thus, Melody duo and Moximate were not effective enough to inhibit the growth of pathogen. Thus, the SAAF was found to be the most effective among all the tested fungicides with the 100 percent mycelial growth inhibition of the pathogen. Nativo, Custodia and Caviet also showed their effectiveness with the growth inhibition of 87.04%, 81.40% and 80.55%, respectively.

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