

Interactive effect of *Glomus fasciculatum* and *Trichoderma* spp. on *Fusarium oxysporum* towards yield and resistance of the medicinal plant *Coleus forskohlii* Briq.

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Received : 17.02.2025

Accepted : 14.06.2025

Published : 29.09.2025

Effect of arbuscular mycorrhizal fungi (AMF) and antagonistic fungi (*Trichoderma* spp.) on the pathogen (*Fusarium oxysporum*) in relation to yield and resistance of a medicinal plant *Coleus forskohlii* was studied. *Glomus fasciculatum* (AMF) treated *Coleus* plants showed maximum (81.84%) mycorrhization, higher amount of leaf phosphate (20.45%) and chlorophyll content (10.72 mg/g). Combined treatments with *G. fasciculatum* + *Trichoderma* spp. + *Fusarium oxysporum* exhibited higher percentage of mycorrhization correspondingly with higher amount of leaf phosphate and chlorophyll content than that of the control plants but that too, was lower than that of the AMF treated plants. Application of *G. fasciculatum* in the soil of the host plants, *C. forskohlii* showed enhancement of plant growth in terms of plant height, number of branching twigs, number of leaflets and fresh weight of tuber. Dual treatment of plants with *G. fasciculatum* and *F. oxysporum* was effective to enhance the plant growth and yield than the pathogen treated and control plants. The interactive effect of *T. harzianum* and *T. viride* individually with the AM fungus, *G. fasciculatum* and the pathogen on growth and yield of *C. forskohlii* was carried out. It is apparent that among the two *Trichoderma* species, integration with *T. harzianum* showed more vigour of *Coleus* plants in respect of plant height, number of branching twigs, leaflets and fresh weight of tubers than performed by *T. viride*.

Keywords : *Coleus forskohlii*, *Glomus fasciculatum*, *Fusarium oxysporum*, growth, *Trichoderma* spp., yield

INTRODUCTION

Coleus forskohlii (Wild.) Briq. is a potentially important medicinal plant of the future, being immensely valued for its pharmacoproperties that have been discovered only recently. Its tuberous roots are found to be rich source of forskolin, which is being developed as a remedy for hypertension, glaucoma, asthma, congestive heart failures, and certain types of cancers.

The plant suffers tremendously from a wilt pathogen, *Fusarium oxysporum*, which results in great economic loss to our country during its cultivation. The mycorrhizal fungus is a specialized member of rhizosphere microorganisms (Huey *et al.* 2020). Arbuscular

mycorrhizal (AMF) fungi refer to the symbiotic association formed by the fungi of family Endogonaceae and roots of higher plants (Kehri *et al.* 2018). Arbuscular mycorrhizal (AM) fungi are known to colonize a number of tropical plants including vegetables (Diagne *et al.* 2020). Naz *et al.* (2019) studied the effect of inoculation of four arbuscular mycorrhizal fungi on growth of chickpea and reported that *G. fasciculatum* inoculated plants showed higher plant height, root length and fresh and dry weight compared to other three AM fungi. Mycorrhizal inoculation with *G. fasciculatum* not only reduced the tomato damping-off disease incidence but also significantly increased the host plant height, total biomass, dry matter and fruit yield (Reddy *et al.* 2006). Akkopru and Demir (2005) used arbuscular mycorrhizal fungi as biocontrol agents against *Fusarium* wilt of tomato. Kavita *et al.* (2003) observed suppression of damping-off in chilli

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following dual inoculation of native arbuscular mycorrhizal fungi and *Azospirillum*. It has been reported that dual application of *G. fasciculatum* and *T. harzianum* caused fewer plant death than single application (Weng *et al.* 2022). Root tissues colonized first by VAM fungi physically exclude a pathogen competing for the same infection site. Bennett *et al.* (2006) recognized that mycorrhizal fungi could affect plant enemies by improving plant nutrition, modifying plant tolerance, or modifying plant defences and in addition mycorrhizal fungi could directly interfere with pathogen, herbivory and parasitism by occupying root space. High chitinase activity of the mycorrhizal tissue inhibits the growth of the pathogen competing for the same infection site and also inhibits the growth of the pathogens in the host system (Tyćkiewicz *et al.* 2022). Singh *et al.* (2007) studied the influence of interaction of different biocontrol agents on suppression of *Fusarium* wilt of *Dalbergia sissoo* and reported that there was least mortality of *Fusarium* infected seedlings in presence of *Trichoderma* spp. and *Glomus* spp. The interactive effect of *Glomus fasciculatum* and antagonistic fungi *Trichoderma* spp. was attempted on the pathogen *Fusarium oxysporum* towards yield and resistance of the medicinal plant *Coleus forskohlii*.

MATERIALS AND METHODS

Preparation of inoculum of AMF

The inoculum of an arbuscular mycorrhizal (AMF) fungus, *Glomus fasciculatum* was obtained from Department of Forestry, Vidyasagar University, West Bengal, India. The multiplication of inoculum of AMF fungus was done on *Zea mays* roots in pots having sterile sand and soil (pH 5.6, organic carbon 0.6%, P content 45 ppm) in 1:1 ratio. A thin layer of mycorrhizal inoculum was placed 4 cm below the grains in pot soil in which maize grains were sown. After 30 days of sowing, the roots of the maize seedlings were chopped off and mixed with pot soil, in which a second crop was raised. Finally, the inocula consisting of both the spores in rhizosphere soil and the colonized root fragment was used for further experiment.

Preparation of cuttings and growth conditions

A soil culture of *Glomus fasciculatum* was propagated as endomycorrhizal species in *Zea mays* as suitable host in pot culture using sterile sand and soil (pH 5.6; organic carbon 0.6%, P content 45 ppm) in 1:1 ratio. 400 gm of rhizosphere soil with AMF-infected root fragments of *Zea mays* was introduced in the dry, loamy, fine-textured soil (pH 5.8; organic carbon 0.6%, P content 45 ppm, total nitrogen 0.03%) of pot culture (2.0 kg of soil per pot) at a depth of 2 inches below the *Coleus* cutting (12-16 cm long comprising 3-4 pairs of leaves) being planted. After two weeks, the cuttings (both AMF infected and non AMF infected) were transplanted.

Pathogens and Biocontrol agents

Isolation of the pathogen (*Fusarium oxysporum*) from the diseased plants and its identification has been confirmed by IARI (Indian Agricultural Research Institute), New Delhi (ITCC No.6933.08). Among the species of *Trichoderma*, *T. viride* was isolated from Mycology and Plant Pathology laboratory, Burdwan University, West Bengal and the other antagonist viz. *T. harzianum* was procured from Indian Agricultural Research Institute (IARI), New Delhi. The pathogen as well as *Trichoderma* strains were grown on potato dextrose agar (PDA) plates for a week at 28° ± 1°C.

Preparation of mycelial mats and spore suspension of the microorganisms

To obtain mycelial mats and spore suspension, 50 ml of potato dextrose broth at pH 7.2 was taken in conical flask and sterilized at 15 lbs. p.s.i. pressure for 15 minutes. The flasks were inoculated with 5 mm inoculum disc of the test pathogen, *F. oxysporum* and the antagonists, *Trichoderma* spp. separately and incubated for 20 days. After the incubation period, mycelial mats and spore suspension were taken.

Transplantation of cuttings and application of pathogen and antagonists

Cuttings were transplanted to non-sterile pots with same soil character as cutting pot. The transplantation was done in 4 sets. Each set consisted of 5 pots. The first set (C) of pots were

transplanted with non infected cuttings from non-inoculated pot and served as control. Likewise the second set (Fo) was transplanted with non-infected cuttings from non AMF treated pot but the spore suspension of *Fusarium oxysporum* was added. In the third set (Gf), plants were raised with cuttings from AMF treated pot and in the fourth set (Gf+Fo) AMF infected cuttings were transferred to non-sterile pots with added *Fusarium oxysporum* spore suspension.

In order to note the effect of antagonists, spore suspension and mycelial mats of two species of *Trichoderma* viz. *T. harzianum* and *T. viride* were directly applied to another sets of Gf + *Fusarium* treated pots by using previously sterilized wheat bran as substrate so as to get Gf + *Fusarium* + *Trichoderma* spp. treated (GFT) pots. Application of two species of *Trichoderma* was done separately.

Estimation of mycorrhization in roots

Standard technique of **Giovannetti and Mosse (1980)** was adopted for estimation of mycorrhization. Root samples from 30 days old plants were collected and were washed with sterilized distilled water. Staining of root samples were carried out following the **Phillips and Hayman (1970)**. Photographs were taken the method of **Philip and Hayman (1970)** using trypan blue in lactophenol as stain. Softened bits of stained root samples were mounted in 50% glycerol for examination under a bright field microscope (Lieca DFC295, version V3, Germany) and photographs.

Estimation of phosphate in host tissue

Microkjeldhal method of Jackson (1973) was employed for determination of total tissue (leaf) phosphate. To estimate the phosphorus, 60% perchloric acid, a combined reagent, H_2O_2 and distilled water were taken. Combined reagent was prepared by mixing the following reagents: 5 (N) H_2SO_4 , potassium antimony tartarate (0.275 g in 100 ml distilled water), ammonium molybdate (4 g in 100 ml distilled water), ascorbic acid (1.76 g ascorbic acid in 100 ml distilled water). This combined reagent is bluish or colourless and

transparent and is prepared just before use. The percentage of phosphate was measured by taking optical density value at 660 nm in a UV- Vis spectrophotometer (Systronics 117). The results were recorded after 30 days of infection

Estimation of chlorophyll in host tissue

In order to determine the amount of chlorophyll in host tissue, method of Mahadevan and Sridhar (1982) was adopted. The data were recorded in terms of O.D. value at 660 nm in a UV- Vis spectrophotometer (Systronics 117) .

Improvement of growth and yield of host plant

Plant growth responses in *Coleus* plants inoculated with *F. oxysporum*, *Glomus fasciculatum* and two *Trichoderma* species were recorded by measuring shoot length and counting the number of branching twigs, leaflets and fresh weight of tubers at 15, 30, 45, 60 and 180 days after infection.

Statistical analysis

Data were expressed as mean±standard deviation. Significant differences among the means were determined by Fisher's least-significant difference test after one-way analysis of variance. Significance of between-treatment means was tested at the 0.05 level of probability using Stat Plus Version 4.8, 2007 software.

RESULTS

Interaction of microorganisms affecting mycorrhizaion

The results (Table 1) indicate that the *Coleus* plant subjected to infection by an AM fungus, *Glomus fasciculatum* showed higher degree of mycorrhization. Consequent upon which the chlorophyll content and the leaf phosphate get increased. The percentage of mycorrhization (Table 1 & 2) was found to be significantly ($P < 0.05$) highest in AMF treated plants (81.84%) and lowest in AMF + pathogen treated plants (45.62%). The two treatments viz. AMF+*F. oxysporum*+*T.harzianum* and AMF+*F. oxysporum*+*T. viride* resulted 71.93% and

68.41% mycorrhization respectively. It is apparent from the result that (Table 1) AMF treated plants showed maximum amount of leaf phosphate (20.45%) followed by combined treatment with AMF+ *F. oxysporum* (11.87%).

Phosphate and chlorophyll contents

The percentage of phosphate was recorded minimum (1.06%) in the plant infected with the pathogen compared to all other treatments. It is also noted (Table 2) that *Coleus* plants treated with AMF+antagonistic fungi + pathogen showed higher percentage of phosphate than that of the control plants but lower than that of the AMF treated plants alone. The effect of two antagonistic fungi exhibited interspecific variation where all the two treatments with species of *Trichoderma* viz. *T.harzianum* and *T. viride* showed accumulation of 19.05% and 17.19% leaf phosphate respectively over the control (7.33%). Chlorophyll content, on the other hand, maintained the same pattern of changes under similar treatments where AMF treated plants alone recorded maximum amount of chlorophyll (10.72 mg/g). Presence of intercellular hyphal network of spores and large populations of arbuscules of the mycorrhizal fungus *G. fasciculatum* in the root cortex of *Coleus* plants infected with AMF

suggesting its suitable harbouring of *G. fasciculatum* (Fig. 1a & 1b).

Interactions affecting growth and yield

The interaction of the AM fungus, *G.fasciculatum* and antagonistic *Trichoderma* species with the pathogen, *F. oxysporum* on growth and yield of *Coleus* plants was investigated. It is evident from the result (Table 3) that application of *G. fasciculatum* (AMF) showed enhancement of plant growth in terms of plant height, number of branching twigs, number of leaflets and fresh weight of tuber. It is also evident that *G.fasciculatum* (Fig.3b) induced significantly increased vigour of *Coleus* plants with increased in days of cuttings were planted than the other treatments (Fig. 3a, Fig. 3c & Fig. 3d). *F. oxysporum* treated plants showed less vigour and also become non-productive consequently. Dual treatment of plants with *G.fasciculatum* and *F.oxysporum* was effective to enhance plant growth and yield than the pathogen treated and control plants. *G. fasciculatum* showed significantly ($P<0.05$) highest of tuber yield (71.60 g/plant) followed by *G.fasciculatum* + *F.oxysporum* treated plants (32.41 g/plant) and control plants (6.60 g/plant) (Fig. 4c, Fig.4b & Fig. 4a) respectively. It is evident from the results that

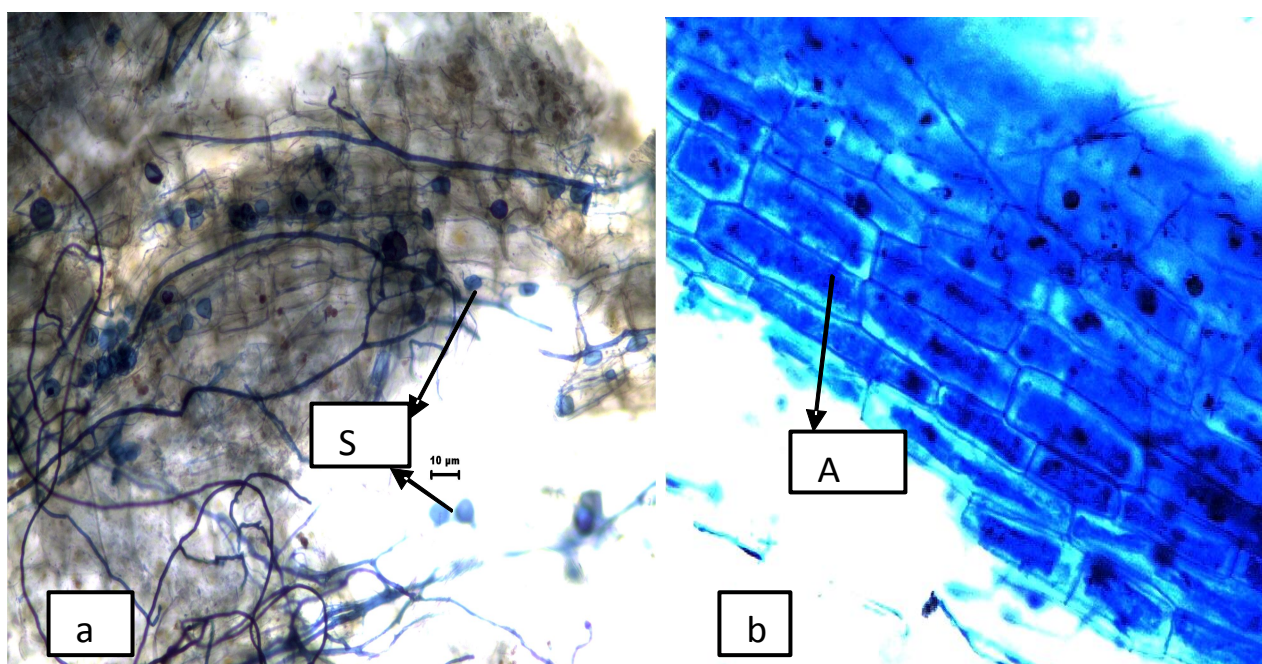


Fig.1: Photographs showing mycorrhizal root colonization of *Coleus forskohlii* (a) intraradical spores [S] and (b) numerous arbuscules within the cortex cells, Bar=50μm.

Table 1: Changes in leaf phosphate, chlorophyll content and mycorrhization in *Coleus* plant by different treatments with AMF and pathogen.

Treatments	Mycorrhization (%)	Phosphate (%)	Chlorophyll (mg/g)
AMF (Gf)	81.84±0.45	20.45±0.57	10.72±0.42
AMF+Fusarium (Gf+Fo)	45.62±0.91	11.87±0.45	9.24±0.03
<i>Fusarium oxysporum</i> (Fo)	0	1.06±0.01	4.03±0.02
Control (C)	0	5.23±0.02	6.20±0.03

Data shows mean $\pm$ standard deviation of 5 replicates, indicate significant differences between the control and treated plants ($P < 0.05$).

Table 2: Changes in leaf phosphate, chlorophyll content and mycorrhization in *Coleus* plant by different treatments with *Glomus fasciculatum*, *Trichoderma* spp. and *Fusarium oxysporum*.

Treatment	Mycorrhization (%)	Phosphate (%)	Chlorophyll (mg/g)
AMF + <i>F. oxysporum</i> + <i>T. harzianum</i>	71.93±0.64	19.05±0.02	10.07±0.03
AMF+ <i>F. oxysporum</i> + <i>T. viride</i>	68.41±0.01	17.19±0.05	8.21±0.01
Control (C)	0	7.33±0.06	2.03±0.02

Data shows mean $\pm$ standard deviation of 5 replicates, indicate significant differences between the control and treated plants ($P < 0.05$).

Table 3 : Effect of *Glomus fasciculatum* and *Fusarium* on growth and tuber yield of *Coleus forskohlii*

Treatment	Days after cuttings planted	Plant height (cm)	Number of Twigs/plant	Number of Leaflets/plant	Fresh weight of tuber (g/plant)
Control	15	10.42±0.10	5±0.54	19±0.54	0
	30	16.40±0.13	10±0.00	31±0.96	0
	45	18.40±0.06	17±0.00	41±0.81	0
	60	22.00±0.70	22±0.00	48±0.00	0
	180	30.71±0.46	28±0.03	60±0.02	6.60±0.24
<i>Glomus fasciculatum</i>	15	13.62±0.04	10±0.57	35±0.57	0
	30	24.40±0.60	16±0.54	49±0.54	0
	45	30.24±0.11	25±0.54	60±0.54	0
	60	45.60±0.98	35±0.54	91±0.54	0
	180	57.31±0.13	44±0.83	110±0.89	71.60± 3.40
<i>Fusarium oxysporum</i>	15	5.21±0.01	2±0.44	5±0.00	0
	30	7.41±0.01	4±0.54	11±0.01	0
	45	9.07±0.03	5±0.54	13±0.03	0
	60	13.42±0.01	6±0.54	19±0.02	0
	180	0	0	0	0
Gf+Fo	15	9.10±0.00	6±0.00	20±0.01	0
	30	15.81±0.01	13±0.02	37±0.04	0
	45	27.18±0.05	19±0.01	44±0.01	0
	60	35.02±0.01	25±0.00	53±0.02	0
	180	40.11±0.00	31±0.00	81±1.09	32.41±0.17

Data shows mean $\pm$ standard deviation of 5 replicates, indicate significant differences between the control and treated plants ($P < 0.05$); Gf=*Glomus fasciculatum*, Fo=*Fusarium oxysporum*

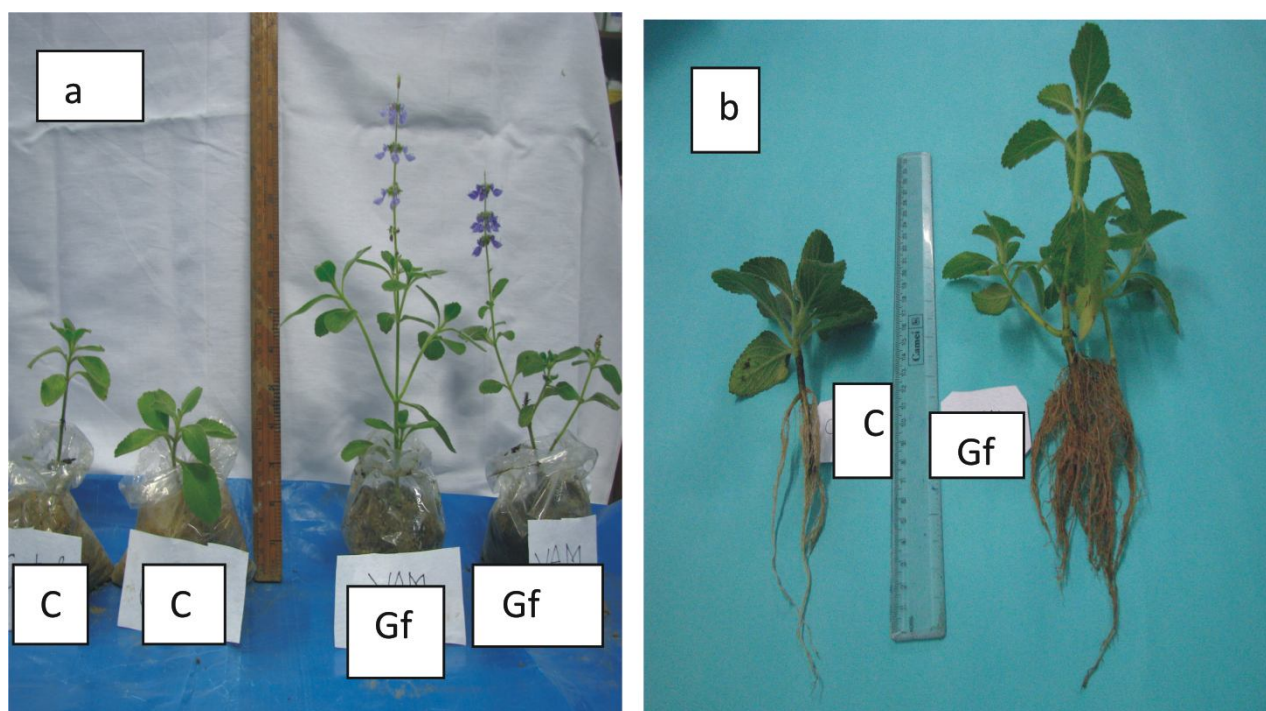


Fig.2: Effect of AM fungus (*G. fasciculatum*) [Gf] on growth parameters (a) increased vigour with flowering stage in inoculated plant and (b) increased number of roots than the control (C).

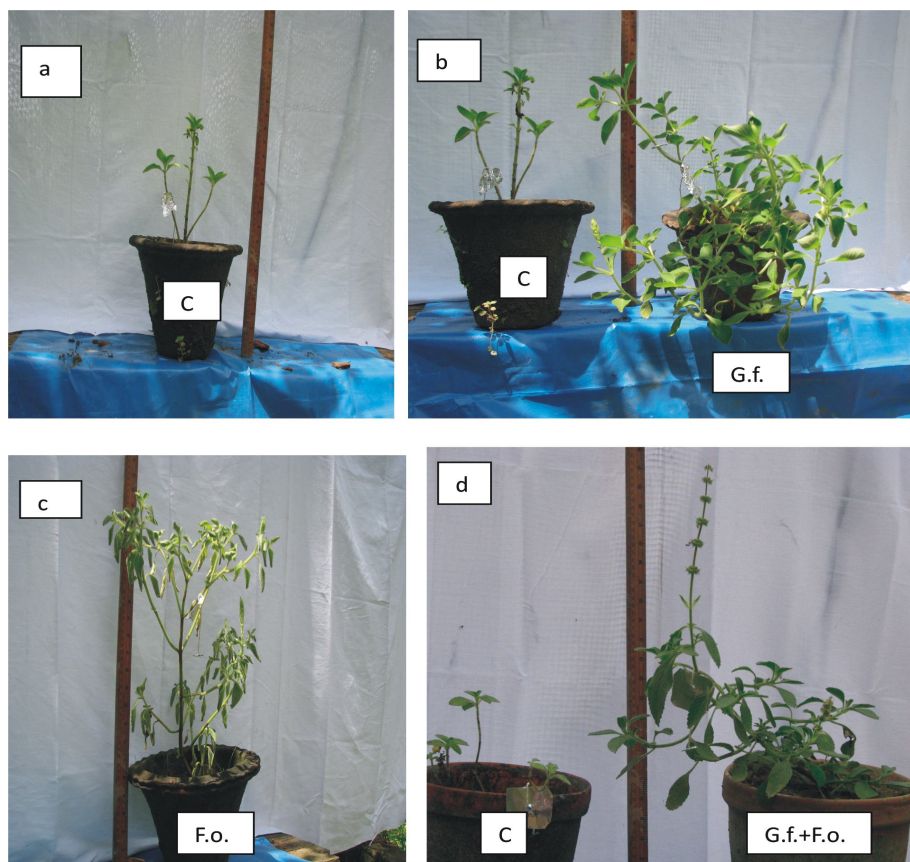


Fig.3: Photographs showing the effect of AM fungus, *G. fasciculatum* on growth and vigour of *Coleus* plants: (a) Control [C], (b) *G. fasciculatum* [G.f.] treated plant, (c) *F. oxysporum* [F.o.] treated plant, and (d) *G. fasciculatum* + *F. oxysporum* [G.f.+ F.o.] treated plant.

Table 4: Effect of *Glomus fasciculatum*, *Trichoderma* spp. and the pathogen on growth and yield of tuber of the plant.

Treatment	Days after cuttings planted	Plant height (cm)	Number of Twigs/plant	Number of Leaflets/plant	Fresh weight of tuber (g/plant)
Control	15	5.10±0.00	5.30±0.00	17±0.00	0
	30	7.42±0.01	10±0.02	29±0.01	0
	45	11.20±0.01	15±0.03	38±0.00	0
	60	19.13±0.03	20±0.02	47±0.01	0
	180	27.37±0.03	25±0.01	61±0.00	6.89±0.24
Gf+Th+Fo	15	11.03±0.02	9±0.53	25±0.03	0
	30	17.08±0.01	14±0.00	40±0.01	0
	45	29.02±0.02	21±0.01	55±0.03	0
	60	38.01±0.00	30±0.02	71±0.01	0
	180	47.30±0.01	37±0.02	90±0.00	48.25±0.08
Gf+Tv+Fo	15	10.22±0.01	7.21±0.00	23±0.00	0
	30	16.33±0.06	13±0.03	38±0.02	0
	45	28.04±0.02	18±0.02	49±0.00	0
	60	35.44±0.01	27±0.00	61±0.00	0
	180	41.41±0.02	32±0.01	83±0.00	40.76±0.05

AMF treatment enhanced the vigour as well as promoted the flowering of plants (Fig. 2a). Besides, mycorrhizal plants showed maximum number of roots in comparison to the control plants (Fig.2b).

The data recorded in Table 4 shows the results of the interactive effect of *T.harzianum* and *T.viride* individually with the VAM fungus, *G. fasciculatum* and the pathogen on growth and tuber yield of *Coleus* plants. It is apparent that among the two *Trichoderma* species, integration with *T. harzianum* showed maximum herbage in respect of plant height, number of twigs, number of leaflets and fresh weight of tuber followed by *T.viride*. Thus integration of *T. harzianum* with the AM fungus is proved to be effective in suppressing the hazardous effect of the pathogen on the plants. It is evident from the result (Table 1) that

mycorrhization, chlorophyll content and leaf phosphate were found to be significantly ($P < 0.05$) higher in plant treated with *Glomus fasciculatum* (AMF) than other treatments. Treatment dually with *G. fasciculatum* and *F. oxysporum* also showed increased chlorophyll content and leaf phosphate compared to the control plants. Integrated treatment with AMF and antagonistic *Trichoderma* spp. against the pathogen also resulted in greater amount of chlorophyll and phosphate than the control plants as well as AMF + pathogen treated plants (Table 2). Among the *Trichoderma* species, *T. harzianum* was found to be superior over the other species. It is apparent from the result that the application of AM fungus alone and dual treatment with AMF + *Trichoderma* spp. were able to suppress more or less the activity of the pathogen, *F. oxysporum*.

DISCUSSION

Findings of the present study are in conformity with the reports of Wang *et al.* (2022) who obtained significant increase in phosphorus in *F.oxysporum* infected tomato plants when inoculated with *Glomus* sp. Chakraborty *et al.* (2008) also recorded enhanced phosphorus level in mycorrhiza treated plants. Disease inhibition by arbuscular mycorrhizal fungi was linked to their ameliorative effects for plant nutrients especially phosphorus content (Ojha, 2008). Variation in phosphorus nutrition influences host-defence-related gene expression (Khan *et al.* 2023). Hazzoumi *et al.* (2022) reported that plant root colonized with *Glomus* sp. showed greater amount of chlorophyll which is in full conformity with the present findings. The increase in total chlorophyll content in AMF inoculated *Coleus* plants may be due to increased uptake of phosphorus, which will increase the photosynthetic activity of the plants and ultimately the chlorophyll content in plants (Khatun, 2020a). Of the different microbes interacting with roots, symbiotic mycorrhizal fungi are the most ubiquitous which actively help the plants in absorbing nutrients under natural conditions. These fungi use some of the root exudates and modify root physiology thereby altering the microbial equilibrium on the root surface (Khaliq *et al.* 2022). Concomitant colonization and infection of roots by mycorrhizal fungi, pathogens and other microbes inevitably interfere with each other activities (George and Ray, 2023). These interactions are of great importance, if maintained or enhanced that could result in biological control of plant pathogens (Lahlali *et al.* 2022). AM fungi are known to impart resistance to the host plant against soil borne wilt pathogen, particularly *Fusarium* spp. (Wang *et al.* 2022). Wang *et al.* (2022) reported that mycorrhization with *Glomus* sp. may be an effective means of management tool in wilt disease caused by *F. oxysporum* and propagules of pathogenic fungi steadily declined in various treatments. Khatun and Chatterjee (2011) reported that an AM fungus *Glomus fasciculatum* systemically induced resistance against wilt disease of *Coleus forskohlii* via production of phenolics, the defence related enzymes phenylalanine ammonia-lyase and polyphenol oxidase, and pathogenesis-related

proteins, and peroxidase in response to *Fusarium oxysporum* infection. Root colonization with *G. fasciculatum* (AMF) amounts to enhancement of plant growth. The plant height, number of branching twigs and leaflets and fresh weight of tuber were increased in AMF treated plant over the other treatments. AMF+*F. oxysporum* treated plants were found to have significant ($P < 0.05$) improvement in terms of growth over the control treatments. The present findings also run parallel with the earlier reports indicating that plants inoculated with *Glomus* sp. exhibited greater vigour in terms of different growth parameters (Sun *et al.* 2022). Khatun and Chatterjee (2011) reported that *G. fasciculatum* reduced the disease severity and promoted plant growth significantly ($P < 0.05$) in single-treated and in combination with *F. oxysporum*-treated plants compared to control plants. The results suggested that *G. fasciculatum* is a potentially effective protection agent against *F. oxysporum*. Gogoi and Singh (2011) reported that AMF were found to be the most excellent for endorsement of favourable growth, nutritional responses and economic yield in *Piper longum* production. Mycorrhizal plants are known to have altered nutritional status, increased photosynthetic rates, altered levels of growth regulating substances and altered pattern of root exudation due to changes in membrane permeability. These physiological changes along with the physical and chemical presence of external hyphae of the mycorrhizal fungi significantly alter the chemical, physical and microbiological composition of the rhizosphere soil (Weng *et al.* 2022). Wang *et al.* (2022) suggested that AM fungi are able to increase nutritional status of the host plant thereby improving resistance against the pathogens. Root tissues colonized first by AMF physically exclude a pathogen competing for the same infection site. High chitinase activity of the mycorrhizal tissue inhibits the growth of pathogen competing for the same infection site and also inhibits the growth of the pathogen (Weng *et al.* 2022). Arbuscular mycorrhizal fungi may limit physiological and microbial modifications in the mycorrhizosphere, competition for space and nutrients, changes in root-system, altering the chemical composition of plant tissues and the activation of plant defense mechanisms (Wahab *et al.* 2023). Bennet *et al.* (2006) recognized that mycorrhizal fungi could



Fig 4 : Photographs showing the effect of AM fungus, *G. fasciculatum* on tuber yield of *Coleus* plants: (a) Control [C], (b) *G. fasciculatum* [G.f.] treated tuber, and (c) *G. fasciculatum* + *F. oxysporum* [G.f.+ F.o.] treated tubers.

directly interfere with pathogen infection, herbivory and parasitism by occupying root space. In addition, mycorrhizal different metabolites and impart resistance to the host tissue against pathogen invasion and mycorrhizal fungi also known to produce antifungal and antibacterial antibiotics (Weng *et al.* 2022). Trindade *et al.* (2019) postulated that the AM fungi indirectly induce changes in host tissues. They stimulate lignifications or development of callosities or lignitubers in the host cells and thus create a physical barrier to check penetration of the pathogen. The increase in amino acids (Matthew *et al.* 2012), sugars (Jin *et al.* 2024), phenolic compounds (Duc *et al.* 2020) and enzyme activity (Wang *et al.* 2022) in the roots of mycorrhizal plants has been suggested as the possible reasons for checking of disease development on mycorrhizal plants. Occurrence of phytoalexins in mycorrhizal interactions may impart a role in biological control of plant diseases (Lahlali *et al.* 2022). It is apparent that in combined treatment of *Coleus* plant with AMF and antagonistic fungi viz. *T. harzianum* and *T. viride* against the pathogen, all growth parameters happened to be more vigorous than AMF + pathogen treated and

control sets. Among the two species of *Trichoderma*, *T. harzianum* exhibited maximum effect in respect of enhanced growth followed by *T. viride*. This result fully complies with the previous findings (Khatun, 2020b) where *T. harzianum* proved its excellency as a most potent antagonistic agent. *Trichoderma* spp. are known to induce plant growth by producing growth regulating factors (Tyœkiewicz *et al.* 2022) and suppressing the activity of pathogenic organisms (Yao *et al.* 2023). Recently it was found to enhance the activity of AM fungus and hence was designated as Mycorrhiza Helper Organism (MHO) [Khaliq *et al.* 2022]. But the information available on the interaction between AM fungi and *Trichoderma* and their consequential effect on the plant growth is meager. Recently it was reported that dual inoculation with *Glomus mosseae* and the Plant Growth Promoting Rhizomicroorganism (PGPR)/(MHO), *Trichoderma harzianum* not only improved growth, biomass yield, P nutrition and andrographolide concentration of kalmegh but also helped in saving 25% of P fertilizer application (Arpana and Bagyaraj, 2007). In the present investigation *Trichoderma* spp. may suppress the growth of the pathogen, *F. oxysporum*, thereby inhibiting the pathogenic stress to the plant and resulting in vigorous plant growth. Effectiveness of different *Trichoderma* species against *F. oxysporum* were reported by Brizuela *et al.* (2023). Synergistic effect of *Glomus* sp. in combination with the antagonistic fungi *Trichoderma* spp. on disease suppression was observed by many workers (Zhou *et al.* 2021, Khuong *et al.* 2023). Martínez-Medina *et al.* (2009) recorded that combined inoculation of *Glomus* sp. and *T. harzianum* was most effective in suppressing root diseases. *Trichoderma* spp. stimulate mycorrhizae formation (lula *et al.* 2021) and also stimulate the growth of the mycelium of *Glomus* sp. (Tyœkiewicz *et al.* 2022). Integration of *Trichoderma* spp. with mycorrhizal fungi improves plant growth and vigour which might have a positive role on suppression of root diseases (Yao *et al.* 2023). Ghosh *et al.* (2017) revealed that *Trichoderma* species are efficient producers of iron-chelating ligands, siderophores which can bind with iron in a high specificity and affinity thus making the iron unavailable for other microorganisms and thereby limiting the growth of the pathogen. Govic *et al.* (2020) reported the

production of hydroxamate and catecholate type of siderophores by mycorrhizal fungi. Thus the siderophores produced by mycorrhizal fungi together with that of antagonistic fungi may further create an iron starvation condition for the pathogen which, in turn, checks the growth of the pathogen. So the present investigation summarizes that the use of mycorrhizal fungi may provide an effective alternative method to control the disease as well as to ameliorate the plant vigour.

DECLARATION

Conflict of Interest. Author declares no conflict of interest.

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