GROWTH, YIELD AND ESSENTIAL OIL PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Ocimum basilicum* L.

by

RAJESWARI E.

(2019-12-002)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"GROWTH, YIELD AND ESSENTIAL OIL PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Ocimum basilicum* L."** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani,

Date: 03·12·2021

RAJESWARI E.

(2019-12-002)

CERTIFICATE

Certified that this thesis entitled **"GROWTH, YIELD AND ESSENTIAL OIL PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Ocimum basilicum* L"** is a record of research work done independently by **Ms. Rajeswari E. (2019-12-002)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place : Vellayani
Date : 03.12.2021

31/12/2021

Dr. DEEPA S. NAIR
(Major Advisor, Advisory committee)
Assistant Professor and Head
Department of Plantation Crops and Spices
College of Agriculture, Vellayani
Thiruvananthapuram

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Rajeswari E. (2019-12-002), a candidate for the degree of **Master of Science in Horticulture** with major in Plantation Crops and Spices agree that the thesis entitled "**Growth, yield and essential oil production responses to microbial elicitation in *Ocimum basilicum* L**" may be submitted by Ms. Rajeswari E. in partial fulfilment of the requirement for the degree.

3/12/2021

Dr. Deepa S. Nair

(Major Advisor, Advisory Committee)
Assistant Professor and Head
Department of Plantation Crops and Spices
College of Agriculture, Vellayani
Thiruvananthapuram

3/12/2021

Dr. Sreekala G.S.

(Member, Advisory Committee)
Assistant Professor
Department of Plantation Crops and Spices
College of Agriculture, Vellayani
Thiruvananthapuram

3/12/2021

Dr. Anith K.N.

(Member, Advisory Committee)
Professor and Head
Department of Agricultural Microbiology
College of Agriculture, Vellayani
Thiruvananthapuram

3/12/2021

Dr. T. Sajitha Rani

(Member, Advisory Committee)
Professor and Head
Instructional Farm
College of Agriculture, Vellayani
Thiruvananthapuram

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	Per cent
°N	Degree North
°E	Degree East
CRD	Completely Randomized Design
et al.	And others
mL	Millilitre
mL^{-1}	Per Millilitre
µm	Micrometre
µg	Microgram
µL	Microlitre
rpm	Rotations per minute
@	At the rate
cm	Centimetre
cm^2	Centimetre square
cm^3	Centimetre cube
min	Minute
h	Hour
m	Metre
g	Gram
cfu	Colony Forming Unit
nm	Nanometre
viz.	Namely
cv.	Cultivar
var.	Variety
Fig.	Figure
T. No.	Table number
mg g^{-1}	Milligram per gram
$plant^{-1}$	Per plant

kg $plot^{-1}$	Kilogram per plot
mL L^{-1}	Millilitre per litre
t	Tonne
ha	Hectare
DMSO	Dimethyl sulphoxide
PE	Pyrocatechol
SE	Standard Error
CD	Critical difference
FYM	Farm yard manure
TCWE	*Trichoderma viride* cell wall extract
PCWE	*Piriformospora indica* cell wall extract
TCF	*Trichoderma viride* culture filtrate
PCF	*Piriformospora indica* culture filtrate
TTC	Triphenyl Tetrazolium Chloride
DAS	Days after sowing
LA	Leaf area
v/w	Volume per weight
Gn %	Germination per cent
S %	Survival per cent
GI	Germination index
MGT	Mean germination time
DIS	Days to initial sprouting
SL	Shoot length
RL	Root length
Sdl L	Seedling length
AI	Allometric index
SVI	Seedling vigour index
IAA	Indole acetic acid
HP	Hydro priming

INTRODUCTION

1. INTRODUCTION

Ocimum basilicum L., commonly known as sweet basil, is a popular aromatic herb belonging to the family Lamiaceae. It is a tetraploid with a chromosome number, 2n=48. The word "basil" originated from the Greek basilikon, which means "royal herb," probably because the plant seemed to have been utilised in the manufacture of royal fragrances by the Greek kings (Maharjan, 2019). It is widely distributed in tropical regions ranging from Central Africa to Southeast Asia. It is widely cultivated for the production of essential oils on several continents throughout the world owing to its diverse economic, therapeutic and aromatic virtues (Egata, 2021). In India, it is mainly confined to Punjab, Himachal Pradesh, Jammu, Uttar Pradesh, Maharashtra, Madhya Pradesh and West Bengal.

The genus *Ocimum* is widely spread in the world's hottest regions, up to 1800 metres above mean sea level. Sweet basil can grow well in a wide range of soils and climatic conditions. It flourishes well in areas with moderate to high rainfall and humidity. Long days and high temperatures have been proven to promote plant growth and oil production. It is a tender annual aromatic herb that grows up to a height of 30–90 cm. The leaves contain glandular hairs that produce highly fragrant aroma compounds and are glabrous in appearance. The white flowers are borne on a long terminal raceme and are mostly cross pollinated by insects. The fruits are blackened nutlets that become mucilaginous when moist (Smitha *et al.*, 2014)

O. basilicum is one of the most widely grown *Ocimum* species for essential oil production. Leaves are the economically valuable component, from which essential oil is produced and utilised as a flavouring ingredient in culinary, fragrance, and pharmaceutical sectors (Makri and Kintzios, 2008).The major aroma compounds include methyl chavicol, linalool, eugenol, 1,8-cineole and methyl cinnamate that can be harnessed to offer a wide range of commercial products (AI-Snafi, 2021).The medicinal value of *O. basilicum* has been mentioned in the classical texts of Sushruta Samhita and Charaka Samhita (1000 B.C) (Pushpangadan and George, 2012). It is used in Ayurvedic, Unani and Folk system of medicine owing to its several therapeutic activities such as anti inflammatory, antimicrobial, antioxidant, cardiac

stimulant, hepatoprotective, immunomodulator, larvicidal activities etc. (Bilal *et al.*, 2012).

Sweet basil has a broad array of industrial applications in pharmacology, aromatherapy, and cosmetic, as well as in traditional medicine. The huge economic potential and health benefits of sweet basil, have developed concern about strengthening the herbage yield and essential oil output of this high-value crop to meet the demand of the aromatic sector. The eco-friendly measures to enhance the farmers' output are given predominance considering its therapeutic value. Fungal inoculants are beneficial for increasing soil fertility, plant development and secondary metabolite production. Fungal inoculants have been shown to have this effect in a variety of therapeutic plant species. (Dojima and Craker, 2016; Rai and Behera, 2019).

Trichoderma and the endophytic symbiotic fungus, *Piriformospora indica* have been designed to boost plant growth, productivity, and disease resistance in a wide range of plant species. (Bae *et al.*, 2011; Varma *et al.*, 2012). *Trichoderma* spp. are well-known fungi with bio-control potential employed in agriculture. It promotes plant growth, nutrient absorption and secondary metabolite synthesis in plants (Rahman *et al.*, 2012a; Khan *et al.*, 2005). Root endophytic symbiotic fungus *P. indica*, isolated from desert soils of Rajasthan has been proved to evoke growth promotion and improve secondary metabolite production in medicinal plants by forming association with roots (Varma *et al.*, 2014; Anith *et al.*, 2018).

The effect of endophytic fungi on plant growth, yield and phytochemical synthesis, though, have been established, information on the effect of fungal derivatives in promoting growth and aroma compounds is very meagre.

In light of the facts presented and enumerated above, the present study entitled "Growth, yield and essential oil production responses to microbial elicitation in *Ocimum basilicum* L." was undertaken with the following objective

- Evaluation of the effect of fungal derivatives on growth, yield and essential oil production in *Ocimum basilicum* L.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Ocimum basilicum L. known as sweet basil, is a tender, aromatic annual herb widely distributed in tropical region. Basil is a good source of essential oils and also has a lot of flavour and aroma compounds. It is a seed propagated crop which grows best under the optimum temperature. Owing to its diverse uses in pharmaceutical and aromatic sectors as well as in indigenous system of medicine, there is a need for ecofriendly strategies for enhancing whole plant yield and essential oil production. Fungal inoculants have a beneficial impact on increasing plant growth and inducing secondary metabolite production in a wide variety of therapeutic plant species (Dojima and Craker, 2016; Rai and Behera, 2019). Priming of seeds with biological derivatives enhanced seed germination and seedling growth attributes. Foliar spray of microbial suspension was observed to give good response with respect to plant growth, yield and enhance essential oil production, in some plant species (Ahlawat *et al.* 2016; Kalboush *et al.*, 2017; Rashnoo *et al.*, 2020).

In the current investigation, the literature related to seed germination, plant growth, yield and essential oil production in response to biopriming and foliar spray with various fungal derivatives in medicinal and aromatic plants are reviewed in this chapter. In addition to medicinal and aromatic plants, the literature pertaining to other crops are also reviewed here.

2.1 BIOPRIMING OF SEEDS WITH FUNGAL DERIVATIVES

Priming is a pre-sowing seed treatment that involves controlling the level of hydration within the seed and regulating the metabolic events needed for seed germination (Bisen *et al.,* 2015). Bio-priming is one of the low-cost, safe and feasible seed priming techniques that would provide a replacement for chemical treatment. It integrates the biological inoculation of seeds with beneficial microorganisms to protect seeds and the physiology of seed hydration (Deshmukh *et al.,* 2020). It enhances the seed viability, seedling vigour index, increases speed of germination, uniformity and mean germination time. It promotes plant growth, biomass and secondary metabolites production. Many biological agents alleviate biotic and abiotic stresses in plants. Fungal endophytes and their derivatives showed a promising effect

on morphological, biochemical, yield and quality parameters in many horticultural crops (Khan *et al.*, 2005; Dolatabadi *et al.*, 2011a). The effects of following fungal derivatives, *viz., T. viride* cell wall extract and culture filtrate, *P. indica* cell wall extract and culture filtrate, in various crops are reviewed here.

2.1.1 Effect of Biopriming of Seeds with *Trichoderma viride* and its Cell Wall Extract

Trichoderma spp. are widely used as biological control agents to protect seed from pathogens. It enhances seed germination and promotes plant growth. The seedling dip using cell wall elicitors of *T. harzianum* for 1 h was effective in managing foot rot in red pepper, caused by *Phytophthora capsici*. This also enhanced total protein, phenol and glucanase activity in 30 days old seedlings. The enzymes produced by *Trichoderma* spp. also have an antagonistic effect on foot rot in black pepper (Diby *et al.*, 2005; Sriram *et al.*, 2010).

In muskmelon, seed treatment with *Trichoderma* strains at 50 % and 100 % concentrations significantly increased the seed germination per cent (95 %), germination index, seedlings emergence and vigour index than the control which showed 60 % germination. The inoculated plants showed improved root-shoot length and shoot diameter (Kaveh *et al.*, 2011).

Bhargava *et al.* (2015) found that biopriming of snapdragon seeds with bioagent, *T. harzianum* at $1x10^5$ cfu mL^{-1} enhanced seed germination percentage (70 %), reduced mean germination time than control plants which showed only 50 % germination. It also increased the number of leaves (8.75), root length (2.70 cm) and shoot length (6.15 cm) in treated plants. Singh *et al.* (2016) revealed that bio-priming of bean seeds with *T. asperellum* increased the number of leaves, shoot length, root length and seedling biomass as well as the plant growth of treated plants over the control.

In ginger, rhizome treatment with *T. viride* followed by soil drenching showed an increase in germination per cent (81 %), yield (10 ton ha^{-1}) and reduced the rhizome rot incidence upto 6.8 % in the field than the untreated plants, which gave

germination per cent (70 %), yield (7 ton ha^{-1}) and level of rhizome rot incidence was noted upto 14.4 % (Tripathi and Singh, 2021).

Singh *et al.* (2021) observed that *T. harzianum* strains are feasible alternatives to combat adverse soil and climatic conditions. The biopriming of okra seeds with *T. harzianum* showed improved plant growth, total chlorophyll content, polyphenol content and ultimately triggered the defense enzyme production.

Ananthi *et al.* (2014) opined that in chilli, biopriming enhanced seed germination, seedling vigour and total biomass. Awad *et al.* (2018) also observed that biopriming with the alcoholic extract of *Trichoderma* spp. increased germination percentage, radicle growth and also induced the defense enzymes against phytopathogens, in tomato seedlings over the control.

Savitha and Sriram (2017) reported that oligosaccharide cell wall elicitors of *Trichoderma* isolates have the potential to elicit induced systemic resistance and defense enzyme production in hot pepper (*Capsicum frutescens* L.), which has ability to reduce the *Phytophthora capsici* infection upto 70 %. The control plants, however, showed 100 % infection.

2.1.2 Effect of Biopriming of Seeds with *Trichoderma viride* and its Culture Filtrate

The fungal metabolites present in the culture filtrate of *Trichoderma* spp. are able to produce volatile compounds, extracellular enzymes and antibiotics in host plants ensuring biocontrol potential to inhibit the pathogen attack and enhancing the plant growth. The metabolic compounds present in culture filtrates have a positive effect on radicle growth and seedling development (Odamtten and Clerk, 1988; Calistru *et al.*, 1997). Moreover, an *in vitro* assay showed *Trichoderma* spp. has great potential to inhibit the fungal pathogens *Aspergillus flavus* and *Fusarium monoliformae.*

Celar and Valic (2005) observed that culture filtrate of *Trichoderma* spp. stimulated the seed germination per cent and rate of germination in the vegetable seeds such as spinach, red beet, chicory and tomato under *in vitro* condition. Bokhari

(2009) revealed that *T. harzianum*, *T. hamatum*, *T. koningii* culture filtrates significantly reduced the reniform and root-knot nematode population in brinjal *var.* black beauty. This was observed in one week old culture and one month old brinjal seedlings.

Tomato seeds treated with *T. harzianum* culture filtrate revealed the nematode inhibition (84 %) in treated plants over the control (21 %) under *in vitro* culture. The soil application of culture filtrate either alone or in combination with salicylic acid controlled the nematode population, and induced the production of related defense enzymes in tomato (Naserinasab *et al.*, 2011).

Mukhopadhyay and Pan (2012) reported that *Trichoderma* spp. culture filtrate showed the highest seed germination percent (84 %) in radish. It also enhanced the leaf area, shoot length, root length, seedling vigour index and seedling biomass under field performance. Rahman *et al.* (2012) reported that *T. harzianum* culture filtrate recorded 100 % germination, enhanced plant growth and yield parameters in bioprimed chilli seeds. It also reduced the level of anthracnose fruit rot disease in chilli field.

In Rangpur lime, biopriming of seeds with culture filtrates of *Trichoderma* spp. increased seedling emergence percentage (90 %) and reduced the mortality level (10 %) in 60 days old seedlings compared to non-treated plants (Ambadkar and Jadhav, 2007). The seed germination and seedling parameters enhanced on biopriming the chilli seeds with culture broth of *T. viride* and *Pseudomonas fluorescens* (Rai and Behara, 2019).

Rahman *et al.* (2020) demonstrated that biopriming of vegetable seeds with 7 % aqueous solution of *T. harzianum* culture filtrate effectively managed the seed borne pathogens caused by *Aspergillus* spp., *Pencillium* spp. and *Fusarium oxysporum* in cucurbits and okra seeds and improved the germination per cent over the control.

Tomato seeds on biopriming with culture filtrate of *T.afroharzianum* isolates showed that it has strong potential to improve the seed germination, radical length, hypocotyl length and seedling vigour index in treated seeds. Under greenhouse

conditions, it acted as plant growth promoting agent and involved in the synthesis of plant defense enzymes (Juan *et al.*, 2021)

2.1.3 Effect of Biopriming of Seeds with *Piriformospora indica* and its Cell Wall Extract

Piriformospora indica, a versatile root endosymbiont has the potential to offer numerous benefits in horticultural crops. It promoted the seed germination, plant growth and yield components and also was a bioprotector against phytopathogens. It also incorporated stress tolerance and resistance mechanism in plants to combat adverse environmental conditions (Gill *et al.*, 2016). According to Cheng *et al.* (2018), seed biopriming with *P. indica* accelerated the speed of germination and seedling development in longan berry.

According to Suthar and Purohit (2012), biopriming with *P. indica* exhibit 70 % root colonization in *in vitro* raised seedlings of *Boswellia serrata.* The seedlings survival rate was also observed to be higher (75 %) in colonized ones compared to control under *in vivo* condition. In *Centella asiatica*, *P. indica* colonized seedlings showed 70 % survival and seedling growth under water stressed conditions compared to control plants which exhibited only 10 % survival (Jisha *et al.*, 2018).

According to Ghabooli *et al.* (2019), biopriming with *P. indica* spore suspension effectively reduced the seed dormancy in *Kelussia odoratissima*, a wild celery. Moreover, the highest seed germination (75 %) and germination rate were also obtained in the treated seeds compared to the control. Vyshakhi and Anith (2021) observed that seed inoculation with *P. indica* has the potential to improve the seedling growth and vigour index of solanaceous vegetables *viz.*, tomato, chilli and brinjal under *in vitro* condition.

Vadassery *et al.* (2009) described that *P. indica* cell wall extract stimulated the seedling growth of the model plant, *Arabidopsis thaliana* under *in vitro* condition. It also elevated the calcium ions level in colonized roots leading to the functioning of signal transduction pathway in an effective manner. In addition to that, the cell wall

extract of *P. indica* treated Chinese cabbage seedlings showed higher growth promotion over the control plants as determined by Lee *et al.* (2011).

Liu *et al.* (2019) observed that the cell wall extract of *P. indica* enhanced the survival rate, seedling growth and total plant biomass of rye grass under greenhouse condition. The inoculated seedlings exhibited higher chlorophyll content and relative water content.

2.1.4 Effect of Biopriming of Seeds with *Piriformospora indica* and its Culture Filtrate

The symbiotic fungi *P. indica* and its culture filtrate showed a promising effect in various medicinally important plants. Prasad *et al.* (2010) reported that Titanium dioxide nano particles coated *P. indica* and its culture filtrate has capability to increase the seed germination and seedling length in broccoli (*Brassica oleracea var. italica*).

In *Chlorophytum* sp., dual inoculation of *P. indica* with *P. fluorescens* improved the survival rate (91.20 %), root -shoot length, total biomass and number of lateral roots formation in the bioprimed tissue cultured plantlets under greenhouse conditions (Gosal *et al.*, 2010).

P. indica treated seeds of cabbage, radish, palak, endive and onion showed the highest seed germination percentage (100 %) at 25 days after planting, good plant growth was also obtained in 3 months old plants. *P. indica* culture filtrate contains fungal exudates, proteins, minerals, enzymes and hormones, that facilitated considerable seed germination, plant growth and yield characteristics. *In vitro* culture assay showed that, culture filtrate at 2.5 mL induced the seed germination, enhanced root and shoot growth of seedlings by breaking the seed dormancy of cabbage, broccoli and beans (Varma *et al.*, 2012)

Athira and Anith (2020) studied the compatibility between *P. indica* and bacterial bioagents *viz., Bacillus velezensis, Bacillus amyloliquefaciens* and *Streptomyces leeuwenhoekii*, and their antagonistic effect against bacterial wilt disease in tomato. *In vitro* assay *via* agar well diffusion and disc diffusion method proved that 100 µL and 10 µL of endophytic culture filtrate has great potential against the

bacterial plant pathogen. These recorded 40 % inhibition in inoculated seedlings of tomato. In field performance, greater plant growth promotion was obtained in both, either combined inoculation of biological agents or 1 % *P. indica* alone treated plants, compared to control.

2.2 EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON PLANT GROWTH AND YIELD

2.2.1 Effect of *Trichoderma viride* and its Cell Wall Extract on Plant Growth and Yield

The biological agent *Trichoderma* spp. showed significant enhancement in morphological and yield parameters in various crops. As a result of biopriming, *T. harzianum* ($1x10^5$ cfu mL^{-1}) mediated snapdragon plants outperformed control plants in terms of plant height (75.94 cm), early blooming (45 days), number of spikes (12.00), number of flowers per spike (21.00) and seed yield (3.68 g) under field performance (Bhargava *et al.*, 2015).

Abdollahi *et al.* (2015) demonstrated that, basil seeds when initially bioprimed with *P. indica* followed by fertigation using copper nitrate at 4 weeks after planting promoted the plant growth parameters *viz.* stem diameter, plant height, number of branches and leaves, and plant biomass. Co-inoculation of *T. tomentosum* and *P. indica* along with different levels of copper nitrate showed a positive response compared to individual application.

According to Musheer and Ashraf (2017), foliar spray of *T. viride* ($4x10^6$ cfu mL^{-1}) given at 30 and 45 days after planting, suppressed the turmeric leaf spot and increased the yield of fresh rhizome (35.33 %) and dry rhizome (39.19 %) in treated plants compared to the control plants.

In turmeric, rhizome treatment along with soil application of *T. asperellum* significantly enhanced the plant height (87.60 cm), rhizome yield (430 g) and reduced the rhizome rot disease from 78.07 % to 14.2 % in inoculated plants. It also triggered the production of defense enzymes (2-3 fold) and phenolic compounds, that inhibit phytopathogens (Vinayarani *et al.*, 2019). Leaf colonization of *T. viride* facilitated

plant growth promotion by regulating the expression of H^+ATPase activity through the activation of Mitogen-activated protein (MAP) kinase6 in the inoculated seedlings of *Arabidopsis* as determined by Guo *et al.* (2020).

According to Musheer *et al.* (2019), foliar application of *T. viride* along with propiconazole and neem cake in turmeric showed maximum plant height (81.03 cm), rhizome girth (19.87 cm), fresh and dry weight of rhizome (4.18 and 2.27 kg $plot^{-1}$). This also reduced the turmeric leaf spot incidence to 17 % compared to control which exhibited 29 % of disease incidence.

In *Salvia miltiorrhiza*, mycelial extract of *T. atroviride* increased the hairy root growth (40 %) and enhanced the synthesis of tanshinone in treated roots. Moreover, the production of dihydrotanshinone (DT) and cryptotanshinone (CT) were enhanced to 35-fold at 300 mg g^{-1} of mycelial extract and 83-fold at 150 mg g^{-1} of mycelial extract, respectively, over the control (Ming *et al.*, 2013).

2.2.2 Effect of *Trichoderma viride* and its Culture Filtrate on Plant Growth and Yield

The fungal biomass and culture filtrate of *Trichoderma* spp. elicit plant growth, yield and phytochemical production in medicinal and aromatic plants. According to Khan *et al.* (2005), foliar application of *T. reesei* culture filtrate showed greater plant growth promotion and yield response. In spearmint, foliar spraying of 100 mg l^{-1} freeze-dried mycelium enhanced the root-shoot numbers (108 % and 39 %), number of leaves (57 %), maximum fresh weight (75 %) in the fungal sprayed plants over the control.

Combined application of microbial consortia (*T. viride* + *P. fluorescens* + *B. subtilis*) as foliar spray exhibited 49.15 % and 42.44 % inhibition of onion leaf blight disease, respectively under both green house and field conditions. This also recorded significant enhancement in plant height and yield in inoculated plants (Karthikeyan *et al.*, 2008).

According to Haque *et al.* (2012), *Trichoderma* enriched biofertilizers exhibited a prominent effect on crop growth and yield components. The combined

application of nitrogen fertilizer along with *Trichoderma* enriched biofertilizers in the ratio of 50:50 in mustard and tomato, increased the yield to 108 % and 203 %, respectively. However, the lowest yield of 81.90 % and 61.82 %, respectively was obtained with NPK alone.

According to Prasad *et al.* (2013), 3 % culture filtrate of *T. harzianum* enhanced the shoot biomass (1.24-fold) and growth index (7.67) in *Centella asiatica* under *in vitro* conditions. It also enhanced the asiaticoside content to 2.35 times over the non-inoculated control.

In *O.basilicum*, the cell pellets of *T. harzianum* (10 mL plant^{-1}) enhanced the total plant biomass to 2.37 fold. While in *O. sanctum*, the culture filtrate of *T. harzianum* (10 mL plant^{-1}) showed higher plant biomass (1.25-fold) after 60 days of planting in treated plants over the control plants (Gupta *et al.*, 2018).

Trichoderma spp. spore suspension and culture filtrate exhibited a strong influence on crop growth, yield and nematode management in tomato. Soil application of *T. harzianum* spore suspension at 1x10^5 cfu mL^{-1} controlled the nematode population (30 %), thereby increasing the plant growth (19-22 %), total biomass (16-20 %) and yield per plant (29 %) followed by *T.hamatum* (13-16 % yield) and *T.virens* (9-13 % yield). But the application of *Trichoderma* spp. culture filtrate at 2 mL pot^{-1} showed a lesser effect (6-11 %) on plant growth, yield and management of root-knot nematode in tomato (Khan *et al.*, 2018).

Soil application of *T. harzianum* and *T. virens* culture filtrates at 50 mL concentration reduced the soft rot of tuber in potato and enhanced the activity of defense enzymes under field conditions. It significantly increased the shoot length, number of shoots and leaves per pit, tuber weight per pit and total yield per pit in treated plants. Furthermore, foliar spraying of 50 mL culture filtrates improved the storage of potato tubers upto 3 months and its quality parameters *viz.* starch content, total carbohydrates and dry matter (Abdel-Gaied *et al.*, 2020).

Sudha *et al.* (2020) revealed that mixed application of bio-inoculants AMF at 5 g plant^{-1} and *Trichoderma* - FYM mixture at 250 g plant^{-1} initially at the time of

planting and 30 days after planting resulted in a substantial increase in plant height, number of leaves and tillers, dry matter production, fresh and dry rhizome yield. This also enhanced the nutrient uptake in ginger.

2.2.3 Effect of *Piriformospora indica* and its Cell Wall Extract on Plant Growth and Yield

Co-inoculation of *P. indica culture* (at hardening stage) followed by *T. harzianum* (at transplanting stage) provided biocontrol potential and plant growth promotion in the micro-propagated black pepper seedlings. It showed maximum number of roots, leaves, plant height and total plant biomass at 60 days after planting in the field (Anith *et al.*, 2011).

Dolatabadi *et al.* (2011a) reported that, seedling inoculation with *P. indica* mycelial culture at 1 % concentration showed the highest plant height (107.67 cm), stem and root dry weight at 150 days after planting in fennel followed by the root endophyte, *Sebacina vermifera* and control plants. But, *S. vermifera* colonized plants increased the umbel production (47 % higher) and 1000 fruit weight of fennel (3 and 16 % higher) than *P. indica* and control plants under greenhouse condition.

P. indica colonization in *Coleus forskohlii* significantly increased the shoot length, root length, root thickness, number of roots, leaves, branches, leaf area, early flowering and number of inflorescence per branch. However, it reduced the forskolin content in treated roots when compared to control plants (Das *et al.*, 2012). Aslam *et al.* (2019) revealed that the root endophyte *P. indica* has a huge potential to promote plant growth and biomass production, improved the phosphorus uptake in soil and induced stress tolerance in plants.

Upadhyaya *et al.* (2013) revealed that, the cell wall extract of *P. indica* induced tuber formation in potato under *in vitro* condition. It mediated the signal transduction mechanism through which calcium ions and lipoxygenase genes are enhanced that triggered the synthesis of respective genes essential for potato tuberization.

Daneshkhah *et al.* (2013) demonstrated that *P. indica* and its derivatives have antagonistic potential to control the cyst nematode population in the colonized roots of *A. thaliana.* Co-inoculation of *P. indica* and its cell wall extract enhanced the defensive enzyme activities such as acid phosphatase, alkaline phosphatase and sulphur oxide dismutase in *Centella asiatica* under *in vitro* condition (Jisha *et al.*, 2019).

In turmeric, rhizome treatment with talc based *P. indica* formulation gave maximum root colonization, number of leaves (100 % higher), number of roots and rhizome yield (12.67 % higher) than the control plants at nine months after planting (Bajaj *et al.*, 2014). Similarly Bhola *et al.* (2017) also reported that inoculation of 2 % fungal biomass enhanced the leaf number, root number, rhizome size and rhizome yield. The *P. indica* colonized plants produced 12 % higher yield as compared to control plants.

Tashackori *et al.* (2016) reported that, mycelial extract of *P. indica* at 1 % concentration enhanced the root growth (1.35 times), root biomass and lignan production in hairy root cultures compared to control. In the opinion of Keramati *et al.* (2016), *P. indica* colonization increased the leaf area and plant biomass production in *O. basilicum* under saline conditions.

According to Jisha *et al.* (2018), *P. indica* and its cell wall extract exhibited greater plant growth promotion in *C. asiatica. P. indica* colonized plants recorded maximum number of leaves, roots, shoot length, root length and plant biomass followed by *P. indica* cell wall extract treated plants at early growth stage and 45 days after inoculation. On the other hand, *P. indica* cell wall extract treated plants had the highest asiaticoside content in roots compared to *P.indica* colonization and control plants.

According to Nair and Sakuntala (2020), *P. indica* and its fungal derivatives *viz.* cell wall extract and culture filtrate have enormous potential on enhancing crop growth, yield and secondary metabolite production. Under *in vitro* condition, *P. indica* colonized seedlings of *Andrographis paniculata* exhibited the highest shoot length, number of leaves, fresh and dry weight after 40 days of treatment. This was followed

by the application of *P. indica* culture filtrate and cell wall extract. But, *P. indica* cell wall extract inoculated seedlings showed maximum andrographolide content at 35 days after treatment over the *P. indica* and its culture filtrate.

2.2.4 Effect of *Piriformospora indica* and its Culture Filtrate on Plant Growth and Yield

Satheesan *et al.* (2012) observed that root colonization by *P. indica* enhanced the total plant biomass and asiaticoside production in *C. asiatica* under *in vitro* condition. Kumar *et al.* (2012) revealed that, the filter-sterilized and autoclaved culture filtrate of *P. indica* at 2 % concentration promoted the hairy root growth and lignan biosynthesis in *Linum album* under *in vivo* condition.

Co-cultivation of medicinally important crop *Aloe vera* along with endosymbiont *P. indica* improved the growth of micropropagated plantlets under *in vitro* condition. *P. indica* colonization assured 100 % survival rate of seedlings. It also enhanced the plant growth and biomass production in pot cultured plants. Maximum aloin content and gel content (16.50 %) were observed in the leaves of colonized plants (Sharma *et al.*, 2014).

In tomato, either co-inoculation or mixed form of *P. indica* and *B. pumilus* improved the plant height, number of leaves, root biomass and shoot biomass at 21 days after planting. Co-cultivation of *P. indica* with *B. pumilus* recorded the highest root colonization at 21 days after planting than the mixed culture and un-inoculated plants (Anith *et al.*, 2015).

In accordance with Su *et al.* (2017), *P. indica* mediated plants showed maximum biomass production, resistant to lodging and early flowering in *Brassica napus*. Furthermore, it brought down the erusic acid and glucosinolate level in treated plants thereby improving macro and micronutrients accumulation.

According to Anith *et al.* (2018), *P. indica* colonization significantly enhanced plant growth and yield components such as number of leaves per plant, leaf area, number of spikes, spike length and berry yield in one year old bush pepper plants compared to non-inoculated control.

The root colonization by *P. indica* showed maximum plant height (58.29 cm), leaf number (105.29 per plant), number of branches (99.43), root number (127.33), root volume (39.33 cm^3) and root length (19.21 cm) at 40 days after planting and induced early flowering (59 days) in African marigold (Noorjahan *et al.*, 2018).

According to Badge *et al.* (2011), application of 15 mL culture filtrate increased the plant height, collar diameter, number of leaves and flowers, root length, root dry weight, number of seeds and seed dry weight in pot grown plants. *P.indica* colonized plants showed maximum total biomass of about 36.70 % and 68 % higher in both the varieties of *Helianthus annus viz,*. Sun gold and Japanese gold. Dolatabadi *et al.* (2011b) observed that, *P.indica* colonization enhanced the number of shoots, root and shoot biomass production of thyme under both *in vitro* and greenhouse conditions.

The plant growth promoting endophytic fungi *P. indica* and its culture filtrate beneficially interact with many medicinal crops *viz. Artemisia, Withania, Spilanthus, Stevia, Coleus* etc. thereby enhancing plant growth and total biomass. It is also involved in the synthesis of phytochemicals in host plants. Inoculation of seedlings with *P. indica* culture filtrate at 15 mL concentration improved the plant growth of *Helianthus annus* and *Phaseolus vulgaris* in pot culture. Moreover, *P. indica* colonized plants also induced plant growth promotion and early flowering in *Artemisia, Curcuma, Chlorophytum* sp., *Coleus* etc. (Varma *et al.*, 2014).

In *Aristolochia elegans*, higher root length, shoot length, leaf length, number of leaves, number of roots and dry weight of roots were obtained in the pot cultured plants inoculated with 15 mL culture filtrate of *P. indica*. It also improved the total plant biomass to 136 % and aristolochic acid content in leaves to 28.80 % over the control plants (Badge *et al.*, 2014).

According to Ahlawat *et al.* (2016), elicitation with 3 % culture filtrate of root endophytic fungus *P. indica* improved the total biomass of *Withania somnifera* in the cell suspension culture. Furthermore, the highest withaferin production and gene expression were also observed in the elicited cultures. The foliar spray of *P. indica* culture filtrate (7.5 mL) at 20, 40 and 60 days intervals after transplanting enhanced

the overall plant growth parameters in both aeroponic system and field grown chicory plants (Rashnoo *et al.*, 2020).

Li *et al.* (2021) reported that, root colonization by *P. indica* showed maximum biomass accumulation in sweet potato. It also triggered jasmonic acid level and defense gene expression against herbivore attack in colonized plants thereby inhibiting the *Spodoptera litura* population in sweet potato field.

The fungal exudates released by culture filtrate had a profound influence on seed germination, plant growth and biosynthesis of secondary metabolites (Adya *et al.*, 2013). Tamalla *et al.* (2014) reported that, biopriming with the fungal endophytes *P. indica* and *T.virens* exhibited positive response on seedling growth attributes in mung bean. But, *P. indica* treated seeds showed significant enhancement in plant height, root length and biomass production.

2.3. EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON ESSENTIAL OIL YIELD

2.3.1 Effect of *Trichoderma* spp. and its Fungal Derivatives on Essential Oil Yield

Essential oils are aromatic chemical compounds obtained from natural resources, mainly used in aromatherapy, perfumery, cosmetics, pharmaceutical and food industries. It consists of mainly terpenoid and aroma compounds, obtained by hydro and steam distillation. The foliar application of *T .reesei* culture filtrate enhanced total carvone level in spear mint followed by its mycelial suspension and spore suspension treated plants (Khan *et al.*, 2005).

Essential oil content, oil yield and chemical composition vary depending upon the environmental and geographic conditions. It also depends on agronomic practices and post harvest operations. Poonkodi (2016) revealed that essential oil obtained from sweet basil was enriched with nutritional and health benefits. Furthermore, it also had antifungal, antibacterial, insecticidal and antioxidant properties.

Co-inoculation of biocontrol agent *T. harzianum* along with *Bacillus* sp. and mycorrhizal fungi effectively managed the root knot nematode population *in O.*

basilicum in the field grown plants. It also enhanced the herbage yield (60 %), oil yield (51 %), seed yield (51 %) over the nematicide (carbofuran) treatment and untreated plants (Tiwari *et al.*, 2017).

Gupta *et al.* (2018) observed the potential of *T. harzianum* culture and its fungal derivatives to increase the essential oil content and its chemical constituents in *Ocimum* sp. In sweet basil *var.* CIM Saumya, maximum essential oil content, linalool and methyl chavicol content were observed in *T. harzianum* cell pellets inoculated plants followed by culture filtrate. However, in holy basil *var.* CIM Angana, the highest essential oil content and its chemical composition were obtained in culture filtrate treated plants.

Shaikh *et al.* (2018) found that *Trichoderma* spp. are the most effective one in increasing essential oil content and its chemical composition in Japanese mint. Hence, the highest menthol content (98.25 %) was obtained in *T. harzianum* treated plants followed by *T. viride* with menthol content (98.07 %). Correspondingly, in lemon grass, *T. viride* elevated the essential oil concentration as well as its chemical constituents such as linalool, geraniol, citral and geranyl acetate in the inoculated plants (Shaikh *et al.*, 2019).

According to Singh *et al.* (2019), dual inoculation of beneficial microbial consortia *T. harzianum* and *Brevibacterium halotolerans* improved the plant height, fresh weight of herb, nutrient absorption, essential oil content (0.81 %) and oil yield (0.9 g $plant^{-1}$) in Japanese mint under both green house and field grown plants. Application of *Trichoderma* spp. and *Glomus* spp. augmented the essential oil production (8-9 kg ha^{-1}) and its chemical composition in *O. basilicum* (Giannoulis *et al.*, 2020).

Similarly Tiwari *et al.* (2021) reported that, combined application of microbial consortia *T. harzianum* and *B. megaterium* resulted in greater plant growth promotion, essential oil production and nutrient accumulation in sweet basil. Moreover, it suppressed the root knot nematode infection in inoculated plants by 72 %.

Soil application of *Trichoderma* isolates and its metabolic compounds such as harzianic acid and 6-pentyl-α-pyrone stimulated the production of volatile organic compounds in olive trees *via* regulating their corresponding biosynthetic pathways (Dini *et al.*, 2021).

2.3.2 Effect of *Piriformospora indica* and its Fungal Derivatives on Essential Oil Yield

Dolatabadi *et al.* (2011a, b) revealed that inoculation with *P. indica* augmented the production of aroma compounds as well as its chemical constituents in fennel. It increased the essential oil content (2.46 %) and its major constituents such as anethole and fenchone level in treated plants. Similarly in thyme, root endophytic fungal species *P. indica* and *S. vermifera* increased the essential oil and thymol content in colonized plants. Maximum essential oil content of 0.53 % was obtained in *S. vermifera* colonization followed by *P. indica* colonization with an essential oil content of 0.41 %.

Das *et al.* (2012) observed root colonization by *P. indica* showed an increased biomass production and enhanced p-cymene level in the essential oil of coleus. *P. indica* colonization enhanced the bioactive phytochemicals and secondary metabolite production in turmeric. When compared to non-colonized plants, it boosted the volatile oil (1.5 %) and curcumin content (3.63 %), which were 21 % and 19 % higher, respectively (Bajaj *et al.*, 2014). The root endophytic fungus, *P. indica* colonized plants recorded the highest essential oil content (0.7 %), oil yield and its chemical constituents such as methyl chavicol (24.78 %) geranial (26 %) and neral (24.88%) and in sweet basil (Keramati *et al.*, 2016).

According to Ghesmati *et al.* (2017), elicitation with *P. indica* cell wall extract at 4 % concentration increased the sesquiterpenoid, valerenic acid content in *Valeriana officinalis* through modulating the biosynthetic genes involved in the sesquiterpene pathway under *in vitro* condition.

Anith *et al.* (2018) reported that *P. indica* colonized black pepper plants recorded maximum oleoresin and piperine content in the berries about 19 % and 24 %

higher than the non-inoculated control plants. Combined inoculation of *P. indica*, *Agrobacterium* sp. and *Enterobacter* sp. increased the essential oil content, oil yield and the main constituents such as geranial, geraniol, geranyl acetate in Moldavian balm (*Dracocephalum moldavica*) under drought stress conditions (Amini *et al.*, 2020).

According to Abdollahi *et al.* (2021), inoculation with *P. indica* improved the total biomass, seed yield, essential oil content and oil yield. It also enhanced the major essential oil compound, anethole (236.85 %) in the field grown anise plants under water stress conditions.

Dual inoculation of root endophyte *P. indica* along with mycorrhizal fungi exhibited maximum essential oil content (0.80 %) and oil yield in pepper mint. It also increased the essential oil constituents *viz.* 1,8 -cineole, menthol, menthone and antioxidant activity under green house conditions (Khalvandi *et al.* 2021).

2.4 EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON BIOCHEMICAL PARAMETERS

2.4.1 Effect of *Trichoderma* spp. and its Fungal Derivatives on Chlorophyll Content

Application of *Trichoderma* spp. and its culture filtrate as soil treatment and foliar spray augmented chlorophyll content, polyphenol content, production of defense enzymes (polyphenol oxidase, peroxidise etc.) and phytohormone production. As a result of that, it increased the synthesis of natural hormone, IAA about 20 ppm in both green house and field grown rice plants (Kalboush *et al.*, 2017).

The combined application of microbial consortium *T. harzianum*, *B. subtilis*, *P. fluorescens* and *Streptomyces griseus* increased the level of biochemical components *viz.*, chlorophyll, total carbohydrates and carotenoids in the *O. basilicum* field grown plants (Metwaly and Abd-El-Sayed, 2018)

According to Musheer *et al.* (2019), combined inoculation of *T. viride* along with fungicide propiconazole and neem cake significantly increased the chlorophyll

content (26.67 mg g^{-1}) and carotenoid content (0.85 mg g^{-1}) in turmeric leaves and curcumin content (0.158 mg g^{-1}) in fresh rhizome. Dual inoculation of endophytic fungus *P. indica* and *T. virens* improved the chlorophyll content in *Stevia rebaudiana* compared to single application under saline conditions (Saravi *et al.*, 2019).

2.4.2 Effect of *Piriformospora indica* and its Fungal Derivatives on Chlorophyll Content

Root colonization by *P. indica* significantly increased the chlorophyll content (8.76 mg g^{-1}) in *Chlorophytum* sp. (Gosal *et al.*, 2010). Similarly in rice, *P. indica* colonized plants showed significant enhancement in chlorophyll and carotenoid content. It also increased proline accumulation in the treated plants to combat salt stress conditions (Jogawat *et al.*, 2013).

Co-cultivation of *Aloe vera* along with *P. indica* significantly improved the antioxidant activity and content of chlorophyll a (101.61 %), chlorophyll b (60.46 %) and total chlorophyll (93.45 %) than the non-inoculated plants (Sharma *et al.*, 2014).

According to Jisha *et al.* (2018), *P. indica* cell wall extract treated plants showed the highest chlorophyll content (chl a and chl b) at 45 days after inoculation in *Centella asiatica* accompanied by *P. indica* colonized plants and un-inoculated control plants. Ghorbani *et al.* (2018) reported that, root colonizing fungus *P. indica* increased the level of photosynthetic pigments *viz.* chlorophyll a, chlorophyll b, carotenoids and induced the proline accumulation in roots to mitigate the salt stress conditions in tomato plants.

According to Liu *et al.* (2019), dual inoculation of *P. indica* and *Xerocomus badius* significantly increased the plant growth and biochemical parameters in *Lolium multiflorum*. *P. indica* colonization showed maximum chlorophyll content (0.86 mg g^{-1} of fresh weight) and relative water content (85 %) followed by *X. badius* with chlorophyll content of 0.77 mg g^{-1} and relative water content of about 80 % higher than the control non-colonized plants.

P. indica culture filtrate (7.5 mL) applied as a foliar spray at 20, 40 and 60 days intervals after transplanting increased chl a, chl b, relative water content,

anthocyanin and proline accumulation in both aerophonic and field grown chicory plants (Rashnoo *et al.*, 2020). Co-inoculation of *P. indica* and *T .virens* improved the physiological parameters in mung bean. However, *P. indica* inoculated plants showed the maximum chlorophyll a, chlorophyll b and total chlorophyll content (Tamalla *et al.*, 2014).

2.4.3 Effect of *Trichoderma* spp. and its Fungal Derivatives on Polyphenol Content

Polyphenols are the naturally occurring organic compounds obtained from the concentrated plant extract. They are the major secondary metabolites distributed abundantly in plants in the form of flavonoids, phenolic acids, tannin compounds. They play a vital role in plant defense systems against insect pests and phytopathogens (Modnicki and Balcerek, 2009).

Sriram *et al.* (2010) revealed that inoculation with *T. harzianum* cell wall glucan elicitors showed the highest phenol content (42 $\mu g\ g^{-1}$) and glucanase activity in red pepper plants in comparison with control plants which showed the lowest phenol content (27 $\mu g\ g^{-1}$).

The bioagent *Trichoderma* spp. and its cell wall fractions, fungal exudates and culture filtrate showed a promising effect on biosynthesis of phytochemical and biochemical components. Inoculation with *T. harzianum* cell pellets and culture filtrate improved the total phenol content and antioxidant activity of *O. basilicum* and *O. sanctum* in the field grown plants (Gupta *et al.*, 2018).

As reported by Sesan *et al.* (2020), foliar application of microbial consortia *T. asperellum* and *T. harzianum* at the concentration of 10^8 cfu mL^{-1} significantly improved the quality components of *P. caerulea* by increasing the antioxidant activity, polyphenol and flavonoid level about 10 % higher than the control plants after 60 days of foliar treatment.

Correspondingly, *Trichoderma* spp. showed antagonistic potential against phytopathogens in okra by enhancing the level of phenolic compounds and defensive

enzymes activities in the field grown plants, enabling them to withstand adverse climatic conditions (Singh *et al.*, 2021).

2.4.4 Effect of *Piriformospora indica* and its Fungal Derivatives on Polyphenol Content

The beneficial root endosymbiont *Piriformospora indica* was involved in regulation of metabolic pathways leading to the biosynthesis of phenylpropanoid derivatives, polyphenolic and flavonoid compounds in plants. The cell wall extract of *P. indica* enhanced the phenolic compounds and lignin production in the elicited hairy root cultures of *Linum album via* modulating their biosynthetic pathway (Tashackori *et al.*, 2018).

In accordance with Jisha *et al.* (2018), *P. indica* and its fungal metabolites have great potential to mitigate stress conditions and enhancing the production of phytochemicals in host plants. Hence, *P. indica* cell wall extract treated plants showed the highest phenolic compounds (26.2 mg g^{-1} of dry matter) and antioxidant activity in *Centella asiatica* followed by *P. indica* colonized plants with phenolic content of 25.10 mg g^{-1} of dry mass.

P. indica colonization alleviates stress conditions in *Artemisia* through which it enhanced the antioxidant activity and phenolic compounds by regulating the biosynthetic pathways *viz.* terpenes, flavonoids and isoprenoids (Rahman *et al.*, 2020).

In *Ficus carica*, elicitation with both *P. indica* cell wall extract and culture filtrate at 2 % concentration enhanced the total phenol content in hairy root culture. The highest flavonoid content was obtained in *P. indica* cell wall extract at 6 % concentration. Moreover, the fungal metabolites increased the major flavonoids such as rutin, quercetin, apigenin and phenolic acids in the elicited cultures (Amani *et al.*, 2021).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The project entitled on "Growth, yield and essential oil production responses to microbial elicitation in *Ocimum basilicum* L." was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2019-2021. The study aimed at the evaluation of the effect of fungal derivatives on growth, yield and essential oil production in *Ocimum basilicum* L. The details of the materials used and methodology adopted for the study are described in this chapter.

The study was carried out in two phases:

Phase 1- Seed priming using fungal derivatives for enhanced germination.

Phase 2- Evaluation of the effect of foliar application of fungal derivatives for growth, yield and essential oil production.

3.1 LOCATION

The study was conducted at the College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. It was located at 8^0 28'28" N latitude and 76^0 57'47" E longitude at an altitude of 28 m above mean sea level.

3.2 SOURCE OF PLANTING MATERIAL

The seeds of *Ocimum basilicum* used for the study were sourced from Anand Agricultural University, Gujarat.

3.3 PHASE I- SEED PRIMING USING FUNGAL DERIVATIVES FOR ENHANCED GERMINATION

3.3.1 Initiation and Maintainence of fungal cultures

The microbial cultures were procured from the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram. The fungal cultures were maintained in potato dextrose agar medium (PDA) at neutral pH (Plate 1). *Trichoderma viride* inoculated plates were incubated at 28°C for 7 days and *Piriformospora indica* plates were incubated at 28°C for 15 days. The mycelial cultures

of both the fungi, after specific incubation period were transferred to potato dextrose broth in 100 mL conical flask (Plate 2) and further, kept in incubated shaker (Jeio Tech, Korea- Model SI-300) at 28°C for 15 days.

3.3.1.1 Preparation of Cell Wall Extract

The mycelial growth of both the fungus *P. indica* and *T. viride* were taken from 15 days old liquid culture. They were separately homogenised using mortar and pestle by adding some water. After that the homogenate were filtered using muslin cloth into the sterile conical flask. The fungal biomass in the muslin cloth was washed several times with water. Then it was transferred to a sterile Petri dish, air dried under laminar airflow and the cell wall extract was recovered (Plate 3 A, B). The cell wall extract (1%) was prepared by suspending 1g of CWE of *P. indica* in 100 mL sterile water and autoclaved for 20 min at 121°C. This autoclaved material releases the active fraction. The autoclaved suspension was subjected to centrifugation for 10 min at 14,000 rpm and filter sterilized through a 0.4 µm membrane filter. It was then stored in refrigerator until further use (Baldi *et al.*, 2009; Jisha *et al.*, 2018).

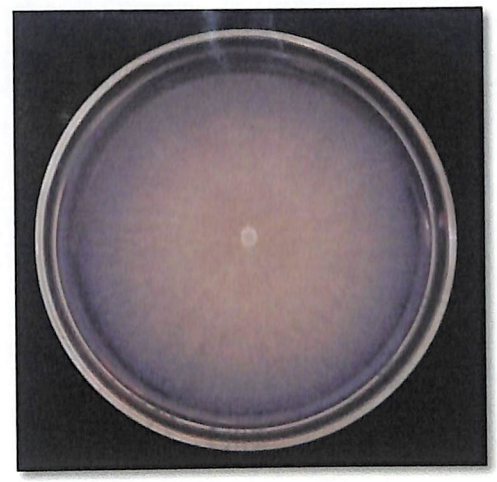
3.3.1.2 Preparation of Culture Filtrate

The mycelial growth of both the fungus *P. indica* and *T. viride* were taken from 15 days old liquid culture, for the preparation of culture filtrate (Plate 3 C, D). They were filtered using muslin cloth into a sterile conical flask. The culture filtrate was collected and centrifuged. The supernatant was taken and filter sterilized through a 0.4 µm membrane filter. For preparing 1% CF, 1 mL of the culture filtrate was suspended in 100 mL sterile water. It was then stored in refrigerator until further use (Baldi *et al.*, 2009; Bagde *et al.*, 2011).

3.3.2 Seed Priming

The seeds of *O. basilicum* were subjected to priming prior to sowing (Plate 4A). The seeds were immersed in various suspensions of fungal derivatives *viz.*, *Trichoderma viride* cell wall extract (TCWE) (1 %), *Trichoderma viride* culture filtrate (TCF) (1 %), *Piriformospora indica* cell wall extract (PCWE) (1 %) and *Piriformospora indica* culture filtrate (PCF) (1 %) and hydro priming for 2 h followed by air drying for 1 h. The air

Trichoderma viride



Piriformospora indica

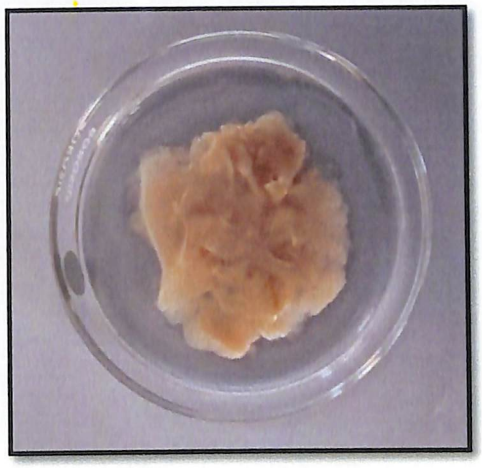
Plate 1. ***Trichoderma viride*** **and** ***Piriformospora indica*** **cultures**

Trichoderma viride **broth**

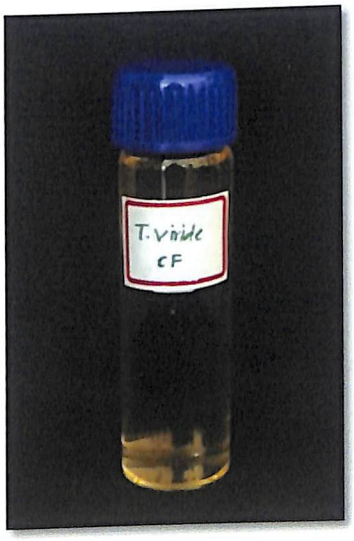
Piriformospora indica **broth**

Plate 2. ***Trichoderma viride*** **and** ***Piriformospora indica*** **broth cultures**

A. TCWE



B. PCWE



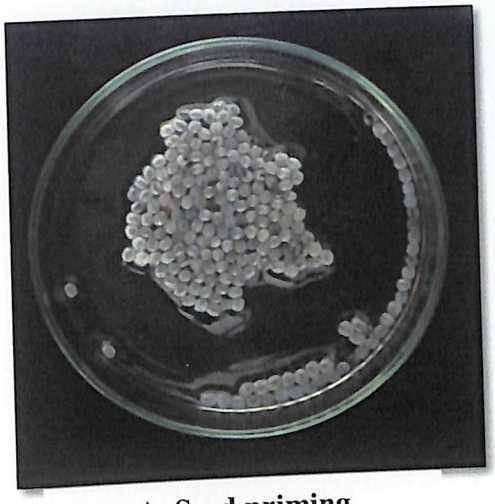
C. TCF

D. PCF

Plate 3. Fungal derivatives of *Trichoderma viride* and *Piriformospora indica*

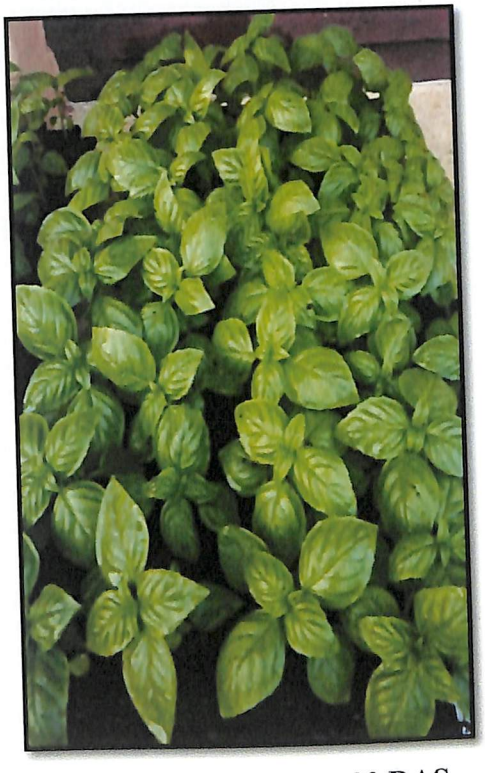
A) *Trichoderma viride* Cell Wall Extract, B) *Piriformospora indica* Cell Wall Extract, C) *Trichoderma viride* Culture Filtrate, D) *Piriformospora indica* Culture Filtrate

Plate 4. Priming of *O. basilicum* seeds



A. Seed priming

B. Seedling growth at 10 DAS



C. Seedling growth at 30 DAS

dried seeds were sown in portrays filled with coirpith and vermicompost in the ratio of 2:1. The seedlings were maintained upto 30 days in portrays to study the effect of priming on germination and seedling growth (Plate 4 B, C). In the experiment, the seeds without any priming were taken as the absolute control.

3.3.2.1 Design of the Experiment

The experiment was laid out in completely randomized block design (CRD) (Panse and Sukhatme, 1985) with six treatments and three replications in each treatment. Each replication comprised of 50 seeds. The treatment details for the first experiment are presented in Table 1.

Table 1. Treatments for seed priming of *O. basilicum* for enhanced germination

Treatment No.	Priming treatments
T_1	*T. viride* cell wall extract (1 %) (TCWE)
T_2	*P. indica* cell wall extract (1 %) (PCWE)
T_3	*T. viride* culture filtrate (1 %) (TCF)
T_4	*P. indica* culture filtrate (1 %) (PCF)
T_5	Hydro priming
T_6	Absolute Control

3.3.3 Observations on the Effect of Priming on Enhanced Seed Germination

3.3.3.1 Seed Viability

Seed viability is the ability of a seed to germinate and produce a normal seedling for some specific period of time under suitable growth conditions. It can be tested by using 2,3,5 - triphenyl tetrazolium chloride.

A colourless solution of 2,3,5-triphenyl tetrazolium chloride (TTC) was used as the indicator. It formed triphenyl formazan, a red, stable and non-diffusible compound in living cells. It was used to differentiate red-coloured living tissues from colourless dead ones. The seeds were thus categorised into viable and non-viable seed classes.

Seeds were conditioned by soaking seeds in water for 1 h. It was done to stimulate respiration and activate the hydrolytic enzyme, dehydrogenase. The seeds should be either dissected or punctured to facilitate solution (TTC) penetration into internal tissues. The seeds were dissected and immersed in a 1% tetrazolium solution at 30-35°C for 12 h at a pH of 6-8. The seeds were evaluated using a stereo microscope (Magnus MSZ-TR, Model-21A0812, Noida) when they were stained. Seeds were identified as viable or dead on the basis of their staining pattern. The embryo completely stained with red was taken as viable seeds, while the embryo, plumule or radicle unstained was termed as non-viable seeds.

$$\text{Seed viability per cent (\%)} = \frac{\text{Number of viable seeds}}{\text{Total number of seeds taken in sample}} \times 100$$

3.3.3.2 Days to Initial Sprouting

The days to initial sprouting was recorded by counting the number of days taken for sprouting as indicated by the emergence of embroyonic leaves from the seeds sown in protrays.

3.3.3.3 Germination per cent

Germination per cent is an estimate of viability of seed population. Seeds of *O. basilicum* were sown in protrays (50 cells) at the rate of one seed per cell and observed for germination upto 15 days. The germination was determined as indicated by plumule protrusion from the seeds. The germination per cent was calculated by the following equation

$$\text{Germination per cent} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds initially sown}} \times 100$$

3.3.3.4 Survival per cent

The survival per cent of seeds was recorded at daily interval from the first day of germination upto the end of the study (30 days in protray).

$$\text{Survival per cent} = \frac{\text{Number of surviving plants at end of the study}}{\text{Number of seeds sown}} \times 100$$

3.3.3.5 Germination Index

The seeds showing plumule protrusion were counted for number of seeds germinated on each day upto 15 days. Germination index was calculated by using the following formula (AOSA, 1983),

$$\text{Germination index (GI)} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots \frac{n - X_n - 1}{Y_n}$$

X_1 - Number of seeds germinated on first day

X_2 - Number of seeds germinated on second day

X_n - Number of seeds germinated on n^{th} day

Y_1 - Number of days from sowing to first count

Y_2 - Number of days from sowing to second count

Y_n - Number of days from sowing to n^{th} count

3.3.3.6 Mean Germination Time

Mean germination time (MGT) is the measure of rate and time-spread of seed germination from the start of imbibition to radicle protrusion. Mean germination time was calculated based on the following equation (Schelin *et al.*, 2003).

$$\text{Mean germination time (MGT)} = \frac{\Sigma fini}{\Sigma N}$$

f_i - Number of seeds germinated on each day

n_i - Number of days from sowing

N - Total number of germinated seeds

The mean germination time was expressed in days.

3.3.3.7 Shoot Length

Three seedlings were randomly selected from each replication of all treatments and the shoot length was measured by using a measuring scale from the collar region to the highest point of the primary stem at 30 days after sowing. Mean length was worked out and expressed in centimetre (cm).

3.3.3.8 Root Length

The root length of the selected three seedlings per replication after uprooting, was measured by using a measuring scale from basal portion to growing tip of the root at 30 days after sowing. The mean length was worked out and expressed in centimetre (cm).

3.3.3.9 Seedling Length

The seedling length of the selected seedlings was determined by adding the shoot and the root length at 30 days after sowing. The mean values were calculated and expressed in centimetre (cm).

3.3.3.10 Allometric Index

The shoot length and root length recorded at 30 days after sowing were used to calculate the value of allometric index by using the formula given by Hosseini *et al.* (2013).

$$\text{Allometric index} = \frac{\text{Root length}}{\text{Shoot length}}$$

3.3.3.11 Seedling Vigor Index

Seedling vigor index is the indicative of seedling health. It was estimated by using the formula as described by Vashisth and Nagarajan (2010),

Seedling vigor index (SVI) = Germination per cent x Seedling length

3.4 PHASE II - EVALUATION OF THE EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON GROWTH, YIELD AND ESSENTIAL OIL PRODUCTION

Thirty days old seedlings of *O. basilicum* obtained from experiment I was transplanted to grow bags. The foliar spray of corresponding fungal derivatives (cell wall extract and culture filtrate) were given to plants at fortnightly intervals from transplanting to 90 days after sowing to evaluate their effect on growth, yield and essential oil production. The non-inoculated seedlings were taken as control. Ten

seedlings from each replication of all the treatments from the first experiment were transplanted and maintained organically.

3.4.1 Design of the Experiment

The experiment was laid out in completely randomized block design (CRD) (Panse and Sukhatme, 1985) with six treatments and three replications. Each replication comprised of 10 plants and totally thirty plants were maintained for each treatment. The foliar application treatment details for the second experiment are depicted in Table 2. The general view of the experimental area is presented in Plate 5.

Table 2. Treatments for foliar application of fungal derivatives for enhancing growth, yield and essential oil production

Treatment No.	Foliar application
T_1	*T. viride* cell wall extract (1 %) (TCWE)
T_2	*P. indica* cell wall extract (1 %) (PCWE)
T_3	*T. viride* culture filtrate (1 %) (TCF)
T_4	*P. indica* culture filtrate (1 %) (PCF)
T_5	Water spray
T_6	Absolute Control

UV stabilized growbags of size 40 cm x 24 cm x24 cm with 600 gauge thickness and 15 kg capacity were filled with potting mixture which contain soil, FYM and sand in the ratio of 2:1:1 and mixture was filled upto $3/4^{th}$ of the bag. The seedlings were planted in the planting hole taken at 5 cm depth. The plants were irrigated daily once upto 30 days, and alternate days thereafter. Staking was provided at 50 days after transplanting (Plate 6). Hand weeding was done as and when required. The plants were evaluated at specified intervals and the observations were recorded. The first cut was made 110 days after sowing. Further, the crop was maintained as ratoon crop upto 60 days after the first harvest, to study their effect on yield parameters *viz.*, leaf biomass, stem biomass and herbage yield.

3.4.2 Observations on the Effect of Foliar Application of Fungal Derivatives on Growth, Yield and Essential Oil Production

3.4.2.1 Plant Growth Parameters

The plant growth parameters were recorded at 30 DAS, 60 DAS, 90 DAS and 110 DAS. Three plants were randomly selected from each replication of all the treatments, tagged and the following observations were recorded.

3.4.2.1.1 Plant height

The plant height was taken from tagged plants from each replication measuring from base of the plant to the tip of main axis of the stem. The mean values were calculated and expressed in centimetre (cm).

3.4.2.1.2 Collar girth

The girth of the collar region was determined by measuring the circumference of the basal stem portion by using a thread and measuring scale. The mean values were calculated and expressed in centimetre (cm).

3.4.2.1.3 Leaf area

The leaves were picked from the tagged plants and leaf area was measured according to Mousavi *et al.* (2011)

$$\text{Leaf area (LA)} = 0.209\ (L^2 + W^2) + 0.25$$

$$L = \text{Leaf length}$$

$$W = \text{Leaf width}$$

The mean area was worked out and expressed in cm^2.

3.4.2.1.4 Number of branches

The number of branches was determined by counting the branches arising from the main stem of the plant and their mean values were recorded.

Plate 5. General view of experimental area

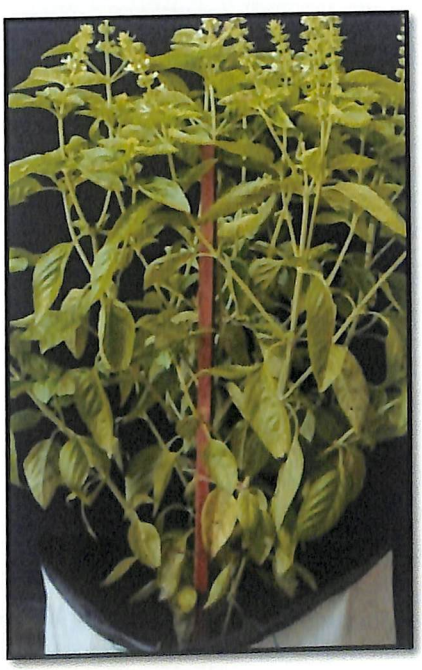


Plate 6. Staking of *O. basilicum* plant at 50 DAS

3.4.2.1.5 Number of flowering shoots

The number of flowering shoots arising from the secondary, tertiary and quaternary branches of the stem was counted separately for each replication of all the treatments and their mean values were recorded.

3.4.2.2 Phenological Parameters

3.4.2.2.1 Days to Flowering

The days to flowering was calculated by counting the number of days taken from sowing to initiation of the first flower in the flowering shoots.

3.4.2.2.2 Days to Fruit set

The days to fruit set was recorded by counting the number of days taken from initiation of the flower to fruit set.

3.4.2.3 Biochemical Parameters

The observations *viz.*, chlorophyll content was taken at 30 DAS, 60 DAS, 90 DAS and at harvest (110 DAS) and polyphenol content was recorded at harvest (110 DAS).

3.4.2.3.1 Estimation of Chlorophyll Content (DMSO Method)

The chlorophyll content of leaf samples was estimated as per the procedure described by Arnon (1949). The leaf samples were collected from the tagged plants and cut into small bits. Leaf bits weighing 0.5 g were placed in test tubes with 5 mL of DMSO (Dimethyl sulphoxide) and kept overnight at room temperature. The sample was then made upto 25 mL with DMSO. The supernatant was collected and the absorbance was measured at 645 nm and 663 nm. The total chlorophyll content was estimated by substituting the data in the given formula and expressed in mg g^{-1} fresh weight of leaf sample.

Total chlorophyll content = $[(20.2 \times A_{645} - 8.01 \times A_{663}) \times V]/W \times 1000$

Where V – Volume of the final chlorophyll extract (mL)

W – Fresh weight of leaf sample (g)

A – Absorbance at specific wavelength (645 nm and 663 nm)

3.4.2.3.2 Estimation of Polyphenol Content

The total phenol content was estimated by using the Folin-Ciocalteau reagent. In an alkaline medium, the phenols react with the oxidising agent phosphomolybdic acid present in Folin-Ciocalteau reagent, producing a blue-coloured complex (molybdenum blue) and the absorbance was read at 650 nm in a UV-Vis Spectrophotometer (Shimadzu Model UV-1900I).

A leaf sample weighing 0.5 g and was ground with 5 mL of 80 % ethanol in a pestle and mortar. The obtained plant extract was centrifuged at 10,000 rpm for 15-20 min. The supernatant was collected and the residue was extracted once again with 5 mL of 80 % ethanol. After centrifuging the extract, the supernatant was collected. The supernatant solution was evaporated to dryness by keeping it in the water bath. Then the residue was dissolved in 5 mL of distilled water. This alcohol-free extract was used to determine the phenolic content in the sample. 0.2 mL of the aliquot from the sample was pipetted into different test tubes and make up to the volume of 3 mL with water. To this, 0.5 mL of Folin-Ciocalteau reagent was added and stirred. After 3 min, add 2 mL of 20 % Na_2CO_3 (sodium carbonate) solution was added to each test tube and thoroughly mixed. Test tubes were placed in water bath for exactly 1 min, cooled and absorbance was measured in a spectrophotometer at 650 nm against a reagent blank.

A standard graph was constructed by using different concentrations of standard catechol solution in the range of 20 -100 µg. Standard stock solution was prepared by dissolving 100 mL catechol in 100 mL water. Working standard was prepared by diluting the standard solution 10 times to obtain 100 µg catechol per mL. From the standard curve, polyphenol content in the test sample was calculated and expressed in mg PE g^{-1} of the sample.

3.4.2.4 Yield Parameters

The yield parameters were recorded at harvest (110 DAS) and 60 days after the first harvest (ratoon crop).

3.4.2.4.1 Leaf Biomass (Fresh and Dry)

The fresh leaves along with petiole were harvested from the tagged plants and weighed using an electronic balance. Then the leaves were dried in hot air oven at 70°C till the constant weight was obtained. The dry weight of the leaf was recorded and the mean values were worked out. The fresh and dry weight of the leaf was expressed in g plant^{-1}.

3.4.2.4.2 Stem Biomass (Fresh and Dry)

The stem portion of the tagged plants was harvested and the fresh weight of the stem was weighed using the electronic balance. The fresh stem was packed, labelled and dried to a constant weight in hot air oven at 70°C. The dry weight was measured and the mean values were worked out. The fresh weight and dry weight of stem was expressed in g plant^{-1}.

3.4.2.4.3 Herbage Yield (Fresh and Dry)

The herbage yield (fresh and dry) was determined by adding the fresh and dry weights of leaf and stem of individual tagged plants. The fresh and dry weights of herbage were calculated and the mean values were worked out. The fresh and dry herbage yield was expressed in g plant^{-1}.

3.4.2.4.4 Root Biomass (Fresh and Dry)

The root portion of the tagged plants was uprooted and the fresh weight of the root was weighed using the electronic balance. The fresh root was packed, labelled and dried to a constant weight in hot air oven at 70°C. The dry weight was measured and the mean values were worked out. The fresh weight and dry weight of root was expressed in g plant^{-1}.

3.4.2.4.5 Total Plant Biomass (Fresh and Dry)

The total plant biomass (fresh and dry) was determined by adding the fresh and dry weights of leaf, stem and root biomass. The fresh and dry weights were measured and the mean values were worked out. The fresh and dry weight of the whole plant was expressed in g plant^{-1}.

3.4.2.4.6 Essential Oil Yield

The leaves were collected at harvest stage, dried under shade condition at room temperature and ground to powder form. 50g of a finely ground leaf sample was taken for distillation. The essential oil was distilled for 3 h by hydro-distillation using Clevenger-type apparatus. The distilled essential oil was then dried using anhydrous sodium sulphate. The oil was collected and sealed in screw cap vials. The essential oil yield of the sample was estimated by using the following formula,

$$\text{Essential oil content (\%v/w)} = \frac{\text{Quantity of essential oil (mL)}}{\text{Weight of the leaf sample (g)}} \times 100$$

Essential oil yield (g plant^{-1}) = Total leaf yield (g plant^{-1}) x Essential oil content of the sample (%)

3.4.2.4.7 Harvest Index

The harvest index was calculated by using the formula,

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

The economic yield was taken as the weight of the above ground portion (leaf, stem and herbage) of plant on dry weight basis. The biological yield was calculated from the weight of the whole plant on dry weight basis.

3.4.3 Incidence of Pest and Diseases

Occurrence of pests and diseases incidence at different stages throughout the crop period was observed and recorded.

3.5 STATISTICAL ANALYSIS

The experiment in the first phase of study was laid out in completely randomized design (Panse and Sukhatme, 1985). The data generated from the study were subjected to analysis of variance (ANOVA) using the statistical package GRAPES version 1.0.0 (Gopinath *et al.*, 2020). The means were compared using critical difference (CD) at 5% level of significance.

RESULTS

4. RESULTS

The present study entitled "Growth, yield and essential oil production responses to microbial elicitation in *Ocimum basilicum* L." was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani. The field experiments were laid out in the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2019 - 2021. The data collected from the field experiment and laboratory analyses were statistically analysed and the results are presented in this chapter.

4.1 PHASE I: SEED PRIMING USING FUNGAL DERIVATIVES FOR ENHANCED GERMINATION IN *OCIMUM BASILICUM*

4.1.1 Effect of Priming Treatments on Seed Germination and Seedling Growth Parameters in *O. basilicum*

The seeds were subjected to various priming treatments using fungal derivatives *viz., Trichoderma viride* cell wall extract (1 %) (TCWE), *Trichoderma viride* culture filtrate (TCF) (1 %), *Piriformospora indica* cell wall extract (1 %) (PCWE), *Piriformospora indica* culture filtrate (1 %) (PCF) and hydro priming to study their effect on seed germination and seedling growth parameters upto 30 days after sowing in *O. basilicum*. The seeds without any priming were taken as the absolute control. The results on the effect of priming treatments on various germination parameters are presented in Table 3 and seedling growth parameters in Table 4.

4.1.1.1 Seed Viability

The seeds sourced from Anand Agricultural University, Gujarat recorded 83 % viability by TTC test (Plate 7).

4.1.1.2 Days to Initial Sprouting

The priming treatments significantly influenced the days to initial sprouting. Treatments T_3 (TCF @ 1 per cent) and T_4 (PCF @ 1 per cent) recorded minimum

number of days (3 days) to initial sprouting. While T_1 (TCWE @ 1 per cent) and the absolute control (T_6) had taken maximum number of days (5 days) to initial sprouting.

4.1.1.3 Germination per cent

Germination per cent exhibited significant variation among the various priming treatments. Priming with PCF @ 1 per cent (T_4) recorded the highest germination of 96 per cent, which was statistically on par with TCF @ 1 per cent (T_3). The lowest germination of 72 per cent was observed in the absolute control (T_6) than all other treatments.

4.1.1.4 Survival per cent

All the seeds that germinated survived till the end of the experiment. Hence, same results as in germination were recorded in survival also. T_4 (PCF @ 1 per cent) showed the maximum survival rate of 96 per cent, that was on par with T_3 (TCF @ 1 per cent). The lowest (72 per cent) survival rate was observed in the absolute control (T_6) than all other treatments.

4.1.1.5 Germination Index

Significant variation was observed among the various priming treatments with respect to germination index. Seeds bioprimed with T_3 (TCF @ 1 per cent) showed maximum germination index (34.50) compared to all other priming treatments. The lowest germination index (17.07) was observed in the absolute control treatment (T_6).

4.1.1.6 Mean Germination Time

The priming treatments significantly influenced the mean germination time also. T_5 (hydro priming) recorded the highest mean germination time (8.65 days) among the various priming treatments. The treatment, T_3 (TCF @ 1 per cent) recorded the lowest (6.29 days) mean germination time.

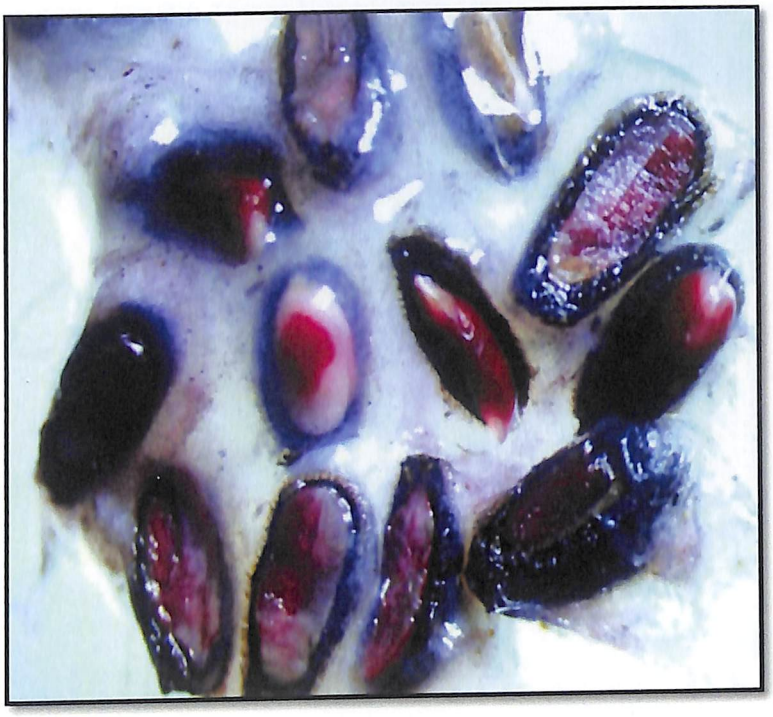


Plate 7. Seed viability test using triphenyl tetrazolium chloride

4.1.1.7 Shoot Length

Significant variation was observed in shoot length after one month of sowing, among the various priming treatments tried. Seedlings from the treatment, T_4 (PCF @ 1 per cent) recorded the highest (21.50 cm) shoot length compared to other priming treatments. The lowest (16.10 cm) shoot length was observed in the absolute control (T_6).

4.1.1.8 Root Length

Root length also exhibited significant variation at one month after sowing, among the various priming treatments tried. Seedlings from the treatment, T_4 (PCF @ 1 per cent) recorded the highest root length of 19.50 cm compared to other priming treatments. The lowest (13.80 cm) root length was observed in hydro priming (T_5) and absolute control (T_6).

4.1.1.9 Seedling Length

The priming treatments showed a significant effect with respect to seedling length (Plate 8). Treatment T_4 (PCF @ 1 per cent) recorded the highest seedling length of 41.00 cm compared to various priming treatments. The lowest (29.90 cm) seedling length was observed in the treatment T_6 (absolute control).

4.1.1.10 Allometric Index

Allometric index also exhibited significant variation among the various priming treatments. The highest (1.07) allometric index was recorded in the treatment, T_2 (PCWE @ 1 per cent) compared to other priming treatments. The lowest (0.81) allometric index was observed in the hydro priming treatment (T_5).

4.1.1.11 Seedling Vigor Index

A significantly higher (39.37) seedling vigor index was recorded in T_4 (PCF @ 1 per cent) than all other priming treatments. The lowest (21.53) seedling vigor index was observed in the absolute control (T_6).

4.2 PHASE II - EVALUATION OF THE EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON PLANT GROWTH, YIELD AND ESSENTIAL OIL PRODUCTION IN *O. BASILICUM*

The results of the effect of various foliar spray treatments using fungal derivatives and water spray on plant growth, yield and essential oil production in *O. basilicum* are presented in this section. The treatment without any foliar application was taken as absolute control.

4.2.1 Effect of Foliar Application of Fungal Derivatives on Plant Growth Parameters in *O. basilicum*

The observations on plant growth parameters *viz.*, plant height, collar girth, number of branches, number of flowering shoots and leaf area were recorded at 30 DAS (at transplanting), 60 DAS, 90 DAS and 110 DAS (at harvest). The data on the effect of foliar application of fungal derivatives on various plant growth parameters are illustrated in this section.

4.2.1.1 Plant Height

The results of the effect of foliar application of fungal derivatives on plant height of *O. basilicum* at different growth stages are depicted in Table 5. Among the various foliar spray treatments, significant variation in plant height was recorded at 30, 60, 90 and 110 DAS (at harvest).

At 30 DAS, plants in the treatment T_4 (PCF @ 1 per cent) recorded the highest plant height (21.50 cm) which was significantly different from other treatments. The lowest plant height (16.10 cm) was observed in the control treatment (T_6), which was statiscally on par with T_1.

At 60 DAS, maximum plant height (42.00 cm) was observed in the treatment T_4 (PCF @ 1 per cent), which was on par with T_3. The control treatment (T_6) exhibited the lowest plant height (25.17 cm) and was on par with T_1 and T_5.

Table 3. Effect of priming treatments on seed germination parameters in *O. basilicum* at 30 DAS

T. No.	Priming treatments	DIS (Days)	Gn (%)	S (%)	GI	MGT (Days)
T_1	TCWE (1%)	5 ± 1^{a}	82.33 ± 2.08^{d}	82.33 ± 2.08^{d}	19.31 ± 0.34^{e}	8.30 ± 0.02^{b}
T_2	PCWE (1%)	4 ± 1^{ab}	90.00 ± 2.00^{b}	90.00 ± 2.00^{b}	26.81 ± 0.41^{c}	7.73 ± 0.12^{c}
T_3	TCF (1%)	3 ± 0^{b}	93.00 ± 1.00^{ab}	93.00 ± 1.00^{ab}	34.50 ± 1.22^{a}	6.29 ± 0.32^{e}
T_4	PCF (1%)	3 ± 0^{b}	96.00 ± 2.00^{a}	96.00 ± 2.00^{a}	33.03 ± 0.99^{b}	6.82 ± 0.08^{d}
T_5	HP	4 ± 1^{ab}	86.00 ± 1.00^{c}	86.00 ± 1.00^{c}	24.00 ± 0.49^{d}	8.65 ± 0.02^{a}
T_6	Absolute control	5 ± 1^{a}	72.00 ± 2.00^{e}	72.00 ± 2.00^{e}	17.07 ± 0.49^{f}	8.02 ± 0.20^{bc}
SEm(±)		0.471	1.009	1.009	0.424	0.096
C.D. (0.05)		1.453	3.109	3.109	1.305	0.294

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; HP - Hydro priming; DIS – Days to initial sprouting; Gn - Germination; S - Survival; GI - Germination Index; MGT - Mean Germination Time. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 4. Effect of priming treatments on seedling growth parameters in *O. basilicum* at 30 DAS

T. No.	Priming treatments	SL (cm)	RL (cm)	Sdl L (cm)	AI	SVI
T_1	TCWE (1%)	16.60 ± 0.17^{cd}	15.00 ± 0.20^{d}	31.60 ± 0.36^{d}	0.90 ± 0.01^{b}	26.55 ± 0.62^{d}
T_2	PCWE (1%)	17.00 ± 0.36^{c}	18.20 ± 0.20^{b}	35.20 ± 0.46^{c}	1.07 ± 0.02^{a}	31.69 ± 1.11^{c}
T_3	TCF (1%)	19.40 ± 0.20^{b}	17.00 ± 0.27^{c}	36.40 ± 0.17^{b}	0.88 ± 0.02^{bc}	34.94 ± 0.57^{b}
T_4	PCF (1%)	21.50 ± 0.95^{a}	19.50 ± 0.30^{a}	41.00 ± 0.82^{a}	0.91 ± 0.05^{b}	39.37 ± 1.48^{a}
T_5	HP	17.00 ± 0.27^{c}	13.80 ± 0.30^{e}	30.80 ± 0.56^{d}	0.81 ± 0.01^{d}	26.49 ± 0.65^{d}
T_6	Absolute control	16.10 ± 0.17^{d}	13.80 ± 0.20^{e}	29.90 ± 0.36^{e}	0.86 ± 0.01^{c}	21.53 ± 0.85^{e}
SEm(±)		0.259	0.143	0.287	0.014	0.541
C.D. (0.05)		0.799	0.442	0.884	0.043	1.666

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; HP - Hydro priming; SL - Shoot Length; RL - Root Length; Sdl L - Seedling Length; AI - Allometric Index; SVI - Seedling Vigour Index.. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

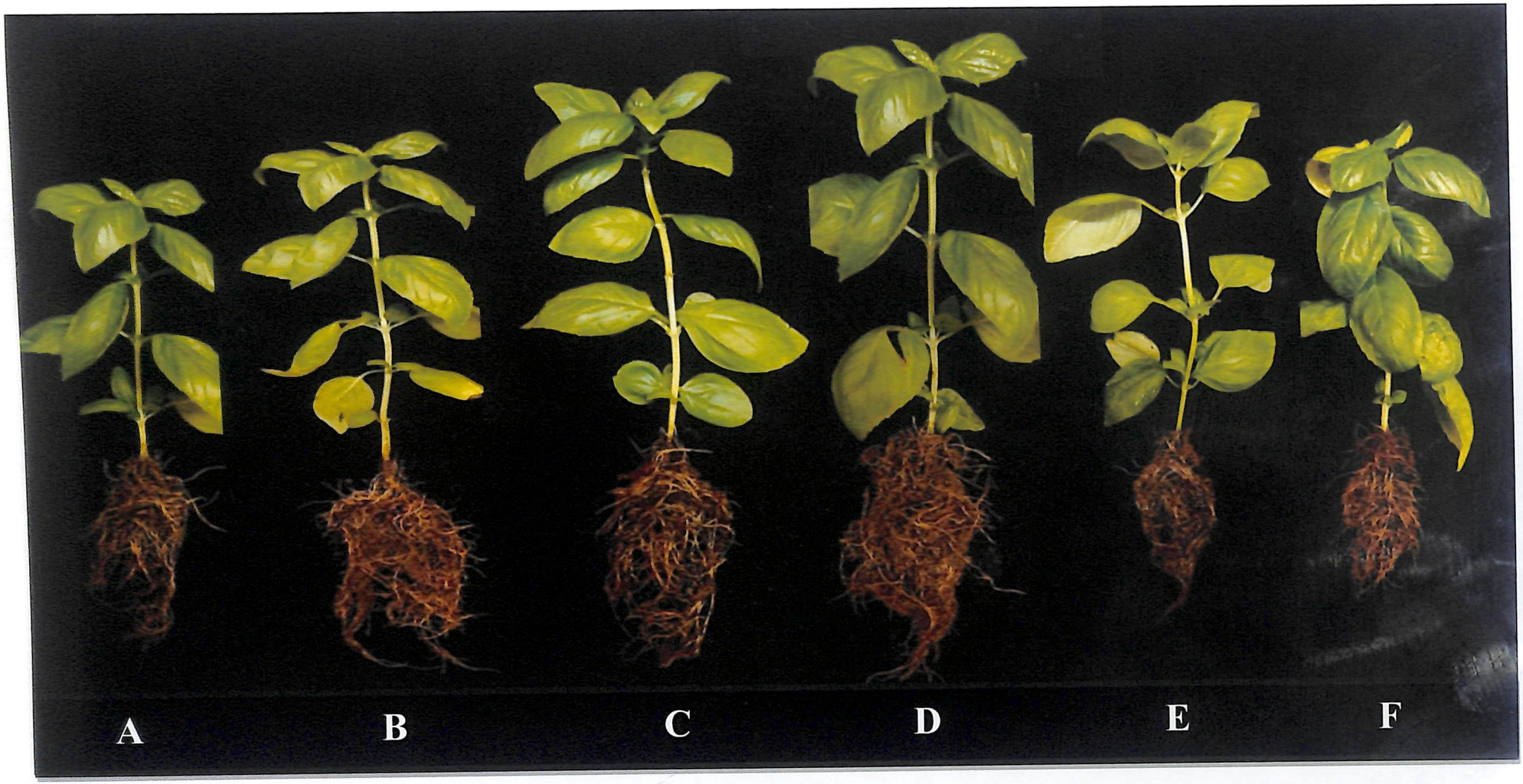


Plate 8. Effect of priming treatments on seedling growth in *O. basilicum* at 30 DAS: A) *Trichoderma viride* cell wall extract, B) *Piriformospora indica* cell wall extract, C) *Trichoderma viride* culture filtrate, D) *Piriformospora indica* culture filtrate, E) Hydro priming, F) Absolute control

At 90 DAS, the highest plant height (76.07 cm) was recorded in treatment T_4 (PCF @ 1 per cent), which was found to be on par with T_3. The treatment, T_5 (water spray) recorded the lowest plant height (54.00 cm) and was on par with T_1 and T_6.

Similarly, at 110 DAS also, T_4 (PCF @ 1 per cent) treatment significantly showed a higher plant height (80.20 cm), which was on par with T_3. Treatment T_5 (water spray) recorded the lowest plant height (54.00 cm), which was on par with T_6. No increase in plant height over 90 DAS was observed in T_5 (water spray) and T_6 (absolute control).

4.2.1.2 Collar Girth

The results of the effect of foliar application of fungal derivatives on collar girth of *O. basilicum* at different growth stages are presented in Table 6. Significant variation was observed in collar girth at 60, 90 and 110 DAS (at harvest). While at 30 days after sowing, the treatments had no significant influence on collar girth.

At 60 DAS, plants from the treatment T_4 (PCF @ 1 per cent) exhibited the highest collar girth (2.73 cm), that was on par with T_2, T_3 and T_5. The lowest collar girth (1.40 cm) was observed in the control treatment (T_6). This was found to be on par with T_1, T_2 and T_5.

At 90 DAS, plants from the treatment T_4 (PCF @ 1 per cent) recorded a significantly higher collar girth (5.67 cm) among the different foliar treatments tried. The lowest collar girth (3.67 cm) was obtained in the control treatment (T_6).

Furthermore, at 110 DAS also, significantly higher collar girth (6.03 cm) was observed in treatment T_4 (PCF @ 1 per cent) compared to other foliar treatments. The plants from control treatment (T_6) exhibited the lowest collar girth (3.73 cm).

4.2.1.3 Leaf Area

The result of the effect of foliar treatments on leaf area is depicted in Table 7. Significant variation in leaf area was observed among the various foliar treatments tried at all stages of observation.

At 30 DAS, T_4 (PCF @ 1 per cent) treatment exhibited the highest (208.59 cm^2) leaf area compared to other foliar treatments. The lowest leaf area (125.00 cm^2) was observed in T_6 (Absolute control).

At 60 DAS also, maximum leaf area (1554.84 cm^2) was observed in T_4 (PCF @ 1 per cent) and the control treatment (T_6) recorded the lowest (640.35 cm^2) leaf area.

Similarly, at 90 and 110 DAS also, significantly higher leaf area (3845.23 cm^2 and 4010.82 cm^2) was obtained in treatment T_4 (PCF @ 1 per cent) and the lowest leaf area (1995.34 cm^2 and 2217.65 cm^2 respectively), was recorded in the control treatment (T_6).

4.2.1.4 Number of Branches

The effect of foliar application of fungal derivatives on number of branches at all stages of plant growth is depicted in Table 8. The main stem did not produce any branches at 30 DAS. However, significant variation was observed in number of branches at 60, 90 and 110 DAS (at harvest).

At 60 DAS, the treatment T_4 (PCF @ 1 per cent) recorded the highest number of branches (19.33), which was statistically on par with T_3 (TCF @ 1 per cent). The lowest number of branches (13.67) was recorded in the control treatment (T_6). This was found to be on par with T_1 and T_5.

Moreover, at 90 DAS also, the treatment T_4 (PCF @ 1 per cent) recorded a significantly higher number of branches (25.67). The control treatment (T_6) recorded the lowest (16.67) number of branches. This was found to be on par with T_1, T_2 and T_5.

At 110 DAS, significantly higher number of branches (28.00) was obtained in T_4 (PCF @ 1 per cent) treatment. The lowest (17.00) number of branches was observed in the control treatment (T_6), which was on par with T_1 and T_5.

4.2.1.5 Number of Flowering Shoots

The result of the effect of foliar application of fungal derivatives on number of flowering shoots at all stages of observation is presented in Table 9. At 30 DAS, no flowering shoots were observed in transplanted *O. basilicum*. However, significant variation was observed in number of flowering shoots at 60 and 90 DAS.

At 60 DAS, the treatment T_4 (PCF @ 1 per cent) recorded a significantly higher (52.00) number of flowering shoots. The lowest (25.50) number of shoots was observed in the control treatment (T_6), which was on par with T_5 (water spray).

At 90 DAS, the highest (95.33) number of flowering shoots was observed in the plants subjected to PCF @ 1 per cent treatment. The lowest (74.33) number of flowering shoots was recorded in the control treatment (T_6), which was found to be on par with T_5 (water spray).

Correspondingly, at 110 DAS also, T_4 (PCF @ 1 per cent) recorded the highest (104.00) number of flowering shoots. The control treatment (T_6) recorded the lowest number of shoots (78.67), which was found to be statistically on par with T_5 (water spray).

4.2.2 Effect of Foliar Application of Fungal Derivatives on Phenological Parameters in *O. basilicum*

The results of the effect of foliar application of various fungal derivatives on phenological parameters *viz.* days to flowering and days to fruit set are depicted in Table 10.

4.2.2.1 Days to Flowering

The foliar spray treatments exhibited a significant influence on days to flowering. Treatment T_4 (PCF @ 1 per cent) recorded minimum number of days (55) to flowering, which was on par with treatment with T_2 (PCWE @ 1 per cent). The treatment, T_5 (water spray) recorded maximum number of days (72.33) to flowering in

O. basilicum. This was found to be on par with T_1 (TCWE @ 1 per cent) and T_6 (absolute control).

4.2.2.2 Days to Fruit set

The influence of various foliar spray treatments on days to fruit set in *O. basilicum* is depicted in Table 10. The results revealed that there was no significant variation with respect to days to fruit set, among the various foliar spray treatments.

4.2.3 Effect of Foliar Application of Fungal Derivatives on Biochemical Parameters in *O. basilicum*

The results on the effect of foliar application of various fungal derivatives on biochemical parameters *viz.*, chlorophyll content and polyphenol content in *O. basilicum* are illustrated in this section. The chlorophyll content (at 30, 60, 90 and 110 DAS) and polyphenol content recorded (at 110 DAS) are depicted in Table 11.

4.2.3.1 Total Chlorophyll Content

The data indicated that various foliar spray treatments tried had a significant effect on total chlorophyll content at all stages of observation in *O. basilicum*.

At 30 DAS, T_4 (PCF @ 1 per cent) and T_3 (TCF @ 1 per cent) recorded maximum total chlorophyll content (0.53 mg g^{-1}). The lowest total chlorophyll content (0.49 mg g^{-1}) was observed in T_5 (water spray) and T_6 (absolute control).

At 60 DAS, a significantly higher total chlorophyll content (1.92 mg g^{-1}) was observed in the treatment T_4 (PCF @ 1 per cent) compared to all other foliar spray treatments. The control treatment (T_6) recorded the lowest total chlorophyll content (1.56 mg g^{-1}).

At 90 DAS also, the highest total chlorophyll content (2.20 mg g^{-1}) was recorded in treatment T_4 (PCF @ 1 per cent). The lowest total chlorophyll content (1.78 mg g^{-1}) was observed in treatment T_5 and T_6.

Table 5. Effect of foliar application of fungal derivatives on plant height in *O. basilicum*

T. No.	Fungal derivatives	Plant height (cm)			
		30 DAS	60 DAS	90 DAS	110 DAS
T_1	TCWE (1%)	16.60 ± 0.17^{cd}	27.30 ± 4.06^{cd}	61.03 ± 6.57^{cd}	62.50 ± 3.39^{c}
T_2	PCWE (1%)	17.00 ± 0.36^{c}	32.93 ± 4.44^{bc}	65.50 ± 2.78^{bc}	69.47 ± 1.46^{b}
T_3	TCF (1%)	19.40 ± 0.20^{b}	38.33 ± 4.21^{ab}	72.00 ± 3.28^{ab}	75.50 ± 1.95^{a}
T_4	PCF (1%)	21.50 ± 0.96^{a}	42.00 ± 5.77^{a}	76.07 ± 3.58^{a}	80.20 ± 1.41^{a}
T_5	Water spray	17.00 ± 0.27^{c}	30.03 ± 3.59^{cd}	54.00 ± 3.28^{d}	54.00 ± 3.28^{d}
T_6	Absolute control	16.10 ± 0.17^{d}	25.17 ± 1.76^{d}	54.27 ± 3.66^{d}	54.27 ± 3.66^{d}
SEm(±)		0.259	2.393	2.340	1.555
C.D. (0.05)		0.799	7.373	7.210	4.791

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 6. Effect of foliar application of fungal derivatives on collar girth in *O. basilicum*

T. No.	Fungal derivatives	Collar girth (cm)			
		30 DAS	60 DAS	90 DAS	110 DAS
T_1	TCWE (1%)	0.80 ± 0.27	1.83 ± 0.35^{bc}	4.50 ± 0.46^{c}	4.60 ± 0.36^{c}
T_2	PCWE (1%)	0.83 ± 0.15	2.13 ± 0.55^{abc}	4.63 ± 0.35^{c}	4.87 ± 0.40^{c}
T_3	TCF (1%)	0.97 ± 0.21	2.43 ± 0.40^{ab}	5.17 ± 0.06^{b}	5.57 ± 0.15^{b}
T_4	PCF (1%)	1.13 ± 0.12	2.73 ± 0.40^{a}	5.67 ± 0.15^{a}	6.03 ± 0.15^{a}
T_5	Water spray	0.77 ± 0.15	2.07 ± 0.42^{abc}	4.43 ± 0.12^{c}	4.50 ± 0.00^{c}
T_6	Absolute control	0.73 ± 0.12	1.40 ± 0.35^{c}	3.67 ± 0.31^{d}	3.73 ± 0.23^{d}
SEm(±)		0.102	0.241	0.161	0.148
C.D. (0.05)		NS	0.743	0.496	0.455

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 7. Effect of foliar application of fungal derivatives on leaf area in *O. basilicum*

T. No.	Fungal derivatives	Leaf area (cm^2)			
		30 DAS	60 DAS	90 DAS	110 DAS
T_1	TCWE (1%)	162.50 ± 0.80^{d}	924.65 ± 0.52^{d}	2272.15 ± 0.57^{d}	2480.14 ± 1.42^{d}
T_2	PCWE (1%)	169.05 ± 0.24^{c}	1090.03 ± 3.17^{c}	2465.11 ± 1.45^{c}	2780.14 ± 1.42^{c}
T_3	TCF (1%)	184.66 ± 0.72^{b}	1278.12 ± 1.68^{b}	3250.72 ± 1.74^{b}	3340.47 ± 2.07^{b}
T_4	PCF (1%)	208.59 ± 0.86^{a}	1554.84 ± 2.19^{a}	3845.23 ± 0.73^{a}	4010.82 ± 1.20^{a}
T_5	Water spray	132.59 ± 0.57^{e}	675.01 ± 1.54^{e}	2056.15 ± 1.04^{e}	2254.00 ± 1.34^{e}
T_6	Absolute control	125.00 ± 0.96^{f}	640.35 ± 1.70^{f}	1995.34 ± 0.71^{f}	2217.65 ± 0.87^{f}
SEm(±)		0.421	1.135	0.649	0.909
C.D. (0.05)		1.299	3.497	1.998	2.800

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 8. Effect of foliar application of fungal derivatives on number of branches in *O. basilicum*

T. No.	Fungal derivatives	Number of branches			
		30 DAS	60 DAS	90 DAS	110 DAS
T_1	TCWE (1%)	-	15.33 ± 0.58^{cd}	17.33 ± 1.16^{c}	18.00 ± 0.00^{d}
T_2	PCWE (1%)	-	16.67 ± 0.58^{bc}	19.00 ± 2.00^{c}	20.67 ± 1.16^{c}
T_3	TCF (1%)	-	18.00 ± 0.00^{ab}	22.33 ± 1.53^{b}	24.00 ± 0.00^{b}
T_4	PCF (1%)	-	19.33 ± 1.16^{a}	25.67 ± 1.16^{a}	28.00 ± 1.00^{a}
T_5	Water spray	-	14.00 ± 2.00^{d}	17.00 ± 1.00^{c}	17.33 ± 1.16^{d}
T_6	Absolute control	-	13.67 ± 0.58^{d}	16.67 ± 0.58^{c}	17.00 ± 0.00^{d}
SEm(±)		-	0.593	0.758	0.451

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 9. Effect of foliar application of fungal derivatives on number of flowering shoots in *O. basilicum*

T. No.	Fungal derivatives	Number of flowering shoots			
		30 DAS	60 DAS	90 DAS	110 DAS
T_1	TCWE (1%)	-	32.00 ± 3.61^{d}	80.67 ± 1.16^{d}	86.00 ± 1.16^{d}
T_2	PCWE (1%)	-	38.67 ± 0.58^{c}	85.33 ± 3.51^{c}	91.67 ± 3.51^{c}
T_3	TCF (1%)	-	45.00 ± 2.00^{b}	90.33 ± 0.58^{b}	98.00 ± 0.58^{b}
T_4	PCF (1%)	-	52.00 ± 2.65^{a}	95.33 ± 0.58^{a}	104.00 ± 0.58^{a}
T_5	Water spray	-	26.67 ± 3.51^{e}	75.00 ± 2.65^{e}	79.33 ± 2.65^{e}
T_6	Absolute control	-	25.50 ± 2.00^{e}	74.33 ± 3.06^{e}	78.67 ± 3.06^{e}
SEm(±)		-	1.503	1.305	1.472
C.D. (0.05)		-	4.632	4.022	4.537

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 10. Effect of foliar application of fungal derivatives on phenological parameters in *O. basilicum*

T. No.	Fungal derivatives	Days to flowering (Days)	Days to fruit set (Days)
T_1	TCWE (1%)	69.00 ± 4.00^{ab}	1.67 ± 0.58
T_2	PCWE (1%)	60.00 ± 5.00^{cd}	1.67 ± 0.58
T_3	TCF (1%)	64.67 ± 3.06^{bc}	1.33 ± 0.58
T_4	PCF (1%)	55.00 ± 4.58^{d}	1.33 ± 0.58
T_5	Water spray	72.33 ± 2.08^{a}	2.00 ± 0.00
T_6	Absolute control	71.67 ± 1.53^{a}	2.33 ± 0.58
SEm(±)		2.082	0.304
C.D. (0.05)		6.414	NS

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

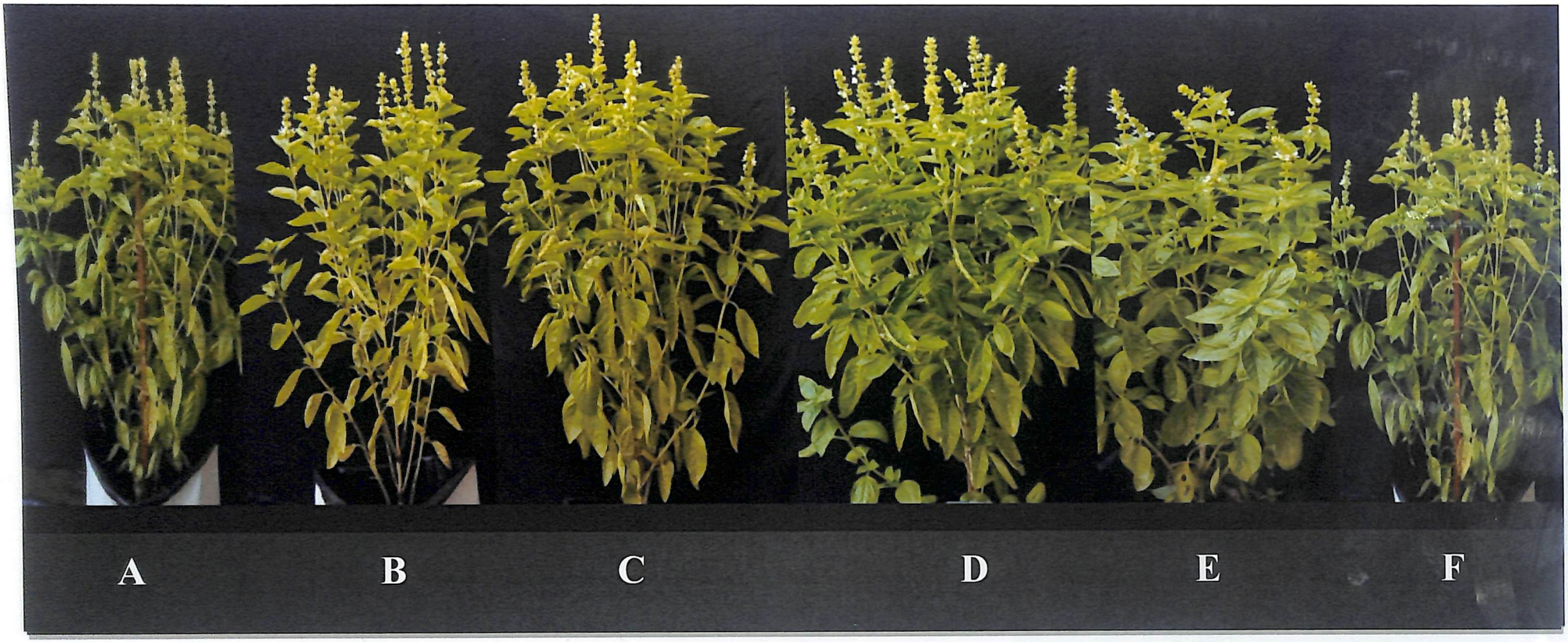


Plate 9. Effect of foliar application of fungal derivatives on herbage yield in *O. basilicum* at harvest: A) *Trichoderma viride* cell wall extract, B) *Piriformospora indica* cell wall extract, C) *Trichoderma viride* culture filtrate, D) *Piriformospora indica* culture filtrate, E) Water spray, D) Absolute control

Similar trend as in 90 DAS was observed at 110 DAS also, the treatment T_4 (PCF @ 1 per cent) showed maximum (1.20 mg g^{-1}) total chlorophyll content. The treatment, T_5 and T_6 recorded minimum total chlorophyll content (0.88 mg g^{-1}).

4.2.3.2 Polyphenol Content

Among the foliar spray treatments tried, the data showed a significant variation in polyphenol content in *O. basilicum* at harvest stage (110 DAS).

Treatment T_4 (PCF @ 1 per cent) recorded the maximum (84.31 mg PE g^{-1}) polyphenol content, which was statistically on par with T_2 (PCWE @ 1 per cent). The lowest (53.22 mg PE g^{-1}) polyphenol content was observed in the control treatment (T_6). This was found to be on par with T_5 (water spray).

4.2.4 Effect of Foliar Application of Fungal Derivatives on Yield Parameters in *O. basilicum*

The data on the effect of foliar application of various fungal derivatives on yield parameters *viz.*, leaf biomass (fresh and dry), stem biomass (fresh and dry), herbage yield (fresh and dry), root biomass (fresh and dry), total plant biomass (fresh and dry) and harvest index are presented in this section. The observations were recorded at harvest stage (110 DAS).

4.2.4.1 Leaf Biomass (Fresh and Dry)

Among the foliar spray treatments, significant variation was observed in total leaf biomass, both on fresh weight and dry weight basis (Table 12). Treatment T_4 (PCF @ 1 per cent) significantly recorded higher fresh (210.00 g) and dry (19.04 g) leaf weight. The treatment, T_6 recorded the lowest fresh (164.67 g) and dry (15.04 g) leaf weight, which was statistically on par with T_5 (water spray) on fresh and dry weight basis.

4.2.4.2 Stem Biomass (Fresh and Dry)

Stem biomass, both fresh and dry, exhibited significant variation among the foliar spray treatments tried. The data is presented in Table 12. The highest stem fresh

weight (135.33 g) was recorded in treatment T_4 (PCF @ 1 per cent), which was on par with T_3. The lowest stem fresh weight (90.00 g) was observed in treatment, T_6 (absolute control) which was found to be on par with T_5 (water spray). The highest stem dry weight (12.21 g) was obtained in treatment, T_4 (PCF @ 1 per cent). The control treatment (T_6) recorded the lowest value of stem dry weight (8.82 g) which was statistically on par with T_5 (water spray).

4.2.4.3 Herbage Yield (Fresh and Dry)

The foliar spray treatments significantly influenced the herbage yield, both on fresh weight and dry weight basis. The data is depicted in Table 12 and Plate 9. Treatment T_4 (PCF @ 1 per cent) exhibited the highest fresh and dry herbage yield (345.33 g and 31.25 g, respectively). The lowest herbage yield in terms of both fresh weight (255.33 g) and dry weight (23.86 g) were recorded in the control treatment (T_6). This was found to be on par with treatment T_5 (water spray).

4.2.4.4 Root Biomass (Fresh and Dry)

Among the foliar spray treatments, significant variation was observed in root biomass with respect to both fresh and dry root biomass. The data is depicted in Table 13 and Plate 10. The maximum fresh root (52.00 g) and dry root (4.63 g) biomass was observed in treatment T_4 (PCF @ 1 per cent). The lowest values in terms of both fresh root (25.00 g) was observed in T_5 and T_6. While the lowest dry root (2.08 g) biomass was recorded in the control treatment (T_6),which was found to be statistically on par with treatment T_5 (water spray).

4.2.4.5 Total Plant Biomass (Fresh and Dry)

The treatments showed significant variation on total plant biomass, both in fresh as well as dry plant biomass. The data is presented in Table 13. Treatment T_4 (PCF @ 1 per cent) recorded the highest total plant fresh weight (397.33 g) and dry weight (35.88 g). The lowest values (280.33 g and 25.95 g, respectively) of fresh and dry total plant biomass were recorded in the control treatment (T_6). This was found to be on par with treatment T_5 (water spray).

Table 11. Effect of foliar application of fungal derivatives on biochemical parameters in *O. basilicum*

T. No.	Fungal derivatives	Total chlorophyll content (mg g^{-1})				Polyphenol content (mg PE g^{-1})
		30 DAS	60 DAS	90 DAS	110 DAS	At harvest
T_1	TCWE (1%)	0.49 ± 0.002^{b}	1.66 ± 0.004^{d}	1.82 ± 0.003^{d}	0.90 ± 0.004^{d}	65.26 ± 0.36^{c}
T_2	PCWE (1%)	0.50 ± 0.001^{b}	1.72 ± 0.002^{c}	1.92 ± 0.002^{c}	0.93 ± 0.001^{c}	83.86 ± 0.51^{a}
T_3	TCF (1%)	0.53 ± 0.002^{a}	1.86 ± 0.004^{b}	2.17 ± 0.001^{b}	0.98 ± 0.002^{b}	73.52 ± 0.75^{b}
T_4	PCF (1%)	0.53 ± 0.004^{a}	1.92 ± 0.002^{a}	2.20 ± 0.003^{a}	1.20 ± 0.002^{a}	84.31 ± 0.82^{a}
T_5	Water spray	0.49 ± 0.002^{c}	1.57 ± 0.002^{e}	1.78 ± 0.001^{e}	0.88 ± 0.003^{e}	53.31 ± 0.39^{d}
T_6	Absolute control	0.49 ± 0.002^{c}	1.56 ± 0.002^{f}	1.78 ± 0.001^{e}	0.88 ± 0.002^{e}	53.22 ± 0.54^{d}
SEm(±)		0.001	0.002	0.001	0.001	0.338
C.D. (0.05)		0.004	0.004	0.004	0.004	1.041

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 12. Effect of foliar application of fungal derivatives on leaf biomass, stem biomass and herbage yield in *O. basilicum* at 110 DAS

T. No.	Fungal derivatives	Leaf biomass (g $plant^{-1}$)		Stem biomass (g $plant^{-1}$)		Herbage yield (g $plant^{-1}$)	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
T_1	TCWE (1%)	175.00 ± 8.00^{cd}	16.13 ± 0.43^{c}	105.33 ± 5.03^{c}	10.06 ± 0.23^{c}	280.33 ± 13.01^{d}	26.19 ± 0.45^{d}
T_2	PCW E (1%)	182.33 ± 2.52^{bc}	18.32 ± 0.28^{b}	114.33 ± 4.04^{b}	10.53 ± 0.29^{c}	296.67 ± 5.69^{c}	28.84 ± 0.42^{c}
T_3	TCF (1%)	188.00 ± 3.61^{b}	18.77 ± 0.08^{ab}	129.00 ± 3.61^{a}	11.13 ± 0.40^{b}	317.00 ± 6.25^{b}	29.90 ± 0.41^{b}
T_4	PCF (1%)	210.00 ± 5.00^{a}	19.04 ± 0.41^{a}	135.33 ± 5.03^{a}	12.21 ± 0.30^{a}	345.33 ± 8.96^{a}	31.25 ± 0.13^{a}
T_5	Water spray	166.67 ± 6.11^{d}	15.41 ± 0.47^{d}	95.00 ± 2.00^{d}	9.07 ± 0.28^{d}	261.67 ± 5.03^{e}	24.48 ± 0.38^{e}
T_6	Absolute control	164.67 ± 10.02^{d}	15.04 ± 0.41^{d}	90.67 ± 1.16^{d}	8.82 ± 0.18^{d}	255.33 ± 10.02^{e}	23.86 ± 0.58^{e}
SEm(±)		3.697	0.214	2.177	0.166	4.981	0.241
C.D. (0.05)		11.391	0.660	6.709	0.512	15.351	0.740

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 13. Effect of foliar application of fungal derivatives on root biomass and total plant biomass in *O. basilicum* at 110 DAS

T. No.	Fungal derivatives	Root biomass (g plant^{-1})		Total plant biomass (g plant^{-1})	
		Fresh weight	Dry weight	Fresh weight	Dry weight
T_1	TCWE (1%)	33.00 ± 2.65^c	2.80 ± 0.23^c	313.33 ± 15.18^c	29.00 ± 0.67^c
T_2	PCWE (1%)	45.33 ± 1.53^b	3.54 ± 0.30^b	342.00 ± 6.00^b	32.39 ± 0.64^b
T_3	TCF (1%)	35.67 ± 3.51^c	3.06 ± 0.23^c	352.67 ± 9.61^b	32.95 ± 0.60^b
T_4	PCF (1%)	52.00 ± 4.00^a	4.63 ± 0.17^a	397.33 ± 6.03^a	35.88 ± 0.13^a
T_5	Water spray	25.00 ± 3.00^d	2.17 ± 0.25^d	286.67 ± 8.02^d	26.64 ± 0.56^d
T_6	Absolute control	25.00 ± 1.00^d	2.08 ± 0.18^d	280.33 ± 9.50^d	25.95 ± 0.63^d
SEm(±)		1.627	0.134	5.526	0.329
C.D. (0.05)		5.013	0.411	17.028	1.015

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 14. Effect of foliar application of fungal derivatives on harvest index in *O.basilicum* at 110 DAS

T. No.	Fungal derivatives	Harvest Index		
		Leaf	Stem	Herbage
T_1	TCWE (1%)	0.56 ± 0.01^{b}	0.35 ± 0.01	0.90 ± 0.01^{c}
T_2	PCW E (1%)	0.57 ± 0.01^{ab}	0.33 ± 0.003	0.89 ± 0.01^{d}
T_3	TCF (1%)	0.57 ± 0.01^{ab}	0.34 ± 0.01	0.91 ± 0.01^{bc}
T_4	PCF (1%)	0.53 ± 0.01^{c}	0.34 ± 0.01	0.87 ± 0.004^{e}
T_5	Water spray	0.58 ± 0.01^{a}	0.34 ± 0.02	0.92 ± 0.01^{ab}
T_6	Absolute control	0.58 ± 0.01^{a}	0.34 ± 0.002	0.92 ± 0.01^{a}
SEm(±)		0.005	0.005	0.004
C.D. (0.05)		0.015	NS	0.010

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

4.2.4.6 Harvest Index

The foliar spray treatments exhibited a significant variation on harvest index. The data is presented in Table 14.

The maximum (0.58) harvest index in terms of leaf yield was recorded in the treatments T_5 and T_6, this was found to be statistically on par with treatments T_2 and T_3. The treatment, T_4 (PCF @ 1 per cent) was recorded minimum (0.53) harvest index.

The harvest index in terms of stem yield, treatments did not show any significant variation among the various foliar spray treatments tried.

The harvest index in terms of herbage yield was observed to be the highest (0.92) in T_5 and T_6. The lowest harvest index (0.87) was observed in treatment, T_4 (PCF @ 1 per cent).

4.2.5 Effect of Foliar Application of Fungal Derivatives on Yield Parameters in Ratoon Crop (60 days after the first harvest) of *O. basilicum*

The data on the effect of foliar application of various fungal derivatives on yield parameters in ratoon crop of *O. basilicum* is depicted in Table 15. The observations *viz.*, leaf biomass (fresh and dry), stem biomass (fresh and dry) and herbage yield (fresh and dry) were recorded at 60 days after the first harvest.

4.2.5.1 Leaf Biomass (Fresh and Dry)

'Significant variation was observed in total leaf biomass with respect to both fresh weight and dry leaf biomass, among the foliar spray treatments applied. The highest leaf biomass (125.33 g and 12.44 g) respectively, in terms of both fresh and dry weight was obtained in treatment T_4 (PCF @ 1 per cent). The lowest fresh leaf of 83.00 g and dry leaf biomass of 8.25 g was recorded both in T_5 and T_6.

4.2.5.2 Stem Biomass (Fresh and Dry)

Among the foliar spray treatments, significant variation was observed in total stem biomass, both on fresh weight and dry weight basis. Treatment T_4 (PCF @ 1 per

cent) recorded a higher fresh (76.00 g) and dry (7.31 g) stem biomass. The lowest value in terms of both fresh (38.00 g) and dry (3.75 g) stem biomass was recorded in the control treatment (T_6).

4.2.5.3 Herbage Yield (Fresh and Dry)

The foliar spray treatments exhibited significant variation on herbage yield, both on fresh weight and dry weight basis. Treatment T_4 (PCF @ 1 per cent) recorded significantly higher herbage yield in terms of both fresh weight and dry weight (201.33 g and 19.75 g, respectively). The lowest fresh (121.00 g) and dry (12.00 g) herbage yield was observed in the control treatment (T_6) and this was found to be statistically on par with T_5.

4.2.6 Effect of Foliar Application of Fungal Derivatives on Essential Oil Production in *O. basilicum*

The results of the effect of foliar application of various fungal derivatives on essential oil content and oil yield are depicted in this section. The observations were recorded at harvest stage (110 DAS).

4.2.6.1 Essential Oil Content (Fresh and Dry Leaf Weight Basis)

As shown in Table 16, the foliar spray treatments showed significant variation on essential oil content, both on fresh and dry leaf weight basis in *O. basilicum*.

On fresh weight basis, treatment T_4 (PCF @ 1 per cent) showed maximum (2.11 per cent) essential oil content compared to other foliar spray treatments. While the water spray (T_5) and control treatment (T_6) recorded the lowest (0.87 per cent) essential oil content.

On dry weight basis, the highest (1.00 per cent) essential oil content was obtained in treatment T_4 (PCF @ 1 per cent), while the lowest (0.40 per cent) essential oil content was recorded in T_5 and T_6.

4.2.6.2 Essential Oil Yield (Fresh and Dry Leaf Weight Basis)

The data on the effect of foliar spray treatments on essential oil yield that showed a significant effect, in terms of both fresh and dry weight of leaf is presented in Table 16.

On fresh leaf weight basis, a significantly higher essential oil yield (443.10 g) was recorded in the treatment T_4 (PCF @ 1 per cent) and the lowest (141.33 g) essential oil yield was observed in the control treatment (T_6). This was statistically on par with T_5 (water spray).

On dry leaf weight basis, the treatment T_4 (PCF @ 1 per cent) recorded the highest essential oil yield (19.04 g) compared to all other foliar spray treatments. The lowest essential oil yield (6.02 g) was observed in control treatment (T_6), which was found to be statistically on par with T_5 (water spray).

4.3. INCIDENCE OF PESTS AND DISEASES

After transplanting, leaf roller was observed at random throughout the crop period, irrespective of all the treatments, which could be controlled by hand picking and application of entomopathogenic fungus, *Beauveria bassiana.* At 80 DAS, the lace bug was observed in the field at random in all the treatments, which could be controlled by the application of neem oil and *Beauveria bassiana.* In the ratoon crop, downy mildew incidence was observed (Plate 11).

Table 15. Effect of foliar application of fungal derivatives on yield parameters in ratoon crop (60 days after the first harvest) of *O. basilicum*

T. No.	Fungal derivatives	Leaf biomass (g plant^{-1})		Stem biomass (g plant^{-1})		Herbage yield (g plant^{-1})	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
T_1	TCWE (1%)	90.67 ± 1.16^{d}	8.98 ± 0.06^{d}	51.67 ± 0.58^{d}	4.87 ± 0.01^{d}	142.33 ± 1.53^{d}	13.85 ± 0.06^{d}
T_2	PCW E (1%)	94.67 ± 1.53^{c}	9.39 ± 0.02^{c}	55.00 ± 1.00^{c}	5.62 ± 0.04^{c}	149.67 ± 0.58^{c}	15.01 ± 0.05^{c}
T_3	TCF (1%)	110.00 ± 2.00^{b}	10.97 ± 0.03^{b}	65.67 ± 2.08^{b}	6.75 ± 0.03^{b}	175.67 ± 4.04^{b}	17.72 ± 0.04^{b}
T_4	PCF (1%)	125.33 ± 0.58^{a}	12.44 ± 0.02^{a}	76.00 ± 2.00^{a}	7.31 ± 0.03^{a}	201.33 ± 2.08^{a}	19.75 ± 0.03^{a}
T_5	Water spray	83.00 ± 3.00^{e}	8.25 ± 0.03^{e}	39.33 ± 1.53^{e}	3.87 ± 0.04^{e}	122.33 ± 2.31^{e}	12.12 ± 0.06^{e}
T_6	Absolute control	83.00 ± 1.73^{e}	8.25 ± 0.02^{e}	38.00 ± 2.00^{e}	3.75 ± 0.01^{f}	121.00 ± 1.00^{e}	12.00 ± 0.03^{f}
SEm(±)		1.054	0.018	0.943	0.015	1.284	0.026
C.D. (0.05)		3.248	0.056	2.904	0.045	3.957	0.078

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Table 16. Effect of foliar application of fungal derivatives on essential oil production in *O. basilicum*

T. No.	Fungal derivatives	Essential oil content (%)		Essential oil yield (g plant^{-1})	
		Fresh leaf	Dry leaf	Fresh herbage	Dry herbage
T_1	TCWE (1%)	1.20 ± 0.17^{bc}	0.50 ± 0.00^{c}	209.20 ± 23.28^{c}	8.07 ± 0.22^{c}
T_2	PCW E (1%)	1.54 ± 0.21^{b}	0.83 ± 0.06^{b}	280.47 ± 34.66^{b}	15.27 ± 1.16^{b}
T_3	TCF (1%)	1.54 ± 0.21^{b}	0.77 ± 0.06^{b}	289.88 ± 42.97^{b}	14.39 ± 1.20^{b}
T_4	PCF (1%)	2.11 ± 0.19^{a}	1.00 ± 0.00^{a}	443.10 ± 41.24^{a}	19.04 ± 0.41^{a}
T_5	Water spray	0.87 ± 0.23^{c}	0.40 ± 0.10^{d}	143.73 ± 35.33^{d}	6.14 ± 1.39^{d}
T_6	Absolute control	0.87 ± 0.23^{c}	0.40 ± 0.00^{d}	141.33 ± 31.79^{d}	6.02 ± 0.16^{d}
SEm(±)		0.120	0.030	20.478	0.512
C.D. (0.05)		0.370	0.093	63.106	1.575

T. No. - Treatment Number; TCWE - *Trichoderma viride* cell wall extract; PCWE - *Piriformospora indica* cell wall extract; TCF - *Trichoderma viride* culture filtrate; PCF - *Piriformospora indica* culture filtrate; DAS - Days after sowing. Each figure represents mean (±SD) of three replications. Figures followed by same letter do not differ significantly ($p>0.05$).

Plate 10. Effect of foliar application of fungal derivatives on root biomass in *O. basilicum* at harvest: A) *Trichoderma viride* cell wall extract, B) *Piriformospora indica* cell wall extract, C) *Trichoderma viride* culture filtrate, D) *Piriformospora indica* culture filtrate, E) Water spray, D) Absolute control

Plate 11. Pests and diseases observed during the study in *O. basilicum*: A) Leaf roller, B) Lace bug, C) Downy mildew

DISCUSSION

5. DISCUSSION

The present study entitled "Growth, yield and essential oil production responses to microbial elicitation in *Ocimum basilicum* L." was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2019-2021. The results obtained in the study are discussed in this chapter.

The seeds of *O. basilicum* were subjected to various priming treatments using fungal derivatives *viz., Trichoderma viride* cell wall extract (1%) (TCWE), *Trichoderma viride* culture filtrate (1%) (TCF), *Piriformospora indica* cell wall extract (1%) (PCWE), *Piriformospora indica* culture filtrate (1%) (PCF) and hydro priming to study their effect on germination and seedling growth parameters. It is transplanted to grow bags at 30 days after sowing and foliar sprays of corresponding fungal derivatives (cell wall extract and culture filtrate) were given to plants at fortnightly intervals from transplanting to 90 days after sowing to evaluate their effect on plant growth, yield and essential oil production. The first cut was made 110 days after sowing. Further, the crop was maintained as ratoon crop upto 60 days after the first harvest, to study their effect on yield parameters. The responses of the effect of various priming treatments on seed germination and seedling growth parameters and further the foliar application of fungal derivatives on growth, yield and essential oil production are discussed here.

5.1 EFFECT OF PRIMING TREATMENTS ON SEED GERMINATION AND SEEDLING GROWTH PARAMETERS IN *O. basilicum*

The different priming treatments significantly influenced the seed germination and seedling growth parameters in *O. basilicum*. In our study, it was observed that biopriming with PCF @ 1 per cent recorded the highest germination per cent and survival per cent that was found to be statistically on par with priming treatment of TCF @ 1 per cent. PCF @ 1 per cent also recorded a significantly higher shoot length, root length, seedling length and seedling vigour index compared to other priming treatments tried. The effect of seed priming using fungal derivatives on seed germination, seedling length and seedling vigour index in *O. basilicum* is given in Fig 1.

In consensus with our study, Varma *et al.* (2012) reported that culture filtrate of *P. indica* @ 2.5 mL increased the seed germination, shoot length and root length in cabbage, broccoli and beans. He also confirmed that *P. indica* primed seeds of cabbage, radish, palak and onion exhibited the highest germination per cent (100 %) at 25 days after planting. According to Vyshakhi and Anith (2021), biopriming with *P. indica* showed a positive effect on enhancing the seedling growth and vigour index in solanaceous vegetables under *in vitro* condition.

The report of Gosal *et al.* (2010) also corroborates with the results of our study in *O. basilicum*. He observed the highest survival rate (91.2 %), and increase in shoot length, root length and total biomass in *Chlorophytum* sp. on biopriming with *P. indica*. A similar effect of *P. indica* was also reported by Jisha *et al.* (2018) in *Centella asiatica* who observed that *P. indica* colonized seedlings showed a 70 per cent survival rate under water stress conditions as against 10 per cent in the control plants. In consensus with our findings with respect to TCF, Rai and Behara (2019) revealed that a significant enhancement in seed germination and seedling growth parameters on biopriming the chilli seeds with culture broth of *T. viride*. Similar effects of TCF were also observed in seeds of radish (Mukhopadhyay and Pan, 2012) and tomato (Juan *et al.*, 2021).

However, it was observed that seeds biopriming with PCF @ 1 per cent inspite of showing a higher germination per cent, recorded a lower germination index and higher mean germination time. While treatment with TCF @ 1 per cent exhibited the highest germination index and lowest mean germination time in *O. basilicum*. In agreement with this result, Kaveh *et al.* (2011) demonstrated that seeds treated with *Trichoderma* strains at 50 % and 100 % concentrations showed a significantly higher germination index in muskmelon. Bhargava *et al.* (2015) also confirmed that *T. harzianum* at $1x10^5$ cfu mL^{-1}concentration reduced the mean germination time in bioprimed seeds of snapdragon.

In our study, we also observed that PCWE gave the highest allometric index, which indicates that root growth is better with PCWE. The PCF has the highest root length and shoot length. This higher shoot length resulted in a lower allometric index

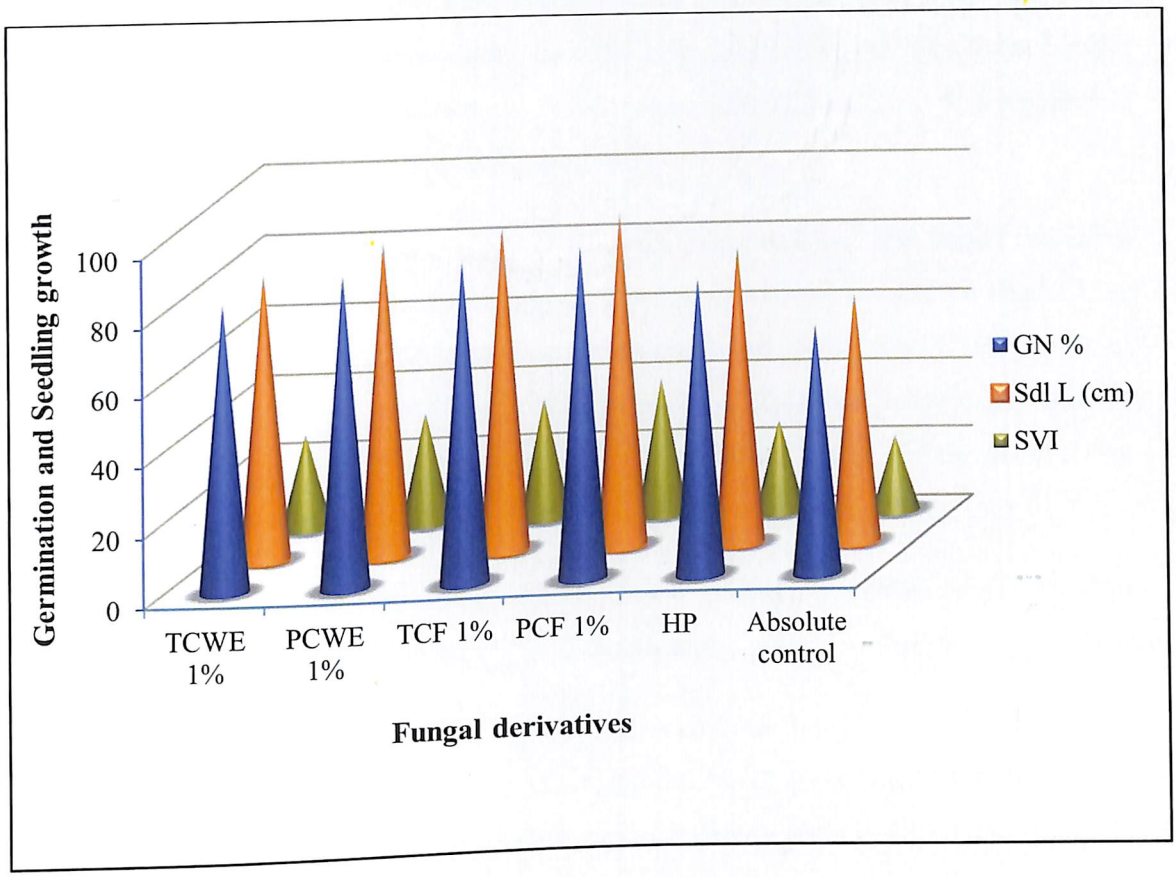


Fig 1: Effect of seed priming using fungal derivatives on germination per cent (GN %) seedling length (Sdl L) and seedling vigour index (SVI) in *O. basilicum*

since it is determined based on root length - shoot length ratio. *P. indica* being a root endophyte, both culture filtrate and cell wall extract gave better rooting but shoot growth was better with culture filtrate compared to cell wall extract. This resulted in higher allometric index in PCWE compared to PCF.

Varma *et al.* (2012) and Adya *et al.* (2013) reported that the fungal exudates, proteins, minerals, hormones and enzymes present in the culture filtrate might have contributed to the positive effect on seed germination and plant growth. The results of our study confirmed the opinion of Tamalla *et al.* (2014) that biopriming with *P. indica* and *T. virens* exhibited positive response on seedling growth attributes in mung bean.

5.2 EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON PLANT GROWTH PARAMETERS IN *O. basilicum*

The foliar spray of different fungal derivatives has significantly influenced the various plant growth parameters *viz.,* plant height, collar girth, leaf area, number of branches and number of flowering shoots in transplanted plants. Significantly higher values with respect to the said growth parameters were recorded in plants subjected to foliar application with PCF @ 1 per cent at all stages of observation. TCF @ 1 per cent also recorded higher plant height at 60, 90 and 110 DAS. The collar girth did not show any significant variation at 30 DAS among the treatments but PCF @ 1 per cent recorded significantly higher collar girth at 60, 90 and 110 DAS. Bhargava *et al.* (2015) and Vinayarani *et al.* (2019) reported that *Trichoderma* inoculated plants significantly enhanced the plant height over the control plants under field performance in snapdragon and in turmeric, respectively. The effect of foliar application of fungal derivatives on plant height in *O. basilicum* is given in Fig 2.

In consensus with our study, Varma *et al.* (2012) observed that the positive effect of *P. indica* and its culture filtrate on enhancing plant growth, total plant biomass and phytochemical production in medicinally important crops, *viz. Artemisia, Withania, Spilanthus, Stevia, Coleus* etc. They also confirmed that application of 15 mL PCF improved the plant height, collar diameter, number of leaves, flowers and roots in *Helianthus annus* and *Phaseolus vulgaris.* Similar results on plant growth

promotion were demonstrated by Badge *et al.* (2011) in *H. annus* and Badge *et al.* (2014) in *Aristolochia elegans.*

The study conducted by Rashnoo *et al.* (2020) in chicory plants also reported the positive influence of PCF foliar application on enhancing plant growth parameters at different plant growth stages. The findings of our study were further corroborated by the observations of Dolatabadi *et al.* (2011a, b) in fennel and thyme, Das *et al.* (2012) in *Coleus forskohlii*, Bhola *et al.* (2017) in turmeric, Anith *et al.* (2018) in bush pepper and Noorjahan *et al.* (2018) in African marigold. They found that *P. indica* colonization significantly enhanced plant growth parameters *viz.*, plant height, number of leaves, number of branches and root biomass.

With respect to leaf area, a significant enhancement was recorded by the foliar application of PCF at all stages of observation. This higher leaf area would result in more photosynthetic efficiency, resulting in higher herbage and oil yield per plant in *O. basilicum.* Keramati *et al.* (2016) confirmed that enhancement in leaf area and plant biomass production in *O. basilicum* under saline conditions by the application of *P. indica.* Similar effects of *P. indica* on enhancing leaf area was also demonstrated by Das *et al.* (2012) in *Coleus forskohlii* and Anith *et al.* (2018) in bush pepper.

In our study, it was observed that the main stem did not produce any branches and flowering shoots at 30 DAS. However, PCF @ 1 per cent exhibited significantly higher number of branches and flowering shoots at 60, 90 and 110 DAS (at harvest). Das *et al.* (2012) observed *P. indica* colonization in *Coleus forskohlii* induced higher percentage of flowering and more number of inflorescence.

As reported by Varma *et al.* (2012), the root endosymbiont, *P. indica* has been identified as a plant growth promoter, bioprotector, biofertilizer, as well as a nutrient uptake and transport agent. It was proven to evoke growth promotion and improve secondary metabolite production in medicinal plants through interaction with roots. Malla *et al.* (2004) also confirmed that *P. indica* contains a huge amount of acid phosphatase that has the ability to solubilise and transfer phosphate from the soil to the plant.

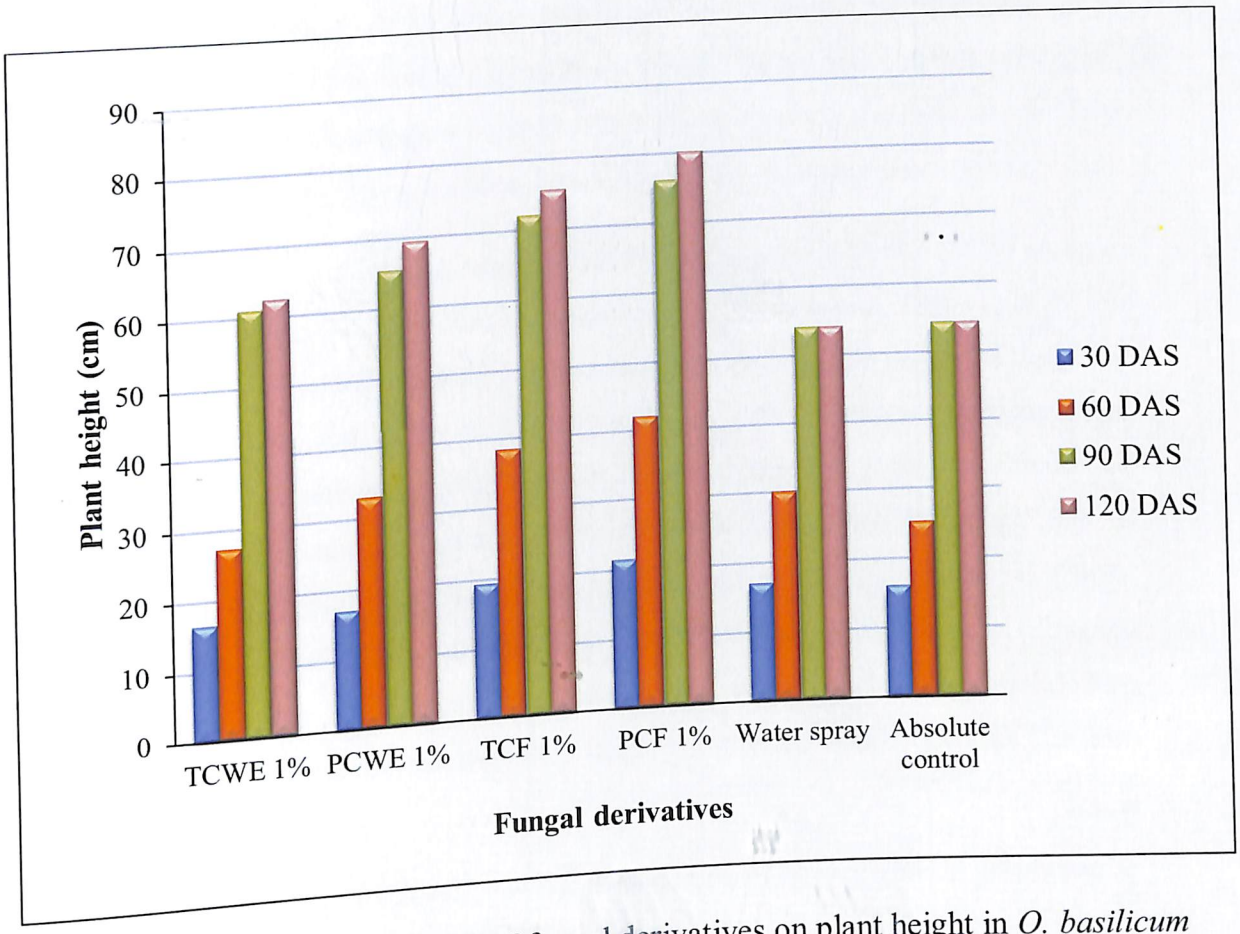


Fig 2: Effect of foliar application of fungal derivatives on plant height in *O. basilicum*

Similar findings on growth promotion were reported by Shahollari *et al.* (2005), Kumar *et al.* (2011) and Aslam *et al.* (2019). They observed that endophytic fungi also promoted phosphate uptake from the soil and growth medium which highly influenced the plant growth promotion by enhancing the phosphate absorption.

In our study, it was demonstrated that *P. indica* culture filtrate (PCF) recorded the highest plant growth promotion compared to other treatments. This might be due to the presence of many known and unknown compounds in the culture filtrate. The study conducted by Badge *et al.* (2010) revealed that it contains carbohydrates, proteins, minerals, hormones, enzymes etc. that exhibited a positive influence on growth and development in plants. They also illustrated that *P. indica* acts as modulator by increasing the nitrate assimilation and starch degradation in plants. Sherameti *et al.* (2005) also confirmed that the growth promoting effect of *P. indica* was associated with a co-regulated activation of nitrate and starch metabolism enzymes. These findings ascertained the results of our study with respect to *P. indica* application in *O. basilicum.*

Tamalla *et al.* (2014) observed that both the fungal endophytes *T. viride* and *P. indica* showed a positive response on seed germination parameters in mung bean. However, they observed that *P. indica* treatments gave better plant growth promotion compared to *T. viride,* as confirmed in our study. The study also revealed that the culture filtrates of both the fungal endophytes, *P. indica* and *T. viride* gave better growth promotion compared to cell wall extract. Similar finding on plant growth promotion by culture filtrate in comparison to cell wall extract of *P. indica* is reported in *Andrographis paniculata* seedlings by Nair and Sakuntala (2020).

5.3 EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON PHENOLOGICAL PARAMETERS IN *O. basilicum*

Among the foliar spray treatments tried, PCF @ 1 per cent had taken least number of days to flowering. This was found to be on par with PCWE @ 1 per cent with respect to days to flowering. However, the foliar spray treatments did not show any significant effect with respect to days to fruit set in *O. basilicum.*

In consensus with our findings, Das *et al.* (2012) revealed that *P. indica* colonization induced early flowering in *C. forskohlii*. The study conducted by Varma *et al.* (2012) in *Artemisia, Curcuma, Chlorophytum* sp. and *Coleus* also reported similar results. Furthermore, the findings of Noorjahan *et al.* (2018) in African marigold and Su *et al.* (2017) in *Brassica napus* also corroborate with the results of our study in *O. basilicum*. Induction of early flowering in *P. indica* inoculated plants might be attributed to the early expression of development modulating genes (Waller *et al.*, 2008; Pan *et al.*, 2017 and Kim *et al.*, 2017).

5.4 EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON BIOCHEMICAL PARAMETERS IN *O. basilicum*

5.4.1 Total Chlorophyll Content

'The results indicated that foliar application with fungal derivatives had a significant effect on total chlorophyll content at all stages of observation. The foliar application with PCF @ 1 per cent recorded the maximum total chlorophyll content at 30, 60, 90 and 110 DAS (at harvest).

The study conducted by Rashnoo *et al.* (2020) revealed that positive effect of foliar application of fungal derivatives on enhancing total chlorophyll content and relative water content in chicory plants. Root colonization by *P. indica* has the potential to increase the level of photosynthetic pigments under salt stress conditions in tomato plants (Ghorbani *et al.*, 2018). Similar results on the effect of *P. indica* on chlorophyll content were also reported by Sharma *et al.* (2014) in *Aloe vera* and Liu *et al.* (2019) in *Lolium multiflorum.* In addition to having higher chlorophyll content, the treatment, PCF @ 1 per cent had a larger leaf area per plant at all stages of observation. Studies conducted by Varma *et al.* (2012) proved that endophytic fungus, *P. indica* is involved in nutrient uptake, mainly nitrogen and phosphorous, in plants and hence, might contribute to higher leaf area.

5.4.2 Polyphenol Content

The findings of the present investigation indicated that foliar application of fungal derivatives had significantly influenced the polyphenol content in *O. basilicum.*

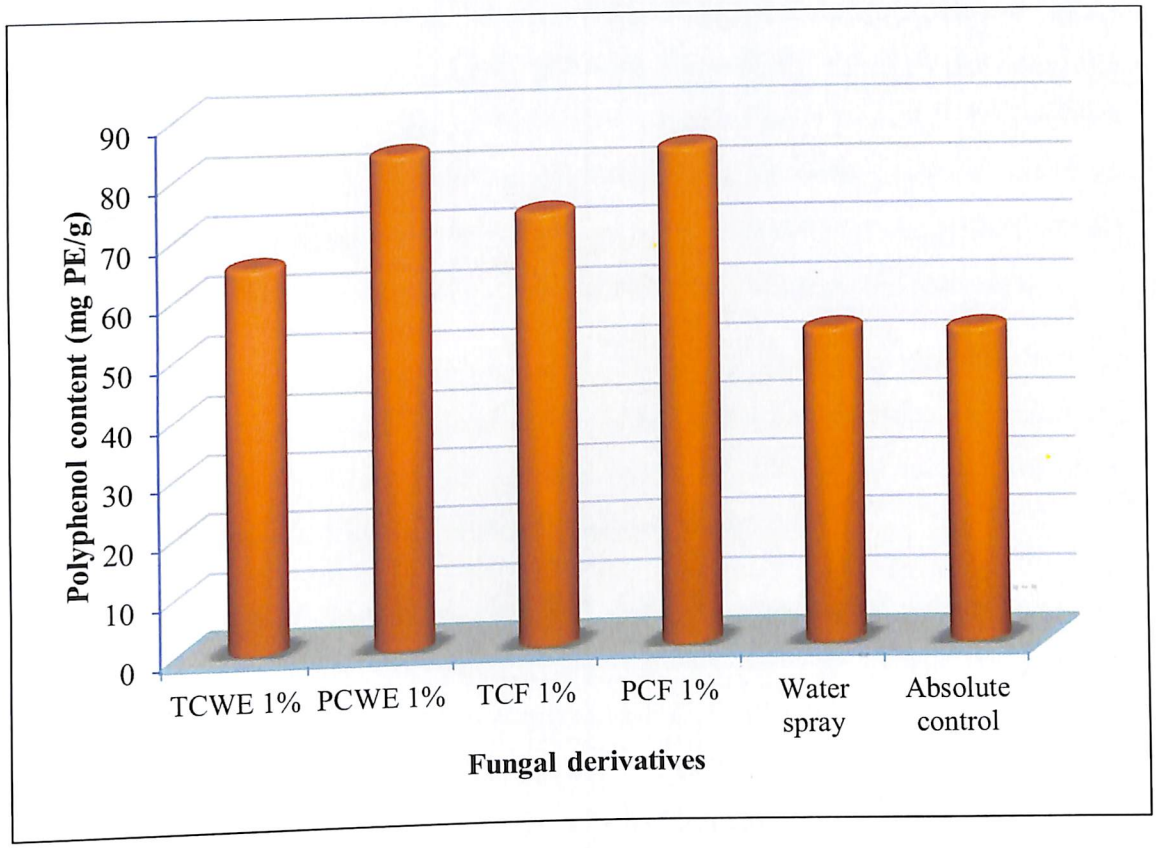


Fig 3: Effect of foliar application of fungal derivatives on polyphenol content in *O.basilicum*

Our study reported that foliar application with PCF @ 1 per cent recorded the highest polyphenol content which was found to be on par with PCWE @ 1 per cent. The effect of foliar application of fungal derivatives on polyphenol content in *O. basilicum* is given in Fig 3. This result was in confirmation with the findings of Rahman *et al.* (2020) in *Artemisia.* They observed that *P. indica* colonization enhanced the phenolic compounds in host plants by regulating the biosynthesis of metabolic pathways.

The study conducted by Amani *et al.* (2021) in *F. carica* reported that both the cell wall extract and culture filtrate of *P. indica* at 2% concentration enhanced the total phenol content in hairy root culture. Moreover, the fungal metabolites were observed to increase the level of major flavonoids present in fig.

The report of Jisha *et al.* (2018) in *Centella asiatica* and Tashackori *et al.* (2016) in *Linum album* also corroborates with the findings of our study in *O. basilicum*. Both PCF and PCWE interaction with *O. basilicum* might have influenced the regulation of metabolic pathways leading to the biosynthesis of phenylpropanoid derivatives, polyphenolic and flavonoid compounds in plants.

5.5 EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON YIELD PARAMETERS IN *O. basilicum*

In our study, it was demonstrated that foliar application of fungal derivatives significantly influenced the yield components *viz.*, leaf biomass, stem biomass, herbage yield, root biomass and total plant biomass. The effect of foliar application of fungal derivatives on herbage yield in *O. basilicum* is given in Fig 4. The plants obtained from the treatment PCF @ 1 per cent recorded the highest leaf biomass, stem biomass, herbage yield, root biomass and total plant biomass. Inspite of higher leaf, stem, herbage and total plant biomass, PCF @ 1 per cent recorded a lower harvest index compared to the control treatment.

In consensus with our findings, Varma *et al.* (2012) reported the positive effect of *P. indica* and its culture filtrate on enhancing plant growth, total plant biomass and secondary metabolite production in medicinally important crops *viz. Artemisia, Withania, Spilanthus, Stevia* and *Coleus*. Similar results were reported by Badge *et al.*

(2011) in *H. annus* and by Badge *et al.* (2014) in *Aristolochia elegans.* They observed that the root biomass, total plant biomass and aristolochic acid content in leaves were increased significantly by the application of PCF. Similar effects of PCF application on total biomass production was demonstrated by Ahlawat *et al.* (2016) in *Withania somnifera.* Foliar application of PCF @ 7.5 mL enhanced the overall plant growth parameters in chicory plants as reported by Rashnoo *et al.* (2020).

In the study, the plants treated with both PCF @ 1 per cent and TCF @1 per cent recorded significantly higher dry leaf biomass. The beneficial impact of *P. indica* colonization on enhancing total plant biomass production was demonstrated by Dolatabadi *et al.* (2011b) in thyme, Das *et al.* (2012) in *Coleus forskohlii* and Satheesan *et al.* (2012) in *Centella asiatica.* According to Khan *et al.* (2005), foliar application of *T.reesei* culture filtrate showed greater plant growth promotion and yield response in spearmint. Prasad *et al.* (2013) also observed that 3 % culture filtrate of *T. harzianum* enhanced the shoot biomass (1.24-fold) and growth index (7.67) in *Centella asiatica* under *in vitro* conditions.

The treatment, PCF @ 1 per cent, also exhibited a significantly higher leaf biomass, stem biomass and herbage yield in terms of both fresh and dry weight, in the ratoon crop. However, the results are comparatively lower than the yield obtained at 110 DAS. This could be attributed to the incidence of downy mildew noticed during the period for which the ratoon crop was maintained.

In our study, it was also observed that foliar application with PCF @ 1 per cent gave the highest root biomass. It proved that root endophytic fungi have enormous potential to increase the root biomass in plants. *P. indica* colonised plant roots and enhanced root length, number of roots, root volume, root thickness, and also the root biomass as reported by Gosal *et al.* (2010) in safed musli, Das *et al.* (2012) in *Coleus forskohlii,* and Noorjahan *et al.* (2018) in *Tagetes erecta.* The results of our study also revealed that though the treatment PCWE @ 1 per cent attained lower values in terms of shoot growth parameters and herbage yield, it gave better root biomass that was comparatively higher than other foliar spray treatments with *Trichoderma* derivatives. This could be attributed to the better root growth obtained in the priming treatment

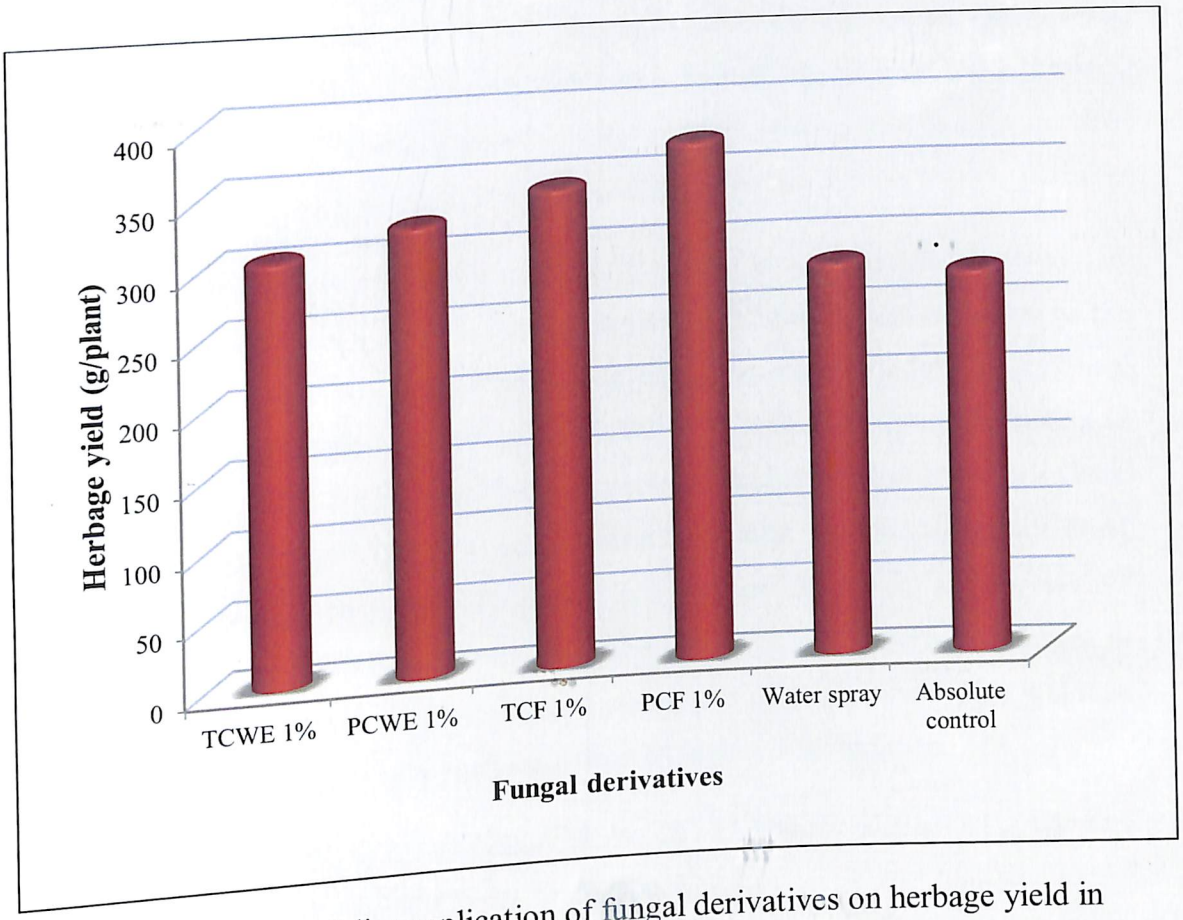


Fig 4: Effect of foliar application of fungal derivatives on herbage yield in *O.basilicum*

with PCWE @ 1 per cent. This is in confirmation with findings of Vadassery *et al.* (2009), who found that PCWE enhanced root growth more than shoot growth. However, Jisha *et al.* (2018) reported a contradictory finding in *Centella asiatica*, wherein the shoot growth was more prominent than root growth.

According to Sirrenberg *et al.* (2007), plant growth was either induced by substances identified in *P. indica* culture filtrate or induced by the organism in the host plants. They explained that plant growth promotion by PCF might be attributed to IAA synthesis in culture filtrate by *P. indica* or *P. indica* mediated production of IAA in the host plants. Moreover, *P. indica* proteins similar to myrosinase associated proteins raise the level of IAA in plants enhancing plant growth. Vadassery *et al.* (2008) were of the view that *P. indica* colonization improved auxin-cytokinin production in plants. They observed that higher levels of cytokinin accumulation in comparison to auxin in *P. indica* colonized plant roots of *Arabidopsis*. This might be the reason that *P. indica* inoculated plants showed higher plant biomass.

5.6 EFFECT OF FOLIAR APPLICATION OF FUNGAL DERIVATIVES ON ESSENTIAL OIL PRODUCTION IN *O. basilicum*

The results of our study indicated that essential oil production in the leaf of *O. basilicum* was significantly influenced by the foliar application of fungal derivatives. The highest essential oil content as well as oil yield, both on fresh and dry weight basis, was recorded in plants subjected to foliar spray treatment with PCF @ 1 per cent. This was followed by PCWE @1 per cent and TCF @ 1 per cent. The effect of foliar application of fungal derivatives on essential oil yield in *O. basilicum* is given in Fig 5. This result was in confirmation with findings of Dolatabadi *et al.* (2011a, b) in fennel and thyme. They revealed that when *P. indica* was inoculated, the essential oil content and its chemical constituents were significantly increased.

In a study conducted by Keramati *et al.* (2016) in *O. basilicum,* it was reported that *P. indica* colonization enhanced essential oil content, oil yield and its chemical constituents such as geranial and estragole. Similar effects of *P. indica* was reported in plants of *Coleus* (Das *et al.*, 2012), turmeric (Bajaj *et al.*, 2014), black pepper (Anith *et al.*, 2018) and peppermint (Khalvandi *et al.*, 2021).

In our study, though the treatment PCWE @ 1 per cent had a lower impact on plant growth parameters and herbage yield, it had a better response to *O. basilicum* essential oil content and yield. The higher polyphenol content observed in this treatment might have resulted in increased essential oil production. The results of the study confirmed the opinion of Ghesmati *et al.* (2017) that *in vitro* elicitation with PCWE @ 4 per cent could enhance the valerenic acid content in *Valeriana officinalis* by modulating the genes involved in the sesquiterpene pathway.

According to the findings, *P. indica* and its fungal derivatives have a huge potential for enhancing phenol compounds and contributing to the biosynthesis of secondary metabolites in host plants. The major components of essential oils are phenol compounds and terpenes. This may be the reason for higher essential oil production in *O. basilicum* due to application of *P. indica.*

In our study, the fresh leaves of *O. basilicum* recorded higher essential oil content and oil yield when compared to dried leaves. According to Baritaux *et al.* (1992), essential oil and its chemical components were dramatically reduced after drying and storage in *O. basilicum*. Hassanpouraghdam *et al.* (2010) also confirmed that drying under warm air leads to the loss of volatile oil. They observed that glandular trichomes involved in essential oil production are thermo-sensitive and volatile in nature. This might be attributed to the reason that the dried sample of *O. basilicum* showed a lower essential oil content and production than the fresh sample in our investigation.

In the first phase of study, PCF @ 1 per cent gave better performance in terms of seed germination, seedling growth and seedling vigour index. The transplanted seedlings from the same treatment when subjected to foliar application with PCF @ 1 per cent at fortnightly intervals gave the highest plant growth, biochemical and yield parameters in the second phase of study. Hence, it can be inferred that biopriming followed by foliar application of the fungal derivative PCF @ 1 per cent would give superior performance in terms of plant growth, yield and essential oil production in *O. basilicum.*

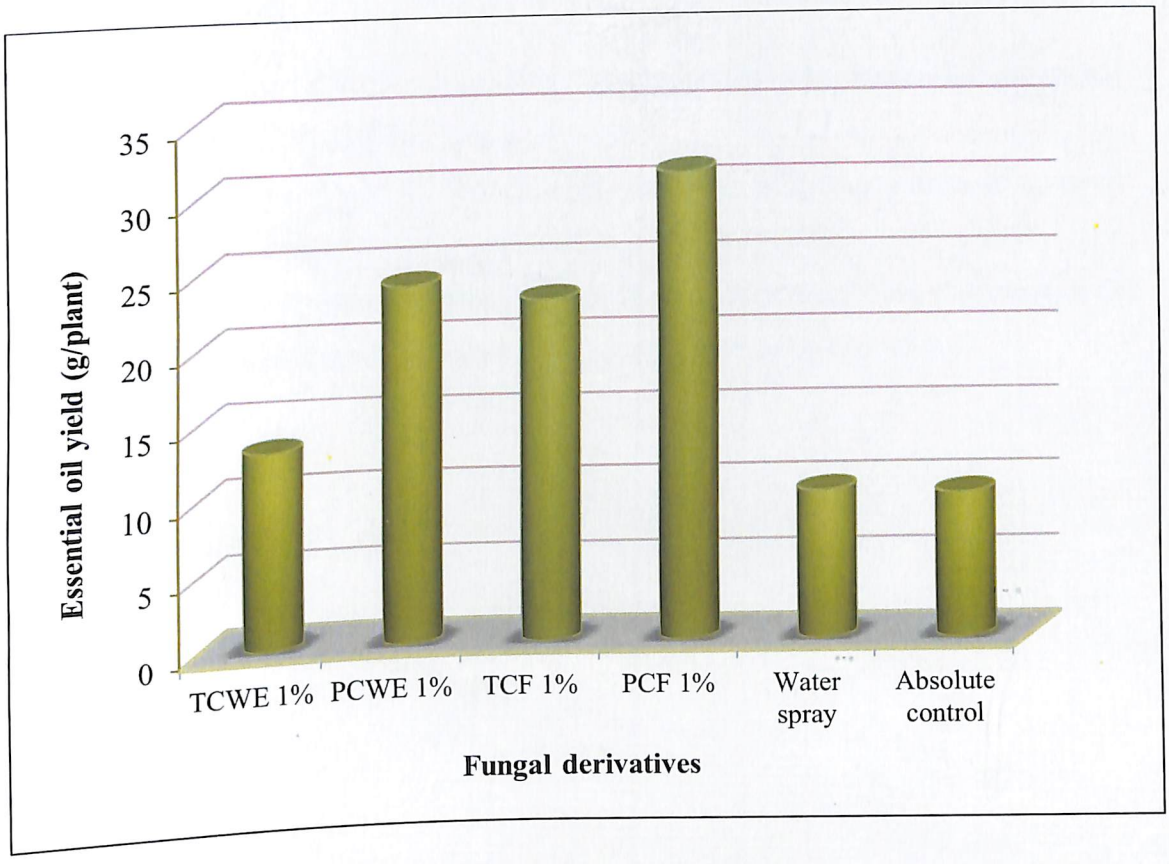


Fig 5: Effect of foliar application of fungal derivatives on essential oil yield in *O.basilicum*

Future line of work

- The effect of seed priming and seedling treatments with fungal derivatives has to be investigated under field conditions.
- The effect of fungal derivatives on the synthesis of specific aroma compounds in *O. basilicum* need to be explored.
- Physiological and molecular analyses of secondary metabolite elicitation in *O. basilicum* by fungal derivatives of *P. indica* have to be carried out.

SUMMARY

6. SUMMARY

The present investigation entitled "Growth, yield and essential oil production responses to microbial elicitation in *Ocimum basilicum* L." was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani. The field experiments were laid out in the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2019-2021. The main objective of the study was to evaluate the effect of fungal derivatives on growth, yield and essential oil production in *Ocimum basilicum* L.

The seeds of *O. basilicum* used for the study were sourced from Anand Agricultural University, Gujarat. The study was carried out in two phases: Phase 1- Seed priming using fungal derivatives for enhanced germination. Phase 2- Evaluation of the effect of foliar application of fungal derivatives for growth, yield and essential oil production. The experiment was laid out in completely randomized block design (CRD) with six treatments and three replications.

In the first phase of study, the seeds of *O. basilicum* were subjected to various priming treatments using fungal derivatives viz., *Trichoderma viride* cell wall extract (1 %) (TCWE), *Trichoderma viride* culture filtrate (1 %) (TCF), *Piriformospora indica* cell wall extract (1 %) (PCWE), *Piriformospora indica* culture filtrate (1 %) (PCF) and hydro priming to study their effect on seed germination and seedling growth parameters upto 30 days after sowing. The seeds without any priming were taken as the absolute control.

In the second phase of study, the 30 days old seedlings of *O. basilicum* were transplanted to grow bags. The foliar spray of corresponding fungal derivatives (cell wall extract and culture filtrate) at 1% concentration were given to plants at fortnightly intervals from transplanting to 90 days after sowing to evaluate their effect on growth, yield and essential oil production. The treatment without any foliar application was taken as absolute control.

The salient findings of the study are summarized in this chapter.

The seed germination parameters *viz.*, days to initial sprouting, germination per cent, survival per cent, germination index and mean germination time were

significantly influenced by the priming treatments. The seeds bioprimed with PCF @ 1 per cent recorded the highest germination per cent (96 %), survival per cent (96 %) and also it had taken less number of days (3 days) to initial sprouting. While the treatment TCF @ 1 per cent exhibited the highest germination index (34.50) and lowest mean germination time (6.29 days) in *O. basilicum*. In regards of seedling development, PCF @ 1 per cent recorded a significantly higher shoot length (21.50 cm), root length (19.50 cm), seedling length (41.00 cm) and seedling vigour index (39.37) compared to other priming treatments tried. The highest (1.07) allometric index was observed in the treatment PCWE @ 1 per cent.

The foliar spray of various fungal derivatives significantly influenced the plant growth parameters, including plant height, collar girth, number of branches, number of flowering branches and leaf area , in transplanted plants, at 30 DAS (at transplanting), 60 DAS, 90 DAS and 110 DAS (at harvest). The plants subjected to foliar application with PCF @ 1 per cent recorded significantly higher values in terms of plant growth parameters at all stages of observation. The foliar spray treatments did not show any significant variation on collar girth at 30 DAS. At 110 DAS, the plants subjected to PCF @ 1 per cent spray recorded the highest plant height (80.20 cm), collar girth (6.03 cm), leaf area (4010.82 cm^2) number of branches (28.00) and number of flowering shoots (104.00).

In terms of phenological parameters, the foliar spray treatments exhibited a significant influence on days to flowering. Treatment with PCF @ 1 per cent recorded minimum number of days (55 days), which was observed to be on par with PCWE @ 1 per cent. The foliar application did not show significant effect on days to fruit set in *O. basilicum*.

The observations on biochemical parameters *viz.*, total chlorophyll content (30 DAS, 60 DAS, 90 DAS and 110 DAS) and polyphenol content (110 DAS) showed significant variation among various treatments at all stages of observation. PCF @ 1 per cent treatment recorded significantly higher (1.20 mg g^{-1}) total chlorophyll content and polyphenol content (84.31 mg PE g^{-1}) at 110 DAS.

The foliar application of different fungal derivatives exhibited a significant variation on the yield components in *O. basilicum* at harvest (110 DAS) and also in ratoon crop (60 days after first harvest). The plants from the treatment PCF @ 1 per cent recorded maximum leaf biomass (210.00 g and 19.04 g), stem biomass (135.33 g and 12.21 g), herbage yield (345.33 g and 31.25 g), root biomass (52.00 g and 4.63 g) and total plant biomass (397.33 g and 35.88 g) respectively, on both fresh weight and dry weight basis.

The foliar spray treatments significantly influenced the harvest index in terms of leaf yield and herbage yield of *O. basilicum.* The harvest index in terms of stem yield did not show any significant variation among the various foliar spray treatments tried. The water spray and absolute control treatment recorded the maximum harvest index in terms of leaf yield (0.58) and stem yield (0.92). While the plants from the treatment PCF @ 1 per cent exhibited a minimum harvest index in terms of leaf yield (0.53) and stem yield (0.87). In the ratoon crop (60 days after the first harvest), the treatment PCF @ 1 per cent exhibited a significantly higher leaf biomass (125.33 g and 12.44 g), stem biomass (76.00 g and 7.31 g) and herbage yield (201.33 g and 19.75 g) respectively, on both fresh weight and dry weight basis.

Among the foliar spray treatments, the plants obtained from the treatment PCF @ 1 per cent recorded the highest essential oil content (2.11 per cent and 1.00 per cent) and oil yield (443.10 g and 19.04 g, respectively) in terms of both fresh and dry leaf weight.

In the first phase of study, PCF @ 1 per cent gave better performance in terms of seed germination, seedling growth and seedling vigour index. The transplanted seedlings from the same treatment when subjected to foliar application with PCF @ 1 per cent at fortnightly intervals gave the highest plant growth, biochemical and yield parameters in the second phase of study. Hence, it can be inferred that biopriming followed by foliar application of the fungal derivative PCF @ 1 per cent would give superior performance in terms of plant growth, yield and essential oil production in *O. basilicum.*

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GROWTH, YIELD AND ESSENTIAL OIL PRODUCTION RESPONSES TO MICROBIAL ELICITATION IN *Ocimum basilicum* L.

by

RAJESWARI E.

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Abstract of the thesis

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ABSTRACT

The present investigation entitled "Growth, yield and essential oil production responses to microbial elicitation in *Ocimum basilicum* L." was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani. The field experiments were laid out in the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2019-2021 with the objective to evaluate the effect of fungal derivatives on growth, yield and essential oil production in *Ocimum basilicum* L.

The seeds of *O. basilicum* used for the study were sourced from Anand Agricultural University, Gujarat. The study was carried out in two phases: Phase 1- Seed priming using fungal derivatives for enhanced germination. Phase 2- Evaluation of the effect of foliar application of fungal derivatives for growth, yield and essential oil production. In the first phase of study, the seeds were subjected to various priming treatments using fungal derivatives viz., *Trichoderma viride* cell wall extract (1 %) (TCWE), *Trichoderma viride* culture filtrate (1 %) (TCF), *Piriformospora indica* cell wall extract (1 %) (PCWE), *Piriformospora indica* culture filtrate (1 %) (PCF) and hydro priming, maintained upto 30 days after sowing. The seeds without any priming were taken as the absolute control. In the second phase of study, the 30 days old seedlings of *O. basilicum* were transplanted to grow bags. The foliar spray of corresponding fungal derivatives (cell wall extract and culture filtrate) at 1 % concentration were given to plants at fortnightly intervals from transplanting to 90 days after sowing. The treatment without any foliar application was taken as the absolute control.

The seeds bioprimed with PCF @ 1 per cent recorded the highest germination per cent (96%), survival per cent (96%) and had taken minimum number of days (3 days) to initial sprouting. While TCF @ 1 per cent exhibited the highest germination index (34.50) and lowest mean germination time (6.29 days). With regard to seedling development, PCF @ 1 per cent recorded a significantly higher shoot length (21.50 cm), root length (19.50 cm), seedling length (41.00 cm) and seedling vigour index (39.37). The highest (1.07) allometric index was observed in the treatment PCWE @ 1 per cent.

At 110 DAS, the plants subjected to foliar application with PCF @ 1 per cent exhibited higher plant height (80.20 cm), collar girth (6.03 cm), leaf area (4010.82 cm^2), number of branches (28.00) and number of flowering shoots (104.00). The same treatment induced early flowering (55 days) in *O.basilicum.*

The foliar spray treatment with PCF @ 1 per cent exhibited significantly higher total chlorophyll content (1.20 mg g^{-1}) and polyphenol content (84.31 mg PE g^{-1}) at 110 DAS. The plants subjected to foliar application with PCF @ 1 per cent recorded maximum leaf biomass (210.00 g and 19.04 g), stem biomass (135.33 g and 12.21 g), herbage yield (345.33 g and 31.25 g), root biomass (52.00 g and 4.63 g) and total plant biomass (397.33 g and 35.88 g) respectively, on both fresh weight and dry weight basis. The same treatment recorded the highest leaf biomass (125.33 g and 12.44 g), stem biomass (76.00 g and 7.31 g), and herbage yield (201.33 g and 19.75 g), on fresh weight and dry weight basis respectively, in the ratoon crop harvested 60 days after the first cut.

PCF @ 1 per cent was also observed to give the highest essential oil content (2.11 per cent and 1.00 per cent) and oil yield (443.10 g and 19.04 g, respectively) in terms of both fresh and dry leaf weight. This is followed by PCWE @ 1 per cent and TCF @ 1 per cent in terms of oil content and yield.

In the first phase of study, PCF @ 1 per cent gave better performance in terms of seed germination, seedling growth and seedling vigour index. The transplanted seedlings from the same treatment when subjected to foliar application with PCF @ 1 per cent at fortnightly intervals gave the highest plant growth, biochemical and yield parameters in the second phase of study. Hence, it can be inferred that biopriming followed by foliar application of the fungal derivative PCF @ 1 per cent would give superior performance in terms of plant growth, yield and essential oil production in *O. basilicum.*