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***In vitro* assay of different fungicides and bio control agents against fungal pathogens of dragon fruit (*Hylocereus* spp.) plantation in Assam**

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Abstract

Dragon fruit (*Hylocereus* spp.) is a commercially lucrative plant with amazing health benefitting qualities which has drawn attention of farmers in every part of the country so too in Assam. The crop has drawn considerable public attention due to its high commercial value. As a result interest in planting and cultivation of this fruit is increasing in the state. However with increase in cultivation of the crop there are reports of diseases such as anthracnose, stem rot and stem gray blight from the state. As such actions are needed to address these diseases for effective, sustainable and practical management strategies. Hence, the present study was conducted to identify efficient fungicide and bio control agent against *Colletotrichum gloeosporioides* OQ274997, *Fusarium oxysporum* OQ274999, *Fusarium dimerum* OQ274998 and *Diaporthe phaseolorum* OQ275000 causing anthracnose, stem rot and stem gray blight disease of dragon fruit to develop suitable integrated disease management strategies. *In vitro* efficacy of fungicides (Trifloxystrobin 25%+Tebuconazole 50%, Azoxystrobin and Copper oxychloride) at three different concentrations (0.05%, 0.1 and 0.2%) and bio control agents (*Trichoderma viride* KF439055, *Pseudomonas fluorescens* KT258013 and *Bacillus subtilis* ON3861930) were carried out against the above pathogens through poisoned food technique and dual culture method. Result revealed that combination fungicide Tebuconazole 50%+Trifloxystrobin 25% WG @ 0.05% and *T. viride* KF439055 significantly lowered mycelia growth of the pathogens and found to be the most promising amongst the fungicide and bio control agents evaluated against the pathogens.

Keywords: Dragon fruit, anthracnose, stem rot, gray blight, fungicide, bio control agent

1. Introduction

Like many other crops, dragon fruit is also attacked by several of economically important diseases, the majority of which causes fungal diseases. Although dragon fruit is a low-maintenance crop, there is a significant and numerous new and first country reports of dragon fruit diseases and their associated pathogen. Disease incidence has arisen with significant impact, likely due to increases in planting acreage and the consequent buildup of pathogen inoculums. Anthracnose is the most destructive and frequently reported fungal disease of dragon fruit. The disease is caused by different species of *Colletotrichum* such as *C. truncatum*, *C. gloeosporioides*, *C. siamense*, *C. fructicola*, *C. aenigma*, *C. karstii* and *C. boninense* (Masyahit *et al.*, 2009; Vijaya *et al.*, 2014; Palmateer *et al.*, 2007; Awang *et al.*, 2010; Abirami *et al.*, 2019; Ma *et al.*, 2014; Pegu *et al.*, 2025) [1, 2, 3, 4, 5, 6]. The fungus *C. gloeosporioides* is the most widely reported and is hosted by *H. undatus*, *H. monacanthus* and *H. megalanthus*. The abundance of viable spores is a huge challenge with diseases caused by *Colletotrichum* spp. In India, 30 per cent of dragon fruit plants were found to be infected by anthracnose and caused yield reduction (Abirammi *et al.*, 2019). *Fusarium* spp. associated with dragon fruit stem rot (Wright *et al.*, 2007; Rita *et al.*, 2016; Hawa *et al.*, 2017 and Mohd *et al.*, 2019, Pegu *et al.*, 2025) [6, 7, 8, 9, 10] and of basal rot (Wright *et al.*, 2007) [7] has been reported. Shakirah *et al.* (2021) [11] reported gray blight on the stem of *H. polyrhizus* in Malaysia caused by *Diaporthe* spp. such as *D. phaseolorum*, *D. tectonendophytica*, *D. arecae*, *D. eugeniae* and *D. hongkongensis*.

2. Materials and Methods

The present investigation entitled “*In vitro* assay of different fungicides and bio control agents against fungal pathogens of Dragon fruit (*Hylocereus* spp.) plantation in Assam” was carried out in the Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam during the year 2023-2024. The details of materials used, procedures followed and techniques

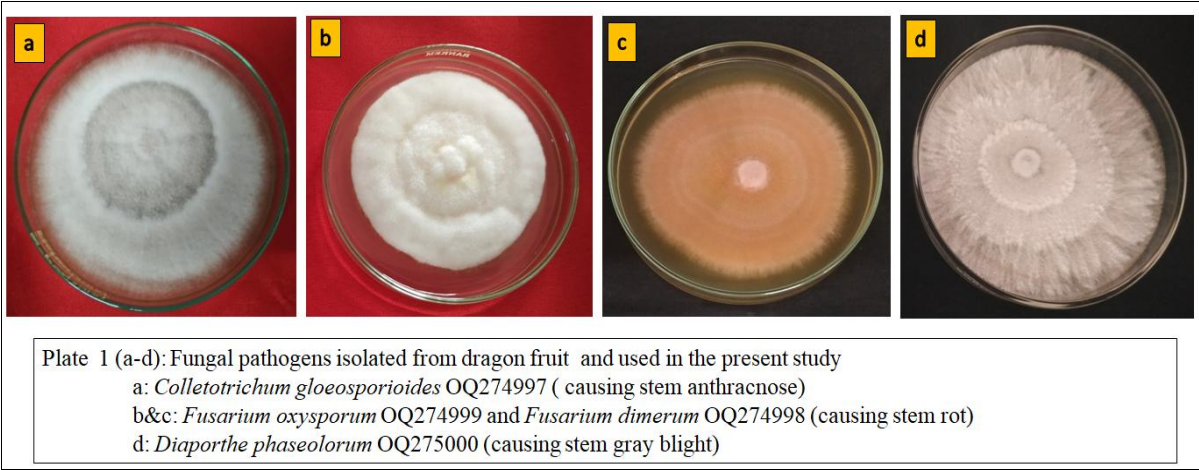
adopted for carrying out the experiments are presented in this chapter

2.1 *In vitro* assay

The pathogens isolated from dragon fruit plantations and used for the *in vitro* assay are presented in the Table 2.1 and Plate 1

Table 2.1: Pathogens selected for the study

Isolated pathogens	Name of the disease	Host plant	Reference
<i>Colletotrichum gloeosporioides</i> OQ274997	Anthrachnose	<i>Hylocereus</i> spp.	Pegu <i>et al.</i> , 2025 ^[6]
<i>Fusarium oxysporum</i> OQ274999	Stem rot		
<i>Fusarium dimerum</i> OQ274998	Stem rot		
<i>Diaporthe phaseolorum</i> OQ275000	Stem gray blight		



2.2 *In vitro* assay of fungicides

Three fungicides viz., combination Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin and Copper oxychloride were used to evaluate its *in vitro* efficacy against the pathogens.

The fungicides used and their concentrations are presented in Table 2.2 Standard poison food technique (Nene and Thapliyal, 1979) ^[12] was followed to assess the efficacy of the fungicide.

Table 2.2: Fungicides used for the *in vitro* assay

Fungicide used	Concentration (%)
Tebuconazole 50% + Trifloxystrobin 25% WG (Nativo)	0.05, 0.1 and 0.2
Azoxystrobin (Amistar)	0.5, 1.0 and 2
Copper oxychloride (COC)	0.1, 0.2 and 0.4

2.3 Preparation of Culture and culture media

The pathogens were cultured on potato dextrose agar (PDA) medium in 9 cm diameter Petri plates and incubated at 25±2 °C temperature for 7 days. Standard PDA medium (30ml) was autoclaved at 121 °C for 15 minutes, cooled to 40±5 °C and then amended with 0.3g/l of Streptomycine sulphate. The required concentrations of the fungicides (30ml) were prepared and added to the media while it was warm and agitated thoroughly to get a uniform distribution of fungicides in the media. For control plates appropriate amount of Streptomycin sulphate (0.3g/l) alone was added. Approximately 15 ml of each of the amended

media was poured aseptically into 9 cm sterilized Petri plates. The same amount of medium without the fungicide was used as control for comparison under the same conditions. Mycelial disc of 5 mm diameter of the fungal pathogen was cut with sterile cork borer and placed aseptically in the centre of each Petri plates containing the poisoned medium as per treatment combination presented in Table 2.3. Inoculated Petri plates were than incubated at 25±2 °C. For each treatment three replications were maintained. The study was arranged in a completely randomized design consisting of 10 treatments and 3 replications.

Table 2.3: Treatment combinations for *in vitro* assay of fungicide against the pathogen

T1	:	Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05% +Pathogen
T2	:	Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.1% +Pathogen
T3	:	Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.2% +Pathogen
T4	:	Azoxystrobin @ 0.5% +Pathogen
T5	:	Azoxystrobin @ 1.0% +Pathogen
T6	:	Azoxystrobin @ 2% +Pathogen
T7	:	Copper oxychloride @ 0.1% +Pathogen
T8	:	Copper oxychloride @ 0.2% +Pathogen
T9	:	Copper oxychloride @ 0.4% +Pathogen
T10	:	Control (Pathogen only)

2.4 Observation recorded

Observation on colony diameter of the pathogen was recorded up to the complete coverage of the control plate by the pathogen. Pathogen colony diameter was measured with a calliper ruler after every 24 hrs of inoculation. Percent inhibition of mycelial growth was calculated using the formula given by Bell *et al.*, 1982 as-

$$\% \text{ MGI} = \frac{\text{MGC} - \text{MGT}}{\text{MGC}} \times 100$$

Where,

MGI = Mycelial growth inhibition

MGC = Mycelial growth in control

MGT = Mycelial growth in treatment

3.7 *In vitro* assay of different bio control agents against the pathogens

2.5 Bio control agents used for the *in vitro* assay

Three bio control agents used to evaluate its *in vitro* efficacy against the pathogens were collected from the Mycology department of Plant Pathology, AAU, Jorhat and are presented in Table 4. Dual culture method (Skidmore and Dickson, 1976) [13] was followed for the assay as per the treatment combinations presented in Table 2.5

Table 2.5: Bio control agents used for the study

Bio control agent used	Source of the Bio control agent
<i>Trichoderma viride</i> KF439055	Mycology, Department of Plant Pathology, AAU, Jorhat
<i>Pseudomonas fluorescens</i> KT258013	
<i>Bacillus subtilis</i> ON386193	

Mycelial disc of the pathogen from seven day old culture grown on PDA were placed on one side of the plate and *T. viride* (KF439055) were placed on the other side of the plate. In case of *B. subtilis* (ON386193) and *P. fluorescens* (KT258013), a line of streak was made on both sides of the Petri plate with the

pathogen at the center and incubated at 25±2°C. Three replications were maintained for each tested pathogens. The pathogen grown as monoculture served as control. The plates were observed daily after 24 h of inoculation till the pathogen grew and covered the plate in the control.

Table 2.6: Treatment combinations for *in vitro* assay of bio control agent against the pathogen

T1	:	<i>T. viride</i> KF439055 + Pathogen
T2	:	<i>P. fluorescens</i> KT258013 + Pathogen
T3	:	<i>B. subtilis</i> ON386193 + Pathogen
T4	:	<i>T. viride</i> KF439055 + <i>P. fluorescens</i> KT258013 + Pathogen
T5	:	<i>T. viride</i> KF439055 + <i>B. subtilis</i> ON386193
T6	:	<i>P. fluorescens</i> KT258013 + <i>B. subtilis</i> ON386193 + Pathogen
T7	:	<i>T. viride</i> KF439055 + <i>P. fluorescens</i> KT258013 + <i>B. subtilis</i> ON386193 + Pathogen
T8	:	Control (Pathogen only)

The study was arranged in a completely randomized design consisting of 8 treatments and 3 replications.

2.7 Observation recorded

The colony diameter of the pathogens were measured and recorded and percent inhibition of mycelial growth was calculated using formula given by Vincet (1947) [14] as-

$$\% \text{ MGI} = \left[\frac{\text{MGC} - \text{MGT}}{\text{MGC}} \right] \times 100$$

Where,

MGI = Mycelial growth inhibition

MGC = Mycelial growth in control

MGT = Mycelial growth in treatment

2.8 Statistical analysis

Completely Randomized Design (CRD) and Fischer's method of analysis of variance (ANOVA) was employed for statistically analysis of the experimental data. Multiple comparisons amongst treatment means were undertaken using DMRT. The analysis of variance (ANOVA) was used to compare the significant difference with the t-test at $p = 0.05$. The standard error of difference between the treatment means (S.Ed.) was calculated using the formula:

$$\text{S.Ed} \pm = \sqrt{\frac{2 \times \text{Error mean square}}{\text{Number of replications}}}$$

The CD (Critical Difference) was performed to test the significance of the difference between pairs of means for various observations by using the following formula: $\text{CD} = \text{S.Ed.} \times t_{0.05}$ Where, S.Ed stands for Standard Error of Difference and $t_{0.05}$ denotes table value of "t" at a 5% probability level.

3. Results and Discussions

3.1 Effect of different fungicides on radial growth of *C. gloeosporoides* OQ274997

Results presented in Table 3.1 showed that all the three fungicides tested against *C. gloeosporoides* OQ274997 inhibited 100 per cent at higher concentrations. Among the fungicides, significantly lowered mycelia growth of the pathogen at lower concentration was recorded in treatment T1 (Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05%) with a per cent radial inhibition of 82.59 followed by treatment T5 (Azoxystrobin @ 1%), T8 (Copper oxychloride @ 0.1%), T4 (Azoxystrobin @ 0.5%) and T7 (Copper oxychloride @ 0.1%) with a per cent radial inhibition of 66.66, 64.07, 55.92 and 40.00 respectively. Comparatively Copper oxychloride was found significantly less effective at a concentration of 0.1 per cent. Combination fungicide Tebuconazole 50% + Trifloxystrobin 25% WG resulted in maximum per cent radial growth inhibition of *C. gloeosporoides* OQ274997 and found most superior over all the

fungicides tested at its lowest concentration and hence selected for further studies. The superior effect of the combination fungicide may be due to the fact that Tebuconazole a triazole fungicide is dimethylase inhibitor which interferes in process of building the structure of fungal cell wall thereby inhibiting the reproduction and further growth of fungus. Moreover, Trifloxystrobin a strobilurin fungicide that blocks the mitochondrial electron transport chain and inhibits energy

production in fungi. Azoxystrobin, a strobilurin group of fungicide and it works on the respiratory system of the fungus, thus depriving the fungus of the energy required to grow and killing the fungus. The results of the present study were in line with the *in vitro* evaluation of Tebuconazole 50% + Trifloxystrobin 25% WG against *C. gloeosporioides* by Ann *et al.* (2017) ^[15] who reported 84.62 per cent mycelia inhibition at 0.2 g/l and complete inhibition of the fungus at 0.4 g/l.

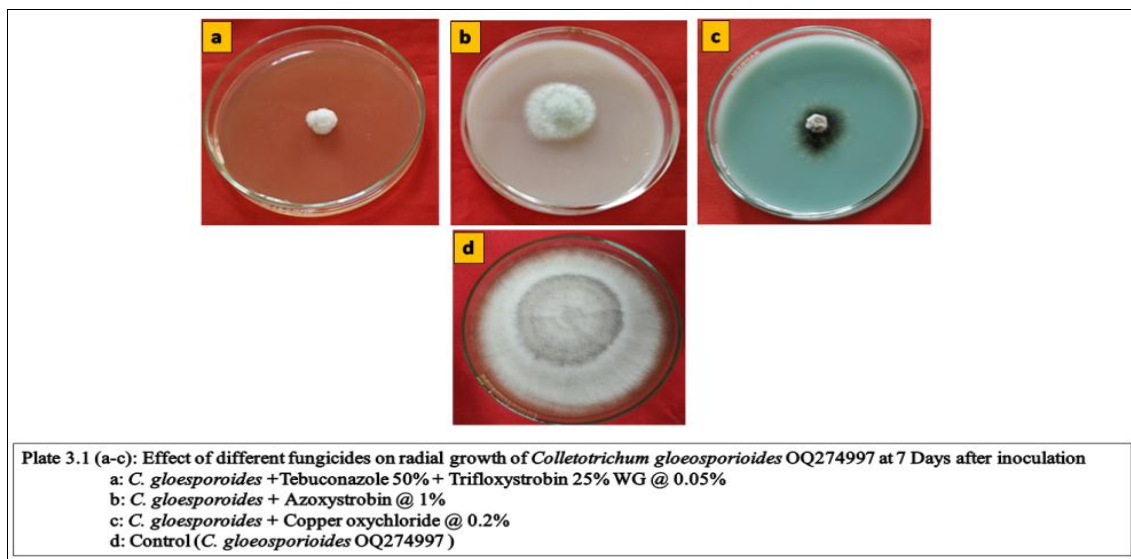


Table 3.1: Effect of different fungicides on radial growth of *C. gloeosporioides* OQ274997 at 7 days after inoculation

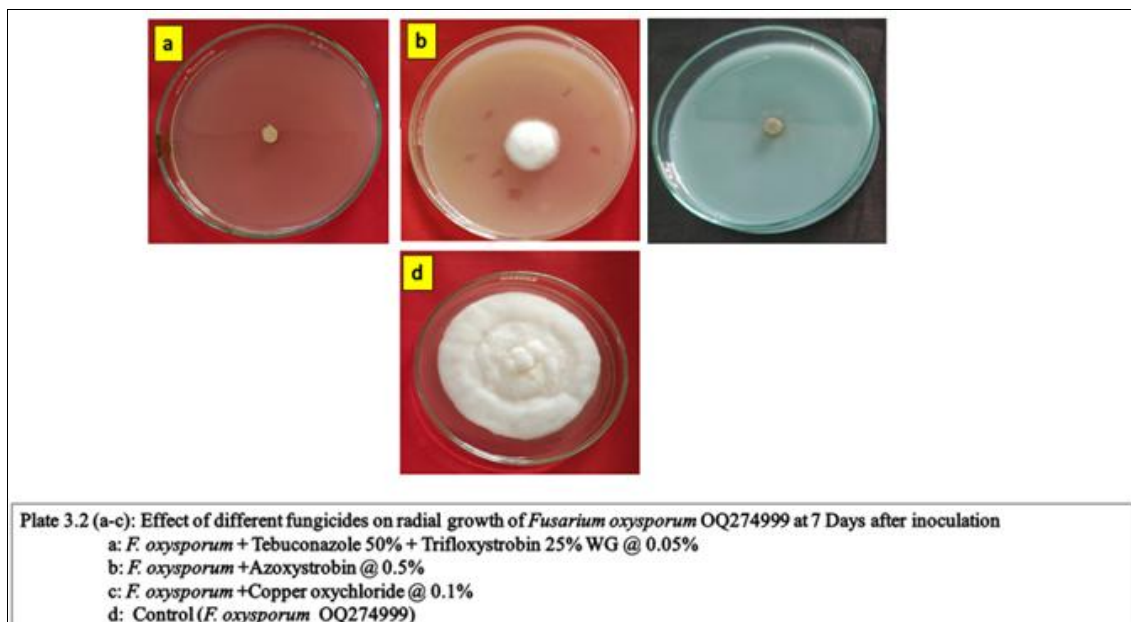
Treatment	Dose (%)	Radial growth mean (mm)*	Radial growth inhibition (%)
T1: Tebuconazole 50% + Trifloxystrobin 25% WG+ <i>C. gloeosporioides</i> OQ274997	0.05	15.66e	82.59
T2: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>C. gloeosporioides</i> OQ274997	0.10	0.00f	100.00
T3: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>C. gloeosporioides</i> OQ274997	0.20	0.00f	100.00
T4: Azoxystrobin + <i>C. gloeosporioides</i> OQ274997	0.50	30.66c	55.92
T5: Azoxystrobin + <i>C. gloeosporioides</i> OQ274997	1.00	21.00d	66.66
T6: Azoxystrobin + <i>C. gloeosporioides</i> OQ274997	2.00	0.00f	100.00
T7: Copper oxychloride + <i>C. gloeosporioides</i> OQ274997	0.10	45.00b	40.00
T8: Copper oxychloride + <i>C. gloeosporioides</i> OQ274997	0.20	23.33d	64.07
T9: Copper oxychloride+ <i>C. gloeosporioides</i> OQ274997	0.40	0.00f	100.00
T10: <i>C. gloeosporioides</i> OQ274997 (Control)	-	90.00a	0.00
SE (d)		2.11	
CD(0.05)		4.44	

*Data are mean of three replications In column mean followed by common letter do not differ significantly at 5% level of probability.

3.2 Effect of different fungicides on radial growth of *F. oxysporum* OQ274999

Result presented in Table 3.2 showed that treatment T1 (Tebuconazole 50%+Trifloxystrobin 25% WG @ 0.05%) completely inhibited the radial growth of *F. oxysporum* OQ274999. This was followed by T7 (Copper oxychloride @ 0.1%) and T4 (Azoxystrobin @ 0.5%) with a per cent inhibition of 77.03 and 70.37 respectively. Hence it is evident from the data presented in Table 3.2 that maximum per cent inhibition of *F. oxysporum* OQ274999 was caused by Tebuconazole 50%+Trifloxystrobin 25% WG @ 0.05 per cent and found

superior over other two tested fungicides. Results of present findings were in close proximity to the *in vitro* evaluation of Tebuconazole 50%+Trifloxystrobin 25% WG carried out by Bhimani *et al.* (2018) ^[16] that significantly inhibited the radial growth of *F. oxysporum*. Patra and Biswas (2016) ^[17] also reported the combination fungicide Tebuconazole 50% + Trifloxystrobin 25% WG as effective fungicides against *F. oxysporum* f.sp. *ciceri*. Tebuconazole 50%+Trifloxystrobin 25% at @ 0.07 per cent evaluated *in vitro* against *F. oxysporum* f.sp. *lycopersici* on PDA significantly inhibited mycelial growth (Ahmad *et al.*, 2021) ^[18]

**Table 3.2:** Effect of different fungicides on radial growth of *F. oxysporum* OQ274999 at 7 days after inoculation

Treatment	Dose (%)	Radial growth mean (mm)*	Radial growth inhibition (%)
T1:Tebuconazole 50% + Trifloxystrobin 25% WG+F. <i>oxysporum</i> OQ274999	0.05	0.00d	100.00
T2:Tebuconazole 50% + Trifloxystrobin 25% WG +F. <i>oxysporum</i> OQ274999	0.10	0.00d	100.00
T3:Tebuconazole 50% + Trifloxystrobin 25% WG +F. <i>oxysporum</i> OQ274999	0.20	0.00d	100.00
T4:Azoxystrobin + F. <i>oxysporum</i> OQ274999	0.50	26.66b	70.37
T5:Azoxystrobin +F. <i>oxysporum</i> OQ274999	1.00	0.00d	100.00
T6:Azoxystrobin +F. <i>oxysporum</i> OQ274999	2.00	0.00d	100.00
T7:Copper oxychloride +F. <i>oxysporum</i> OQ274999	0.10	20.66c	77.03
T8:Copper oxychloride +F. <i>oxysporum</i> OQ274999	0.20	0.00d	100.00
T9:Copper oxychloride+ F. <i>oxysporum</i> OQ274999	0.40	0.00d	100.00
T10:F. <i>oxysporum</i> OQ274999 (Control)	-	90.00a	0.00
SE (d)		0.61	
CD(0.05)		1.29	

3.3 Effect of different fungicides on radial growth of *F. dimerum* OQ274998

Results pertaining to Table 3.3 showed that all the three tested fungicides completely inhibited the growth of *F. dimerum* OQ274998 at higher concentrations. However treatment T1 (Tebuconazole 50%+Trifloxystrobin 25% WG @ 0.05%) showed 100 per cent mycelial inhibition of the pathogen at its lower dose as compared to other fungicides. These was followed

by T7 (Copper oxychloride @ 0.1%) and T4 (Azoxystrobin@0.5%) with a per cent inhibition of 74.81 and 60.74. Hence, the data presented in Table 3.3 showed that maximum per cent inhibition of *F. dimerum* OQ274998 was recorded in Tebuconazole 50%+Trifloxystrobin 25% WG @ 0.05 per cent and found superior over the other two tested fungicides.

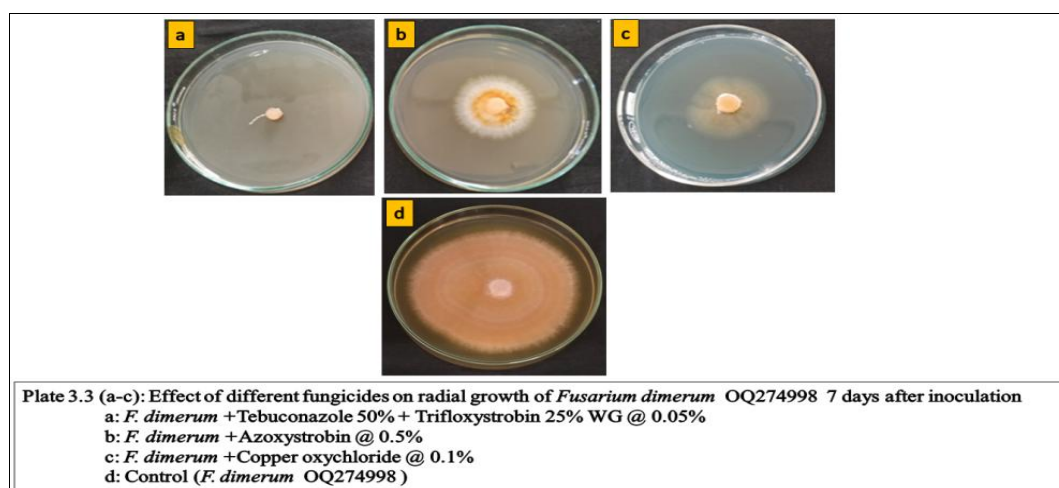


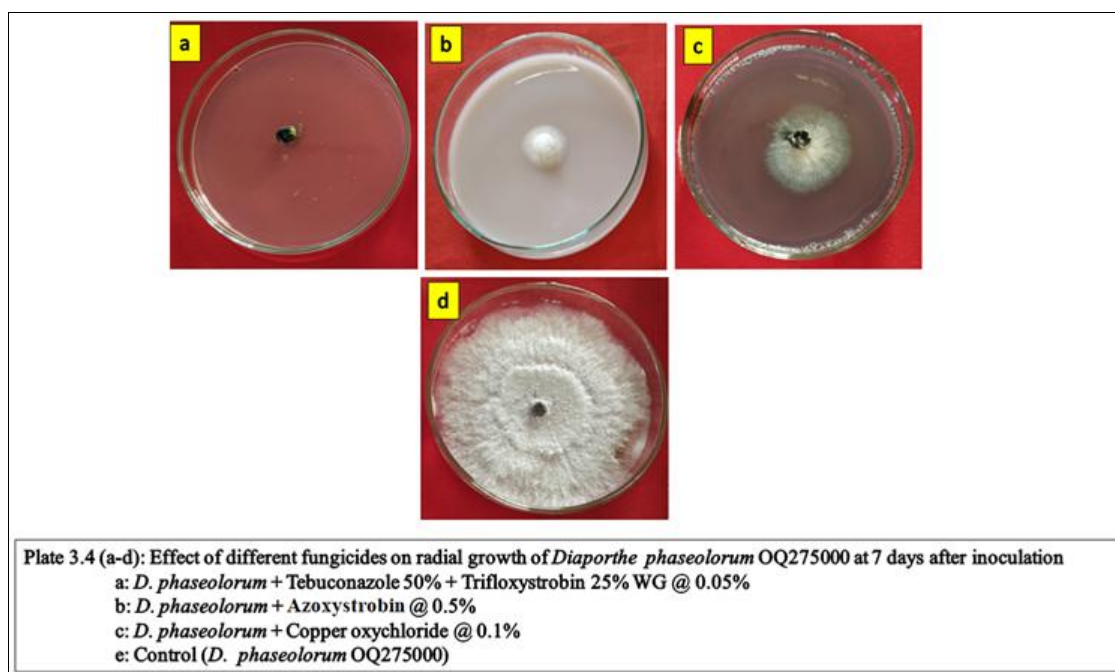
Table 3.3: Effect of different fungicides on radial growth of *F. dimerum* OQ274998 at 7 days after inoculation

Treatment	Dose (%)	Radial growth mean (mm)*	Radial growth inhibition (%)
T1: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>F. dimerum</i> OQ274998	0.05	0.00d	100.00
T2: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>F. dimerum</i> OQ274998	0.10	0.00d	100.00
T3: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>F. dimerum</i> OQ274998	0.20	0.00d	100.00
T4: Azoxystrobin + <i>F. dimerum</i> OQ274998	0.50	35.33b	60.74
T5: Azoxystrobin + <i>F. dimerum</i> OQ274998	1.00	0.00d	100.00
T6: Azoxystrobin + <i>F. dimerum</i> OQ274998	2.00	0.00d	100.00
T7: Copper oxychloride + <i>F. dimerum</i> OQ274998	0.10	22.66c	74.81
T8: Copper oxychloride + <i>F. dimerum</i> OQ274998	0.20	0.00d	100.00
T9: Copper oxychloride + <i>F. dimerum</i> OQ274998	0.40	0.00d	100.00
T10: <i>F. dimerum</i> OQ274998 (Control)	-	90.00a	0.00
SE (d)		1.08	
CD(0.05)		2.28	

3.4 Effect of different fungicides on radial growth of *D. phaseolorum* OQ275000

Results pertaining to Table 3.4 showed that all the three fungicides tested significantly inhibited the mycelial growth of *D. phaseolorum* OQ275000 at its lower concentrations. However treatment T1 (Tebuconazole 50%+Trifloxystrobin 25% WG @ 0.05%) showed 100 per cent mycelial inhibition of *D. phaseolorum* OQ275000 followed by T7 (Copper oxychloride @

0.1%) and T4 (Azoxystrobin @ 0.5%) with per cent radial inhibition of 67.03 and 65.92 respectively. It was also observed that with increasing concentration of all fungicides, per cent inhibition of *D. phaseolorum* OQ275000 also increased. Hence, Tebuconazole 50%+Trifloxystrobin 25% WG @ 0.05 per cent was found superior over the other two tested fungicides.

**Table 3.4:** Effect of different fungicides on radial growth of *D. phaseolorum* OQ275000 at 7 days after inoculation

Treatment	Dose (%)	Radial growth mean (mm)*	Radial growth inhibition (%)
T1: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>D. phaseolorum</i> OQ275000	0.05	0.00	100.00
T2: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>D. phaseolorum</i> OQ275000	0.10	0.00	100.00
T3: Tebuconazole 50% + Trifloxystrobin 25% WG + <i>D. phaseolorum</i> OQ275000	0.20	0.00	100.00
T4: Azoxystrobin + <i>D. phaseolorum</i> OQ275000	0.50	30.66b	65.92
T5: Azoxystrobin + <i>D. phaseolorum</i> OQ275000	1.00	0.00	100.00
T6: Azoxystrobin + <i>D. phaseolorum</i> OQ275000	2.00	00.00	100.00
T7: Copper oxychloride + <i>D. phaseolorum</i> OQ275000	0.10	29.66c	67.03
T8: Copper oxychloride + <i>D. phaseolorum</i> OQ275000	0.20	0.00	100.00
T9: Copper oxychloride + <i>D. phaseolorum</i> OQ275000	0.40	0.00	100.00
T10: <i>D. phaseolorum</i> OQ275000 (Control)	-	90.00a	0.00
SE (d)		0.33	
CD(0.05)		0.70	

4 *In vitro* assay of bio control agent against the pathogen

4.1 Effect of different bio control agents on radial growth of *C. gloeosporioides* OQ274997 at 7 days after inoculation

Results presented in Table 4.1 showed that all the tested three bio control agents inhibited the radial growth of *C. gloeosporioides* OQ274997 with variations in their per cent inhibition. Treatment T7 (*T. viride* KF439055 + *P. fluorescens* KT258013+B. subtilis ON386193) showed the highest per cent inhibition of radial growth of *C. gloeosporioides* OQ274997 with a per cent inhibition of 85.55, followed by T5 (*T. viride* KF439055+B. subtilis ON386193), T4 (*T. viride* KF439055+*P. fluorescens* KT258013) and T1 (*T. viride* KF439055) with a per cent inhibition of 84.44, 84.07and 83.70 respectively. However the lowest per cent inhibition of the radial growth of the pathogen was recorded in T2 (*P. fluorescens* KT258013) followed by T3 (*B. subtilis* ON386193) and T6 (*P. fluorescens* KT258013+B. subtilis ON386193) with a per cent inhibition of 42.59, 54.81 and 57.40 respectively. *T. viride* KF439055 resulted in maximum per cent growth inhibition (83.70%) and appeared to be most superior over individual effect of *P. fluorescens* KT258013 and *B. subtilis* ON386193 and were at par with T7, T4 and T5. It was evident that *C. gloeosporioides* OQ274997 progress its growth until it comes in contact with the bio control agent thereafter after which the growth terminates. Clear zone of inhibition was observed in between *T. viride*

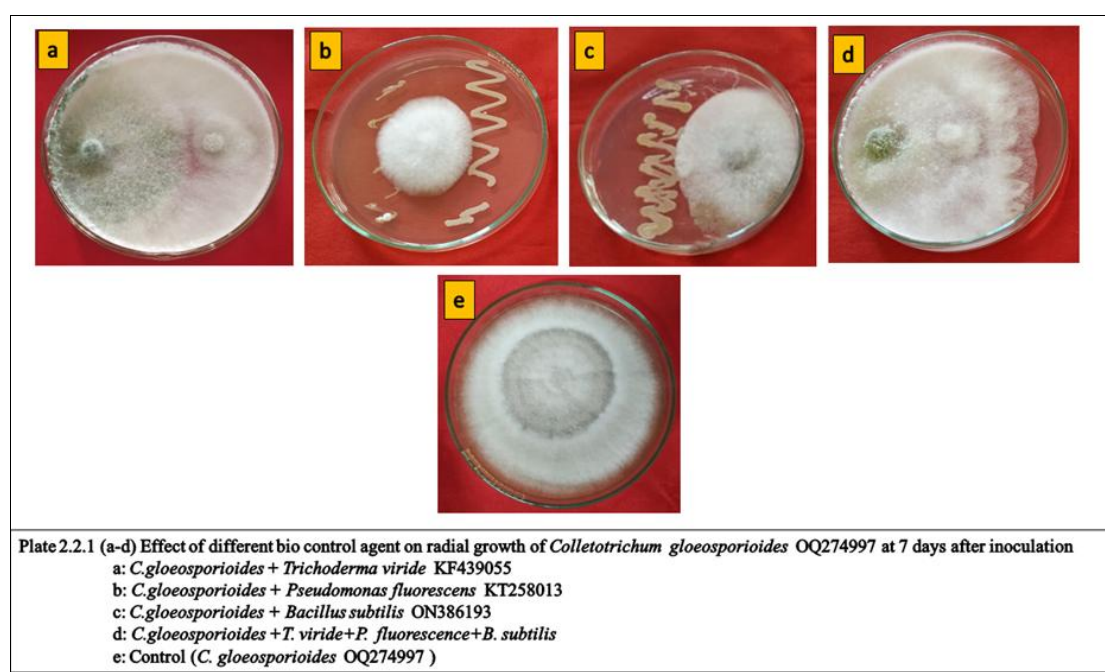
KF439055 and *C. gloeosporioides* OQ274997 indicating that *Trichoderma* restrict further growth of the pathogen upon contact and it continued its growth over the pathogen.

Observation on mycelium from the zone of interaction of *T. viride* KF439055 and pathogen under light microscope revealed lysis of the pathogen hyphae. Our results were in line with the *in vitro* evaluation carried out by Valenzuela *et al.* (2015) [19] who reported that *T. viride* inhibited the growth of *C. gloeosporioides* by 60 per cent. The results from the present findings showed that the fast growing *T. viride* KF439055 caused more growth inhibition of the pathogens which might be due to mycoparasitism and competition for space and nutrients. Some contrasting interactions were noticed in the experiments in which *T. viride* KF439055 showed antagonism by overgrowing the pathogen and reducing its growth although *P. fluorescens* KT258013 and *B. subtilis* ON386193 were still growing in treatment T4, T5 and T7. This might be due to chitinolytic enzymes produced by *Trichoderma* spp. which are reported biologically more active than enzymes of other microorganism and more effective against a larger spectrum of fungi (Lori *et al.*, 1993) [20] an is well known biological agents that have significant antagonistic activity against several plant pathogenic fungi.

Table 4.1: Effect of different bio control agent on radial growth of *Colletotrichum gloeosporioides* OQ274997 at 7 days after inoculation

Treatment	Radial growth Mean (mm)*	Radial growth inhibition (%)
T1: <i>T.viride</i> KF439055+ <i>C. gloeosporioides</i> OQ274997	14.66e	83.70
T2: <i>P. fluorescens</i> KT258013+ <i>C. gloeosporioides</i> OQ274997	51.66b	42.59
T3: <i>B.subtilis</i> ON386193+ <i>C. gloeosporioides</i> OQ274997	40.66c	54.81
T4: <i>T.viride</i> KF439055+ <i>P. fluorescens</i> KT258013+ <i>C.gloeosporioides</i> OQ274997	14.33e	84.07
T5: <i>T.viride</i> KF439055+B. <i>subtilis</i> ON386193+ <i>C. gloeosporioides</i> OQ274997	14.00e	84.44
T6: <i>P. fluorescens</i> KT258013+B. <i>subtilis</i> ON386193+ <i>C. gloeosporioides</i> OQ274997	38.33d	57.40
T7: <i>T.viride</i> KF439055 + <i>P. fluorescens</i> KT258013+B. <i>subtilis</i> ON386193+ <i>C. gloeosporioides</i> OQ274997	13.00e	85.55
T8: <i>C. gloeosporioides</i> OQ274997 (Control)	90.00a	0.00
SE(d)	1.02	
CD (0.05)	2.19	

*Data are mean of three replications In column mean followed by common letter do not differ significantly at 5% level of probability



4.2 Effect of different bio control agents on radial growth of *F. oxysporum* OQ274999 at 7 days after inoculation

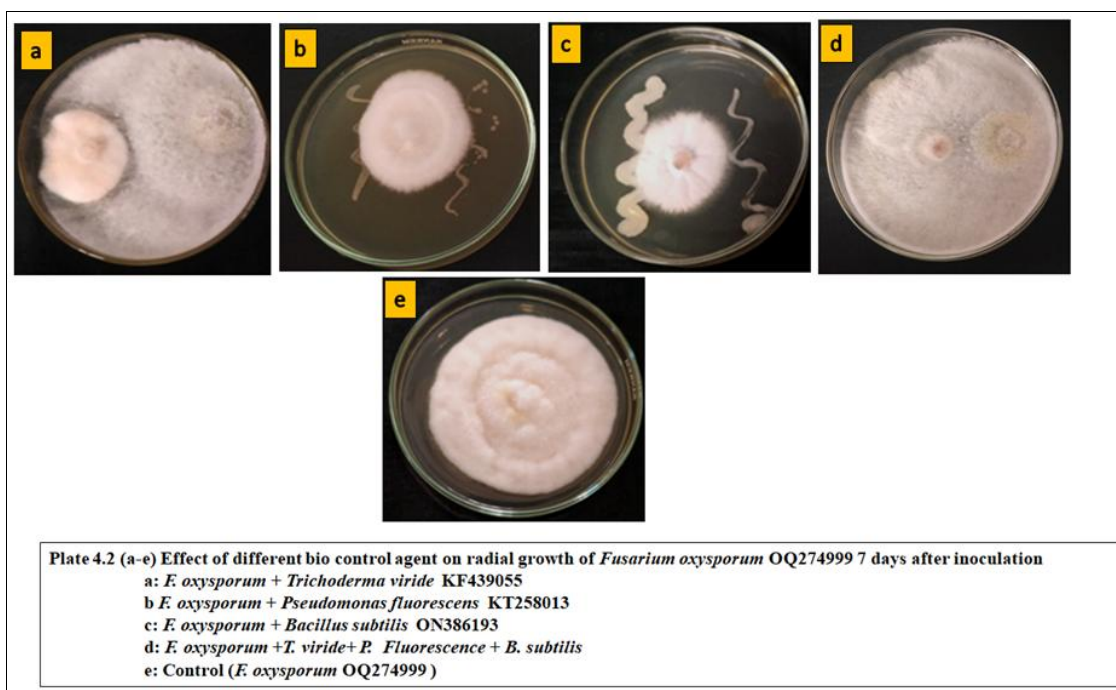
The result presented in Table 4.2 showed that treatment T5 (*T. viride* KF439055 + *B. subtilis* ON386193) and T7 (*T. viride* KF439055 + *P. fluorescence* KT258013 + *B. subtilis* ON386193) showed the highest inhibition per cent of 72.59 followed by T4 (*T. viride* KF439055 + *P. fluorescence* KT258013), T6 (*P. fluorescence* KT258013+ *B. subtilis* ON386193) and T3 (*B. subtilis* ON386193) with a per cent inhibition of 70.74, 63.33 and 61.11 whereas the lowest radial growth inhibition of *F. oxysporum* OQ274999 was in T2 (*P. fluorescens* KT258013) with a per cent mycelia inhibition of 50.37 respectively. However, treatment T1 (*T. viride* KF439055 alone) showed radial inhibition per cent of 70.00 and were at par with T4, T5 and T7 and hence selected for further studies. The

formation of inhibition zones between *T. viride* KF439055 and *F. oxysporum* OQ274999 was evident in the present study which might be due to production of secondary metabolites. The result of the present investigation was in accordance with the results reported by Abhiram and Masih (2018) ^[21] who stated that *T. viride* inhibited the growth of *F. oxysporum* by 71 per cent in dual culture plate technique. *Trichoderma* spp. act against pathogens through a combination of hyperparasitism, competition for space and nutrients and antibiosis (Whipps and Lumsden, 1991; ^[22] Calistru *et al.*, 1997) ^[23]. Similarly Rehman *et al.* (2013) ^[24] also showed the efficacy of *T. viride* against *F. oxysporum* f. sp. *ciceris* with a per cent mycelia inhibition of 81 respectively. Cherkupally *et al.* (2017) ^[25] have also evaluated the efficacy of *T. viride* against *F. oxysporum* f.sp. *melongenae* with a per cent inhibition of 78.88 respectively.

Table 4.2: Effect of different bio control agent on radial growth of *F. oxysporum* OQ274999 at 7 days after inoculation

Treatment	Radial growth Mean (mm)*	Radial growth inhibition (%)
T1: <i>T.viride</i> KF439055+ <i>F. oxysporum</i> OQ274999	27.00d	70.00
T2: <i>P. fluorescens</i> KT258013+ <i>F.oxysporum</i> OQ274999	44.66b	50.37
T3: <i>B.subtilis</i> ON386193+ <i>F.oxysporum</i> OQ274999	35.00c	61.11
T4: <i>T. viride</i> KF439055 + <i>P. fluorescence</i> KT258013+ <i>F. oxysporum</i> OQ274999	26.33d	70.74
T5: <i>T. viride</i> KF439055+ <i>B. subtilis</i> ON386193+ <i>F. oxysporum</i> OQ274999	24.66d	72.59
T6: <i>P. fluorescence</i> KT258013+ <i>B. subtilis</i> ON386193+ <i>F. oxysporum</i> OQ274999	33.00c	63.33
T7: <i>T. viride</i> KF439055+ <i>P. fluorescence</i> KT258013+ <i>B.subtilis</i> ON386193+ <i>F. oxysporum</i> OQ274999	24.66d	72.59
T8: <i>F. oxysporum</i> OQ274999 (Control)	90.00a	0.00
SE(d)	2.55	
CD (0.05)	5.46	

*Data are mean of three replications In column mean followed by common letter do not differ significantly at 5% level of probability



4.3 Effect of different bio control agents on radial growth of *F. dimerum* OQ274998 at 7 days after inoculation

The results presented in Table 4.3 showed that the tested bio control agent and their combination had significant effect in reducing the radial growth of the pathogen. Treatment T7 (*T. viride* KF439055+*P. fluorescence* KT258013+*B. subtilis* ON386193) showed the highest per cent inhibition of 85.55 followed by T5 (*T. viride* KF439055+ *B. subtilis* ON386193),

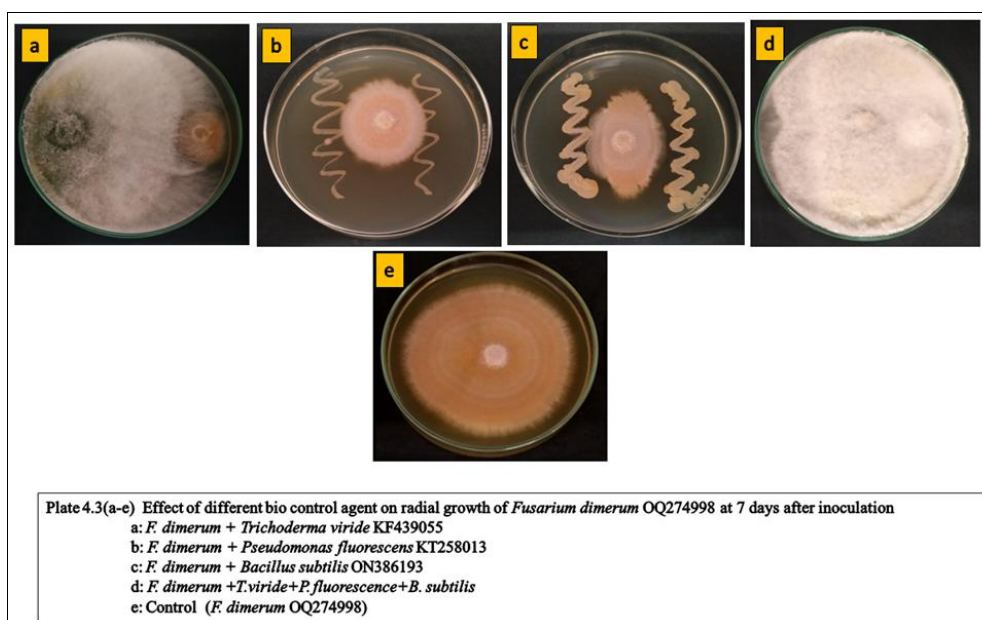
T4 (*T. viride* KF439055+ *P. fluorescence* KT258013) and T1 (*T. viride* KF439055) with a per cent inhibition of 82.96, 82.59 and 81.85 respectively. Among the three bio control agents *T. viride* KF439055 was found most promising because it alone showed radial growth inhibition of 81.85 per cent of the pathogen and were at par with T4 and T5. The clear zone of inhibition observed in between *T. viride* KF439055 and *F. dimerum* OQ274998 in dual culture indicates that *T. viride* KF439055 restricted further growth of *F. dimerum* OQ274998. The hyphae of the antagonist along with the pathogen at the meeting point

were observed under microscope and found that free lysis of pathogen hyphae. intermingling and coiling of hyphae leading to malformation and

Table 4.3: Effect of different bio control agent on radial growth of *F. dimerum* OQ274998 at 7 days after inoculation

Treatment	Radial growth Mean (mm)*	Radial growth inhibition (%)
T1: <i>T.viride</i> KF439055+ <i>F. dimerum</i> OQ274998	16.33e	81.85
T2: <i>P. fluorescens</i> KT258013+ <i>F. dimerum</i> OQ274998	50.00b	44.44
T3: <i>B.subtilis</i> ON386193+ <i>F. dimerum</i> OQ274998	38.00c	57.77
T4: <i>T. viride</i> KF439055 + <i>P. fluorescence</i> KT258013+ <i>F. dimerum</i> OQ274998	15.66e	82.59
T5: <i>T. viride</i> KF439055 + <i>B. subtilis</i> ON386193+ <i>F. dimerum</i> OQ274998	15.33e	82.96
T6: <i>P. fluorescence</i> KT258013+ <i>B. subtilis</i> ON386193+ <i>F. dimerum</i> OQ274998	28.33d	68.51
T7: <i>T. viride</i> KF439055+ <i>P. fluorescence</i> KT258013+ <i>B.subtilis</i> ON386193+ <i>F. dimerum</i> OQ274998	13.00f	85.55
T8: <i>F. dimerum</i> OQ274998 (Control)	90.00a	0.00
SE(d)	1.36	
CD (0.05)	2.91	

*Data are mean of three replications In column mean followed by common letter do not differ significantly at 5% level of probability



4.4 Effect of different bio control agents on radial growth of *D. phaseolorum* OQ275000 at 7 days after inoculation

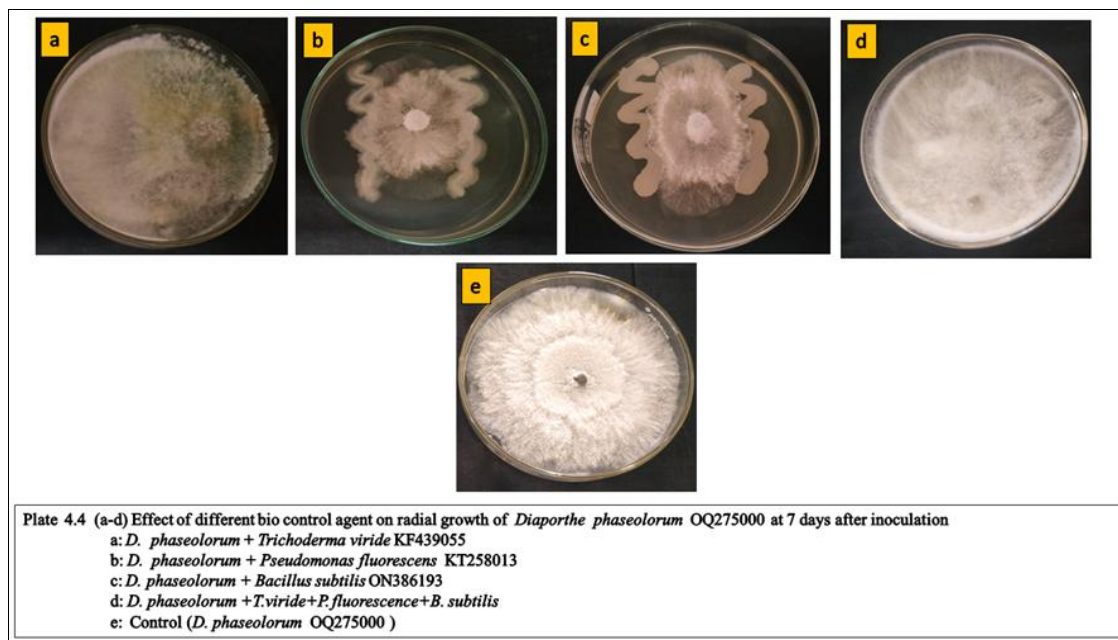
Result presented in Table 4.4 showed that the entire tested bio control agents and their combination inhibited the mycelial growth of the pathogen with variation in their per cent inhibition. Treatment T7 (*T. viride* KF439055+*P.fluorescence* KT258013+*B. subtilis* ON386193) showed the highest inhibition per cent of 87.40 followed by T5 (*T. viride* KF439055+ *B. subtilis* ON386193), T4 (*T. viride* KF439055+ *P. fluorescence* KT258013) and T1 (*T. viride* KF439055) with a per cent inhibition of 84.07, 82.96 and 82.59. These were followed by treatment T6 (*P. fluorescence* KT258013+*B. subtilis* ON386193), T3 (*B. subtilis* ON386193) and T2 (*P. fluorescens*

KT258013) with radial per cent inhibition of 64.44, 64.07 and 47.03. However, treatment T1 (*T. viride* KF439055 alone) showed radial inhibition per cent of 82.59 and were at par with treatment T5 and T6. *T. viride* KF439055 overgrew *D. phaseolorum* OQ275000 and covered the entire medium surface. The result of the present study were in close proximity of Jakatimath *et al.* (2017) [26] who stated that fungal bio agents are better than bacterial bio agents in inhibiting the growth of *P. vexans*. Our results were in line with the *in vitro* evaluation of *T. viride* against *P. vexans* that showed strong antagonistic property against the pathogen (Thesiya *et al.*, 2019) [27]. Kathal (2001) [28] also reported the inhibitory property of *T. viride* toward *P. vexans* under *in vitro* condition.

Table 4.4: Effect of different bio control agent on radial growth of *D. phaseolorum* OQ275000 at 7 days after inoculation

Treatment	Radial growth Mean (mm)*	Radial growth inhibition (%)
T1: <i>T.viride</i> KF439055+ <i>D.phaseolorum</i> OQ275000	15.66d	82.59
T2: <i>P. fluorescens</i> KT258013+ <i>D.phaseolorum</i> OQ275000	47.66b	47.03
T3: <i>B.subtilis</i> ON386193+ <i>D.phaseolorum</i> OQ275000	32.33c	64.07
T4: <i>T. viride</i> KF439055 + <i>P. fluorescence</i> KT258013+ <i>D.phaseolorum</i> OQ275000	15.33d	82.96
T5: <i>T. viride</i> KF439055 + <i>B. subtilis</i> ON386193+ <i>D.phaseolorum</i> OQ275000	14.33d	84.07
T6: <i>P. fluorescence</i> KT258013+ <i>B. subtilis</i> ON386193+ <i>D.phaseolorum</i> OQ275000	32.00c	64.44
T7: <i>T. viride</i> KF439055+ <i>P. fluorescence</i> KT258013+ <i>B.subtilis</i> ON386193+ <i>D.phaseolorum</i> OQ275000	11.33e	87.40
T8: <i>D.phaseolorum</i> OQ275000 (Control)	90.00a	0.00
SE(d)	1.32	
CD (0.05)	2.82	

*Data are mean of three replications in column mean followed by common letter do not differ significantly at 5% level of probability



Conclusion

In vitro evaluation of three different fungicides viz., Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin and Copper oxychloride at three different concentrations were conducted for their efficacy against the pathogens through poisoned food technique and result revealed that combination fungicide Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05% was found to significantly lowered mycelia growth of *C. gloeosporioides* OQ274997, *F. oxysporum* OQ274999, *F. dimerum* OQ274998 and *D. phaseolorum* OQ275000 pathogens at lower concentration. Similarly *in vitro* screening of three bio control agents viz., *T. viride* KF439055, *P. fluorescens* KT258013 and *B. subtilis* ON386193 against *C. gloeosporioides* OQ274997, *F. oxysporum* OQ274999, *F. dimerum* OQ274998 and *D. phaseolorum* OQ275000 were carried out through dual culture method and result revealed that *T. viride* KF439055 resulted in maximum per cent radial growth inhibition of the tested pathogens and appeared to be most superior over individual effect of *P. fluorescens* KT258013 and *B. subtilis* ON386193.

Recent researches on this crop in different parts of the country are particularly focusing on its physico-chemical characteristics, great potential health benefits and nutritional value. However, its scientific report of various diseases of the crop is still lacking. Moreover dragon fruit growers in Assam are importing planting materials from various part of the country and there is a need for disease monitoring and pathogen identification to ensure plant bio security as pest and disease incidence can have significant constraints in production of the crop. Accurate pathogen identification is important for formulations of effective disease management strategies. The findings of the present research investigation revealed that Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05 per cent and *T. viride* KF439055 were superior in inhibiting the growth of the tested pathogens and can be incorporated in the future management strategies against the pathogens in the field condition.

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