IJBPAS, October, 2020, 9(10): 2647-2665

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF ENDOPHYTIC SYMBIOTIC FUNGUS *(PIRIFORMOSPORA INDICA)* FORMULATION ON GROWTH STAGES OF CORIANDER

PUNDIR RK[1*], GUPTA V[1], KUMAR S[2], KAMBOJ R[1] AND JAIN P[3]

1: Department of Biotechnology, Ambala College of Engineering and Applied Research, Ambala, Haryana, (India)

2: Department of Agriculture, Faculty in Agriculture, MMU, Sadopur, Ambala, Haryana, India

3: Department of Biotechnology, University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra, Haryana (India)

***Corresponding Author: Ram Kumar Pundir: E Mail: drramkpundir@gmail.com**

Received 8th Feb. 2020; Revised 5th March 2020; Accepted 8th April 2020; Available online 1st Oct. 2020

https://doi.org/10.31032/IJBPAS/2020/9.10.5219

ABSTRACT

In this study the effect of endophytic symbiotic *Piriformospora indica* formulation on growth stages of coriander was determined. The various parameters such as the shoot and root length, length of branches, numbers of leaves and branches, numbers of roots and antimicrobial activity and pyhtochemical analysis of seed extract were investigated. The overall increase in plant biomass and seed yield was found. The treated fungus plants also promoted early germination and increase antibacterial activity of coriander against *E.coli* and *Pseudomonas* sp. No anti-yeast activity was observed. The above finding suggested that the *P. indica* formulation may be used as a plant growth promoter to enhance not only yield but also medicinal value of the plant. Further studies are needed to evaluate the other parameters which were not observed in this study.

Keywords: antimicrobial, endophytic, fungus, growth, *Piriformospora indica,* phytochemical

INTRODUCTION

Coriander (*Coriandrum sativum* L), or dhania, is an annual herb belongs to the family *Apiaceae*. All plant parts of coriander are edible, mostly fresh leaves and the dried

seeds parts are especially used as a spice. This plant has both nutritional and medicinal values. Raw coriander leaves have water, carbohydrates, protein, and fat. The nutritional profile of coriander seeds is different from the fresh stems or leaves. Leaves are particularly rich in vitamin A, C and K, with dietary minerals. Although seeds have lower content of vitamins, they do provide significant amounts of dietary fiber, calcium, selenium, iron, magnesium and manganese **[1]**.

A plant growth promoting root endophytic fungus, *Piriformospora indica*was discovered by Verma *et al.* **[2]**. It belongs to the *Hymenomycetes* (*Basidiomycota*), with a relatively close relationship to Rhizoctonia and *Sebacina.* The hyphae colonize the plant root and show inter- and intracellular structures (vesicles and hyphal differentiations like arbuscules). Chlamydospores, fungal spores are formed inside the root tissues and externally into the environment. It is already shown that *P. indica*has a wide host range among monocots and dicots, including legumes **[3]**.

*P. indica*is a wide-host root, colonizing endophytic fungus which allows plants to grow under extreme physical and nutrient condition. It functions as a plant promoter and biofertilizer in nutrient deficient soils, as a bioprotector against biotic and abiotic stresses including root and leaf pathogens and insect invaders, inducing early flowering, enhanced seed production and stimulation of active ingredients in plants. Positive increments are established for many plants of medicinal and economic importance **[4]**. In brief, *P. indica* can be utilized as a plant promoter, bio-fertilizer, bioprotector, bioregulator, and biotization agent **[5]**. Recently, a mycorrhizal association between *P. indica* and rice seedlings provided a multifaceted protection to rice plants under osmotic stress **[6]**.

The over-use of synthetic commercial chemical fertilizer can result in negative effect such as leaching, pollution of water resources, destruction of microorganisms and friendly insects, crop susceptibility to disease attack, acidification or alkalization of the soil or reduction on soil fertilizer- thus causing irreparable damage to all overall system. The use of biological inoculants instead of chemical fertilizers to avoid these environmental and health related problems. Keeping in view the above points, the effect of *P. indica* formulation on growth stages of coriander along with the antibacterial and anti-yeast activity and phytochemical analysis of coriander seeds was carried out.

MATERIALS AND METHODS

Association of fungus formulation

Seeds of coriander were purchased from Ambala, Haryana (India). Healthy seeds were selected and crushed. Seeds were treated by mixing with *P.indica* formulation obtained from AIMT, AMITY University, Noida, U.P., India in presence of Gur solution as adhesive agent and kept for overnight drying before sowing **(Figure 1)**.

Seed sowing

Seeds were sowed after 12 hours of treatment. Seeds were sowed in 4 tubs (1 ft x 1 ft) containing 3 Kg of soil in Lab with classification, NT (Non Treated), T (Treated with *Piriformospora indica* formulation), F (Soil supplemented with chemical fertilizer, FF (Treatment with *Piriformospora indica* formulation and soil supplemented with chemical fertilizer). In each tub 10 seeds of coriander were sowed and irrigated with tap water.

Growth parameters: The growth parameters evaluated were seed germination, average length of shoot, average length of branches, average length of root and root thickness, root and shoot biomass, yield and Chlorophyll content, antimicrobial activity and photochemical analysis of seed extract. The length parameters were measured in cm. Determined parameters for the underground parts of the plant were root number, length and thickness of roots at the time point of three months grown plants.

Determination of moisture content of shoot and root

Coriander plants were harvested. Then fresh shoots and roots were weighed by electronic balance. Roots and shoots were calculated per plant in gram. Then shoots and roots were dried in an oven at 65^{o}C for 3 days. The oven dried sample of shoots and roots were weighted by electronic balance. The oven dried shoots and roots were calculated per plant in gram **[7]**.

Formula W=W1- W2/W1X100

W=% Moisture content

W1 = Fresh weight W2 = Dry weight

Assessment of Seed Yield

The seeds of each sample were weighed by electronic balance. Weight of seeds was calculated in gram.

Assessment of Root Colonization: Staining of Root Samples and Microscopic Examination

The root segments were harvested from 30 and 90 day-old plantlets and were washed thoroughly in running tap water, cut into 1.0 cm pieces and treated overnight with 10% KOH solution at room temperature. Thereafter, the roots were washed 3-5 times with sterilized distilled water and treated with 1% HCl for 3-4 min before staining

with cotton blue in lacto phenol. The stained root segments were examined microscopically (X40). The roots of control plants were also processed in similar way **[8]**.

Chlorophyll Estimation

For chlorophyll estimation, 1.42 g of puree was taken with 80 % acetone (V/V) following centrifugation. 50 ml supernatant extract was collected in 50 ml volumetric flask. 10 ml of this sample was taken in 3 flasks of 25 ml each. In the first flask, volume was made up to 25 ml by 80 % acetone. In the second flask, 1 ml of 0.5 M oxalic acid in acetone was added and mixed. It was then allowed to stand for 90 min. The flasks volume was then made up to 25 ml with 80 % acetone. In the third flask, 0.32 ml of 12 N HCL was added and the flask was allowed to stand for 30 min, following which 0.23 ml of ethanolamine was added and volume was made up with 80 % acetone. Percent absorbance of clear pigment solution was measured at both 664 and 646.5 nm using UV/VIS spectrophotometer **[9]**.

Concentrations of chlorophyll-a, chlorophyll-b and total chlorophyll was calculated using equations (1 to 3), respectively.

Chlorophyll–a = 30.5 X *A*664.0 + 3.81 X *A*646.5 …(1)

Chlorophyll–b = 36.1 X *A*646.5 –10.0 X *A*664.0 …(2)

Total chlorophyll = Chlorophyll–a + Chlorophyll–b …(3) **[9]**

Preparation of Extracts

Extraction methods were employed to extract the plant powder of coriander seeds using different polarity solvents from non-polar namely chloroform, petroleum ether, ethanol and methanol. 5g of air-dried plant powder was soaked in 25ml of organic solvents, methanol separately for 24 h in tubes at room temperature. Extracts were filtered through the whatman filter paper No.1. The filtrate was allowed to evaporate at room temperature and each filterate was collected in tubes separately. Condensed extracts were stored in air- tight containers at $4^{o}C$ till further investigation **[10]**.

Determination of total Phenolic and Flavonoid Content of coriander leaves

Total phenolic content

Total phenolic compounds were determined by the Folin-Ciocalteu method. The extract samples(1ml) were mixed with Folin-Ciocalteu reagent (250µl, 3.5ml with distilled water) for 5 min and aqueous Na_2C0_3 (4ml,1M) was then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric methods at 765 nm. The standard curve was prepared by

1,2,3,4 and 5 mg/ml solution of Gallic acid in methanol. Total phenol value was expressed in terms of Gallic acid equivalent **[11]**.

Total Flavonoid Content

Total flavonoid content of the extract was determined according to reported methods in literature **[12]**. 0.5ml of sample solution was mixed with 2ml of distilled water and subsequently with 0.15 ml 5% of $NaNO_2$ solution. After 6 min incubation, 0.15 ml of 10% AlCl3 solution was added and allowed to stand for 6 min, following by adding 2 ml of 4% NaOH solution to the mixture. The mixture was made up to 5ml with methanol and mixed well. The absorbance was measured at 510 nm after incubation for 15 min. The total flavonoids content was expressed in milligrams of gallic acid equivalents per gram of extract **[13]**.

Determination of Moisture Content of seeds

Weighed about 1.5 gm of the seed sample in to a weighted flat and thin porcelain dish dried in the oven at 100^{o}C. Cooledindesiccators and observed. The loss in weight was recorded as moisture content **[14-17]**.

Formula W=W1- W2/W1X100

W=% Moisture content

W1 = Fresh weight W2 = Dry weight

Phytochemical Investigation of metanolic extract of coriander seeds

Preliminary phytochemical screening was performed to identify phytochemical in the Methanol extract of seed used in the study. The solvent extracts obtained earlier were subjected to preliminary phytochemical tests for identifying the class of compounds **[15, 16, 17]**.

Test for Alkaloids

The small portion extracts were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate) and Dragendorffs reagent (orange brown precipitate).

Test for Saponin

About 1 ml of extract was diluted with distilled water to 20 ml and shaked in graduated cylinder for 15 minutes. One cm layer of foam indicated presence of saponin.

Test for Steroids

2ml of chloroform was taken and 2ml of methanolic extract was added followed by 2ml of concentrated H_2SO_4 and shaked well. Chloroform layers appearing red color and acid layer showing greenish yellow fluorescence color indicates the presence of steroids.

Test for Tannin

Few drops of 5% $FeCl_3$ solution were added to 2-3 ml of methanol extracts. Formation of yellow color indicates the presence of tannin.

Test for Cardiac- glycosides

1ml of glacial acetic acid was added to 2ml of methanolic extract followed by addition of few drops of $FeCl_3$ solution and concentrated H_2SO_4. Development of blue-green color indicates the presence of cardiac-glycosides.

Test for Poly- urinoides

Methanolic extract was added drop wise into a test tube containing 10 ml of C_2H_5OH and the test tubes were observed for the appearance of violet or blue precipitate.

Test for Terpenoids (Salkowski test)

5 ml of extract was mixed in 2 ml of chloroform, and then 3 ml concentrated $H_2SO_4$was carefully added to form a layer. A reddish brown coloration formed at the interface indicated presence of terpenoids.

Test for reducing sugar

1ml of test solution was added to 1ml of Fehling A and 1ml Fehling B and boiled on water bath for 5 min.Yellow, then brick red precipitates indicate the presence of reducing sugar.

Screening of coriander seed extract for Antimicrobial activity

Antibacterial activity by Agar well diffusion method

To determine the antibacterial activity of seeds and roots, nutrient agar was used as culture medium. The antibacterial activity of coriander extract in methanol solvent was determined by agar well diffusion method **[18, 19, 20]**. The Nutrient Agar medium (25 ml) was poured into petri dishes and kept in the Laminer Air Flow for solidifying of the agar. After the medium gets solidified, perti plates containing 25 ml of nutrient agar media were inoculated with 100 µl of diluted cultures of the different microbes on different perti plated by the spread plate technique and were allowed to dry in the laminar flow. Wells were cut out from agar plates using a sterilized stainless borer and filled with 100 µl of the extract. The plates inoculated with different bacteria were incubated at 37^oC up to 24 hrs and diameter of any resultant zone of inhibition was measured.

Antifungal activity by Agar well diffusion method

To determine the antifungal activity of seeds and root, Potato Dextrose Agar medium was used as culture medium. Then evaluation of antifungal activity was performed using standard agar well diffusion method. 50 µl of *Candida sp*. was aseptically introduced and spread using cotton swabs on surface of sterile PDA medium plates. Wells were cut out from agar plates using a sterilized

stainless borer and filled with 100 µl of the extracts. Plates were incubated at 30°C for 2 days allowing for diffusion of the solution and diameter of any resultant zone of inhibition was measured **[21]**.

RESULTS

Seed germination was observed after 17 day of sowing in Treated (T), 18 days in FF and 20 days in NT and F as shown in **Table 1 and Figure 2**. The average length of shoot was observed maximum in 10.2cm in treated (T) followed by 9.6 cm in NT, 8.2cm in FF and Minimum in 8.0cm. The same pattern was in average length of branches, average length of root and root thickness **(Table 1 and Figure 2)**.

Plant Biomass (weight of shoot and root)

The overall weights of colonized roots and shoots were higher than the weight of the non-colonized controls **(Table 3 and 4)**.

Seed yield

The maximum average weight of seed (yield) obtained was found in this sequence T > FF > NT > F in the field grown coriander seeds of ten plants. The Maximum average weight of yield obtained was found in this sequence T > NT > FF >F in the lab grown coriander seeds of ten plants **(Table 5)**.

Root colonization:

Inoculation of coriander roots with *P.indica* was accompanied by changes in the morphology of the root and fungal staining of *P.indica* showed mycelia with chlamydospore **(Figure 6)**.

Chlorophyll content:

Chlorophyll a and chlorophyll b and total chlorophyll contents were 36% higher in the leaves of the colonized plant as compared with the non- colonized plants **(Table 6)**.

Determination of total phenol and flavonoid contents of coriander leaves

Total phenol compound, as determined by Folin-Ciocaltue method, is reported as gallic acid equivalents. Total phenol content of the coriander (methanol extract) varied largely from 126 to 278 µg/ml. Among these extracts, treated with *P. indica* showed the highest content of phenol compounds (278µg/ml) followed by addition of fertilizer (248µg/ml), treated with *P.indica* and addition of fertilizer (158µg/ml) and Non-treated (126µg/ml). The maximum phenol content was observed in plant treated with *P. indica* formulation **(Table 7)**.

The total flavonoids content reported as mg gallic acid equilalent/ml of extract. Extracts of plants treated with *P. indica* showed the highest content of flavonoids compounds (99µg/ml) followed by extracts of plants with use of fertilizer (95µg/ml), non-treated (93µg/ml) and treated with *P.indica* and fertilizer (60µg/ml) **(Table 7)**.

Determination of the moisture content of seeds

The moisture content was recorded 7.78% in Treated plant with *P. indica* followed by 7.96% in FF, 8.24% in NT and maximum in 8.88 in F treated seeds as compared to specification 9.0% **(Table 8)**.

Phytochemical screening

Phytochemical screening of the metanolic coriander seed extract showed the presence of tannin, phenol, glycosides and reducing sugar and absence of all other tested phytochemical constituents such as alkaloids, saponin, steroid, flavonoids, poly –urinoides and terpenoids **(Table 9)**.

The plant growth was measured by considering various parameters shoot height, root height, root diameter, Length of branches, Numbers of branches, Numbers of roots, Numbers of leaves and Leaf area.The maximum growth of plants was found in treated with *P.indica* (T), followed by non-treated (NT), treated with *P.indica* and soil supplemented with chemical fertilizer (FF), and minimum plant growth was found only soil supplemented with chemical fertilizer (F).The plant biomass measured in dry weight of root and shoot. The maximum plant biomass was found in coriander seeds treated with *P.indica* (T), (NT), (FF) and minimum plant biomass was found only soil supplemented with chemical fertilizer (F).Seed yield was measured in weight of seeds. The maximum seed yield was found in coriander seeds treated with *P.indica* (T), followed non- treated (NT), then treated with *P.indica* and addition of fertilizer (FF) and minimum seed yield was found only soil supplemented with chemical fertilizer (F).The antimicrobial activity of coriander methanolic seed extract was determined against *E.coli* and *P.aeruginosa* (bacteria) *C.albicans* (yeast) by using agar well diffusion method. The maximum activity was recorded in the obtained from fungus treated (16mm) followed by NT (12mm) and minimum in soil supplemented with fertilizer (10mm) **(Table 10)**.

Table 1: Seeds germination days of lab grown Coriander

Experiment	NT	T	F	FF
Seeds germination after	20 days	17 days	20 days	18 days

Table 2: Influence of *P.indica* on growth parameters of lab grown coriander plants

S. No.	Experiments	Parameters			
		Average length of shoot (c.m.)	Average length of branches (c.m.)	Average length of root (c.m.)	Root thickness (c.m.)
1.	NT	9.6	7.0	5.2	0.6
2.	T	10.2	7.4	5.8	0.8
3.	F	8.0	6.6	4.2	0.5
4.	FF	8.2	6.8	4.8	0.5

Table 3: Influence of *P.indica* on growth parameters of lab grown Coriander plants (weight of shoot)

S. No.	Treatment	W1 Fresh wt (gm)	W2 Dry wt (gm)	W3 Moisture content	% Moisture Content
1.	NT	1.88	0.58	1.30	69.14
2.	T	2.00	0.64	1.36	68.00
3.	F	1.42	0.44	0.98	69.01
4.	FF	1.50	0.42	1.08	72.00

Table 4: Influence of *P.indica* on growth parameters of lab grown Coriander plants (weight of root)

S. No.	Treatment	W1 Fresh wt (gm)	W2 Dry wt (gm)	W3 Moisture content	% of Moisture content
1.	NT	0.42	0.10	0.32	76.19
2.	T	0.50	0.11	0.39	78.00
3.	F	0.30	0.08	0.22	73.33
4.	FF	0.32	0.08	0.24	75.00

Table 5: Influence of *P.indica* on seed yield of grown coriander plants

S. No.	Treatment	Average wt. of seeds (g.m) (Lab)
1.	NT	1.8
2.	T	2.0
3.	F	1.0
4.	FF	1.2

Table 6: Chlorophyll content of leaves of Coriander Plants

S. No.	Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
1.	NT	17.59	3.49	21.09
2.	T	24.86	3.87	28.73
3.	F	15.27	2.75	18.02
4.	FF	15.79	3.11	18.90

Table 7: Total phenol and flavonoid contents of coriander seeds in methanol extracts

S. No.	Treatment	Phenol content (µg/ml)	Flavonoids content (µg/ml)
1.	NT	126	93
2.	T	278	99
3.	F	248	95
4.	FF	158	60

Table 8: Moisture content of coriander seeds

S. No	Treatment	Specification	Moisture content of Coriander
1.	NT	NMT- 9.0	8.24
2.	T	NMT-9.0	7.78
3.	F	NMT-9.0	8.88
4.	FF	NMT-9.0	7.96

NMT = Not More Than

Table 9: Phytochemical screening of coriander seed methanolic extract

S. No.	Test for active constituents	Methanol extracts
1.	Alkaloids	-ve
2.	Saponin	-ve
3.	Steroid	-ve
4.	Tannin	+ve
5.	Flavonoids	-ve
6.	Phenol	+ve
7.	Glycosides	+ve
8.	Poly –urinoides	-ve
9.	Terpenoids	-ve
10.	Reducing sugar	+ve

Table 10: Antimicrobial activity of coriander seeds

Spices	Extract	*E. coli* sp. (zone of inhibition in millimeter)	*Pseudomonas* sp. (zone of inhibition in millimeter)	*Candida* sp.
Coriander (NT)	Methanol	12	10	-
Coriander (T)	Methanol	16	12	-
Coriander (F)	Methanol	10	12	-
Coriander (FF)	Methanol	10	12	-

Figure 1: Coriander seeds treated with *Piriformospora indica* formulation

Figure 2: Seeds germination of lab grown Coriander

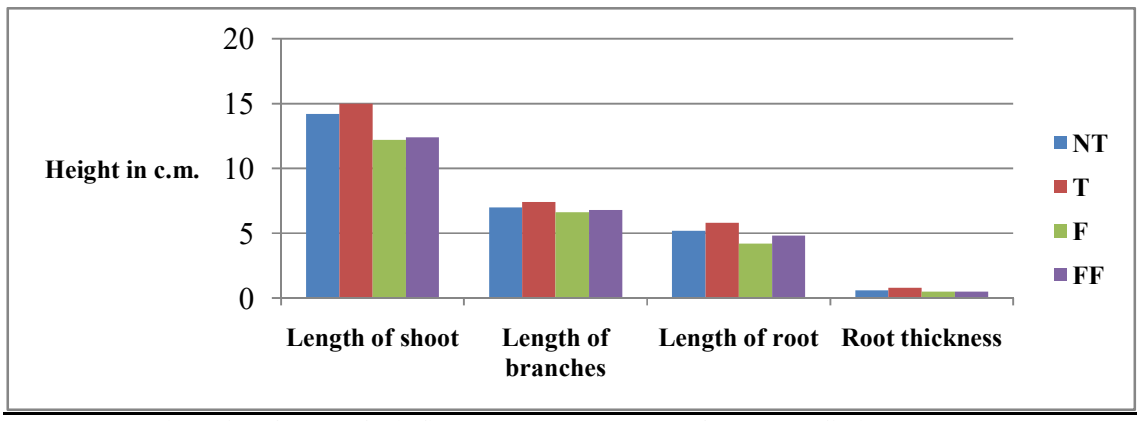


Figure 3: Influence of *P.indica* on growth parameters of lab grown Coriander plants

(a) **(b)**

Figure 3.1: Growth of (a) non- treated plants of Coriander as control (b) Growth of Coriander plants treated with *P. indica* formulation

(a) **(b)**

Figure 4: (a) Growth of Coriander plants treated with in soil *P. indica* formulation and soil supplemented fertilizer in with chemical fertilizer in lab condition (b) Growth of Coriander plants supplemented with chemical fertilizer

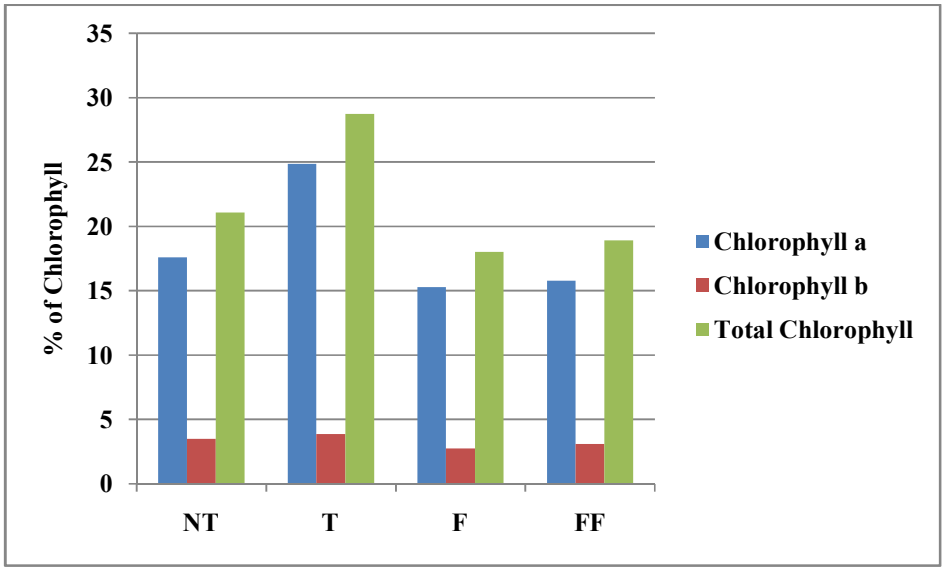
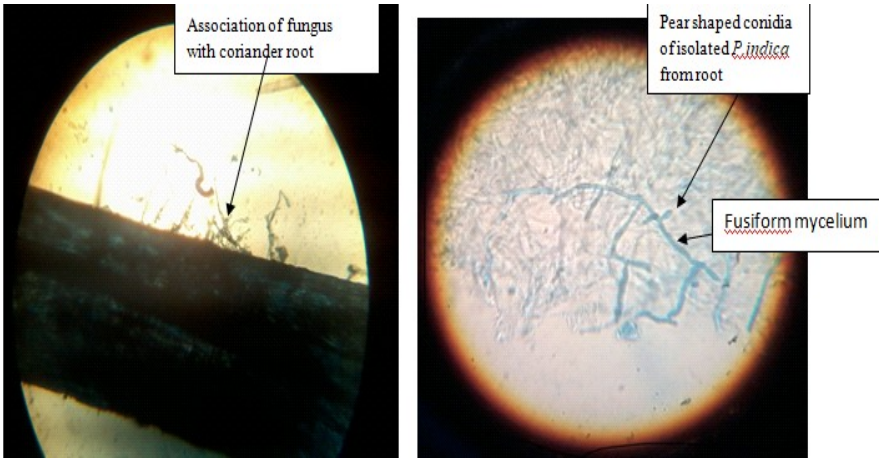
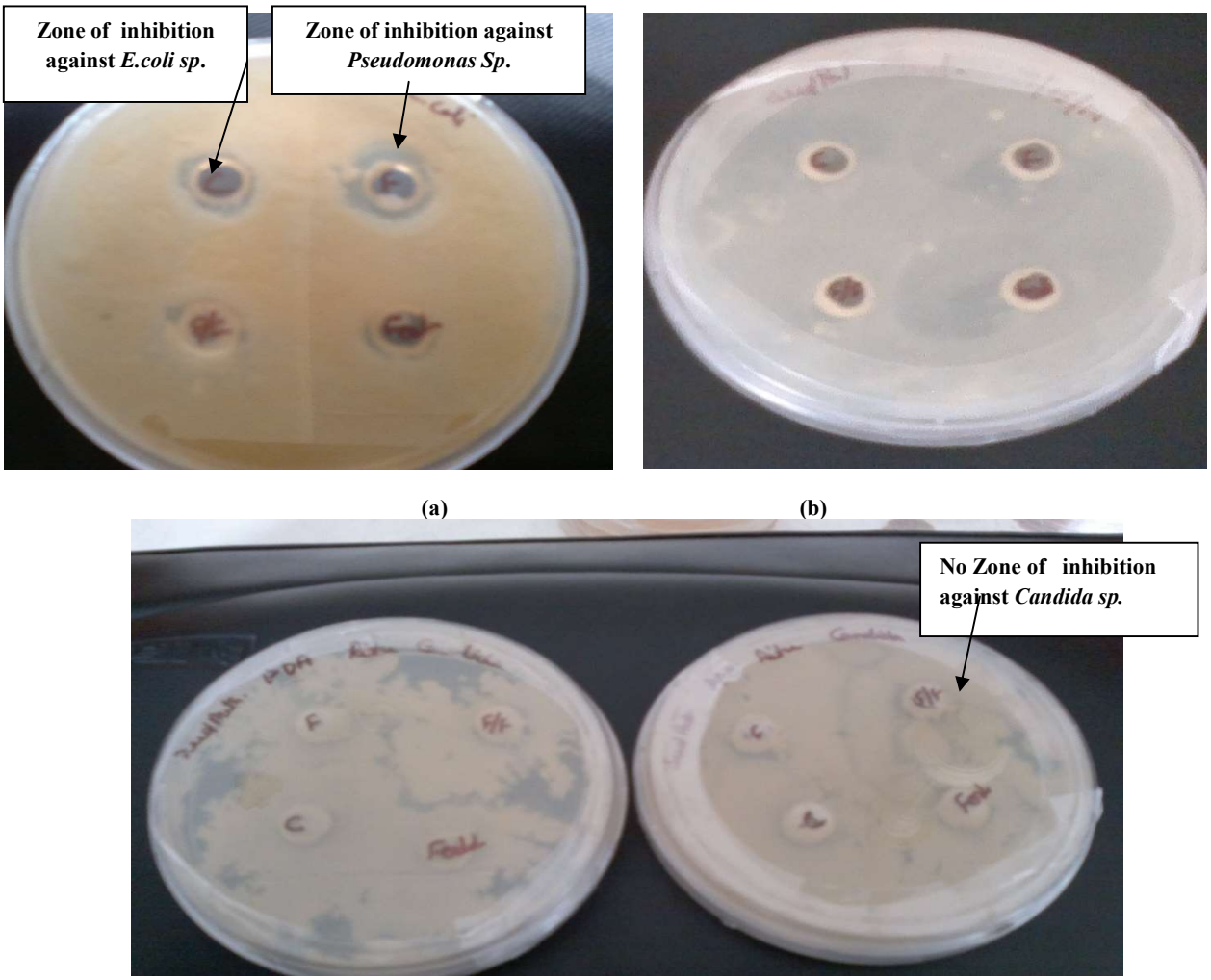


Figure 5: Chlorophyll content of leaves of Coriander Plants



(a) **(b)**

Figure 6: (a) Showing surface association of fungus (*P. indica*) with root of coriander plant (b) Fungal Staining of *P.indica* isolated from colonized root of coriander plant showing pear shaped conidium and fusiform mycelium.



(a) **(b)**

(c)

Figure 7: Antibacterial activity against (a) *E. coli* and (b) *Pseudomonas* sp. (c) Antifungal activity against *Candida* sp.

DISCUSSION

In the present study, the effect of endophytic symbiotic fungus *(Piriformospora indica)* formulation on growth stages of coriander was observed. The maximum seed germination shoot height, root height, numbers of leaves, numbers of root, root diameter and leaf area was found in the pattern T > FF > NT > F in field and T > NT > FF > F in lab grown coriander plants. The authors observed that *P.indica* enhanced the growth of the plant as compared with the non-colonized plants which is also reported previously about the growth enhancing capabilities of *P.indica* and thus support the present study **[22-29]**.The authors also observed that *P. indica* not only induced a faster development of the aerial part of the plant, but also caused early maturation with respect to flowering that is similar with finding of Achatz *et al*. **[29]**. The possible explanation for the faster development of *P.indica* colonized roots compared with the non-colonized plants during all stages of growth could be due to the earlier expression of developmentally regulated genes **[30]**.

The increased growth of *P. indica*-colonized plants may be associated with an enhanced nutrient uptake (especially ofphosphorus and nitrogen) from the soil, as observed for mycorrhizal associations. In particular, *P.indica* seems to promote phosphorous and nitrogen uptake from the soil. The role of phytohormones in *P.indica*-induced reprogramming of coriander development needs to be further investigated. Like in mycorrhizal symbiosis **[31, 32]** increase in leaf area and chlorophyll levels may result in increased carbon assimilation in *P.indica*-colonized plants, which is the basis for faster development and higher biomass production **[29, 33]**. Interestingly, *P.indica* strongly induced early and vigorous flowering in Coriander. Similar findings were also reported previously and thus support our data **[24, 28, 32, 34-36]**.

Different studies have emphasized the importance of nutrient phosphorus on the impact on bud formation and development, the number of flowers, the size of the pollen grain and seed formation. It has been demonstrated in case of tomatoes that phosphorus promotes the formation of flowers and increases the mass of the fruit, seed count and pollen count of the plant as well as the average pollen production of each individual flower. It also observed a strong effect on the inflorescence, raising the question whether they are synthesized as defense compounds **[37, 38]** or simply synthesized because of a better supply of the plant with nutrition. In case of AMF

symbiosis, stimulation of secondary metabolites is specific for the AMF species **[37, 38]**. Thus, the role of *P. indica* and environmental factors for the production of secondary metabolites need to be analyzed in detail **[39, 40]**.

Biomass of the aerial parts of coriander including the levels of the medicinally important secondary metabolites can be substantially stimulated by co-cultivation with *P.indica* under field conditions. The authors suggest the use of *P.indica* in sustainable agriculture for crop yield improvement.

Phytochemical screening of the coriander seed metanolic extract showed the presence of tannin, phenol, glycosides and reducing sugar and absence of all other tested phytochemical constituents such as alkaloids, saponin, steroid, flavonoids, poly –urinoides and terpenoids. The results are in accordance with the findings of the other authors who have studied these spices **[41-43]**.The antimicrobial activity of coriander methanolic seed extract was determined against *E.coli* and *P.aeruginosa* (bacteria) *C.albicans* (yeast) by using agar well diffusion method. The maximum activity was recorded in the obtained from fungus treated (16mm) followed by NT (12mm) and minimum in soil supplemented with fertilizer (10mm). In another study, that bacterial activity coriander was determined against five pathogenic strains in which four bacteria *Enterobacter aerogenes, Klebsilla pneumoniae, Vibrio cholerae* and *Salmonella typhi* gram negative and positive bacteria. The activity of *C.sativum* may be due to the presence of secondary metabolites Glycosides, Tannin, Phenol etc. that results were similar with our study.

CONCLUSION

It can be concluded that the *Piriformospora indica* formulation showed positive effects on the growth of coriander plant in vitro. The overall increase in plant biomass and seed yield was found. The treated fungus plants also promoted early germination and increase antibacterial activity of coriander against *E. coli* and *Pseudomonas* sp. No anti-yeast activity was observed. Presence of flavonoid and phenolic contents exhibited the presence of pharmacological potential of this plant. The above finding suggested that the *P. indica* formulation may be used as a plant growth promoter to enhance not only yield but also medicinal value of the plants. Further studies are needed to evaluate the other parameters which were not observed in this study.

ACKNOWLEDGEMENT

The authors are thankful to Management of Ambala College of Engineering and Applied Research, Ambala, Haryana (India) and Director, UIET, KUK, Haryana, India for encouraging us to write this manuscript as a research article. Authors are also thankful to Prof. Ajit Varma, AIMT, AMITY University, Noida, UP (India) for providing Fungal formulation and encourage writing manuscript.

REFERENCES

[1] Nutritional Data. Coriander seed spice, nutritionaldata.self.com, Accessed.08:10. 2013.

[2] Verma S, Varma A, Rexer KH, Hassel A, Kost G *et al*., *Piriform-ospora indica,* gen. et sp. nov., a new root-colonizing fungus. *Mycologia*. 90, 1998, 896-903.

[3] Varma A., I. Sherameti I, Tripathi S, Oelmüller R, The Symbiotic Fungus *Piriformospora indica*: Review. The Mycota. Eds Hock B., Fungal Associations, Springer, 9, 2012.

[4] Bagde US, Prasad R, Varma A, Interaction of Mycobiont: *Pirifor-mospora indica* with Medicinal plants and plants of Economic importance. Afr. J. Biot. 9(54), 2010, 9214-9226.

[5] Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N, *Piriformospora indica*: Potential and Significance in Plant Stress Tolerance. *Front. Microbiol*, 7, 2016, 332.

[6] Saddique MAB, Ali Z, Khan AS, Rana IA, Shamsi IH, Inoculation with the endophyte *Piriformospora indica* significantly affects mechanisms involved in osmotic stress in rice. *Rice*, 11, 2018, 34.

[7] Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri AK, Varma A, The root endophyte fungus *Piriform-ospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. *Plant Signal. Behav*. 7(1), 2012, 1–10.

[8] Vyas S, Nagori R, Purohit.SD, Root Colonization and Growth Enhancement of Micropropageted *Feronia limonia* (L) Swingle by *Piriformospora indica* – A Cultivable Root Endophyte. *Intern. J. Plant Develop. Biol*., 2008, 128 – 132.

[9] Seema, Keshav A, Thermal Degradation of Coriander Leaves: Kinetic Modeling. *Int. J. Chem. Sci*., 8(5), 2010

[10] Kaur GJ, Arora DS, Antibacterial and phytochemical screening of *Anethum graveolens, Foeniculum vulgare* and *Trachyspermum ammi*. *BMC Complement Altern Med*. 9(30), 2009, 1-10.

[11] Zengin G., Aktumsek A, Guler GO. 2011. Antioxidant properties of methanolic extracts and fatty acid composition of *Centaurea urvillri* DC. Subsp., *hayekiana* Wagenitz. *Rec. Natural Prod*., 5(2), 2011, 123-132.

[12] Maheshwari JK, Singh KK, Saha S, Ethanobotany of tribals of Mirzapur Dirstrict, Uttar Pradesh. Economic Botany Information Service. NBRI, Lucknow, 1986.

[13] Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bekhradnia AR, Antioxidant and free radical scavenging activity of *H. officinalis* L. Var, Angustifolicus, *V. odorate, B. hyrcana* and *C. speciosum*. *Pak J. Pharmacy Sci.* 1, 2010, 29-34.

[14] Krebs KG, Heunsen D, Wimmer H, In Thin Layer Chromatography-A Laboratory Handbook, edited by Stahl E, 2nd Ed., ELBS, London, 1969.

[15] Kokate CK, Practical Pharmacognsoy, 4th reprint edn, Vallabh Prakashan, New Delhi, 1999, 122-125.

[16] The Indian Pharmacopoeia, 4th Ed., The Controller of Publications, Government of India, New Delhi, 1996, 53-54.

[17] Khandelwal KR, Practical Pharmacognsoy, 10th Ed., Nirali Prakashan, Pune, 2003, pp. 162-165.

[18] Parekh J, Chanda S, *In vitro* antimicrobial activity of the crude mrthanol extract of *Wood fordia fruticosa* Kurz. Flower (*Lythaceae*). *Brat. J.Microbiol*. 38, 2007, 204 – 207.

[19] Lokhande PD,Gawai KR, Kodam KM, Kuchekar, BS, Antimicrobial activity of Extracts of *Piper longum. J. Pharmacol. Toxicol*. 2, 2007, 574 – 579.

[20] Mahesh B, Satish S, Antimicrobial activity of some important medicinal plants against plant and human pathogens. *World J. Agri. Sci*. 4, 2008, 839 – 843.

[21] Jain P, Nafis G, Antifungal activity and phytochemical analysis of aqueous extracts of *Ricinus communis* and *Punica granatum. J. Pharm. Res*. 4(1), 2011, 128 – 129.

[22] Oelmüller R, Sherameti I, Tripathi S, Varma A, *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis. 49, 2009, 1-17.

[23] Varma A., Savita V, Sahay SN, Butehorn B, *et al*., *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Appl. Environ. Microbiol.*, 65, 1999, 2741-2744.

[24] Rai M, Acharya D, Singh A, Varma A, Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhiza*, 11, 2001, 123-128.

[25] Rai MK, Varma A, Pandey AK, Antifungal potential of Spilanthes calva after inoculation of *Piriformospora indica*. *Mycoses*, 47, 2004, 479-481.

[26] Peškan-Berghofer T, Shahollari B, Giong P, Hehl S, Market C, Blanke V et al. 2004. Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmatic reticulum and at the plasma membrane. *Physiol. Plant*.122, 2004, 465-77.

[27] Rai M, Varma A, Arbuscular mycorrhiza-like biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica Nees. Electronic Journal of Biotechnology*, 8(1), 2005, 107-112

[28] Rai MK, Andrade-Linares DR, Vons Bargen S, Bandte M, Büttner C, Grosch R et al., Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza*. 20, 2010, 191-200.

[29] Achatz B., von Rüden S, Andrade D, Neumann E, Pons-Kühnemann J, Franken P, et al., Root colonization by *Piriformospora indica* enhances grain yield in barley under diveerse nutrient regimes by accelerating early plant development. *Plant Soil*. 333, 2010, 59-70.

[30] Waller F, Mukherjee K, Deshmukh SD, Achatz B, Sharma M, Schäfer P *et al*., Systemic and local modulation of plant responses by *Piriformospora indica* and related *Sebacinales* species. *J. Plant. Physiol*., 165, 2008, 60-70.

[31] Yano-Melo AM, Saggin Júnior OJ, Lima-Filho JM, Melo NF, Maiai L, Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets. *Mycorrhiza*., 9, 1999, 119-123.

[32] Ceccarelli, N, Curadi M, Martelloni L, Sbrana C, Picciarelli P, Giovannetti M, Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. *Plant Soil*. 335, 2010, 311-23.

[33] Jurkiewicz A., Ryszka P, Anielska T, Waligórski P, D. Białońska, K. Góralska, *et al*. Optimization of culture conditions of *Arnica Montana* L.: Effects of Mycorrhizal fungi and competing plants. *Mycorrhiza*., 20, 2010, 293-306.

[34] Barazani O, Benderoth M,Groten K, Kuhlemier C, Baldwin IT, *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in Nicotiana attenuate. *Oecologia*, 146, 2005, 234-243.

[35] Kumari R, Kisan H, Bhoon YK, Varma A, Colonization of cruciferous plants by *Piriformospora indica* . *Curr. Sci.* 85, 2003, 1672- 1674.

[36] Gaur A, Adholeya A, Diverse response of five ornamental plant species to mixed indigenous and single isolate arbuscular mycorrhizal inocula in marginal soil amended with organic matter. *J. Plant Nutr*., 28, 2005, 707-723.

[37] Copetta A, Lingua G, Berta G, Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. *Mycorrhiza*, 16, 2006, 485-494.

[38] Zubek S, Stojakowska A, Anielska T, Turnau K, Arbuscular mycorrhizal fungi alter thymol deriveative contents of *Inula ensifolia* L. *Mycorrhiza*, 20, 2010, 497- 504.

[39] Graven EH, Webber L, Benias G, Vente RM,Gardner JB, Effect of soil type and nutrient status on the yield and composition of Tagetes oil (*Tagetes minuta* L). *J. Essent. Oil Res*., 3, 1991, 303-307.

[40] Arrebola ML, Variation in the yield and composition of the essential oil of *Thymus serphylloides* Bory subsp *gadorensis* (Pau) Jalas. *J. Essent. Oil Res*., 7, 1995, 369-374.

[41] Vangalapati M, Satya SN, Prakash SDV, Avanigadda S, A Review on pharmacological activities and clinical effects of cinnamon species. *Res. J. Pharm. Biol. Chem. Sci*., 3(1), 2012, 653-663.

[42] Mekha MS, Rajeshwari CU, Shobha RI, Andallu B, Phytochemical constituents in methanolic extract and various fractions of methanolic extract of ajwain (*Trachyspermum ammi* L.) seeds. Novus *Int. J. Anal. Innov*., 1(2), 2012, 20-33.

[43] Chauhan B, Kumar G, Ali M, A Review on phytochemical constituents and activities of *Trachyspermum ammi* (L.) Sprague fruits. *Am. J. Pharmtech. Res*., 2(4), 2012, 329-340.