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In vitro fungicidal sensitivity of *Colletotrichum* spp. isolated from seven different hosts

Pranjali Sinha[1], Jahaar Singh[2], Neelum Chouksey[3] and A.K. Singh[4]

[1,2,4]Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur 497 001, C.G., India
[3]Department of Agriculture Statistics, RMD College of Agriculture and Research Station, Ambikapur, IGKV, Raipur 497 001, C.G., India

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ABSTRACT

Colletotrichum (teleomorph *Glomerella*) is an important plant microbe that affects agricultural and plantation crops across the world. It adheres to the hemi-biotrophic mode of feeding, in which both biotrophic and necrotrophic stages occur sequentially. Pathogenic variability among *Colletotrichum* species affecting various crops as mango (anthracnose), strawberry (fruit rot), chilli (anthracnose), turmeric (leaf blight), soybean (pod blight), bean (anthracnose) and sugarcane (red rot). These naturally infected seven crops were collected from different growing regions of Chhattisgarh. The assay with four fungicides namely Chlorothalonil 75 % WP, Difenoconazole 25 % EC, Azoxystrobin 18.2 % EC and Tebuconazole 50 % + Trifloxystrobin 25 % w/ w were checked at four concentration 50,100,250 and 500 ppm with all the fungal culture and effective amount of control was observed especially with Difenoconazole 25 % EC fungicide.

Key words: *In vitro assay, Fungicides, Colletotrichum spp.*

Introduction

Genus *Colletotrichum* (teleomorph *Glomerella*) is one of the major plant pathogens of agricultural and plantation crops worldwide. The primary distribution, of this pathogen, lies on the various crops grown in tropical, subtropical and temperate areas (Hyde *et al*, 2009). Various species of this genus as *C. gloeosporiodes* (anthracnose of mango), *C. falcatum* (red rot of sugarcane), *C. curcumae* (leaf spot of turmeric), *C capsici* (anthracnose on chilli) and *C. truncatum*(pod blight of soybean) are more important, among the all-other species, and belong to Kingdom- Fungi, Phylum- Ascomycota, Class– Coelomycetes, Order-Melanconiales, and Family-Melanconiaceae (Hawksworth *et al*., 1995). In later stage, after the infection, the hyphae develop and spread in the tissues leading to killing of the host cell. Thus, this genus follows the hemi-biotrophic mode of nutrition where sequential occurrence of the biotrophic and nectotrophic phases take place (Cannon *et al.,* 2012). Fungus *Colletotrichum* produces characteristic acervuli and it causes losses at all stages of growth, as in anthracnose of beans, but few species of C*olletotrichum* may cause tremendous loss by damaging fruits, reducing yields through destruction of blossoms or by affecting leaves and stems. In mango, anthracnose is an important disease that affects plants by killing inflorescence, causing spots on leaves, and dark brown to black decay spots on the fruits at the ripening stage (Agrios, 1969).

([1]Research Scholar, [2]Asst. Professor, [3]Associate Professor, [4]Professor)

In addition, the lack of resistant cultivars of the diverse crop, effective fungicides, and botanicals, to manage anthracnose, has been a concern for both scientists and farmers. As a result, finding effective fungicide(s) and plant-based antifungal compounds, to combat this disease, is critical (Ranjitha *et al.,* 2019). A management strategy, *in-vitro,* involving the use of fungicides and biological agents to control anthracnose and blight, cause by various species of *Colletotrichum*, in diverse hosts, has been reported but complete control is not yet achieved either in any host pathogen interaction. Evaluation of fungicides, *in vitro,* is a handy tool to screen-out large number of fungicides at different concentrations. In the present study, laboratory evaluation of fungicides, by poison food technique for various fungicides evaluated with different concentrationsand confrontation assay by biological agents revealed significant results.

Material and Methods

Fungus *Colletotrichum* spp. studied in this investigation, were isolated from seven different host naturally infected with anthracnose during a survey carried out in *kharif* (2020 - 2021). Pods of soybean (CoA, Raipur), matured chilli fruit, turmeric leaves, sugarcane stem, mango & strawberry leaves (KVK, Ambikapur) and matured pods of bean (Surajpur) regions of Chhattisgarh. The individual symptoms, from the plant parts, for isolation, of the fungus was examined directly by placing infected part on the stereoscopic microscope. Only those infected parts were selected showed typical symptom for presence of conidia. The fungus cultures were purified using single spore isolation on 2 % agar medium while being maintained on potato dextrose agar (PDA) medium. Single spore isolation was used to subculture, at intervals of 15 days, and samples were stored at a low temperature (4!).

Pathogenicity tests of the fungus was proved (i) on young seedlings grown in between wet blotting papers (ii) on stem cuttings, fruits after making a fissure on the stem or fruits and inoculating with mycelium along with small piece of agar (Wijesekara, 2005) and re-isolation was carried out.

Four different fungicides, including Chlorothalonil 75% WP (T_1), Difenoconazole 25 % EC (T_2), Azoxystrobin 18.2 % EC (T_3) and Tebuconazole 50 % + Trifloxystrobin 25 % w/w (T_4) at 50, 100, 250 and 500 ppm concentration, were tested, *in vitro,* to determine the sensitivity/tolerance by different species of *Colletotrichum* via Poisoned Food Technique. 100 ml stock solution (1000µg/ ml) of each fungicide was prepared in sterilized distilled water in 500 ml flask containing 100 ml of sterilized melted PDA, so as to get final concentration of 50 ppm, 100 ppm, 250 ppm and 500 ppm. For calculation of amount of stock solution to be added to PDA to get above concentrations, the formula $C_1V_1 = C_2V_2$ was respectively, and V_1 is the volume (ml) of stock solution to be added to the measured volume (V_2) of PDA. Each Petri plate was centrally inoculated with 5mm discs cut from the seven days old test fungal culture, each PDA contained a different dose of chemicals. Unamended PDA plates served as check. Three replications were maintained for each treatment. Plates were incubated at 28 ± 1°C and colony diameter was measured when the check plates were fully covered with mycelial growth of test fungus. The plates were then sealed with paraffin. Percent inhibition of the mycelium growth was calculatedusing the formula:

$$I = C - T/C \times 100$$

Where,

I = Percent inhibition

C = Radial growth in check in cm

T = Radial growth in treated plates in cm

Statistical analysis was carried out in CRD factorial design with three factors namely seven *Colletotrichum* spp, four fungicidal treatments and four concentrations with three replications.

Results

Data depicted, in Table 1, revealed that the most effective fungicide was Difenoconazole 25 % EC (T_2) followed by Azoxystrobin 18.2 % EC (T_3), respectively. Tebuconazole 50 % + Trifloxystrobin 25 % w/w (T_4) followed by Chlorothalonil 75% WP (T_1) were least effective over the other fungicides but were statistically at par to each other.

The interaction study among all seven *Colletotrichum* spp and four fungicides reveal that the highest inhibition was recorded in C_3 with fungicide T_2 (Plate 3 E) followed by T_3, respectively. However, among all four fungicides, T_2 showed the highest inhibition with C_4 (Plate 4 G), C_2 (Plate 2 C), C_5 (Plate 5 I), C_6 (Plate 6 K) and C_7 (Plate 7 M), but in C_4 and C_1 (Plate 1 A) maximum inhibition was recorded by fungicide T_1. Least inhibition in C_6 (Plate

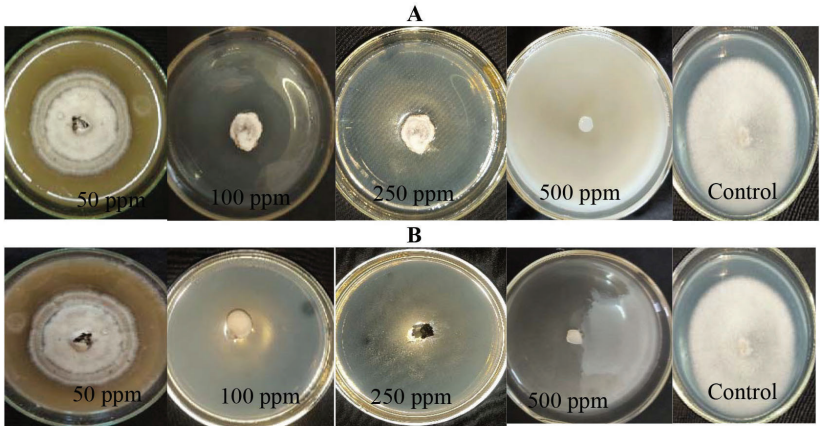

Plate 1. Effect of fungicides at different concentration on radial growth of *C. capcisi* (C_1) *A. Difenoconazole* 25 % EC (T_2) with highest effectivity and B. Tebuconazole 50 %+ Trifloxystrobin 25 % w/w (T_4)with least effectivity

Table 1. Efficacy of four different fungicides on growth of *Colletotrichum* spp at 50, 100, 250 and 500 ppm

Species	Crop	Sample	Fungicides	Growth inhibition (%) at different ppm			
				50	100	250	500
C.capsici	Chilli	C_1	T_1	60.2(0.64)	80.7(0.94)	72.1(0.81)	97.7(1.50)
			T_2	57.8(0.45)	84.7(1.02)	91.0(1.22)	100(1.50)
			T_3	43.8(0.61)	72.0(0.80)	84.6(1.03)	100(1.50)
			T_4	55.2(0.58)	76.9(0.89)	79.0(0.92)	100(1.48)
C. acutatum	Turmeric	C_2	T_1	53.6(0.56)	80.7(0.93)	77.1(0.88)	96.9(1.50)
			T_2	70.8(0.78)	82.7(0.97)	86.5(1.05)	100(1.48)
			T_3	65.3(0.71)	74.0(0.84)	82.7(0.97)	100(1.50)
			T_4	40.6(0.41)	84.9(1.03)	76.3(0,87)	100(1.48)
C.truncatum	Soybean	C_3	T_1	54.1(0.57)	81.7(0.96)	92.3(1.18)	97.5(1.50)
			T_2	91(1.14)	90.3(1.21)	100(1.57)	100(1.47)
			T_3	87(1.05)	83.0(0.98)	91.3(1.05)	100(1.50)
			T_4	80.3(0.93)	78.0(0.89)	86.9(1.15)	100(1.48)
C. lindemuthianum	Bean	C_4	T_1	60(0.64)	84(1.00)	93.3(1.27)	97.5(1.50)
			T_2	48.3(0.50)	87.3(1.06)	93.0(1.27)	100(1.24)
			T_3	36.3(0.37)	86.7(1.05)	89.7(1.11)	100(1.50)
			T_4	26.3(0.26)	40.1(0.41)	80(0.93)	100(1.50)
C. falcatum	Sugarcane	C_5	T_1	32.6(0.33)	56(0.59)	86(1.04)	97.5(1.50)
			T_2	70.7(0.78)	66(0.72)	94.7(1.30)	100(1.50)
			T_3	60.7(0.65)	72(0.80)	92(1.24)	100(1.50)
			T_4	55.9(0.59)	72.7(0.81)	90(1.17)	100(1.48)
C. gleosporoides	Mango	C_6	T_1	40.9(0.42)	68.2(0.75)	56.0(0.59)	59.0(0.63)
			T_2	55.3(0.58)	70.0(0.77)	76.0(0.86)	86.6(1.05)
			T_3	48.0(0.50)	64.7(0.70)	70.0(0.77)	73.0(0.88)
			T_4	46.3(0.48)	55.0(0.58)	75.3(0.85)	77.6(0.88)
C. fragariae	Strawberry	C_7	T_1	44.4(0.46)	55.2(0.58)	51.9(0.54)	83.6(0.99)
			T_2	64.2(0.69)	58.3(0.62)	86.9(1.05)	92.8(1.20)
			T_3	53.5(0.56)	72.5(0.81)	79.7(0.92)	91.7(1.24)
			T_4	57.7(0.61)	68.2(0.75)	74.3(0.83)	92.4(1.18)
Control		-	-	0.0	0.0	0.0	0.0

	Factor A (Sample)	Factor B (Fungicide)	Factor C (Concentration)	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
CD at 5%	0.036	0.030	0.027	0.080	0.071	0.060	0.160
CV=13.0							

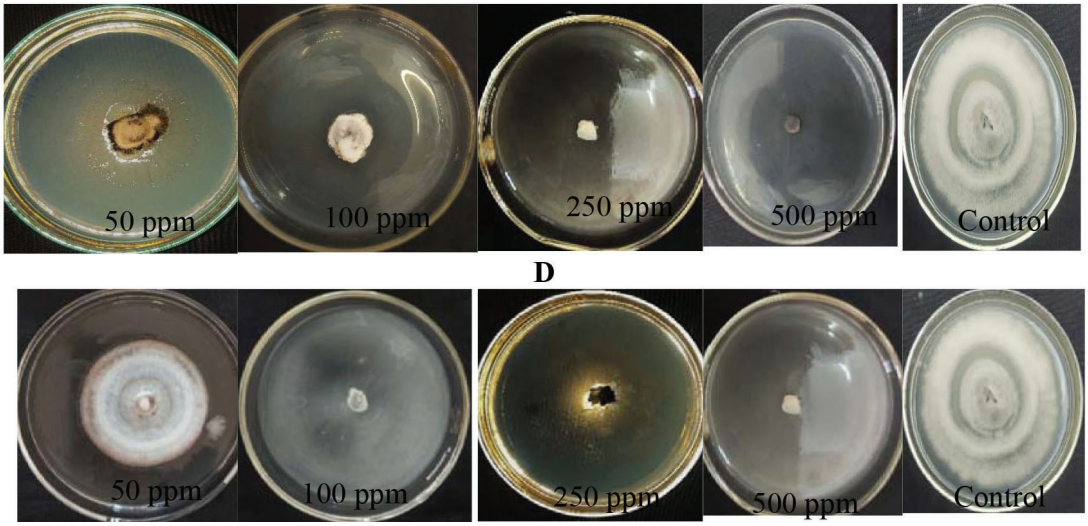

Plate 2. Effect of fungicide at different concentration on radial growth of *C. acutatum* (C_2) *C. Difenoconazole* 25 % EC (T_2) with highest effectivity and D. Tebuconazole + Trioxystrobin25 % w/w (T_4) least effectivity

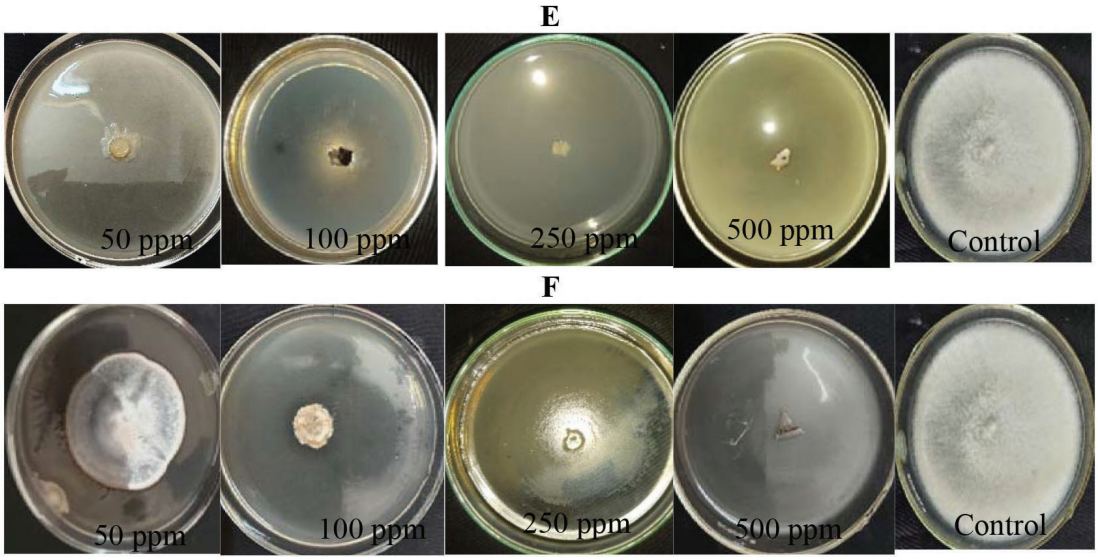

Plate 3. Effect of fungicide at different concentration on radial growth of *C. truncatum* (C_3) *E. Difenoconazole* 25 % EC (T_2) with highest effectivity and F. Chlorothalonil 75 % WP (T_1) with least effectivity

6 L), C_7 (Plate 7 N), C_5 (Plate 5 J) and C_3 (Plate 3 F) was recorded with T_1; in C_2& C_1 by T_4 (Plate 2 D &1 B) and in C_4 by T_3 (Plate 4 H).

The interaction among all three factors as *Colletotrichum* spp., fungicide and concentration showed that the radial growth of five *Colletotrichum* spp. C_1, C_2, C_3, C_4 and C_5 was completely (100%) inhibited by all four fungicides at 500 ppm (except C_6 and C_7) which was statistically at par to C_3 with T_2 at 250 ppm. Significant inhibition was observed inC_5 by T_2 at 250 ppm with mean growth inhibition of 94.7 %. Subsequently, C_4 at 250 ppm with T_1 & T_2, C_7 at 500 ppm with T_3, C_5 at 250 ppm with T_3, C_1 at 250 ppm with T_2, C_3 at 100 ppm with T_2, C_7 at 500 ppm with T_2& T_4, C_3 at 250 ppm with T_1, C_5 and C_3 at 250 ppm with T_4 and C_3at 50 ppm with T_2 fungicide where all statistically at par to each other. The lowest inhibition was found at T_4 with C_4 at 50 ppm (26.3 %) followed by T_1 with C_5 (32.6 %) at same level of concentration.

Out of the four concentrations tested, 500 ppm showed maximum mean per cent inhibition of

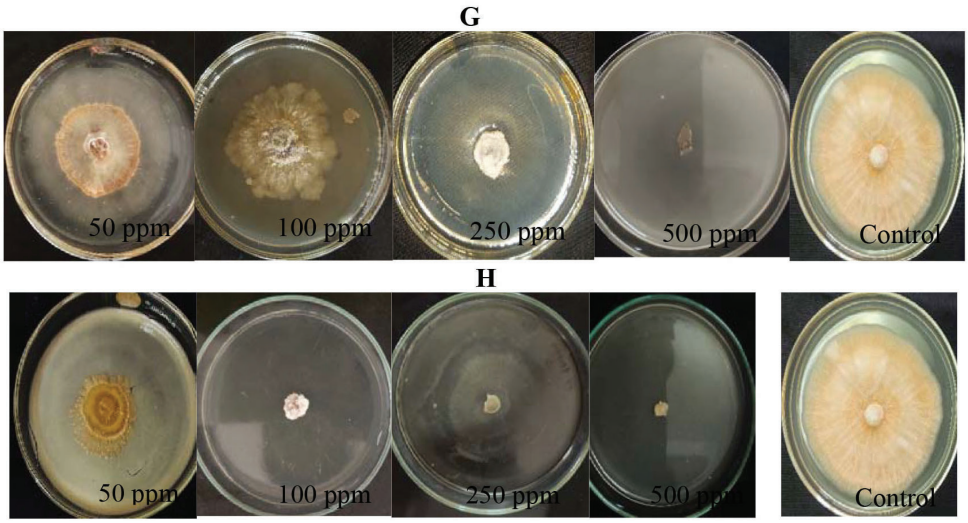

Plate 4. Effect of fungicide at different concentration on radial growth of *C. lindemuthianum* (C_4) G. Chlorothalonil 75 % WP (T_1) with highest effectivity and H.Tebuconazole 50 % + Trifloxystrobin 25 % w/w (T_4) least effective fungicide

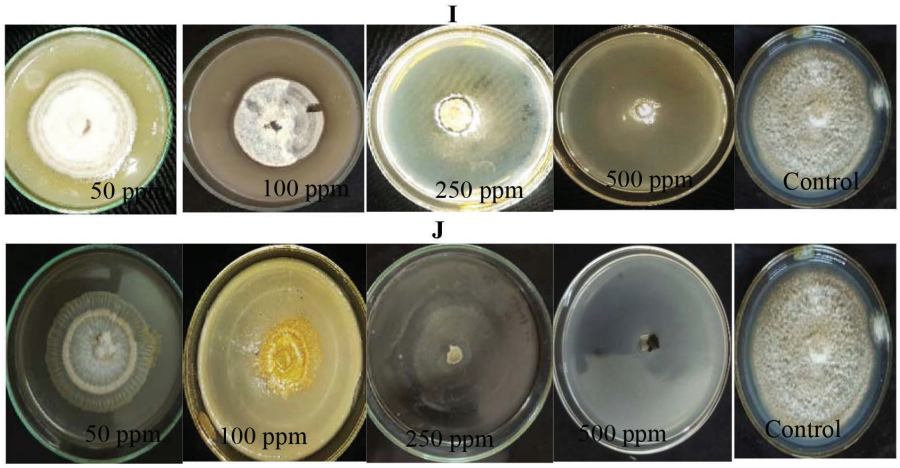

Plate 5. Effect of fungicide at different concentration on radial growth of *C. falcatum* (C_5) I. Difenoconazole 25 % EC (T_2) with highest effectivity and J. Chlorothalonil 75 % WP (T_1) with least effectivity

mycelial growth as all test samples and is found superior over other three concentration. As the concentration of fungicide is being increased, there is subsequently higher inhibition of mycelial growth. Thus, it exhibits a linear relationship between increase in concentration of fungicide and inhibition of mycelial growth.

Discussion

Few fungicides are effective against anthracnose, caused by *Colletotrichum* spp. and emerging resistance makes the search for chemical alternatives more relevant. In previous studies, the *C. acutatum* species were more tolerant to fungicide compared to *C. gleosporoides* (Jayasinghe and Fernando 1998; Greer *et al.*, 2011; Munir *et al.*, 2016) which was contradictory to the result obtained where lower mycelial inhibition was obtained in *C. gleosporoides* (86.6 %) as compared to *C. acutatum* (100 % inhibition) with Difenoconazole 25 % EC at 500 ppm. However, sensitivity of four fungicides Chlorothalonil 75 % WP, Difenoconazole 25 % EC, Azoxystrobin 18.2 % EC and Tebuconazole 50 % + Trifloxystrobin 25 %

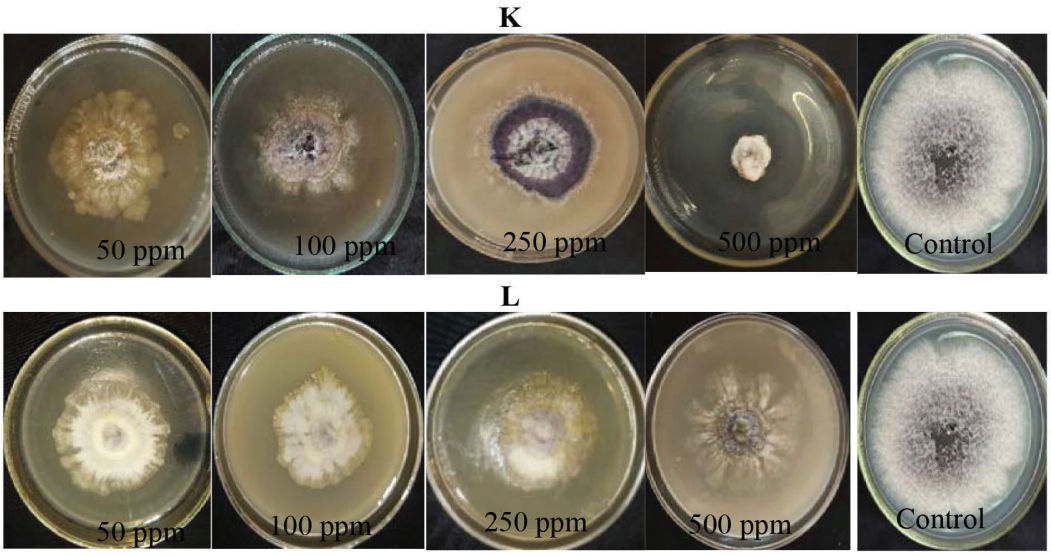

Plate 6. Effect of fungicide at different concentration on radial growth of *C. gleosporoides* (C_6) K. Difenoconazole 25 % EC (T_2) with highest effectivity and L. Cholorothionil 75 % WP (T_1)with least effectivity

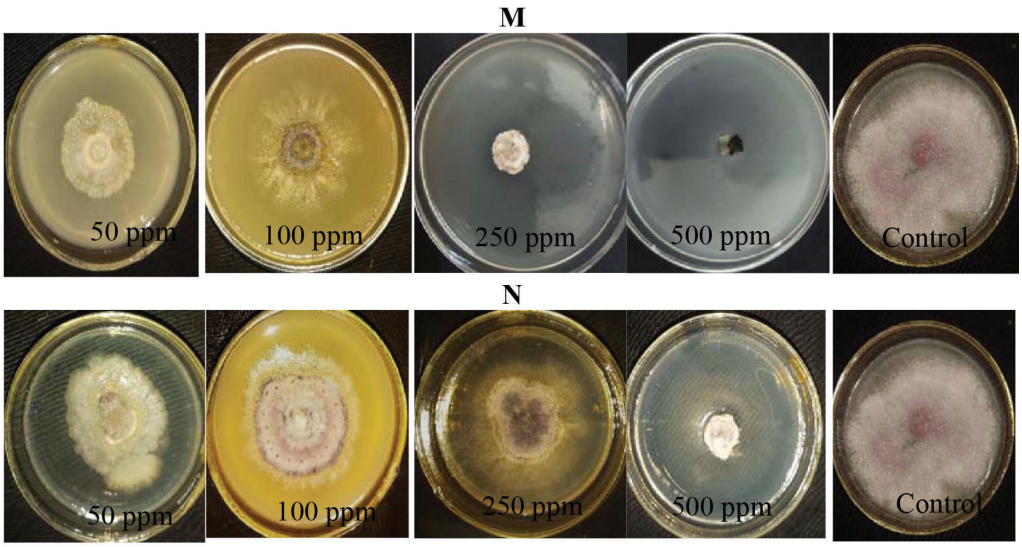

Plate 7. Effect of fungicide at different concentration on radial growth of *C. fragariae*(C_7) M. Difenoconazole 25 % EC (T_2) with highest effectivity and N. Cholorothionil 75 % WP (T_1)with least effectivity.

w/w against *Colletotrichum* spp. were in accordance with the result and indicated that sensitivity to Difenoconazole 25 % EC of *Colletotrichum* spp. was consistently high against *C. fructicola*, *C siamense*, *C. truncatum*, *C. nymphae* and *C. fioriniae* from peach at EC_{50} value (Chen *et al.,* 2016; He *et al.,* 2019); *C. gleosporoides* from mango and pomegranate (Ranjitha *et al.,* 2019; Mahesh *et al.,* 2020); and *C. scovillei*, *C. truncatum*, *C. fruncticola* and *C aenigma* from chilli (Shi *et al.,* 2021) and least inhibition by Chlorothalonil 75% WP against *C. gleosporoides* (Ekbote, 2002). Also, at higher concentration most of the fungicides inhibited maximum mycelial growth but decreased with reduced concentration (Sudhakar, 2000; Kenny *et al.,* 2012).

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