Original Research Article

Efficacy of Botanicals and Biocontrol Agents Against Leaf Spot and Blossom Blight Pathogen of Marigold: A First Report from Longleng District of Nagaland.

ABSTRACT

Aims: To study about etiology and organic disease management strategies of *Fusarium oxysporum* induced blossom blight in marigold (*Tagetes* spp.), cultivated in and around Longleng district of Nagaland. The experiment aimed to formulate an effective organic disease management module to combat the disease and assess the efficacy of plant extracts and biocontrol agents in suppressing fungal growth

Study design: A completely randomized design (CRD) was employed for laboratory-based trials, and an exploratory survey was conducted during the winter season of 2024 to monitor disease incidence in marigold-growing areas around Longleng district of Nagaland.

Place and Duration of Study: Field survey has been carried out in Longleng district of Nagaland. All the laboratory works has been carried out in laboratory of Department of Plant Pathology, Assam Agiculture University, Jorhat, Assam. Duration of research is 6 months.

Methodology: The tested phytoextracts at 10% and 20% concentrations included leaves of neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), garlic (*Allium sativum*), and other local plant species. Biocontrol agents such as *Trichoderma viride*, *Trichoderma harzianum*, and *Pseudomonas fluorescens* were collected from local fields and research institutions for evaluation. Disease incidence and severity were measured based on percent disease intensity (PDI).

Results: The study demonstrated that garlic extract, at both tested concentrations, achieved complete inhibition (100%) of fungal growth. Additionally, several other plant extracts exhibited notable antifungal properties. Among the biocontrol agents, *Pseudomonas fluorescens* showed a strong antagonistic effect, inhibiting *Fusarium oxysporum* by 82.65%. These findings underscore the potential for incorporating

Commented [H9]: Make uniformity in font style

Commented [H1]:

Commented [H2]: Effect of .....

Commented [H3]: Write authority of fungi

Commented [H4]: Agricultural

Commented [H5]: The phytoextracts were tested at 10% and 20% concentrations which included

Commented [H6]: Percent Disease Intensity (PDI)

Commented [H7]: *Pseudomonas fluorescens* showed a strong antagonistic effect against *Fusarium oxysporum* (82.65%).

phytoextracts and biocontrol agents into effective organic disease management strategies for marigold cultivation
Conclusion: It is recommended that studies focus on optimizing the application of garlic extract, exploring its effectiveness in field conditions, and evaluating its potential in combination with other biocontrol agents or plant extracts.

Keywords: [Marigold, Fusarium oxysporum, Phytoextracts, Bioagents, Efficacy]

1. INTRODUCTION

India has a long tradition of flower cultivation. Encouragement for the commercially potent floriculture has yielded in the blossoming of this field into a viable agri-business option. With globalization and free market economy, floriculture has attained an industrial status and gained tremendous momentum around the world. With changing life styles and increased urban affluence, floriculture has assumed a definite commercial status both in domestic and international market (Kaur et al.,2022).

Marigold was one of the earliest cultivated flowers. Today Marigold is one of the important commercial flowers grown worldwide. In India, it accounts for more than half of the nation's loose flower production. Major growing states are Karnataka, Maharashtra, Gujarat, Haryana, Andhra Pradesh, Uttar Pradesh, Odisha, West Bengal, Tamil Nadu, Jammu and Kashmir, Chhattisgarh, Puduchery , Andaman and Nicobar islands and different parts of North eastern states like Meghalaya, Nagaland etc (Gupta et al.,2022). Marigold has gained popularity because of its adaptability to various soil and climatic condition, longer blooming period and beautiful flower having long shelf-life. I t is the important flower used for floral decorations, religious offerings and for making garlands and flower baskets (Mehta et al.,2022).

This flower is native to South-Western Asia as well as Western Europe and the Mediterranean. The marigold is also known as Mary bud, pot marigold. ruddes. golds, holigold, gold bloom, Herb of the Sun and the garden marigold. (Suryavansi & Parvez,2014)

The marigold is also known as the *Tagetes* and belong to Asteraceae (Compositae) family and is a genus that includes about 56 species with a mixture annuals and perennials, but the annual is definitely more common than the perennial flower. It is a diploid plant having chromosome number 2n=24. These flowers have blooms that include colours like gold, orange, white and yellow. The recorded history of marigolds begins with the Aztecs in Mexico, where the flowering plant was used in religious ceremonies and as an herbal medicine. Marigold plant was named for the Virgin Mary -"Mary's Gold in 17th century.

There are different varieties of the marigold plant and they are divided into four basic species. These include African/American marigold *(T. erecta*), French marigold(American origin)- *T patula* , Triploids, and single marigold *(T. tenuifolia*). The two most popular types of marigolds are African or Mexican *(Tagetes erecta)* and French *(Tagetes patula)* marigolds. (Mir et al.,2019).

India's 'flower power' continues to bloom with the country emerging as the second largest growerof flowers around the world, surpassed only by China. About 2,33,000 hectares across the country was used for floriculture, producing 17,29,000 metric tonnes (MT) of loose flowers and 76,732 lakh cut flowers. Andhra Pradesh leads in loose flowers production with 2,24,410 MT cultivated over 34,850 hectares. followed by Karnataka at 2,07,500 MT cultivated in 29,700 hectares and Tamil Nadu with 3,12,970 MT grown in 28,700 hectares. Total area under flower production in different

Commented [H8]: From the study,it is recommended to optimize the application.....

Commented [H10]: Imake it italic

Commented [H11]: Make it italic

Commented [H12]: Make it italic

Commented [H13]: Make it italic

Commented [H14]: pot marigold,ruddes, golds,

Commented [H15]: Make it italic

Commented [H16]: largest grower of

Commented [H17]: Metric Tonnes

countries of the world is 3.05.105 ha. Half ofthe world's cut flower production is from Europe and marigold ranked seventh on the list of the most popular garden plants in world but it ranked 2nd in India. (Kaur et al.,2022). Total area under marigold production in India 55,890ha and production as loose flower is 511.31MT and cut flower is 4.25 lakh but in Odisha, total area under marigold is 2625ha and production is 2,40,031 MT. So floriculture is one of the fast emerging and rapidly growing sector in Odisha. (De et al.,2019)

Though marigold plant is potential plant in pest management, it is affected by several disease like Fusarium *wilt (Fusarium oxysporum)*, Veticillium wilt *(Verticillium dahliae),* leaf spot *(Alterariaaria tagetiea),* Blight *(Alternaria tenuissima),* leaf spot and bud rot of *marigold (Alternaria alternata)*, leaf and flower blight *(Alternaria zinnia),* bacterial leaf *spot (Pseudomonas syringae pv. tageli,\).* southern bacterial wilt or brown rot *(Pseudomonas solanaeearum),* Botrytis *blight (Botrytis cinerea),* Septoria leaf spot *(Septaria ageticola),* wilt and stem Rot *(Phytophthora cryptogea)*, collar Rot *(Phytophthora* sp., *Pythium* sp.). leaf spot and blight *(Alternaria, Cercospora and Septaria* sp.), powdery mildew *(Oidium* spp.) Flower Bud Rot *(Alternaria dianthi),* Damping Off *(Pythium spp.).* Damping *off (Rhizoctonia solani),* Flower bud rot *(Alternaria dianthi).* collar and root *rot (Pellicularia jilamentosa, Pythium ultimum,* (*Sclerotinia sclerotiorum),* leaf blight of fig-marigold (*Rhizoctonia solani*) and a new species of Alternaria on seeds of French marigold *(Alternaria patula sp. nov).* viral diseases like Aster Yellows, Mosaic(cucumber mosaic cucumovirus), little leaf and witches' broom disease (pytoplasma). Insects like *aphids (Aphis gossypii),* bud *caterpillars (Helicoverpu armigera* and *Phycita sp.),* Japanese beetles, leafhopper, plant bug and mite. (Gurjar et al.,2019).

Marigold (*Tagetes* spp.), which is a popular bedding plant, can be used as a cover crop. Marigold produces a substance called alpha-terthienyl, which can aid in the reduction of root-knot nematodes and other disease promoting organisms, such as fungi, bacteria, insects, and some viruses. (Hooks et al.,2010).

Out of several disease and insect pest attack, wilt disease (stem rot, black stem, aster wilt) caused by *Fusarium oxysporum*, is the most serious disease of Marigold in home-yard plantings in Longleng and over most of the world.

In view of severity of marigold disease, it was selected for further research to know the etiology and to formulate organic management strategies. For this efficacy of different plant extracts as well as biological agents have been tested against the causal organism *in vitro*).

2. MATERIAL AND METHODS

2.1. Site of survey for disease occurrence and location of laboratory research work

The survey for disease occurrence was conducted in 2024 winter in several marigold fields across different villages within Longleng district, where farmers grow marigold for traditional purposes. The marigold plants in these areas were observed to exhibit symptoms of leaf spot and blossom blight, both of which are known to be caused by *Fusarium oxysporum*. The sites selected for the survey were primarily located in areas with temperate conditions conducive to the development of fungal diseases. Farmers in these areas reported significant crop loss due to these diseases, which prompted the need for this study. The survey aimed to assess the prevalence and severity of the diseases and evaluate potential biocontrol and plant extract-based solutions, which align with the region's traditional agricultural practices of avoiding chemical pesticides.

Commented [H18]: of the

Commented [H19]: second

Commented [H20]: Make it italic

Commented [H21]: Make it italic

Commented [H22]: Write authority of all the pathogen.

Commented [H23]: Observed for symptoms of

All the Laboratory research works have were carried out in the laboratory of Department of Plant Pathology, Assam Agriculture University, Jorhat, Assam.

Commented [H24]: Assam Agricultural University

2.2. Isolation, characterisation, identification and pathogenicity test of the causal fungus:

Commented [H25]: characterization

The affected Marigold samples such as leaves and flower buds showing disease symptoms were collected from the different locations in and around Longleng during the period of survey. Standard isolation techniques were followed to isolate the fungus involved. On thorough examination of morphological and cultural characters to identify the causal organism had been done which were then subjected to pathogenicity test following the guidelines of Kock's postulates.

Commented [H26]: to isolate the insighted fungus.

Commented [H27]: Koch's postulates

2.3. Preparation of Crude extracts

The fresh plant parts were collected from field and washed with tap water followed by distilled water. The plant parts were dried for few minutes. 100 g of each plant part was weighed and ground using grinder with addition of equal volume (w/v) of ethanol. These extracts were then filtered through double layered muslin cloth and kept in 100 ml conical flasks. The content was mixed thoroughly and centrifuged at 5500 rpm for 10 minutes and the supernatant was filtered through Whatman filter paper no.1 and after filtration the contents were used for further study.

2.4. Poisoned food technique

The bio-efficacy of plant extracts (Table-1) were evaluated by poisoned food technique (Nene and Thaplial, 1973) in two different concentrations i.e. 10% and 20%. Required amount of crude extracts were mixed with 90 and 80 ml of sterilized molten potato dextrose agar medium so as to get 10% and 20% concentration respectively in laminar airflow chamber. Control was maintained without any plant extracts. 20 ml of media was poured into petridishes and allowed to solidify. 8 mm culture disc was put on the middle of the solidified petridishes. All the Plates were incubated at room temperature. Mycelia growth measurement was taken when maximum growth was observed in control Plate. The growth of mycelium on other Plates was compared with the control plate. The efficacy of plant extracts was expressed as percentage inhibition of mycelia growth over control. The per cent inhibition over control was calculated according to formula of Vincent as follows.

Commented [H28]: air flow

Commented [H29]: Petri dishes

Commented [H30]: Petri dishes

Commented [H31]: Mycelial

Commented [H32]: Mycelia growth was measurement when fungal growth was completely covered control plate.

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

Commented [H33]: Tag reference for formula

Where, I = Percent inhibition of radial growth

C= Radial growth on control plate

T= Radial growth on treatment plate

Table-1 : List of plant extracts used in management of the causal fungus

All the experiments were conducted in Completely randomized design (CRD) and statistical analysis was done by using online statistic software, OPSTAT. (**14.139.232.166/opstat/default.asp**

Sl. No.	Common name	Scientific name	Plant parts used	Concentrations used
1.	Tulsi	*Ocimum sanctum*	Leaf	10% and 20%
2.	Sadabahar	*Vinca rosea*	Leaf	10% and 20%
3.	Adhatoda	*Adhatoda vasica*	Leaf	10% and 20%
4.	Morning glory	*Ipomea* spp.	Leaf	10% and 20%
5.	Begonia	*Vitex negundo*	Leaf	10% and 20%
6.	Custard apple	*Annona reticulate*	Leaf	10% and 20%
7.	Big-sage	*Lantana camara*	Leaf	10% and 20%
8.	Neem	*Azadirachta indica*	Leaf	10% and 20%
9.	Milk weed	*Calotropis gigantean*	Leaf	10% and 20%
10.	Karanj	*Pongamia pinnata*	Leaf	10% and 20%
11.	Bael	*Aegle marmelos*	Leaf	10% and 20%
12.	Onion	*Allium cepa*	Bulb	10% and 20%
13.	Garlic	*Allium sativum*	Clove	10% and 20%

2.5 In vitro evaluation of biocontrol agents

The efficiency of biocontrol agents against the causal organism of disease was tested by dual culture method. Biocontrol agents like *Tricoderma viridae* , *Trichoderma harzianum, Trichoderma hamatum, Pseudomonas spp.* and *Bacillus spp* were collected from different places as mentioned below (Table-2) and were tested against the pathogen. The fungal antagonists were grown in potato dextrose agar to get fresh and active culture for the experiment.

Table-2 : List of bio agents with their place of collection

Sl. NO.	Bio control agents	Place of collection

Commented [H34]: Put proper reference

Commented [H35]: Make it italic

Commented [H36]: Were tested

Commented [H37]: Trichoderma viride

Commented [H38]: *Bacillus spp.*

1	*Trichoderma viride*	Rhizospheric soil of Marigold, Hukphang Village, Longleng, Nagaland
2	*Trichoderma harzianum*	Rhizospheric soil of Marigold , Pongo village, Longleng, Nagaland
2	*Trichoderma hamatum*	From Rhizosphere of Marigold, Longleng, Nagaland.
4	*Pseudomonas fluorescens*	Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar,Odisha
5	*Bacillus subtilis*	Central Tropical Mushroom Research and Training centre, AAU, Jorhat,Assam

2.6. Dual culture method

About 20 ml of potato dextrose media was poured into petridishes and allowed to cool down. The fungal mycelial disc (5mm) was transferred to one end of the Plate and fungal antagonist culture disc placed opposite to it leaving 5-6 mm distance from the periphery of the plates.In case of bacterial antagonist, spore suspension of bacteria was mixed in the molten media at 38^{0}C and thoroughly mixed and plated immediately. Eight mm fungal disc was put in the centre after solidification and cooling. The radial growth of the fungus was measured. Fungal disc was also put in the petriplate without bacterial suspension as control. Each treatment was replicated three times. The inoculated Plates were incubated at room temperature. After five days, observations were taken. The efficacy of bio-control agents were expressed as percentage inhibition of mycelia growth over control. The Per cent inhibition over control was calculated according to Vincent's formula as follows

Commented [H39]: Petri dishes

Commented [H40]: Petri plate

Commented [H41]: plates

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

Where, I = Percent inhibition of radial growth

C= Radial growth on control plate

T=Radial growth on treatment

3. RESULTS AND DISCUSSION

3.1. SURVEY OF DISEASE INCIDENCE

The survey indicated the presence of five foliar as well as soil borne fungal diseases namely, Leaf spot (*Alternaria tagetica*), Powdery mildew (*Oidium spp.*), Botrytis *Blight* (*Botrytis cineraria*). Blossom Blight (*Fusarium oxysporum*) and wilt (*F.oxysporum*). However, the most commonly encountered disease was diagonised as Blossom blight of marigold caused by *Fusarium oxysporum* affecting leaf, flowers and flower buds (Fig-1). The same organism was found to be associated with the wilt disease in few instances. Low to moderate incidence of powdery mildew and botrytis blight was recorded (Table-3) at all the location survey.

Commented [H42]: diagnosed

Commented [H43]: were recorded (Table-3) at all the surveyed locations.

Table 3 : Survey on disease incidence of marigold

Sl.NO.	Name of the disease	Causal organism	Percent Disease Intensity (PDI)
1	Leaf spot and blossom blight	*Fusarium oxysporum*	52.3%
2	Wilt	*Fusarium oxysporum*	17.6%
3	Botrytis Blight	*Botrytis cinerea*	43.4%
4	Powdery Mildew	*Oidium spp.*	12.3%
5	Leaf spot	*Alternaria tagetica*	37.9%

Commented [H44]: Sr. No.

Fig-1.Blossom blight of marigold caused by *Fusarium oxysporum*

3.2 lsolation, characterisation, identification and pathogenicity of the causal fungus:

In marigold, *Fusarium* blossom blight of the floral organs is rather uncommon and has not been reported from the marigold growing areas of the country particularly from Nagaland. The affected samples were collected from the growing areas in and around Longleng during the period of survey. Standard isolation techniques were followed to isolate the fungus involved. On thorough examination of morphological and cultural characters. the causal fungus was identified as *Fusarium oxysporum.* The fungus produced light pink to white cottony growth on potato dextrose agar plates and macro as well as microconidia. The taxonomic position of the fungus has been given below.(Booth C, 1971)

Scientific Classification

Kingdom - Fungi
Division - Ascomycota
Class - Sordariomycetes
Order - Hypocreales
Family - Nectriaceae

Commented [H45]: Resize the imge properly

Commented [H46]: characterization

Commented [H47]: Tissue isoaltion method was followed

Commented [H48]: characters, the causal fungus

Genus - Fusarium

Species - F.oxysporum

Commented [H49]: Make it italic

3.3.Symptomatology

The disease symptom was observed mostly in the flower and flower buds of the host plant. The petals were discoloured light brown to black initially and flowers droop. Usually the disease was seen to affect all the petals of the flower, but partial infection was also common at few locations. As the disease advanced pinkish-white mycelial mat was often observed on the affected floral parts. In severe condition the flowers drooped down wards and the plants exhibited a sickly appearance. The plants having affected flowers didn't exhibit the wilt syndrome as observed in the survey.

3.4. Pathogenicity test

The Pathogenicity of the test fungus was proved on potted marigold plants following the postulations demonstrated by Robert Koch (1887) and the procedure described under "materials and methods". Test fungus was inoculated by pinpricking leaf epidermis and by swapping the spore suspension on the injured leaves with a cotton swab. The inoculated plants were covered with polythene bags in order to maintain Favourable microclimate for disease appearance. The inoculated plants started to exhibit the typical disease symptoms on the floral parts at 7 days of inoculation and the flowers completely blighted at 10 days of inoculation. However, the controlled plants maintain for the purpose of comparison did not show any.

Commented [H50]: to maintain favourable

Commented [H51]: after 7 days of inoculation

Commented [H52]: However, the controlled plants maintained without any inoculation were served as control, did not show any symptoms.

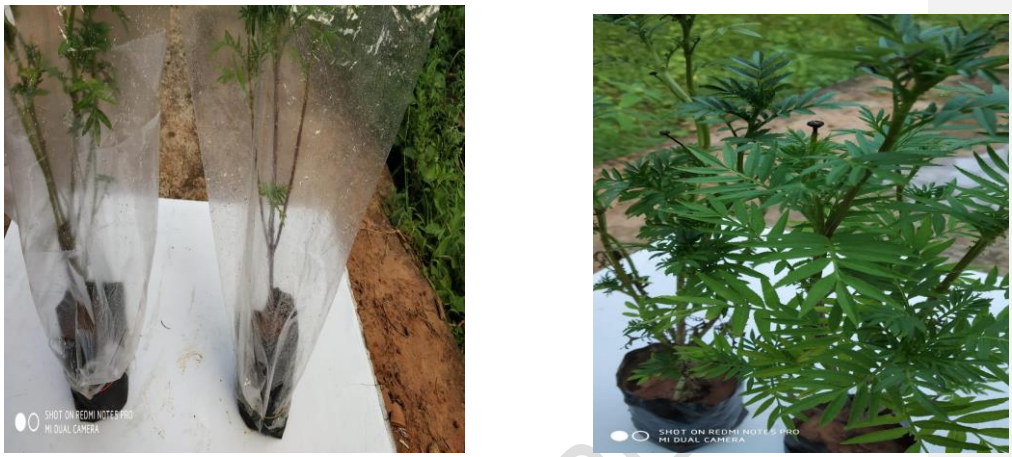

Fig.2 Pathogenicity test on healthy plants following Koch's Postulates.

3.5. Morohological studies

The relevant morphological character were studied from 7 days old petriplate culture after isolation of the pathogen from the affected plant part. Theavailable literature was referred for taxonomic identification of the pathogen as an when required. The detailed cultural characteristics of the species was studied.The fungus produced hyaline, sickle shaped and septatae macro conidia and oval to oblong micro conidia with or without septation. The dimension of the macro conidia (Fig-3) was in the range of 12.25-19.83µmx1.39-3.73µm and the dimension of micro conidia (Fig-4) 4.84-8.73µmx2.13-3.60µm as observed from the spore measurement studies.

Commented [H53]: Morphological studies

Commented [H54]: Petri plates

Commented [H55]: septate

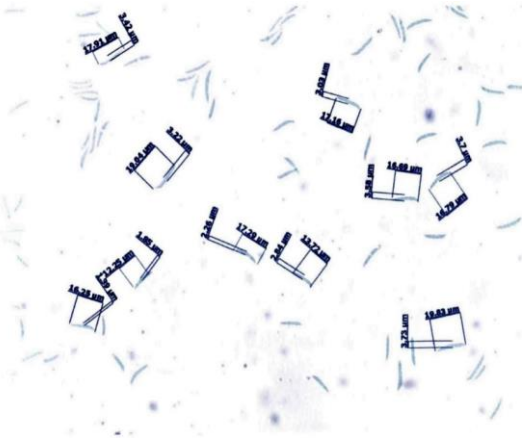

Fig.3 :Macro conidia of *Fusarium oxysporum*

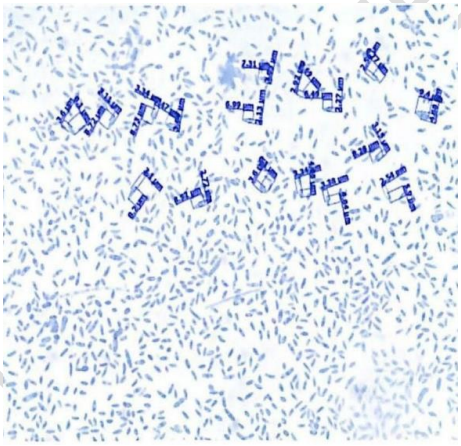

Fig.4: Micro conidia of *Fusarium oxysporum*

3.6.Efficacy of different phytoextracts in inhibiting the radial growth of *Fusarium oxysporum*

Among the tested phytoextracts at two different concentrations, it was observed (Table-4)that the extracts at twenty per cent were significantly superior over ten per cent concentration. The garlic extract was found to be superior in comparison to the other tested extracts showing cent per cent inhibition of diametric growth of both the concentrations. Among the extracts at ten per cent concentration Garlic-T_{13} (100%) was found to be most effective followed by Sadabahar-T_2 (98.49%) and Onion-T_{12} (95.56%). Complete inhibition of radial growth was also retarded by extracts of Sadabahar-T_2 (100%), Basanga-T_3 (100%), Begonia-T_5 (100%), Custard apple-T_6 (100%), Karanj-T_{10} (100 %) as well as Onion (100%) at twenty per cent concentration. Bael plant extract was found to be least effective (68.85%) against radial growth of *Fusarium oxysporum*.

Our p study is in concurrence with the findings of Kamdi et al., (2012) and Hossain et al. (2013). The inhibitory effect of garlic bulb crude extract on fungal growth is primarily attributed to allicin, which serves as the main antibacterial, antifungal, and antiviral compound. (Miron et al., 2000).

Table-4: Effect of Phytoextracts on mean diametric growth and percent inhibition over control

Treatment	Plant Extracts	Plant part used	Mean diametric Growth(mm)		Per cent inhibition over control	
			Concentration (%)		Concentration (%)	
			10	20	10	20
T_1	Tulsi (Ocium sanctum)	Leaf	25 (5.05)	5.93 (2.52)	67.67	92.27

Commented [H56]: fungal growth at both the concentrations

Commented [H57]: Among the extracts, Garlic-T_{13} (100%) was found to be most effective at ten per cent concentration followed by

Commented [H58]: Our study is in concurrence

Commented [H59]: Make it italic

Commented [H60]: Make it italic

Commented [H61]: Make it italic

Commented [H62]: per cent

Commented [H63]: Mean colony diameter

T2	Sadabahar *(Vincarosea)*	Leaf	1.17 (1.23)	0 (0.71)	98.49	100
T_3	Basanga (*Adhatodavasica)*	Leaf	10.67 (3.34)	0 (0.71)	86.21	100
T_4	Morning glory(*Ipoma* spp.)	Leaf	16.57 (4.13)	3.4 (1.94)	78.58	95.57
T_5	Begonia (*Vitex negundo*)	Leaf	5.43 (2.43)	0 (0.71)	92.97	100
T_6	Custard apple (*Annona reticulate*)	Leaf	18.6 (4.37)	0 (0.71)	75.95	100
T_7	Big-sage (*Lantana camara*)	Leaf	23.3 (4.88)	2.27 (1.65)	69.87	97.05
T_8	Neem (*Azadirachtaindica*)	Leaf	16.4 (4.11)	8.77 (3.04)	78.79	88.58
T_9	Milk weed (*Calotropis gigantea*)	Leaf	18.4 (4.35)	9.6 (3.17)	76.21	87.49

T_{10}	Karanj *(Pongamia pinnata)*	Leaf	13.57 (3.75)	0 (0.71)	82.46	100
T_{11}	Bael (*Aegle marmelos*)	Leaf	37.33 (6.15)	10.77 (3.35)	51.72	85.97
T_{12}	Onion (*Allium Cepa*)	Bulb	3.43 (1.98)	0 (0.71)	95.56	100
T_{13}	Garlic (*Allium sativum*)	Clove	0 (0.71)	0 (0.71)	100	100
T_{14}	Control		77.33 (8.82)	76.77 (8.79)		
SE(m) ±			0.096	0.116		
CD at 0.05			0.281	0.339		

values in the parentheses indicate √transformed values

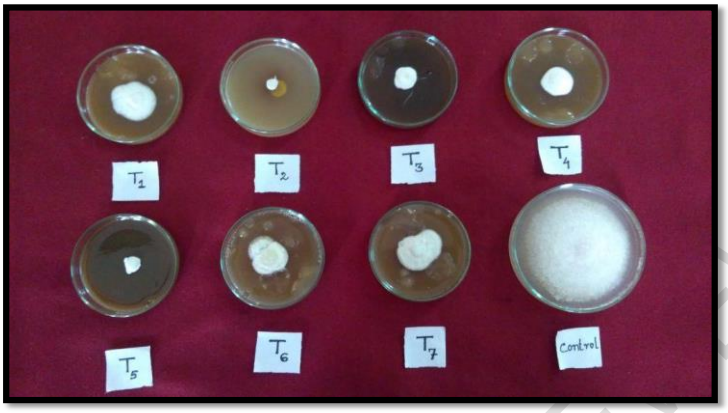

Fig.5: T1: Tulsi, T2: Sadabahar, T3: Adhatoda, T4: Morning glory, T5: Begonia, T6: Custard apple, T7: Big-sage, Control

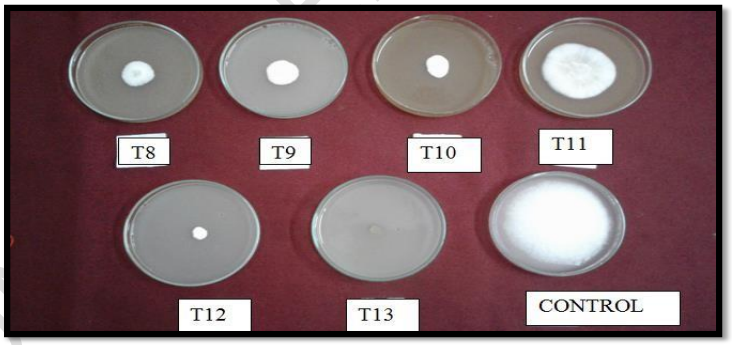

Fig.:6 T8: Neem, T9: Milk weed, T10: Karanj, T11: Bael, T12: Onion, T13: Garlic,Control

(Efficacy of plant extracts against F.oxysporum 10% concentration)

Commented [H64]: Make it italic

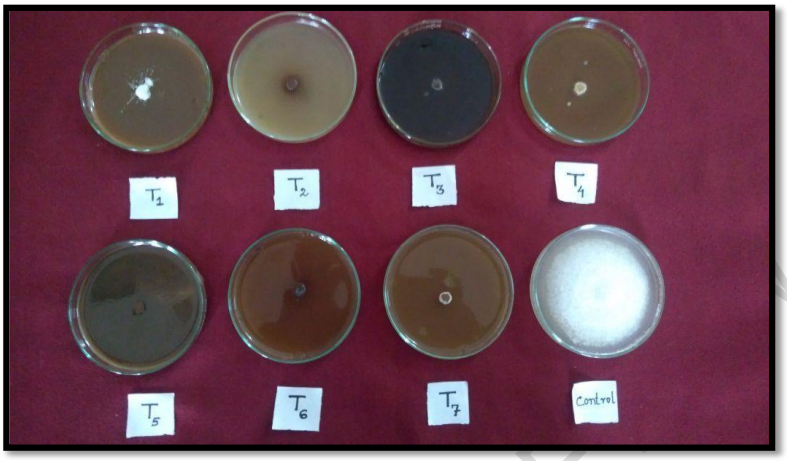

Fig.7- T1: Tulsi, T2: Sadabahar, T3: Adhatoda, T4: Morning glory,

T5: Begonia,T6:Custard apple, T7: Big-sage, Control

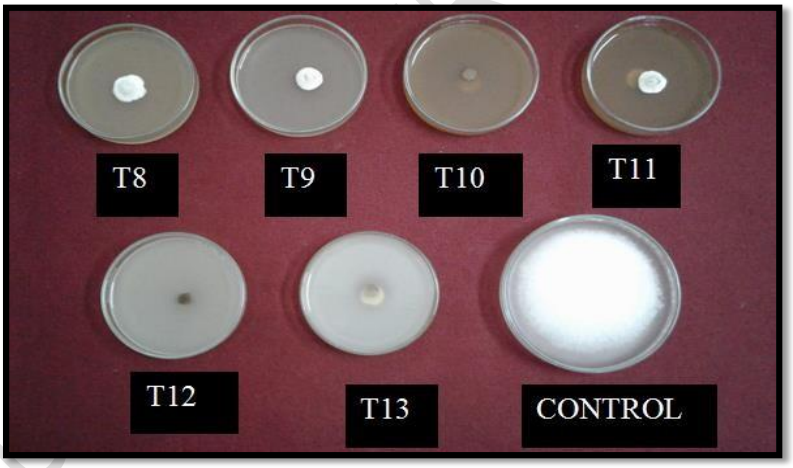

Fig.8: T8: Neem, T9: Milk weed, T10: Karanj, T11: Bael, T12: Onion,
T13: Garlic,Control
(Efficacy of plant extracts against *F.oxysporum* 20%concentration

3.7.Efficacy of fungal and bacterial antagonists against radial growth of *F.oxysporum*

The antagonistic effects of various fungal and bacterial antagonists were evaluated (Table-5) for their impact on the diametric growth of *Fusarium oxysporum*. All fungal bioagents similarly reduced the pathogen's growth, with *Trichoderma harzianum* (T3) showing the highest inhibition, reducing diametric growth by 33.48%, followed by *Trichoderma hamatum* (29%) and *Trichoderma viride* (27.75%) in dual culture. Among bacterial antagonists, *Pseudomonas fluorescens* was the most effective, reducing radial growth by 82.65%, followed by *Bacillus subtilis* (59.65%). Similarly, Kumar et al., (2017) studied the efficacy of plant extracts, bioagents, and fungicides against *Fusarium udum* and reported that among the bioagents, seed treatment with *Trichoderma viride* resulted in the highest control of wilt incidence.

Commented [H65]: In earlier section it is Bacillus spp. and here It is Bacillus subtilis

.

Table-5: Effect of Bio agentss on mean diametric growth and percent inhibition over control

Commented [H66]: Bio agents

Commented [H67]: per cent

Treatments	Fungal bioagents	Mean colony diameter (mm)	Per cent Inhibition
T_1	*Trichoderma viride*	27.33	27.75
T_2	*Trichoderma harzianum*	25.17	33.48
T_3	*Trichoderma hamatum*	26.83	29.07
T_4	Control	37.83	

Treatments	bacterial bioagents	Mean colony diameter (mm)	Per cent inhibition
T_1	*Pseudomonas fluorescens*	13.33	82.65
T_2	*Bacillus subtilis*	31	59.65
T_3	Control	76.83	

Fig.9- T1: *Trichoderma viride*, T2: *Trichoderma harzianum*, T3: *Trichoderma hamatum*, T4:Control

(Efficacy of different fungal antagonists against *F.oxysporum)*

Fig.10- T1: *Pseudomonas fluorescens,* T2: *Bacillus subtilis,*

(Efficacy of different bacterial antagonists against *F.oxysporum).*

4. CONCLUSION

This research contributes valuable insights into sustainable alternatives for managing *F. oxysporum* in marigold cultivation. The use of garlic extract as a biocontrol agent not only offers an effective solution to the disease but also aligns with the region's agricultural practices, promoting environmentally friendly pest management. For future research, it is recommended that studies focus on optimizing the application of garlic extract, exploring its effectiveness in field conditions, and evaluating its potential in combination with other biocontrol agents or plant extracts. Additionally, there is a need for more extensive field trials to assess long-term effects and practicality in local farming systems. For farmers in Longleng, the application of garlic extract could serve as a promising, low-cost, and sustainable approach to managing *F. oxysporum*, improving marigold production while reducing reliance on harmful chemicals. Educational efforts to disseminate these findings and train farmers in its proper use would be beneficial for enhancing agricultural practices and promoting environmental sustainability in the region.

Commented [H68]: phytoextract

Commented [H69]: disease

COMPETING INTERESTS

The authors declare that there are no competing interests related to the research presented in this paper. There are no financial, personal, or professional relationships with individuals or organizations that could have influenced or biased the results of this study. No funding, patents, or consultancies related to the work were involved, and the authors have not received any honoraria or paid expert testimony. The research was conducted independently, and all necessary ethical guidelines were followed.

REFERENCES

1. Kaur, M., Bhat, A., Sadik-Zada, E. R., & Sharma, R. (2022). Productivity Analysis and Employment Effects of Marigold Cultivation in Jammu, India. Horticulturae, 8(3), 263. https://doi.org/10.3390/horticulturae8030263

Commented [H70]: Kaur, M.; Bhat, A.; Sadik-Zada, E. R. & Sharma, R.

Commented [H71]: Make it italic

2. Gupta, Y. C., Panwar, S., Banyal, N., Thakur, N., & Dhiman, M. R. (2022). Marigold. Floriculture and Ornamental Plants, 1–23. https://doi.org/10.1007/978-981-15-3518-5_1

3. Kumar, H., & Singh, R. (2013). ECONOMIC ANALYSIS OF FRESHWATER AQUACULTURE PRODUCTION: A COMPARATIVE ANALYSIS OF DIFFERENT PRODUCTION SYSTEMS. Russian Journal of Agricultural and Socio-Economic Sciences, 13(1), 49–55. https://doi.org/10.18551/rjoas.2013-01.06

4. Suryavanshi, N., & Parvez, Dr. R. (2014). Rose and Marigold Flower Harvester's & Their Income. IOSR Journal of Agriculture and Veterinary Science, 7(5), 49–51. https://doi.org/10.9790/2380-07524951

5. Mir, R. A., Ahanger, M. A., & Agarwal, R. M. (2019). Marigold: From Mandap to Medicine and from Ornamentation to Remediation. American Journal of Plant Sciences, 10(02), 309–338. https://doi.org/10.4236/ajps.2019.102024

6. Kaur, M., Bhat, A., Sadik-Zada, E. R., & Sharma, R. (2022). Productivity Analysis and Employment Effects of Marigold Cultivation in Jammu, India. Horticulturae, 8(3), 263. https://doi.org/10.3390/horticulturae8030263

7. Hellin, J., & Fisher, E. (2019). Climate-Smart Agriculture and Non-Agricultural Livelihood Transformation. Climate, 7(4), 48. https://doi.org/10.3390/cli7040048

8. Rojas-Sandoval, J. (2018). Tagetes erecta (Mexican marigold) [dataset]. In CABI Compendium. CABI Publishing. https://doi.org/10.1079/cabicompendium.52641

Commented [H72]: Make it italic

Commented [H73]: Make it italic

9. Hooks, C. R. R., Wang, K.-H., Ploeg, A., & McSorley, R. (2010). Using marigold (Tagetes spp.) as a cover crop to protect crops from plant-parasitic nematodes. Ap plied Soil Ecology, 46(3), 307–320. https://doi.org/10.1016/j.apsoil.2010.09.005

10. Potato blight and unborn babies. (1973). Nutrition & Food Science, 73(2), 10 –10. https://doi.org/10.1108/eb058559

11. Colletotriauxins AD, New Plant Growth Inhibitors from the Phytopathogenic Fung us Colletotrichum gloeosporioides. (n.d.). https://doi.org/10.1021/acs.jafc.3c0314 3.s001

12. Roy, P., & Lal, A. A. (2024). Efficacy of bio-agents and organic amendment again st fusarium wilt (Fusarium oxysporum f. sp. ciceri) of chickpea (Cicer arietinum L.). International Journal of Advanced Biochemistry Research, 8(10S), 587–591. htt ps://doi.org/10.33545/26174693.2024.v8.i10sg.2536

13. Md, M. H., Nilufar, H., Farjana, S., Shah, M. N. I., Md, S. I., & Md, K. A. B. (2013). Integrated management of Fusarium wilt of chickpea (Cicer arietinum L.) caused by Fusarium oxysporum f. sp. ciceris with microbial antagonist, botanical extract and fungicide. African Journal of Biotechnology, 12(29), 4699–4706. https://doi.or g/10.5897/ajb2013.12503

14. Miron, T., Rabinkov, A., Mirelman, D., Wilchek, M., & Weiner, L. (2000). The mod e of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. Biochimica et Biophysica Acta (BBA) - Biomem branes, 1463(1), 20–30. https://doi.org/10.1016/s0005-2736(99)00174-1

Commented [H74]: Make it italic

Commented [H75]: Make it italic

15. Kumar, S., Singh, D., Yadav, J. K., Kumar, S., & Verma, S. K. (2017). Efficacy of Plant Extracts, Bioagents and Fungicides against *Fusarium udum* Causing Pigeonpea Wilt. International Journal of Current Microbiology and Applied Sciences, 6(8), 2652–2660. https://doi.org/10.20546/ijcmas.2017.609.327

16. Seaward, M. R. D. (1986). The British Ascomycotina. An annotated checklist. By P. F. Cannon, D. L. Hawksworth and M. A Sherwood-Pike. Slough: Commonwealth Mycological Institute/Commonwealth Agricultural Bureaux, in collaboration with the British Mycological Society. 05 1985. pp. viii + 302. ISBN 0 85198 546 7. Prices (including postage): £55.00 (CAB member countries), S100.00 (non-member countries); £35.00 reduced rate to members of the British Lichen Society. [Available from: Commonwealth Agricultural Bureaux, Farnham Royal, Slough SL2 3BK]. The Lichenologist, 18(3), 298–299. https://doi.org/10.1017/s0024282986000427

17. K. A., A. (2014). Effect of Combination among Bioagents, Compost and Soil Solarization on Management of Strawberry Fusarium Wilt. American Journal of Life Sciences, 2(6), 39. https://doi.org/10.11648/j.ajls.s.2014020602.16

18. Abu El-Souod, S., Awadalla, O., Assawah, S., Mahmoud, Y., & El-Debaiky, S. (2017). Studies on the Sclerotia of Some Species in The Genus Aspergillus. Egyptian Journal of Botany, 57(2), 395–404. https://doi.org/10.21608/ejbo.2017.683.1036

19. Elhalag, K. M., Al-Anany, M. S., & Megahed, A. A. (2025). Efficacy of plant extracts and bio-fertilizers for the control of bacterial wilt and soft rot agents in potato. Journal of Plant Pathology. https://doi.org/10.1007/s42161-024-01825-7

20. Bachkar, D., Kolase, S., Bhujbal, M., Thakare, C., Doltade, S., Khatal, M., & Daingade, N. (2021). In vitro efficacy of different fungicides against Fusarium incarnat

Commented [H76]: No need to mention prices

um causing fruit rot of papaya (Carica papaya L.). International Journal of Chemical Studies, 9(1), 1485–1488. https://doi.org/10.22271/chemi.2021.v9.i1u.11437

21. Gote, S., Ghante, P., Mete, V., Kmable, A., & CS, G. (2021). To evaluate in-vivo efficacy of fungicides, bio-agents and phyto-extracts against sudden death syndrome (wilt) of soybean caused by Fusarium oxysporum f. sp. virguliforme. International Journal of Chemical Studies, 9(1), 3281–3283. https://doi.org/10.22271/chemi.2021.v9.i1at.11740

22. Malik, N., Habib, A., Malik, M. K., Zubair, M., Ali, S., Tufail Khan, Q. A., Qamar, H., Hanif, K., Iqbal, S., & Anwar ul Haq, M. (2021). Efficacy of Different Plant Extracts on Fusarium Wilt caused by Fusarium oxysporum in Chillies under Field Condition. International Journal of Advanced Research in Biological Sciences (IJARBS), 8(12), 118–122. https://doi.org/10.22192/ijarbs.2021.08.12.012

23. Tampakleima Chanu, W., Sinha, B., Chakrapani, K., Thangjam, B., Chaurasia, N. K., & Herojit Singh, Sh. (2024). Eco-Friendly Management of Fusarium oxysporum f.sp. pisi inciting wilt of Pea in vitro. Journal of Mycopathological Research, 62(1), 177–183. https://doi.org/10.57023/jmycr.62.1.2024.177