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TRICHODERMA SPP. AS BIOLOGICAL CONTROL AGENTS TO MANAGE THE LEAF SPOT DISEASE OF *EUCALYPTUS CAMALDULENSIS* CAUSED BY *CURVULARIA LUNATA*

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Abstract

Eucalyptus is attacked by various fungal and bacterial pathogens that have caused huge economic losses globally. Leaf spot disease of *Eucalyptus camaldulensis* caused by *Curvularia lunata* was reported in Shorkot Irrigated Forest Plantation Punjab, Pakistan. In the current study, various Trichoderma spp. i.e., *T. viride, T. harzianum, T. Koningii, T. Asperellum,* and *T. Virens* were evaluated during *invitro*. I*nvivo* studies applied ($1x10^5$, $1x10^6$, $1x10^7$ conidia/mL of water) to manage the leaf spot of *Eucalyptus camaldulensis* caused by *Curvularia lunata* in the Research Area, Department of Forestry and Range Management, University of Agriculture, Faisalabad (UAF), Pakistan during (2021-22). The findings revealed that highest fungal growth was inhibited during *invitro* study by *Trichoderma viride* (16.693 mm) followed by *Trichoderma harzianum* (20.737mm), *Trichoderma Koningii* (24.467mm), *Trichoderma Virens* (24.841mm), *Trichoderma Asperellum* (24.841mm) as compared to control (32.246mm) respectively. While, the least disease incidence (17.968%) was observed with the combined application of *T. viride*+ *T. harzianum* followed by *T. viride* (27.221%), *T. harzianum* (31.374%) as compared to control (43.663%) in the greenhouse experiment. The minimum disease incidence (18.134%) was observed with the combined application of *T. viride*+ *T. harzianum* followed by *T. viride* (18.351%), *T. harzianum* (25.294%) when compared with control (36.885%) respectively when applied in the field experiment. The data were analyzed by using the statistical software Minitab-2018 and interpreted accordingly.

Keywords: Disease Management, Curvularia Lunata, *Trichoderma Viride, Trichoderma. Harzianum Trichoderma. Koningii, Trichoderma. Asperellum* and *Trichoderma. Virens.*

INTRODUCTION

Eucalyptus belongs to the family Mytraceae, and second largest genus with more than 700 species (Brooker and Kleinig, 2006; Gizachew, 2017). Current eucalyptus plantation covers at least 25 million hectares of the world's area (Martins et al., 2022). It is used for oil extraction, pulp and paper, insect repellent, natural insecticide, charcoal, mining, fuel wood, tracks, plywood, poles, furniture, and building construction. Eucalyptus oils are used in perfume and cosmetic industries, and bark is used for tanning (Qi, 2002; Bakkali

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et al., 2008). Eucalyptus is attacked by various fungal and bacterial pathogens that cause huge economic losses. Leaf spot of *Eucalyptus camaldulensis* is the most destructive, which is caused by *Curvularia lunata* (Wang et al., 2022). Curvularia is a diverse group of filamentous fungi belonging to the family Pleosporaceae (Pleosporales, Ascomycota) widely present in various regions (Ariyawansa et al., 2015; Marin-Felix et al., 2020). Approximately 250 species were recorded in MycoBank (http://www.mycobank.org, August 2022), and most of them are destructive pathogens that cause 60% of economic losses to the agriculture sector. (Tan et al., 2018; Cui et al., 2020; Wang et al., 2022). It produces brown multi-septate conidia, usually with light terminal cells and inordinately enlarged intermediate cells (Sivanesan, 1987; Marin-Felix et al., 2020). Members of this genus showed different modes of action in plants and animals, i.e., saprophytic, endophytic, and also pathogenic (Madrid et al., 2014; Manamgoda et al., 2015; Raza et al., 2019). Broad ranges of plant hosts are associated with the Curvularia, especially the family Poaceae, which affected the most staple crops like rice, maize, wheat, and sorghum (Kusai et al., 2016; Aslam et al., 2019; Zhang et al., 2020). In March 2021- Leaf spot disease symptoms were observed on *Eucalyptus camaldulensis* in the Shorkot and Changa Manga irrigated forest plantation with approximately 21510 acres. The large area of the Eucalyptus field was found sick with leaf spot disease caused by Curvularia lunata, which produces round to oval spots with 0.5 to 2.0 mm diameter and appears dark brown. Leaves color turned yellow (chlorosis), leading to cell death (necrosis). Curling of leaves and defoliation are the characteristic symptoms of leaf spots of *E. camaldulensis* (Old et al., 2002; Balmelli et al., 2013). The pathogen remains dormant in the plant residues under favorable environmental conditions, which initiates the primary infection. Pathogen produces conidia asexually, and secondary infection is initiated after dispersal in the field (Li et al., 2004).

Management of diseases through biological control agents is one of the most important techniques compared to other conventional methods (Gupta, 2018). Excessive and unsystematic use of fungicides for managing plant diseases causes resistance in pathogens toward fungicides that negatively affect humans, animals, and wildlife (Gupta, 2019). Bio-control agents suppress the number of pathogens by the induction of natural and induced antagonists that occur by manipulating the microenvironment, which boosts the activity of antagonists (Stirling and Stirling, 1997). That's why biological control (BCAs) is considered the most promising approach in recent decades to minimize human and environment-related hazards. Microbial biocontrol agents (BCAs) are bacterial and fungal strains isolated from the endosphere, rhizosphere, and phyllosphere that play a critical role in managing plant pathogenic organisms. Microbial antagonists and biocontrol agents stop the infection of the host plant pathogen by developing a pathogen in the host plant (Heydari and Pessarakli, 2010; Chandrashekara et al., 2012). The primary mechanism to manage the disease is to act primarily upon the pathogens. However, various direct and indirect mechanisms include antibiosis (In which antibiotic and inhibitory metabolites are secreted by antagonist), mycoparasitism (In which antagonist acquires all nutrients from the fungal host), induced resistance (initiation of defense

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response against plant pathogens), and growth enhancement (BCAs boost up the plants growth and reduce the disease by secreting microbial hormones such as indoleacetic acid and gibberellic acid. As a result of altering the transcriptome and proteome mechanism that enhanced the nutrient uptake, plant growth and ultimately devolved the pathway for resistance of plants (Singh, 2014; Zhang et al., 2014; Deketelaere et al., 2017)

Trichoderma belongs to the genus ascomycetic fungi present in the soils and known as a biological control agent against phytopathogens (Romão-Dumaresq et al., 2012; El-Hassan et al., 2013; Carvalhofilho et al., 2018). In the current study, various species of *Trichoderma,* like *T. viride, T. harzianum T. Koningii, T. Asperellum,* and *T. Virens* were evaluated against the *Curvularia lunata.* However, a comprehensive study was conducted to elucidate the etiology of this disease on *Eucalyptus camaldulesnsis*. The objectives of the current study were to identify the pathogen on a morphological basis and manage the disease by applying various Trichoderma spp to control the leaf spot disease of *E. camaladulensis.*

MATERIAL AND METHODS

The present study was conducted in the Postgraduate Research Area, Department of Forestry and Range Management, University of Agriculture Faisalabad (UAF). Lab experiments were performed in the Department of Plant Pathology Postgraduate laboratory from 2020 to 2021. Geographically, Faisalabad is situated in central Punjab (Pakistan), having a height from sea level (150m), at 72.08 to 73^{o}E longitude and 30.35 to 31.47^{o}N Latitude. The climatic conditions during summer (April-September) are, i.e., maximum temperature 45^{o}C, and minimum temperature 27^{o}C, while maximum temperature during winter (November-February) is 29^{o}C and minimum temperature is 6^{o}C, respectively.

Collection of diseased samples, isolation, purification, and identification of pathogen

Samples of leaf spot disease of *Eucalyptus camaldulensis* were collected from Shorkot and Changa Manga irrigated forest plantations of Punjab, Pakistan. The plants with typical symptoms of leaf spot were put in brown bags (9"×12") and brought into the Postgraduate laboratory of the Department of Plant Pathology to isolate, purify, and identify the pathogen. Diseased leaf samples were surface sterilized using 1% sodium hypochlorite solution, and the samples were cut with the help of a sterilized seizer. The diseased samples and some healthy portions (2-3 mm) were cut with the sterilized seizer, dipped into sodium hypochlorite solution for 30 seconds, and washed thrice with distilled water. The samples were dried on sterilized tissue paper and placed on the Petri plates containing (5mm) potato dextrose agar (PDA) media. The process used the laminar flow (ESCO laminar flow horizontal cabinet) to avoid contamination. The samples were placed in an incubator at 25°C±1 for 48-72 hours (Santos et al., 2018). After the appearance of fungal growth, purification was done by shifting single hyphae into a new Petri Plate containing (5mm) PDA media. Pathogen identification was done using a stereo-

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microscope based on morphological characteristics (size and shape of spores) and taxonomic (growth pattern and color). The pathogen was preserved by preparing the slants by adding 1.5ml PDA in the vial then putting a loop of culture and placing at 25°C±1. When the fungal growth appeared, slants were kept at 4°C for further use (Soesanto et al., 2011; Mukunda et al., 2012).

Fig 1: Leaf Spot symptoms of *E. camaldulensis* under field conditions

Pathogenicity Test

Six months old plants of *E. camldulensis* were established in the experimental area, Department of Forestry and Range Management, University of Agriculture Faisalabad (UAF). Plants were grown in pots (18cm) under greenhouse conditions where the temperature was 25-30°C with 80-85% relative humidity. Disease confirmation was done by applying inoculums at (1×10^5 conidia/mL) adjusted with the help of a hemocytometer (BEL©, Model L.24) in the morning when maximum stomata were open through syringe method and foliar application (Materić et al., 2015). The disease symptoms appeared on plants after 12-15 days of inoculum application, and then samples were brought to the laboratory. The pathogen was isolated and re-isolated to fulfill the requirements of Koch's Postulate (French and Hebert, 1982).

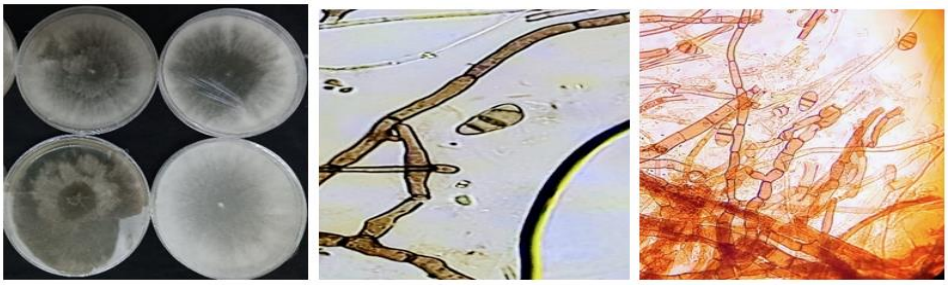

Fig 2: Morphological characteristics of *Curvularia lunata* caused leaf spot of *E. camaldulensis*

***In-vitro* management of leaf spot disease of *Eucalyptus camaldulensis* through biological control agents**

Different bio-control agents were used for the management of leaf spots of *E. camaldulensis,* and the study was designed under Completely Randomized Design

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(CRD). The pathogen was grown on Potato dextrose agar (PDA), and seven days old culture was used for the experiment. A dual culture technique was used to determine the growth against different *Trichoderma spp.* The fungal spore concentrations were adjusted with the help of a haemocytometer (BEL©, Model L.24), and data were measured after 2, 4, and 6 days to determine the growth of the pathogen suppressed by Trichoderma species. (Abreu, 2019). The six treatments T_0 = Control, T_1 = *Trichoderma viride*, T_2 = *Trichoderma harzianum*, T_3 = *Trichoderma Koningii*, T_4 = *Trichoderma Asperellum*, T_5 = *T. harzianum+T. asperellum* were developed and applied at ($1x10^5$, $1x10^6$, $1x10^7$ conidia/mL of water) spore concentrations were counted with the help of a hemocytometer (BEL©, Model L.24).

Management of leaf spot disease of *Eucalyptus camaldulensis* through biological control agents under greenhouse and field conditions

T. viride and *T. harzianum* which expressed significant results, were evaluated alone and in combination at ($1x10^5$, $1x10^6$, $1x10^7$ conidia/mL of water) spores concentrations for management of leaf spot of *E. camaldulensis* and the study was designed under Completely Randomized Design (CRD). One-year-old plants were established in the pots (18cm), and greenhouse temperature and relative humidity were 25-30°C and 80-85%. After 24 hours of inoculum application (1×10^5 conidia/mL), Trichoderma species were applied, and spores concentrations were adjusted with the help of a hemocytometer (BEL©, Model L.24). The control plants were only treated with distilled water. The disease symptoms appeared after 12-15 days of inoculum application, and disease incidence data were recorded after 07, 14, and 21 days of inoculation (Abreu, 2019).

Trichoderma viride and *Trichoderma harzianum,* which showed better results during the In-vitro study, were evaluated alone and in combinations for management of leaf spot of *E. camaldulensis* under field conditions in which *T. viride* and *T. harzianum* applied at three spores concentrations ($1x10^5$, $1x10^6$, $1x10^7$ conidia/mL of water). Experiment was designed under Randomized Complete Block Design (RCBD). Trichoderma species were applied after 24 hours of inoculums (1×10^5 conidia/mL), through a foliar application on the one-year-old plants with three different spore's concentrations. The inoculum was applied in the morning due to the maximum number of stomata being open. The spores concentration was adjusted through a hemocytometer (BEL©, Model L.24). Disease symptoms were observed within 12-15 days of inoculum application. The data were recorded after one week of intervals to find the disease incidence (Abreu, 2019).

RESULTS

Invitro management of leaf spot disease of *Eucalyptus camaldulensis* through biological control agents

Among all the treatments highest fungal growth was inhibited during *in-vitro* study by *Trichoderma viride* (16.693 mm) followed by *Trichoderma harzianum* (20.737mm), *Trichoderma Koningii* (24.467mm), *Trichoderma Virens* (24.841mm), *Trichoderma*

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Asperellum (24.841mm) as compared to control (32.246mm) respectively (Table 1 and Fig. 3). The interaction between treatments and concentration indicated that the maximum fungal growth suppressed by *T. viride* 22.178, 17.356, and 10.544 mm followed by *T. harzianum* 26.756, 20.689, and 14.767 mm, *T. Virens* 28.156, 22.911 and 15.467 mm, *T. Asperellum* 29.000, 25.367, and 20.156 mm, *T. Koningii* 29.256, 25.544, and 18.600 mm as compared to control (32.246 mm) when applied at $1x10^5$, $1x10^6$, and $1x10^7$ conidia/mL of water respectively (Table 2 and Fig. 4). The interaction between treatments and days showed that least fungal growth was observed by *T. viride* (14.511mm) applied after 30 days, (16.500mm) 20 days (19.067mm), 10 days followed by *T. harzianum* (18.944, 20.533, 22.733mm), *T. Virens* (20.21, 22.111, 24.211 mm), *T. Asperellum* (23.233, 24.933, 26.356 mm) and *T. Koningii* (22.700, 24.244, 26.456 mm) as compared to control (34.230, 32.353, and 30.153 mm) as shown in Table 3 and Fig. 5

Table 1: Impact of various *in-vitro* bio-control agents on colony growth

Sr. No.	Treatments	Colony growth (mm)
T_o	Control	32.246a
T_1	*Trichoderma viride*	16.693f
T_2	*Trichoderma harzianum*	20.737e
T_3	*Trichoderma Koningii*	24.467c
T_4	*Trichoderma Asperellum*	24.841b
T_5	*Trichoderma Virens*	24.841b

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

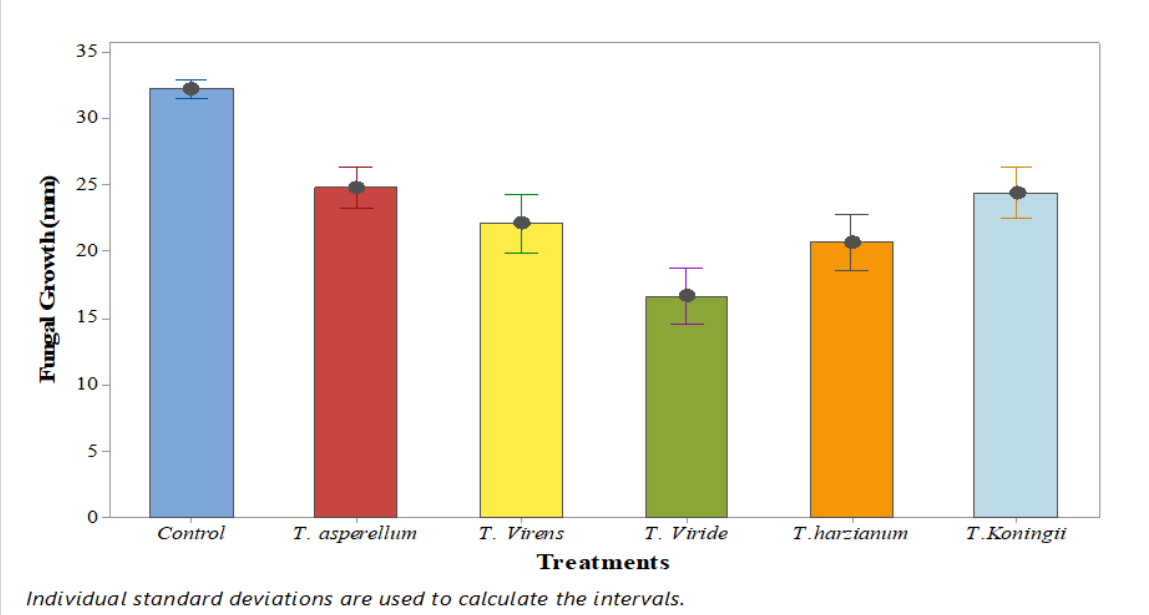

Fig 3: *In-vitro* evaluation of different bio control agents against leaf spot of eucalyptus

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Table 2: Impact of various bio-control agents and their concentrations on colony growth

Treatments	**Colony Growth** (mm)		
	Concentrations (conidia/mL)		
	$1x10^5$	$1x10^6$	$1x10^7$
Control	32.246a	32.246a	32.246a
Trichoderma viride	22.178g	17.356j	10.544m
Trichoderma harzianum	26.756d	20.689h	14.767l
Trichoderma Koningii	29.256b	25.544e	18.600i
Trichoderma Asperellum	29.000b	25.367e	20.156h
Trichoderma Virens	28.156c	22.911f	15.467k

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

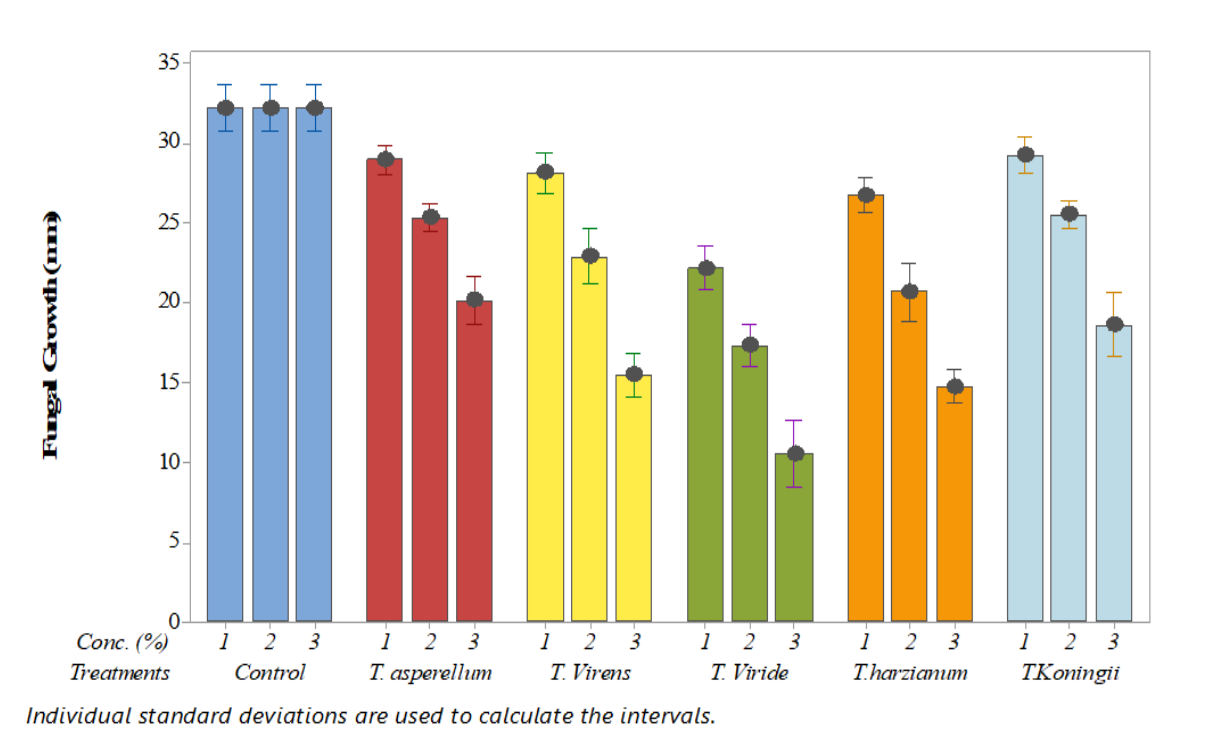

Fig 4: *In-vitro* evaluation of bio-control agents and interaction between treatments and concentrations on the fungal growth of *E. camaldulensis*

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Table 3: Impact of bio-control agents on treatments and days on the colony growth

Treatments	Colony growth (mm)		
	Days (D)		
	D_1	D_2	D_3
Control	30.153c	32.353b	34.230a
Trichoderma viride	19.067j	16.500k	14.511l
Trichoderma harzianum	22.733gh	20.533i	18.944j
Trichoderma Koningii	26.456d	24.244f	22.700gh
Trichoderma Asperellum	26.356d	24.933e	23.233g
Trichoderma Virens	24.211f	22.111h	20.211i

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

D_1 = After two days

D_2 = After four days

D_3 = After six days

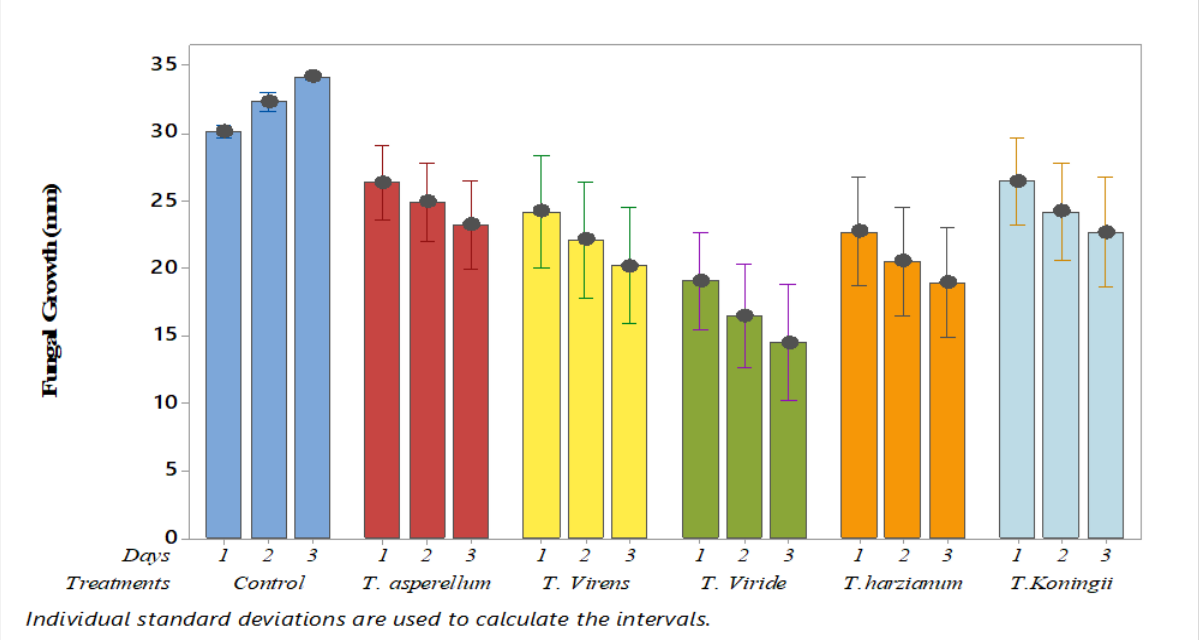

Fig 5: *In-vitro* evaluation of bio-control agents and their interaction between (T × D) days on fungal growth of *E. camaldulensis*

Management of leaf spot disease of *Eucalyptus camaldulensis* through biological control agents under greenhouse conditions

The minimum disease incidence (17.968%) was observed with the combined application of *T. viride*+ *T. harzianum* followed by *T. viride* (27.221%), *T. harzianum* (31.374%) as

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compared to control (43.663%) as shown in Table 4 and Fig 6. The interaction b/w treatments and concentration exhibited the lowest disease incidence with the combined application of *T. viride*+*T. harzianum* (26.281, 17.809, 9.813%) applied @ $1x10^5$, $1x10^6$, and$1x10^7$ conidia/mL of water followed by *T. viride* (33.089, 27.321, 21.253%), *T. harzianum* (37.382, 31.846, 24.894%) as compared with control (43.222, 43.633, and 44.133%) as shown in Table 5 and Fig 7. The interaction b/w treatment and days indicated that minimum disease incidence was observed with the combined application of *T. viride*+*T. harzianum* (20.794%), after 10 days (17.923%), 20 days (15.186%), and 30 days, followed by *T. viride* (29.207, 27.423, 25.033%), *T. harzianum* (33.531, 31.280, 29.311%) as compared to control (40.456, 43.567, 46.967%) (Table 6 and Fig 8)

Table 4: Impact of various bio-control agents on disease incidence of *E. camaldulensis*

Sr. No.	Treatments	Disease Incidence (%)
T_0	Control	43.663a
T_1	*Trichoderma viride*	27.221c
T_2	*Trichoderma harzianum*	31.374b
T_3	*T. viride*+ *T. harzianum*	17.968d

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

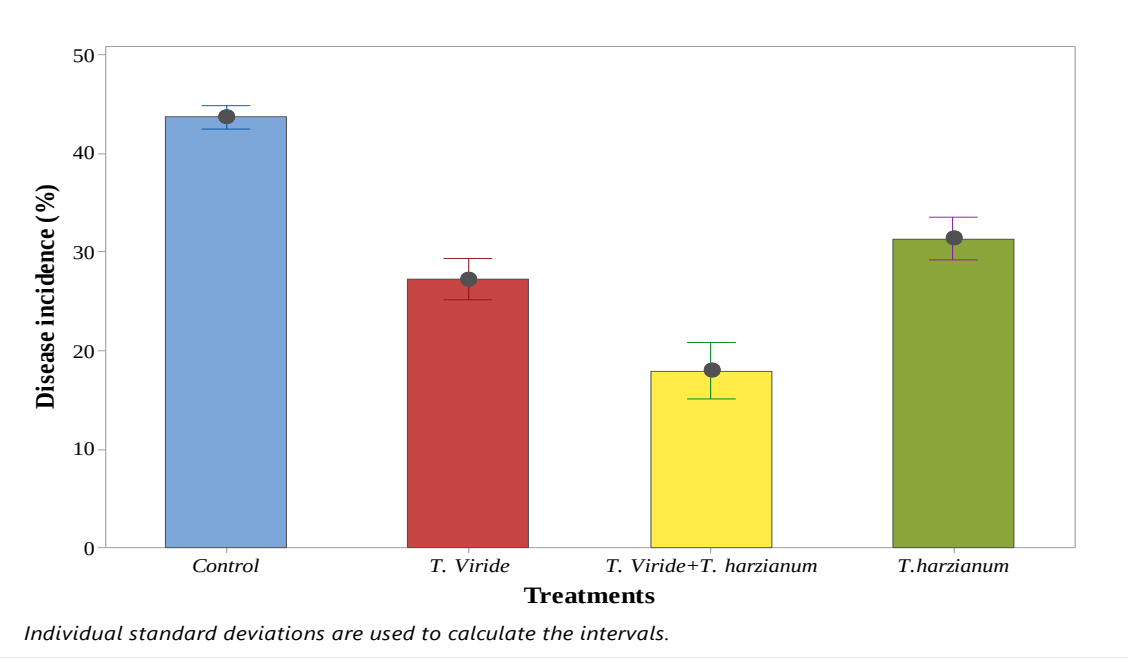

Fig 6: Evaluation of different bio-control agents against leaf spot of eucalyptus under greenhouse conditions

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Table 5: Impact of various bio-control agents and their concentrations on disease incidence of *E. camaldulensis*

Treatments	Disease Incidence (%)		
	Concentrations (conidia/mL)		
	$1x10^5$	$1x10^6$	$1x10^7$
Control	43.222b	43.633ab	44.133a
Trichoderma viride	33.089d	27.321f	21.253i
Trichoderma harzianum	37.382c	31.846e	24.894h
T. viride+T. harzianum	26.281g	17.809j	9.813k

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

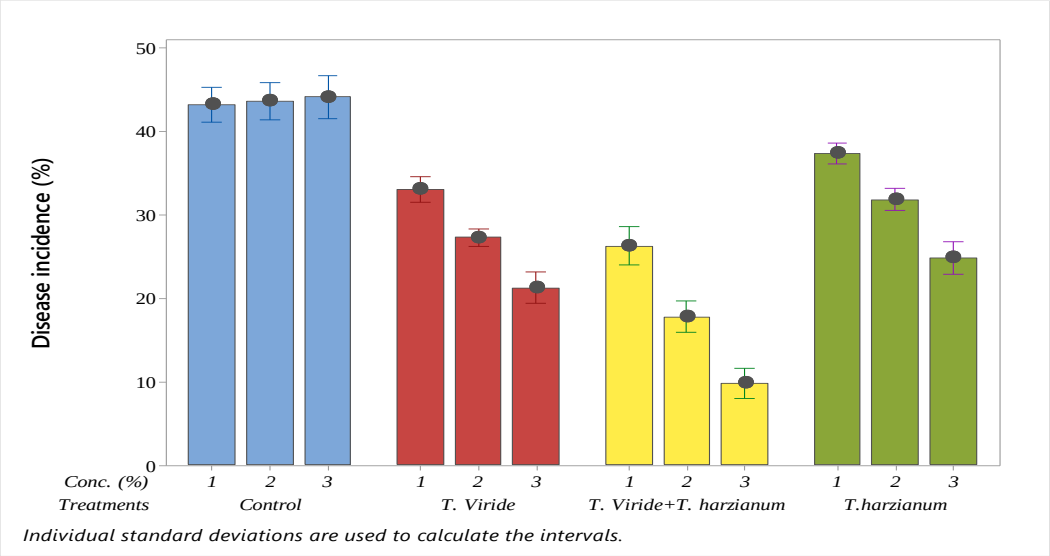

Fig 7: Evaluation of bio-control agents and their interaction between treatments and concentrations on disease incidence under greenhouse conditions

Table 6: Impact of bio-control agents on disease incidence of *E. camaldulensis* under greenhouse conditions

Treatments	Disease Incidence (%)		
	Days (D)		
	D_1	D_2	D_3
Control	40.456c	43.567b	46.967a
Trichoderma viride	29.207f	27.423g	25.033h
Trichoderma harzianum	33.531d	31.280e	29.311f
T. viride+T. harzianum	20.794i	17.923j	15.186k

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

D_1 = After ten days, D_2 = After twenty days, D_3 = After thirty days

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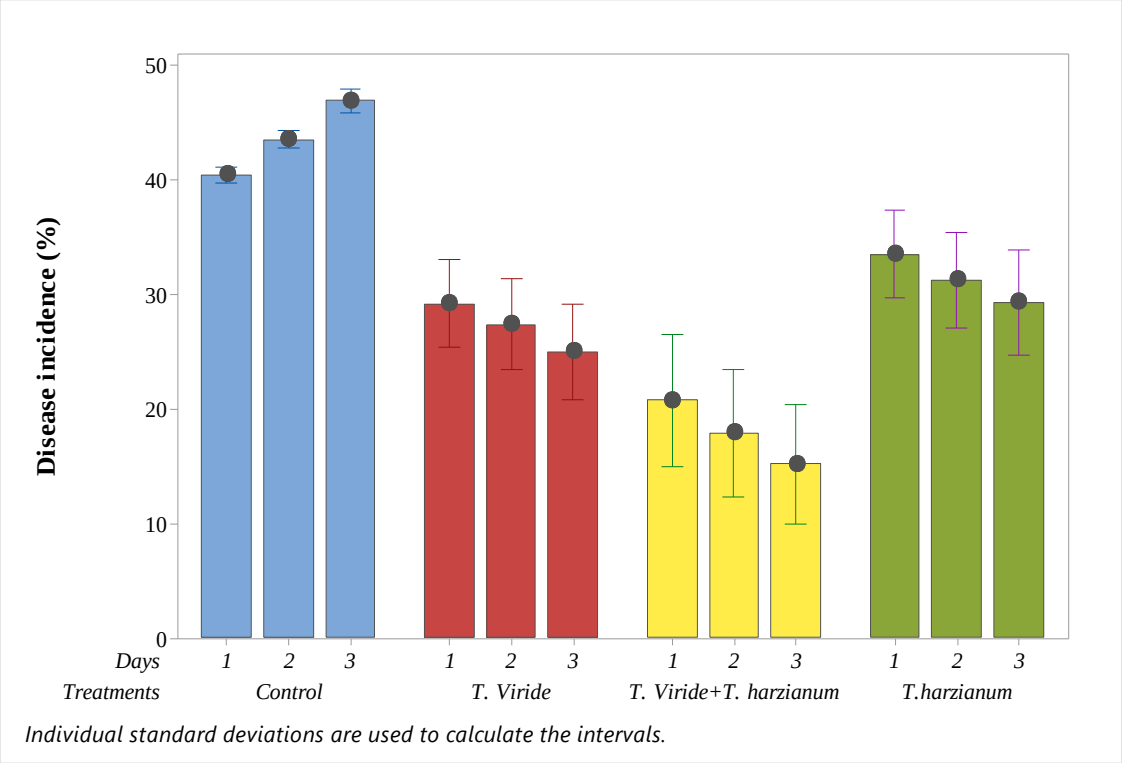

Fig 8: Evaluation of bio-control agents and their interaction between (T × D) days on disease incidence of eucalyptus under greenhouse conditions

Management of leaf spot disease of *Eucalyptus camaldulensis* through biological control agents under field conditions

Among all the treatments, minimum disease incidence (18.134%) was observed with the combined application of *T. viride*+ *T. harzianum* followed by *T. viride* (18.351%), *T. harzianum* (25.294%) as compared with control (36.885%) respectively (Table 7 and Fig. 9).

The interaction b/w treatments and concentration exhibited the lowest disease incidence with combined application of *T. viride*+*T. harzianum* 26.473, 17.836 and 10.093% applied @ $1x10^5$, $1x10^6$, and$1x10^7$ conidia/mL of water followed by *T. viride* 25.563, 17.623, and 11.867%, *T. harzianum* 31.021, 25.787, and 19.073% as compared with control 32.311, 37.089, and 41.256 % respectively (Table 8 and Fig. 10).

The interaction b/w treatment and days indicated that minimum disease incidence was recorded with the combined application of *T. viride*+*T. harzianum* (20.820%), after 10 days (17.993%), 20 and (15.589%) and 30 days followed by *T. viride* (20.587, 18.224, 16.242%), *T. harzianum* (27.213, 25.487, 23.181%) as compared to control (35.489, 36.944, 38.222%) as shown in (Table 9 and Fig. 11).

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Table 7: Impact of various control agents on disease incidence of *E. camaldulensis*

Sr. No.	Treatments	Disease Incidence (%)
T_o	Control	36.885a
T_1	*Trichoderma viride*	18.351c
T_2	*Trichoderma harzianum*	25.294b
T_3	*T. viride+ T. harzianum*	18.134c

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

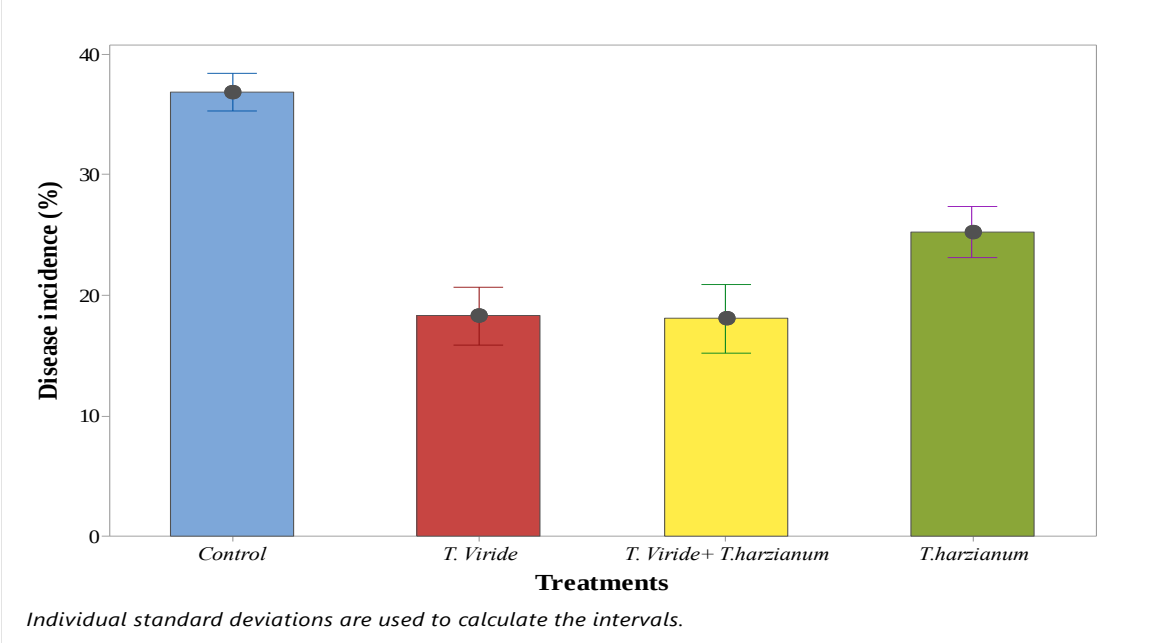

Fig 9: Evaluation of different bio-control agents against leaf spot of eucalyptus under field conditions

Table 8: Impact of various bio-control agents and their concentrations on disease incidence of *E. camaldulensis* under field conditions

Treatments	Disease Incidence (%)		
	Concentrations (conidia/mL)		
	$1x10^5$	$1x10^6$	$1x10^7$
Control	32.311c	37.089b	41.256a
Trichoderma viride	25.563f	17.623h	11.867i
Trichoderma harzianum	31.021d	25.787ef	19.073g
T. viride+T. harzianum	26.473e	17.836h	10.093j

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test (P ≤ 0.05)

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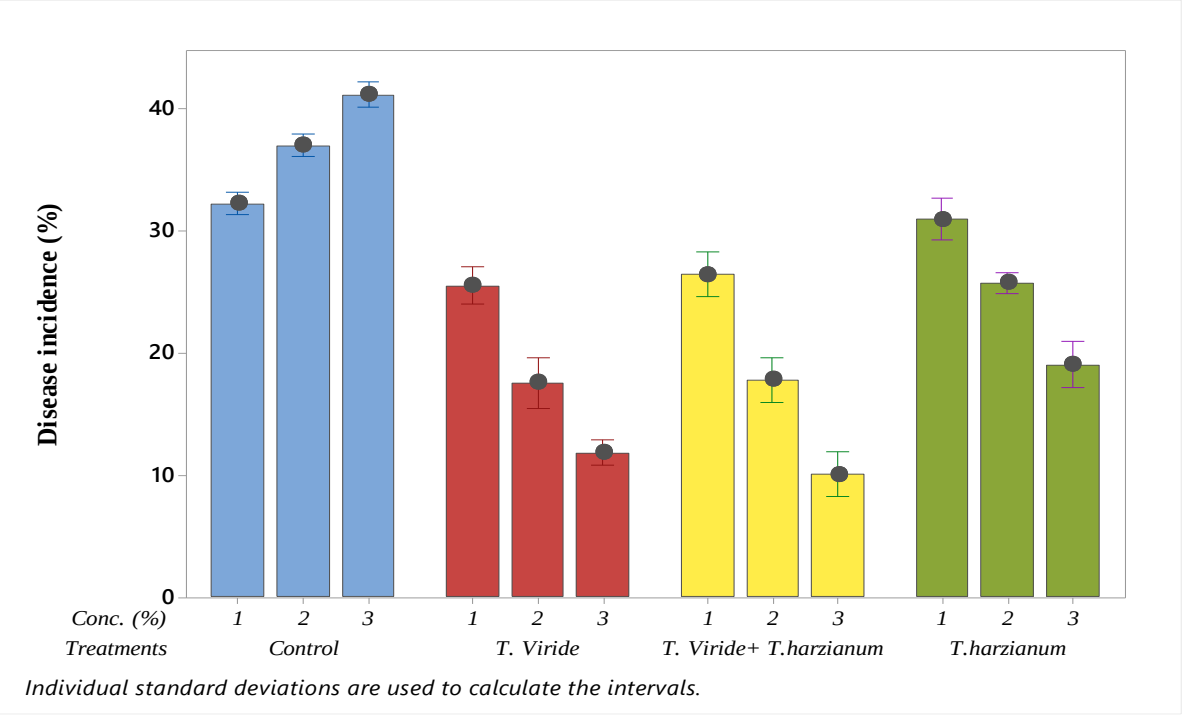

Fig 10: Evaluation of bio-control agents and their interaction between treatments and concentrations on disease incidence of *E. camaldulensis*

Table 9: Impact of bio-control agents on disease incidence of *E. camaldulensis* under greenhouse conditions

Treatments	Disease Incidence (%)		
	Days (D)		
	D_1	D_2	D_3
Control	35.489c	36.944b	38.222a
Trichoderma viride	20.587g	18.224h	16.242i
Trichoderma harzianum	27.213d	25.487e	23.181f
T. viride+T. harzianum	20.820g	17.993h	15.589i

The mean values having similar letters do not differ significantly while different letters are significantly differ among each other as analyzed through LSD test ($P \leq 0.05$)

D_1 = After ten days

D_2 = After twenty days

D_3 = After thirty days

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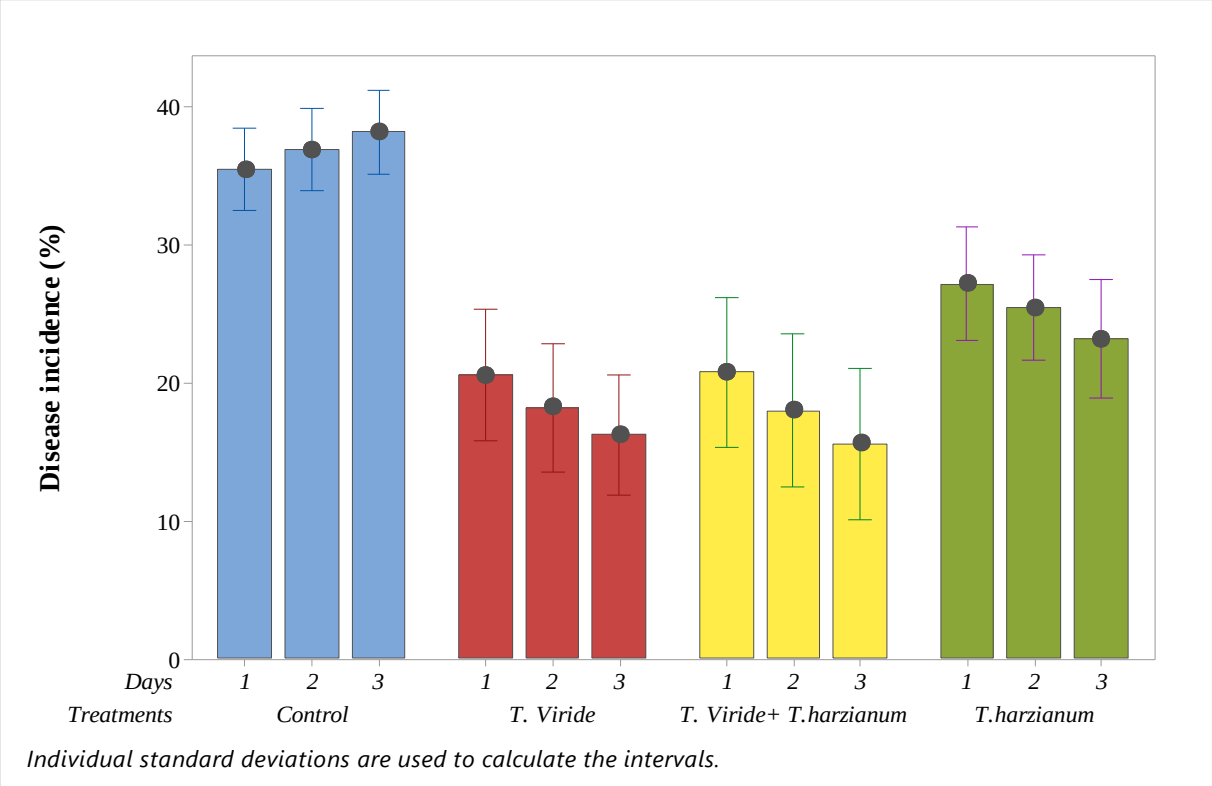

Fig 11: Evaluation of bio-control agents and their interaction between (T × D) days on disease incidence of *E. camaldulensis* under field conditions

DISCUSSION

The current research describes a fungal pathogen *Curvularia lunata* caused leaf spot disease of *Eucalyptus camaldulensis.* Some commercially important species for the biological control of phytopathogens in Trichoderma geneus include *Trichoderma harzianum*, *Trichoderma virens* (Romão-Dumaresq et al., 2012). The activities of anti-phytopathogen include niche exclusion, antibiosis, induced resistance, and mycoparasitism (Bae, 2011). Antibiosis mechanism produces antimicrobial compounds that suppress the growth of phytopathogen, and more than 100 antimicrobial compounds have been identified from Trichoderma spp (Vinale et al., 2008; Reithner et al., 2011). Trichoderma spp. Competing with plant pathogens for nutrients, spreading toxins, and secretes antibiotics to stop pathogen multiplication through hyperparasitisms (Cook, 1985). Trichoderma spp. Degraded the cell wall of phytopathogen by producing cell wall degrading enzymes during the mycoparasitism process (Reithner et al., 2011). Elliott et al. (2009) conducted a study on leaf spots of eucalyptus to manage the diseases through biological control agents. Trichoderma spp. is used due to its great potential as a growth promoter and to develop colonies endophytically in eucalyptus. Elliott et al. (2009) during the in-vitro evaluation of Trichoderma spp. Through dual culture assay, the growth of P.

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ramorum was reduced. Archana, (2008) conducted a study against *Curvularia penniseti* pathogen to determine bio-agents phytoextract and it was reported that about 53% of growth was inhibited by *Trichoderma viride* and 47% *Trichoderma harzianum.* Gliocladium and Trichoderma are filamentous fungi evaluated against *Rhizoctonia, Sclerotinia,* V*erticillium, Alternaria, Botrytis*, *Monilinia*, *Phytophthora, Phythium colletotrichum, Diaporthe,* and *Fusarium.* Trichoderma spp. can invade by producing spores, antibiotics, and fast growing than various other biological control agents (Woo et al., 2006).

These suppress the pathogen by parasitizing them by competing for space and nutrients by modifying the environment, enhancing the growth and defense mechanism of the plants. Trichoderma species produce lytic enzymes that break down the pathogen cell wall by secreting secondary metabolites like polyketides, alkaloids, terpenoids, and peptaibols that develop resistance against the disease (Carvalhofilho et al., 2018). Trichoderma spp. can invade by producing spores, antibiotics, and fast growing compared to various other biological control agents (Woo et al., 2006). The metabolites of different Trichoderma spp. were tested against Phytophthora as a biocontrol agent, and approximately 128 isolates were identified against anti-Phytophthora by using liquid culture of ethyl acetate extracts. *Pestalotiopsis, Cylindrocladium, Rhizoctoniasolani, Botrytis cinerea,* and *Hainesia spp.* are the plant pathogenic fungi used against harmful pathogens. The root shoot cuttings of *Eucalpytus grandis* were prepared and kept for 20 to 25 days at highly humid conditions and light temperatures, favoring these plant's pathogenic fungi (Maciel et al., 2012). As a biological controlling agent, Trichoderma species are the most studied fungi against various diseases. In various studies, Trichoderma also enhanced plant growth (Saba et al., 2012; El-Hassan et al., 2013). Trichoderma spp. has been recognized as a hormone producer and nutrient that dissolves like phosphate in plants (Hoyos-Carvajal et al., 2009; Carvalho et al., 2011). *Trichoderma* spp. Colonized endophytically in different plant tissues (Silva et al., 2006; Chaverri et al., 2011). Remarkably, the environment associated with the plant is important, as it permits numerous elements to show their influence on the arrangement and structure of microbial populations associated with parts of the plant (Sanogo et al., 2002).

Mansilla, (1992) identified the most notorious pest *Gonipteruss cutellatus*, from Spain, which is now called *Gonipterus platensis*. The control of *Gonipterus* was done by using Anaphes nitens parasitoid by spreading it at a specific time; however, it caused defoliation due to the low-intensity spread of Anaphes. (Imtiaj and Lee, 2008) Gliocladium and Trichoderma are filamentous fungi evaluated against *Rhizoctonia, Sclerotinia,* V*erticillium, Alternaria, Botrytis*, *Monilinia*, *Phytophthora, Phythium colletotrichum, Diaporthe,* and *Fusarium.* Trichoderma spp. can invade by producing spores, antibiotics, and fast growth compared to various other biological control agents (Woo *et al.,* 2006). *Gliocladium* is a commercially important and widely used biological control agent due to its long-term viability and abundant production against pathogens. (Sutton *et al.,* 1997). Trichoderma spp. has potential in agriculture, conservation, and preservation of natural

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isolates of fungi in culture collection, as well as in the selection of Ecological diversity. *Trichoderma*s pp. also provides the source of information on the functioning of the genetic material of biological control agents in the future.

CONCLUSION

The *Eucalyptus camaldulensis* is the most planted genus in the world, and it is also present in various Irrigated Forest Plantations in Punjab Pakistan. Leaf spot disease of *E. camaldulensis* was identify caused by *Curvularia lunata.* In recent decades, biological control agents have been considered a useful management strategy to reduce the chemical hazards to humans and the environment. In the current study, various Trichoderma spp. were evaluated under in vitro and in vivo conditions. The findings revealed that *Trichoderma viride* performed better in the lab. Experiment as compared to other species. The experiment was also performed in the greenhouse in which two best species were evaluated alone and in combination with the combined application of *Trichoderma viride+T. Harzianum showed better results than* other species. Similar findings were recorded from a field experiment in which combined application of *T. viride+T. Harzianum showed better results than* other species. The use of Trichoderma spp. Provides a basis for developing sustainable and effective management strategies for leaf spot disease of *E. camaldulensis.*

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