



Influence of *Trichoderma harzianum* (NFCCI 2241) on growth of some *Ocimum* species

K. Shobha¹ and Y. Rahel Ratnakumari*²

¹ M.Sc. student, Anwarul Uloom College, Hyderabad, India.

E-mail: shobhakagithi997@gmail.com

² Corresponding author: Department of Botany, Osmania University, Hyderabad.

E-mail: rahel1984@gmail.com

Abstract

Basil plant is important in the traditional Ayurveda, Unani and Siddha systems of medicine. Basil leaves are used in making sauces, soups, beverages and as flavouring agent. The whole plant is used to cure cold, headache, cough, colic pain, digestive disorders, fever etc. Root colonization by *Trichoderma* spp. also frequently enhances yield and crop productivity. The present study is carried out to know the effect of NFCCI 2241 (*T. harzianum*) on growth of the basil varieties in *in vivo*. For this experiment following basil varieties are taken. *Ocimum basilicum* L. is commonly known as Genovese green Basil (*O. basilicum* variety-1) and Sweet Basil (*O. basilicum* variety-2) is another variety in *O. basilicum*. *Ocimum tenuiflorum* L. has two varieties and those are Rama Tulsi (*O. tenuiflorum* variety-1) and Krishna Tulsi (*O. tenuiflorum* variety-2). In present study NFCCI 2241 treated *Ocimum* species plants showed more shoot length, root length, leaf area and biomass compared to the control treatment. When the application of NFCCI 2241, biomass enhanced in *O. tenuiflorum* (variety-1) 139% and in *O. tenuiflorum* (variety-2) 267.51%. NFCCI 2241 treated *O. basilicum* (variety-1) plants biomass enhanced up to 109.38% and in *O. basilicum* (variety-2) 152.90% compared to the control treatment.

Keywords: *Ocimum basilicum*, *Ocimum tenuiflorum*, *Trichoderma harzianum*

Introduction

Ocimum genus commonly known as basil or tulsi belongs to Lamiaceae family and comprises of 63 species. It is a native plant to tropical and sub-tropical regions of the world (Kalita et al., 2018; Walters and Currey, 2015). Basil plant is important in the traditional Ayurveda, Unani and Siddha systems of medicine. It is grown as an aromatic and medicinal plant throughout the world for its essential oil production. The essential oil of basil contains terpenoids and phenylpropanoids, linalool and eugenol, and estragole, methyl cinnamate, methyl chavicol, 1,8-cineole, neral, geraniol, and

caryophyllene oxide (Ashok et al., 2018, Askarinia et al., 2019; Raina et al., 2016; Sunita, 2016).

Ocimum basilicum L. also known as Holy basil or sacred basil or Queen of herbs is native to India and cultivated widely as a sacred plant. It holds a prominent place in India and is worshipped by Hindus as a holy plant. It is also widely cultivated in South East Asian countries (Alhasan et al., 2020; Hanumanthaiah et al., 2020). It is widely used in cosmetic and pharmaceutical industries for its varied medicinal properties (Smitha and Vandana, 2019). *O. basilicum* is commonly known as sweet basil and Genovese is another variety in *O. basilicum*.

Ocimum tenuiflorum L. has two varieties and those are *O. tenuiflorum* variety-1 (Rama Tulsi) and *O. tenuiflorum* variety-2 (Krishna Tulsi). Krishna Tulsi and Rama Tulsi are having similar chemical constituents and common medicinal properties (Das and Vasudevan, 2006). The leaves of basil are used in making sauces, soups, salad, beverages and as flavouring agent. The whole plant is used for treating cold, headache, cough, colic pain, digestive disorders, fever, kidney complaints, bronchitis, malaria, skin disease, arthritis, eye diseases, diarrhoea, fertility, diabetic, nerves, sharpen memory, ulcers, mouth infections, and insect bites. Its oil is used directly on skin to treat acne (SafaaM et al., 2015).

The need for increasing crop productivity and essential oil production has led to an excessive use of chemical fertilizers, creating serious environmental pollution. Due to the importance of medicinal value of the medicinal plant chemical fertilizers/pesticides usage is not recommended as small amount of chemical residue traced in the by-products/active compounds are not utilized for pharmaceutical purpose. Hence, the use of bio-pesticides or biofertilizers as an alternative to chemical based fertilizers/pesticides in agricultural production is rapidly increasing due to public concerns about human health, productivity, yield, safety of agri-food products, consumed and impact to the environment. *Trichoderma* spp. have been widely studied, and reported to have an ability to protect plants, growth enhancer, stimulant of natural

resistance and as well as to act as soil amendments at a low dosage. *Trichoderma* spp. is free-living fungi that are commonly found in soil and root ecosystems. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to biotic/abiotic stresses and uptake and use of nutrients, increased carbohydrate metabolism, photosynthesis, and phytohormone synthesis (Harman et al., 2004; Stewart and Hill, 2014).

Hence, the present study is carried out to know the effect of the *Trichoderma harzianum* on growth of the basil varieties in pot experiments.

Materials and Methods

Plant material for in vivo experiment: *Ocimum basilicum* – variety-1 (Genovese green Basil), and *O. basilicum* – variety-2 (Sweet Basil); *O. tenuiflorum* - variety-1 (Rama Tulasi) Seeds were purchased from Omaxe world finest Herb Seeds company, and *O. tenuiflorum* - variety-2 (Krishna Tulasi) were from Pyramid seeds company, Hyderabad, India.

***Trichoderma harzianum* (NFCCI 2241):** *T. harzianum* is isolated from mint growing area soils, Hyderabad. The fungal strain is deposited in National Fungal Culture Collection Institute (NFCCI) Pune, India.

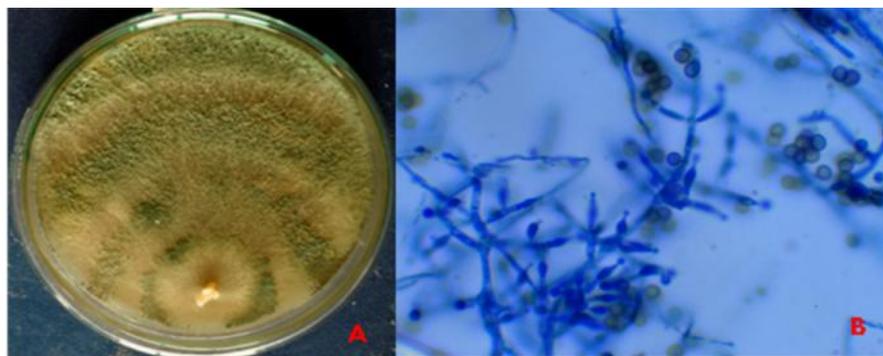


Fig.1. A. NFCCI 2241 (*Trichoderma harzianum*) Culture plate and B. Microphotograph

Mass culture of *Trichoderma harzianum* (NFCCI 2241) preparation: Mass cultures were prepared on Sorghum and Sand (1:3) medium. Sorghum and Sand (1:3) was filled in 2 kg polypropylene bags up to one third. Bags were autoclaved twice at 15 lb pressure. Five mm diameter discs of actively growing mycelium

of each *T. harzianum* isolate was cut from the margin of 3 days old culture of *T. harzianum* (NFCCI 2241) and inoculated into the sorghum and sand contained bags. These bags were incubated at $27 \pm 20^{\circ}\text{C}$ for 30 days (Singh and Singh, 2004).

Pot experiments: Soil application method was used for application of *T. harzianum* to plants. About 20g of *T. harzianum* inoculum having 2×10^7 cfu/g (colony forming units) were mixed with the soil in each pot and without any fungus was added to control pots. The pots were kept in natural conditions for the growth of plants. For each treatment, three replicates were prepared. The experiment was conducted with four varieties of basil plants with two treatments i.e., NFCCI 2241 treatment and control treatment.

Seed germination: For this study 30 seeds per each variety of basil were taken for one pot. For each variety of basil two treatments are kept for this study. Calculate the germination of seeds and percentage germination in the *T. harzianum* (NFCCI 2241) treated pots and control treatment pots.

Analysis of biomass, shoot, root length and leaf area of basil varieties: The shoot length, root length and biomass were recorded after the harvest of crop.

The 3rd and 4th leaves area was recorded and average data was presented in the results.

Results

Seed germination of *Ocimum* spp. varieties when treated with NFCCI 2241: The present study is carried out to know the influence of NFCCI 2241 (*Trichoderma harzianum*) against the seed germination of *Ocimum tenuiflorum*-variety-1 (Rama Tulasi), *Ocimum tenuiflorum*-variety-2 (Krishna Tulasi), *Ocimum basilicum*-variety-1 (Genovese green Basil), and *Ocimum basilicum*-variety-2 (Sweet Basil). Four varieties of *Ocimum* species showed maximum germination when compared to the control treatments. So, the results are clearly proved that, NFCCI 2241 has positive effect on seed germination of all 4 varieties of *Ocimum* species. The maximum percentage of seed germination was 88.33% observed in *O. basilicum*-variety-1, and minimum seed germination was 53.33% in *O. tenuiflorum*-variety-2 (Fig.2).

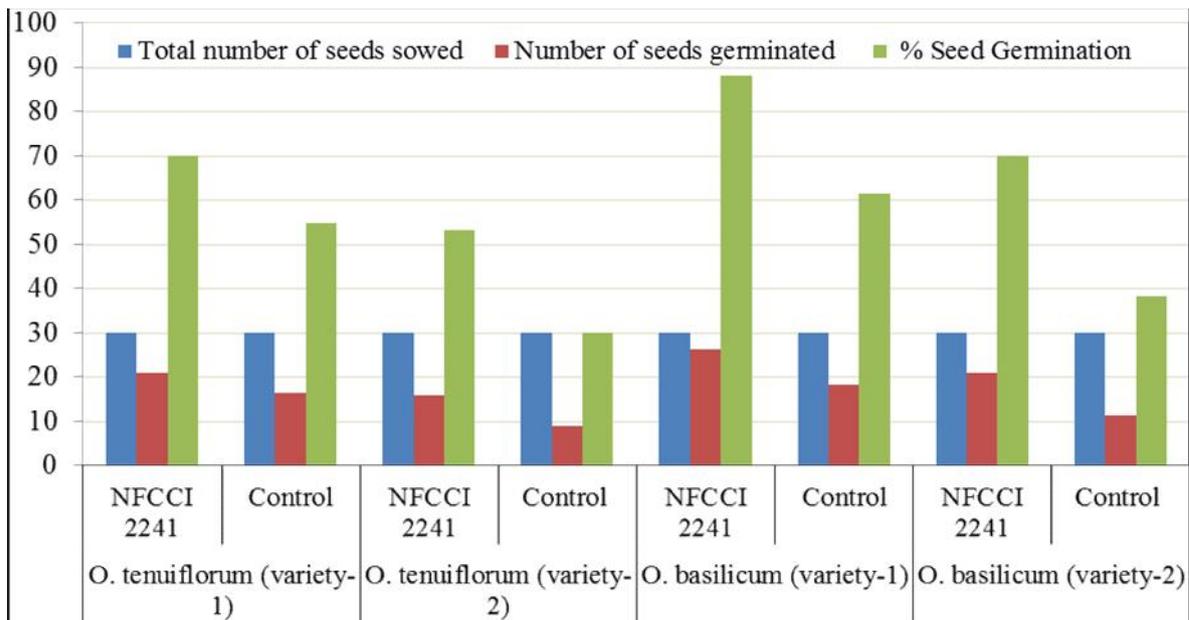


Fig.2. Seed germination of Basil plants treated with NFCCI 2241 (*Trichoderma harzianum*) & control treatment

Plant length and Biomass when treated with NFCCI 2241 and comparison with control: The application of NFCCI 2241 to the *Ocimum* plant varieties enhanced plant length and biomass when compared with control treatment. Among the four varieties of *Ocimum* spp., *O. tenuiflorum* (variety-2) showed maximum shoot and root length of 35.90cm and 30.45cm respectively. In control treatment the shoot and root length was 24.63cm and 14.63cm

respectively. Minimum shoot and root length was 24.18 cm and 20.73 cm respectively in NFCCI 2241 treated *O. basilicum* (variety-2) whereas in control treatment of *O. basilicum* (variety-2) plants the shoot and root lengths observed was 17.27cm and 17.00cm respectively (Fig.3). All the four *Ocimum* varieties showed enhanced biomass in NFCCI 2241 treated pots when compared with control treatment. Maximum biomass was recorded in *O. basilicum* (variety-2)

with 7.92 gms and minimum in *O. tenuiflorum* (variety-1) with 2.79 gms. When compared to control treatment, the two varieties of *O. tenuiflorum* (variety-2) treated with *T. harzianum* (NFCCI 2241) showed 145.76% increases in shoot length and 267.51% increase in biomass. In the same manner, when compared to control, the two varieties of *O. basilicum*

(variety-2) treated with *T. harzianum* (NFCCI 2241) showed 140% increases in shoot length and 152.90% increase in biomass (Fig.4). Hence, this results proved that all varieties of *Ocimum* plants showed positive effect in the growth and biomass when treated with NFCCI 2241 than control.



Fig.3. *Ocimum* species plants shoot and root length

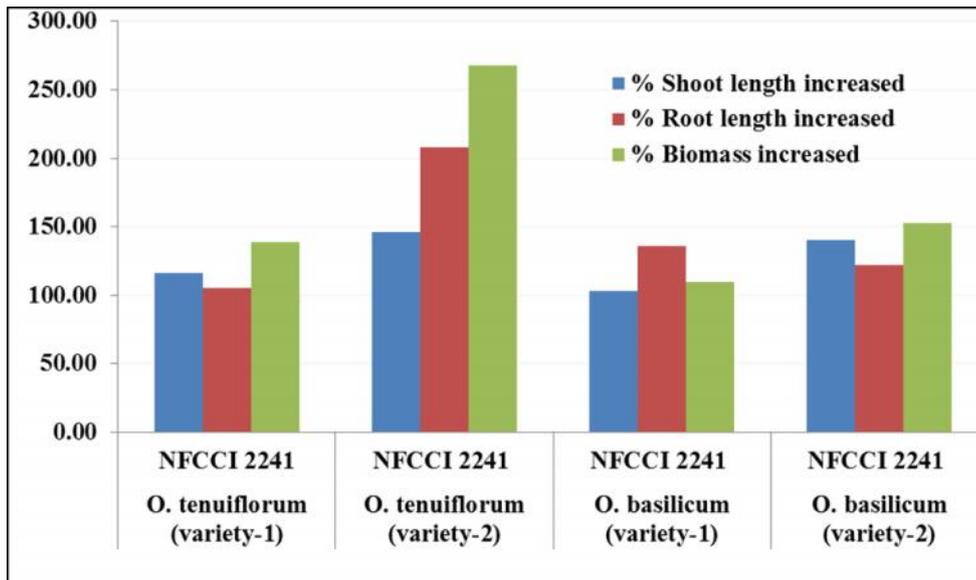


Fig. 4. Plant Shoot length, Root length and Biomass enhanced by NFCCI 2241 when compared with control.

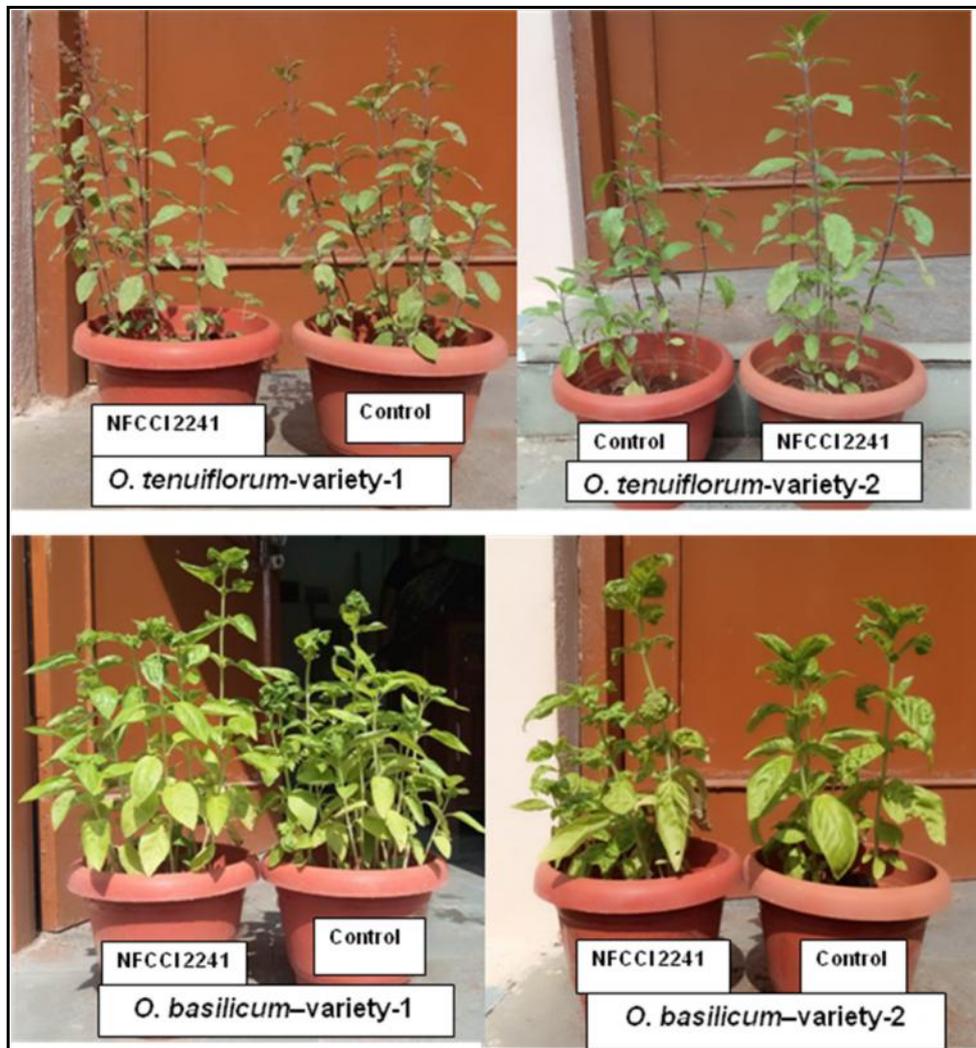


Fig. 5. Pot experiments of Basil varieties

Leaf area of *Ocimum* plants in treated with NFCCI 2241 and control treatments:

Maximum leaf area of 3rd and 4th was observed in *O. tenuiflorum*, (variety-1) when treated with NFCCI 2241 than *O. tenuiflorum* (variety-1) was 218.00mm²

and 255.00mm² respectively when compared to control treatment. Among the two varieties of *O. basilicum*, *O. basilicum* (variety-2) treated with NFCCI 2241 showed maximum leaf area of 3rd leaf with 336.67mm² and 4th leaf area with 379.17mm² in comparison to control.

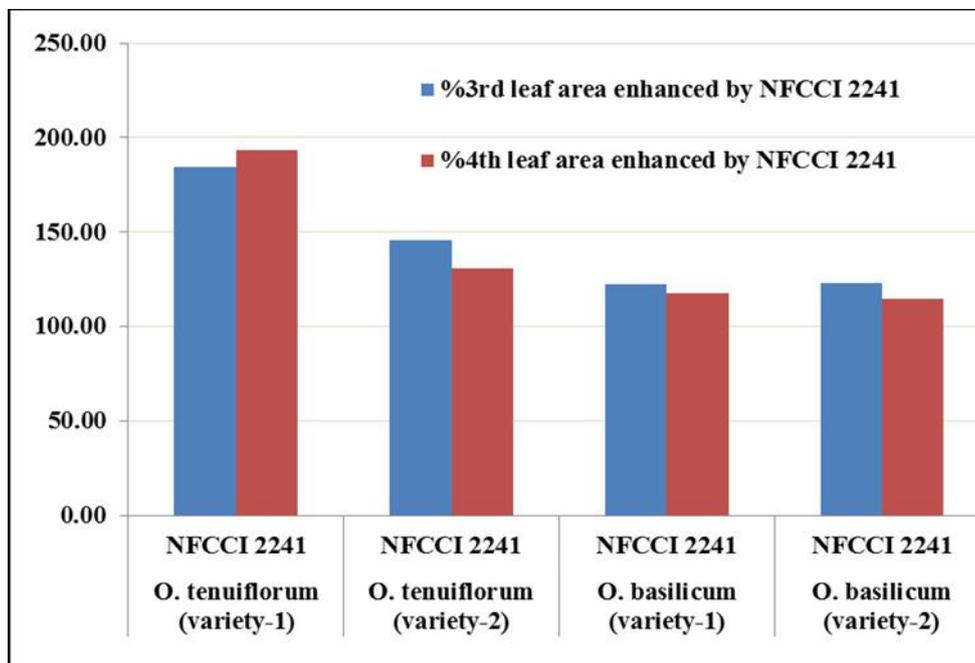


Fig. 6. Leaf area of *Ocimum* plants when treated with NFCCI 2241 and control treatments

Discussion

The present study proved that four varieties of *Ocimum* plants having positive effect when treated with NFCCI 2241. When treated with NFCCI 2241 (*T. harzianum*) more shoot length and root length, biomass were observed compared to control treatment. *Trichoderma* species are widely reported as plant growth promoters (Ousley et al., 1993; Vessey, 2003; Whipps, 2001; Yedidia et al., 2000). *Trichoderma* species promote nutrient uptake by secreting organic acids to dissolve minerals and activate nutrients in the soil, increased carbohydrate metabolism, photosynthesis and phytohormone synthesis (Altomare et al., 1999; Shores et al., 2010). *Trichoderma* secretes cell wall-degrading enzymes such as chitinases, cellulases, proteinases and produces secondary metabolites in the rhizosphere to improve nutrient cycling and enzyme activity in the soil. Yedidia et al., (2001) reported that, *Trichoderma* colonised in root system by application of *T. asperellum* to the cucumber that has been showed enhance the availability of Phosphate and Ferrous to plants, with significant increases in biomass, shoot

length and leaf area. Arpana, (2000) revealed maximum biomass in studied influence of *Glomus mosseae* and *T. harzianum* on medicinal plant *Andrographis paniculata* (Kalmegh). Enhanced biomass and yield observed when *T. harzianum* (NFCCI 2241) is applied to *Mentha arvensis* (Rahel Ratnakumari et al., 2014). The treatment with *T. hamatum* or *T. koningii* increased the productivity of lettuce, tomato and pepper plants up to 300%. Thus, the growth promotion probably due to production of plant hormones or increased nutrients uptake of the plant (Altomare et al., 1999; Chet et al., 1993), strengthens the plant defence mechanism (Shores and Harman, 2008; Woo and Lorito, 2007; Zimand et al., 1996). Plant seeds treated with the spores of *Trichoderma* spp. increased the rate of seed germination (Benitez et al., 1998). In present study *Ocimum* species plants showed a significant variation in growth when the application of NFCCI 2241 (*T. harzianum*). When application of NFCCI 2241 to *O. tenuiflorum* (variety-2) showed maximum shoot length and root length compare to *O. tenuiflorum* (variety-1).

When compared to control treatment, *O. tenuiflorum* (variety-2) treated with *T. harzianum* (NFCCI 2241) showed 145.76% increases in shoot length. When application of NFCCI 2241 to *O. basilicum* (variety-2) showed maximum shoot length and biomass compare to *O. basilicum* (variety-1). When compared to control, *O. basilicum* (variety-2) treated with *T. harzianum* (NFCCI 2241) showed 140% increases in shoot length. When the application of NFCCI 2241, biomass enhanced in *O. tenuiflorum* (variety-1) 139% and in *O. tenuiflorum* (variety-2) 267.51%. NFCCI 2241 treated *O. basilicum* (variety-1) plants biomass enhanced up to 109.38% and in *O. basilicum* (variety-2) 152.90% compared to the control treatment.

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Conflicts of interest: There are no conflicts of interest.

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