PLANT GROWTH PROMOTING AND ROOT COLONIZATION CAPABILITY OF *Trichoderma longibrachiatum* (DT1) IN *Piper nigrum* L.

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Abstract: The role of *T. longibrachiatum* in promoting growth of the plant has been studied on susceptible (Panniyur-7) and resistant (Karimunda) *P. nigrum* cuttings. A good development in the growth (root numbers, shoot length and weight of roots and shoot) was recorded in the treated cuttings on 25th day when compared to control (uninoculated). Maximum number of roots developed in susceptible DT1 treated (ST) and treated along with *P. capsici* inoculated (STP) (33.66 in both) when compared to control and inoculated. Similar increase was found in resistance DT1 treated (RT) and RTP (40.66 in both) when compared to control. The length of the shoot increased in ST and STP (32.63 and 32.66 respectively) when compared to control and inoculated. Similar increase was noted in RT and RTP (37.7 cm in both). The root fresh weight in ST, STP, RT and RTP increased (1.90, 1.92, and 1.93 in both respectively) when compared to control and inoculated. An increase in root dry weight was also found. Similarly shoot fresh weight increased in ST, STP, RT and RTP (7.01, 6.94, and 6.95 in both respectively). There was also an increase in dry weight.

Key Words- *Trichoderma longibrachiatum* (DT1), *Phytophthora capsici* L. Panniyur 7, Karimunda, *Piper nigrum* L.

INTRODUCTION

The 'King of Spices', black pepper popularly known as black gold is an important revenue crop among the spices grown in India especially in the Western Ghats of South India (Abbasi *et al*., 2009). But there has been economic loss to the planters as there is a serious threat to black pepper cultivation due to repeated occurrence of foot rot/quick wilt disease caused by *Phytophthora capsici* L. during rainy season (Dagade, 2003). *Phytophthora capsici* L. is a soil borne pathogen and they have a complex behavioral pattern and biochemical constituents (Gaigale *et al*., 2011). Hence it is difficult to control the disease by cultural practices and fungicide application. Therefore the use of biocontrol agents to combat the disease is necessary.

Trichoderma are fast-growing, highly adaptable to symbiotic relationship with plant roots, thus making them an important biocontrol agent. Apart from their biocontrol ability they can colonize and penetrate the root tissues of the plant initiating a series of morphological and biochemical changes in the plant which help in plant defense response (Saba *et al*., 2012). *Trichoderma spp*. can be an alternative against soil borne pathogens as most of the species promote plant growth, nutrient uptake and induce plant defense enzymes in response to biotic and abiotic stresses (Zehra, *et al*., 2017) facilitate root colonization, the coordination of defense mechanisms (Hermosa *et al*., 2012). The use of chemical fungicides is detrimental to human and animal health as well as other rhizospheric soil microbes. The increasing demand for organic products has led to the use of biological agents in controlling crop diseases and promoting plant growth.

Trichoderma longibrachiatum is one such fungus in the genus Trichoderma which is a soil fungi found all over the world which has been used as a biocontrol agent and plant growth promoter (Zhang *et al*., 2014;

Sobowale, 2010; Lee *et al*., 2016). *Trichoderma asperellum*, *T. harzianum*, *T. longibrachiatum* and *T. reesei* are among the species with promising strains for biocontrol purposes (Maymon *et al*., 2004; Blaszczyk *et al*., 2014) but no studies have been carried out on the potential of *T. longibrachiatum* in promoting plant growth of *P. nigrum* and its root colonization ability. Hence in the present study we have focused on it.

MATERIALS AND METHODS

The plant cultivars/varieties

Two varieties of *Piper nigrum*, Panniyur-7 and Karimunda collected from Pepper Research Station (Kerala Agriculture University), Panniyur, Kerala already screened for its susceptibility and resistance respectively in the Botanical Garden, Department of Studies in Botany, University of Mysore, Mysore, Karnataka were used for the study. These cultivars were used for studying the growth parameters, root colonization as well as enzyme assay studies.

The zoospore suspension

Phytophthora capsici L. isolated from the soil of diseased vine isolated from a plantation in Kanthoor village, Madikeri taluk of Kodagu district, Karnataka was cultured on Carrot Agar Medium (Indira and Smitha, 2013). The profusely growing 7-day old cultures were cut into pieces, flooded with sterile distilled water and kept for incubation for 72 hrs. at 24^0C. Then they were placed at 5^0C for 1 hr. and incubated for 72 hr. at 24^0C for 30-60 mins. (Larkin *et al*., 1995). The suspension was then filtered through two layers of muslin cloth to remove the hyphal and sporangial debris. The zoospore suspension containing $1x10^5$ propagules/ml was counted using haemocytometer (Anith *et al*., 2002., Du, 2013).

The *Trichoderma longibrachiatum* (DT1) spore suspension

T. longibrachiatum isolated from the rhizospheric soil of black pepper plantation in Kanthoor village, Madikeri taluk of Kodagu district, Karnataka was used for inoculum preparation. DT1 was cultured on Potato Dextrose Agar (PDA) media for up to 7 days and a 100ml inoculum containing 1.5×10^8 CFU/ml spore suspension was prepared (Zhang *et al*., 2014).

Sample collection for growth parameter as well as enzyme assay studies

Six cuttings i.e. about three each were dipped in DT1 spore suspension for 10 mins and planted in polythene nursery bags (16x25 cm) filled with sterilized field soil, compost and sand (1:1:1) under green house. The zoospore suspension was then added to 10cm holes made near the roots of the cutting. Zoospore infection was done to three DT1 treated cuttings and separate three non treated ones. One set (three cuttings) were maintained as control without treatment or inoculation. The cuttings were left to acclimatize until new leaf emerged.

Growth parameter

Cuttings were removed from the soil on 25th day; the roots were washed under running tap water to remove soil debris and further used for growth parameter and root colonization. The cuttings were analyzed for the number of roots, fresh and dry weights of the root and shoot, shoot length.

Root colonization by *Trichoderma longibrachiatum* (DT1)

The treated *P. nigrum* cuttings of susceptible and resistant varieties with DT1 treated as well treated along with *P. capsici* inoculated (25^{th} day) were uprooted, roots washed under running tap water and dried using tissue to remove water. The roots were cut into small pieces and plated on Potato Dextrose Agar (PDA) media and incubated at room temperature to confirm the growth of DT1 from the roots.

Endophytic root tissue colonization by *Trichoderma longibrachiatum* (DT1)

The colonized *P. nigrum* roots were washed under running tap water. They were cut into 1cm pieces and put in a beaker containing 10% KOH solution and heated for 5-10 min. The solution was then discarded and the roots washed in 1% HCl for 3 min and the HCl discarded. The roots were then stained with lactophenol trypan blue and observed under light microscope (Adriansyah, 2016).

RESULTS

Plants treated with *T. longibrachiatum* (DT1) were left to acclimatize until new leaf emerged (20 days) and those inoculated with *P. capsici* were uprooted on 25th day and used for the above experiments.

Growth Parameters

DT1 significantly affected the number of roots, shoot length and their fresh and dry weights **(fig. 1 and 2)**. After treatment and before inoculation with the pathogen *P. capsici* the cuttings were left to acclimatize in green house until new leaf emerged (20 days). After 20 days pathogen was inoculated and readings recorded after 25 days post pathogen inoculation (Dpi). *Piper nigrum* rooted cuttings, both susceptible (Panniyur-7) and resistant (Karimunda) dipped in DT1 spore suspension and planted showed increase in root number and similar increase was recorded in treated along with pathogen inoculated cuttings when compared to control and inoculated ones. Likewise there was a minute difference in shoot length of susceptible inoculated cuttings when compared to control, but the treated ones showed similar increase in length. The resistant cuttings also showed increase in shoot length when compared to control and inoculated **(fig. 3A)**. The treated combined with inoculated cuttings showed increase in fresh and dry weights of roots and shoots when compared to control, but the susceptible cuttings inoculated with *P. capsici* showed decrease in weights **(fig. 3B)**.

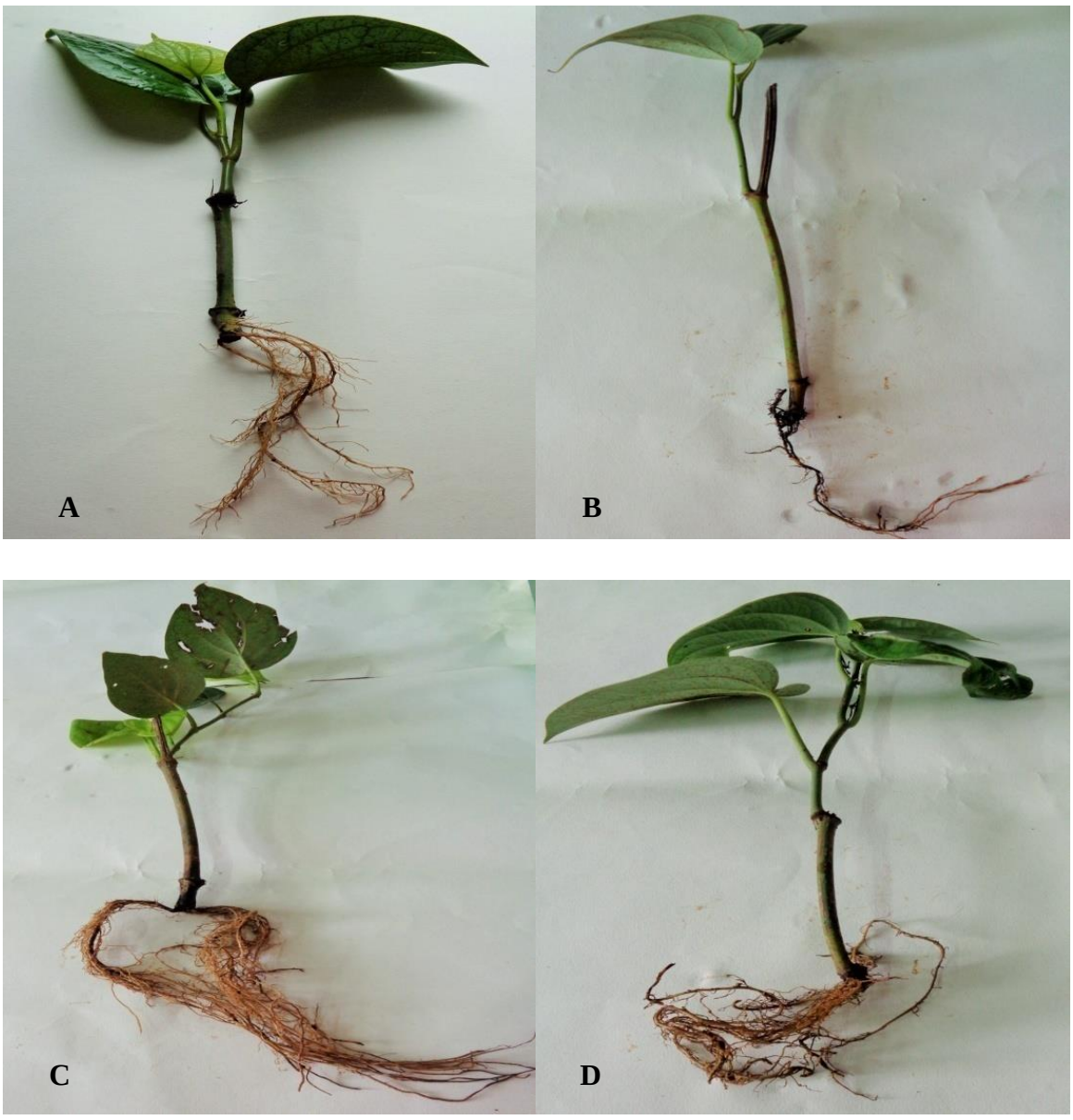


fig. 1. Growth parameter of Susceptible (Panniyur 7) *P. nigrum* cuttings (25th day). (A) Control (uninoculated), (B) *P. capsici* inoculated, (C) *T. longibrachiatum* treated, (D) Treated+*P. capsici* inoculated.

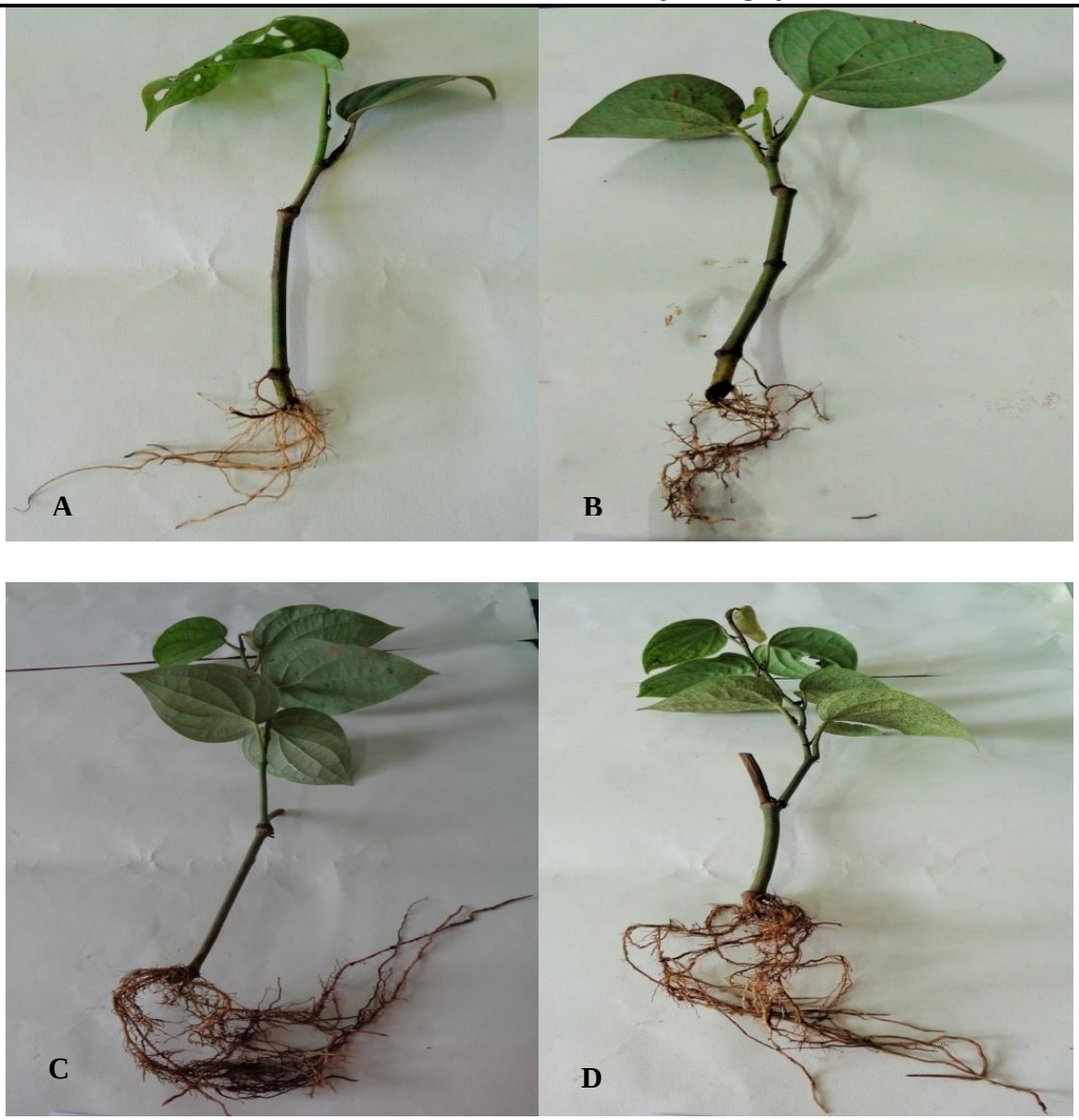


fig. 2. Growth parameter of Resistant (Karimunda) *P. nigrum* cuttings (25th day). (A) Control (uninoculated), (B) *P. capsici* inoculated, (C) *T. longibrachiatum* treated, (D) Treated+*P. capsici* inoculated.

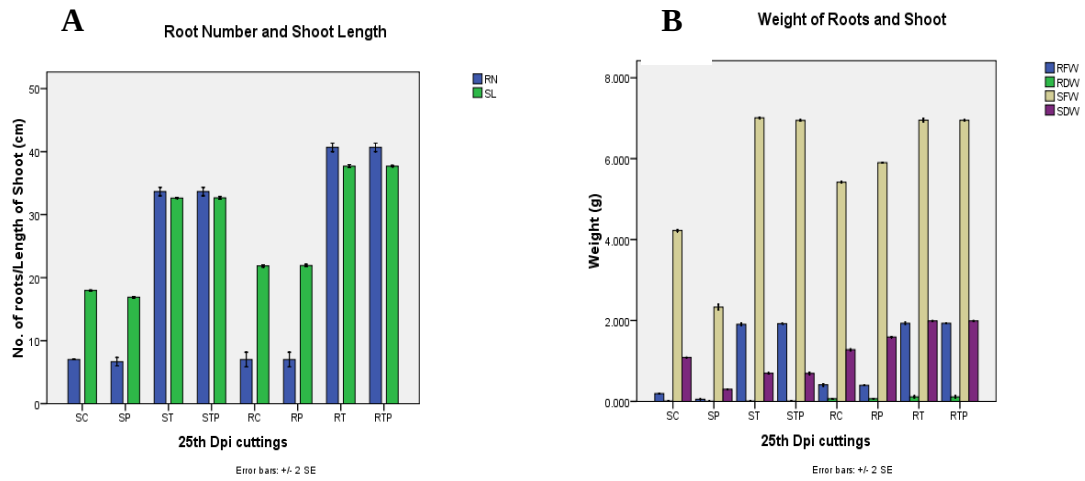


fig.3. Growth parameters of susceptible (Panniyur-7) and resistant (Karimunda) varieties/cultivars of *Piper nigrum* L. (A) Root number and shoot length (B) Root and shoot weight. Where RN=No. of roots, SL=Shoot Length, SFW=Shoot fresh weight, RFW=Root fresh weight, SDW=Shoot dry weight and RDW=Root dry weight. Mean values were of 3 replicates. T Bars represent Standard error (p<0.0).

Root colonization

Trichoderma longibrachiatum was reisolated from the treated and treated along with *P. capsici* inoculated root segments of susceptible and resistant *P. nigrum* cuttings. This proved that they were successfully able to colonize the roots of *P. nigrum* **(fig. 4 and 5)**.

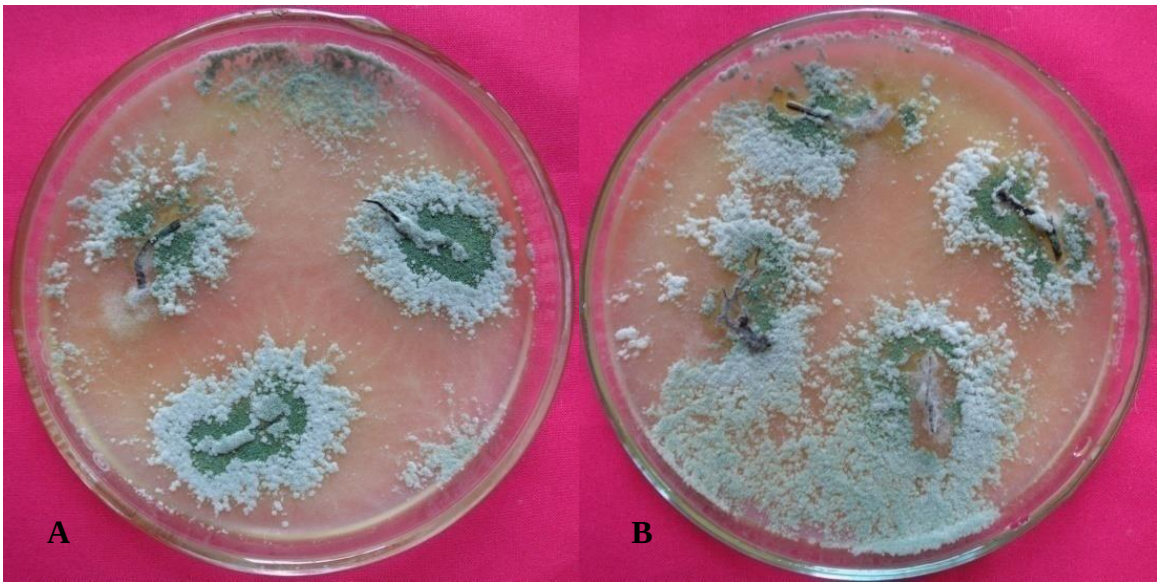


fig. 4 . Root colonization by *T. longibrachiatum* (A) susceptible and (B) resistant *Trichoderma* treated.

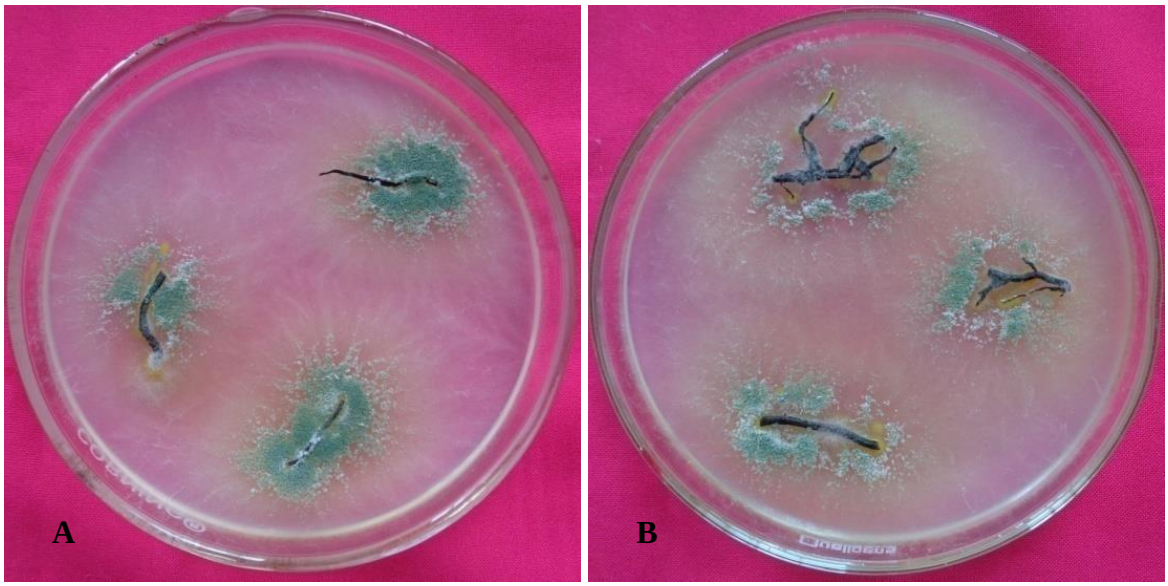


fig. 5 . Root colonization by Trichoderma. (A) susceptible and (B) resistant *T. longibrachiatum* treated+*P. capsici* inoculated root segments of *P. nigrum* cuttings.

Endophytic root tissue colonization by *Trichoderma longibrachiatum* (DT1)

Coiling of DT1 in the endophytic tissues of *P. nigrum* treated with DT1 as well as those treated and inoculated with *P. capsici* was observed in the 25th day plants of both susceptible and control cutting whereas no trace of DT1 was observed in the control plants **(fig. 6 and 7)**.

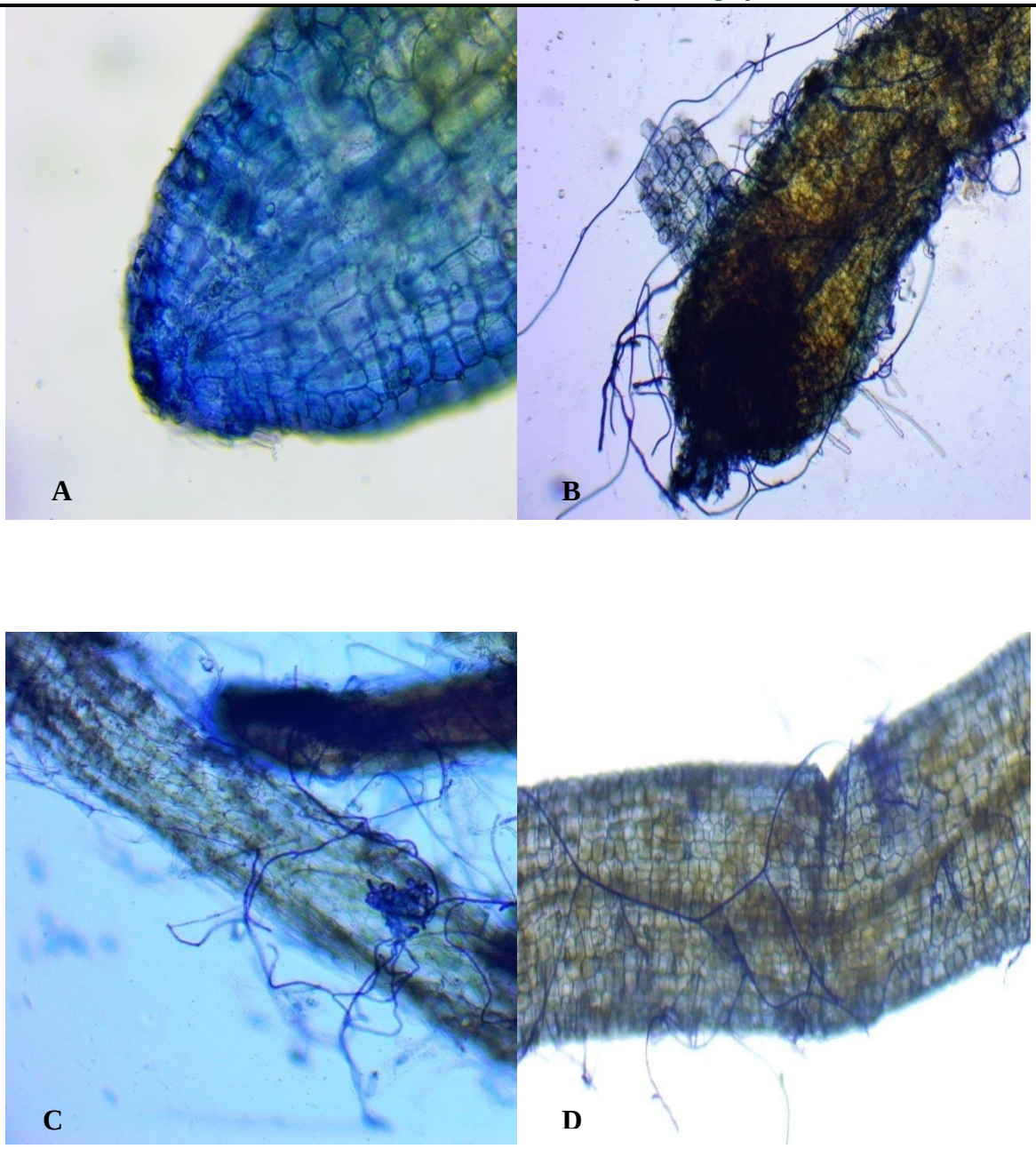
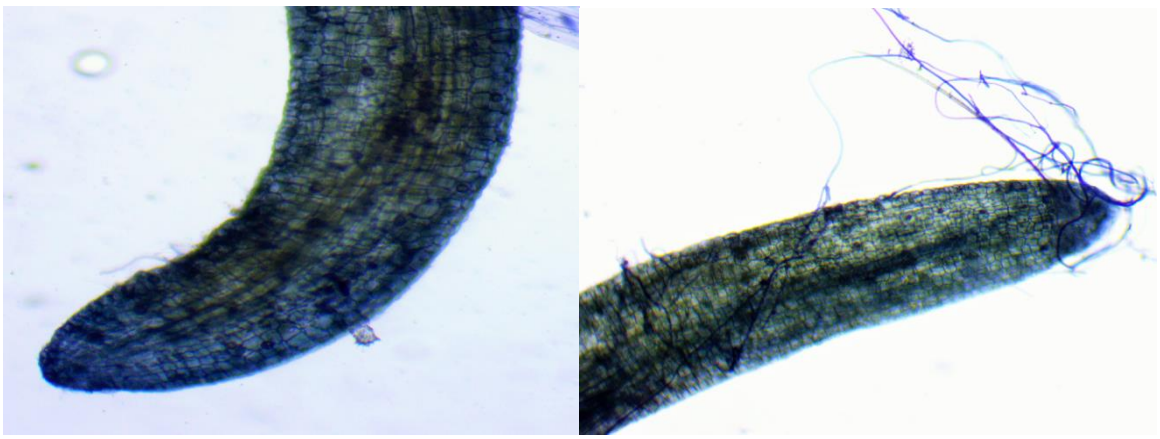


fig. 6. Colonization of *T. longibrachiatum* in the endophytic root tissues of susceptible (Panniyur 7) *P. nigrum* cuttings on 25th day. (A) Control (no presence of Trichoderma), (B) *T. longibrachiatum* colonization in treated as well as *P. capsici* inoculated roots, (C) Colonization in treated roots (D) Presence of coiling hyphae.



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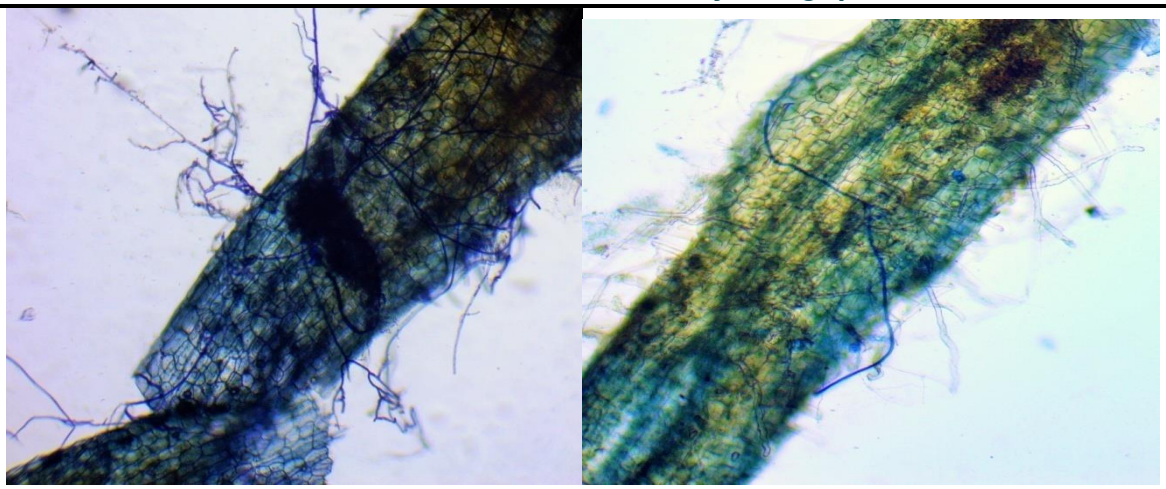

fig. 7. Colonization of *T. longibrachiatum* in the endophytic root tissues of resistant (Karimunda) *P. nigrum* cuttings on 25th day. (A) Control (no presence of Trichoderma), (B) *T. longibrachiatum* colonization in treated as well as *P. capsici* inoculated roots, (C) Colonization in treated roots (D) Presence of coiling hyphae.

Discussion

Trichoderma longibrachiatum (1.5×10^7 spores/ml) treated cucumber seedlings after inoculation with second stage *Meloidogyne incognita* juveniles increased the growth of seedlings 10 weeks after inoculation (Zhang *et al*., 2014). Cadusafos concentration of 1.7mg/kg soil and *T. longibrachiatum* (10^8conidia/ml) caused best growth of zucchini plants (Sokhandani *et al*., 2016). *T. longibrachiatum* was able to promote growth of bean plant, but not as well as *T. harzianum*. Wheat seedlings inoculated with *T. longibrachiatum* (1.5×10^4 and 1.5×10^8 spores/ml) increased plant height, root length and plant biomass (Zhang *et al*., 2014).

T. harzianum (MTCC 5179) (talc formulation 3.5g/3kg soil) was inoculated to the soil and after 120 days the single node *P. nigrum* cuttings showed an increase in fresh and dry weights of roots and shoot and height of plant (Umadevi *et al*., 2017). *P. nigrum* var. Pournami developed high number of roots in *T. harzianum* (P-26) applied potting mixture along with application of *Pseudomonas fluorescens* thrice when compared to Subhakara, both of which are high yielding varieties. Similarly there was an increase in plant height also (Thankamani *et al*., 2005). Studies have shown that the root colonization of *Trichoderma spp*. accumulates antimicrobial compounds and several proteinaceous elicitors in plant roots (Saravanakumar *et al*., 2016). They can colonize and grow on plant root system, thus increasing growth of the plant and also protect the roots from pathogenic infection (Harman *et al*., 2004) and in order to do so they must colonize the plant roots which involves the ability to recognize and adhere to the roots, penetrate the plant and withstand toxic metabolites produced by the plant in response to invasion (Viterbo and Chet, 2006).

Conclusion

The present study proves that *T. longibrachiatum* promotes growth of *P. nigrum* L. plants and also it has the ability to colonize the roots and form a symbiotic relationship with the plant. Hence they can be used as a biofertilizer. Although findings on *Trichoderma spp.* promoting growth of *P. nigrum* are available, there has been no research on the use of *T. longibrachiatum* in growth promotion of *P. nigrum.*

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