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Impact of *Piriformospora indica* on growth and yield parameters of groundnut [*Arachis hypogea* (L.)]

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Abstract
Piriformospora indica an axenically cultivable mycelium of recently defined family sebacinaceae enhances several growths promotional and several other biochemical traits with significant output by establishing stable associations with a wide range of host plant. The aim of the current study was to study the impact of *P. indica* with *Arachis hypogaea* (L.) roots in a pot culture conditions. The forty five days old *in vitro P. indica* treated and controlled plants transplanted to pot culture experiment. The root colonization was confirmed by microscopy and PCR assay. The considerable positive effects on the different growth and yield parameters of host plant have been recorded. Two growth parameters plant height (32.53±0.7cm) and total leaf area (5.92±0.37 cm^2/plant) significantly influenced by *P. indica* inoculation with the host plant compared to rest of the parameters under present investigation. All the yield parameters viz. number pods $plant^{-1}$ (17.31±2.3), number of seeds $plant^{-1}$ (37.36±2.4), hundred seed weight (69.16±7.9 g/seed), shelling percentage (67.33±3.6 %) and pod yield (19.4±2.2 g $plant^{-1}$) found significant over controlled plants.

Keywords: *Piriformospora indica*, *Arachis hypogaea* (L.), co-cultivation, growth enhancement.

Introduction
Groundnut [*Arachis hypogaea* (L.)], is an most important edible oilseed crop belongs to family leguminosae and sub family Papillionace and cultivated in of tropical and subtropical regions. Groundnut is an important source of lipid (44-56%), protein (22-30%), (Savage, *et al*., 1994) [21] vitamins, minerals and some important active component viz., anti cancerous beta-sitosterol (Awad, *et al*., 2000) [3]. It has not only a oil crop but also a food as well as fodder crop. The area, production and productivity of groundnut in India is 26.20 MH, 25.25 MT and productivity of 968 kg/ha, respectively. (Anonymous, 2016) [2]. *P. indica* is an axenically cultivable mycelium belongs to the recently defined family sebacinaceae, order Sebacinales Glomeromycota (Weiss, *et al*., 2004, 2011) [30, 31]. It is discovered in the Thar Desert, India (Verma, *et al*., 1998; Varma, *et al*., 1999) [28, 25] from the roots of xerophytes. With the advent of apparently stable associations with a wide range of plant hosts i.e. more than 12 families and 24 species (Lou, *et al*., 2007; Franken, 2012) [15, 7], this root-endophytic fungus found a potent symbiont which mimics arbuscular mycorrhiza and attributes enormous growth promoting aspects. The association of *P. indica* with host plant enhances plant growth and increase productivity. Numerous host plants have been studied and recorded the beneficial impact of *P. indica*. viz., increased biomass, early flowering and enhanced yield (Varma, *et al*., 1999; Barazani, *et al*., 2005; Peskan-Berghofer, *et al*., 2004; Vyas, *et al*., 2008) [25, 4, 18, 29], abiotic and biotic resistance (Jogawat, 2013 *et al*; Lin, *et al* 2019) [10, 14]. *P. indica* colonization also confer several other striking benefits to the host plant such as in pharmaceutically important medicinal plants an enhanced concentration of active ingredients have been observed. Increased iron and sugar content in sugarcane (Varma, *et al*., 2013) [27] also recorded. The perusal of research and literature reveled that interaction study between *P. indica* and *A. hypogaea* has not been studied previously. In this review, the present investigation was carried out to know effect of *P. indica* on growth aspects of groundnut plant in pot culture experiment.

Materials and Methods
In vitro co-cultivation of *P. indica* with groundnut plant roots
P. indica strain was grown on modified Kaefer medium (Hill and Kaefer, *et al*., 2001) [8] at 25°C in the dark. (Verma, *et al.*, 1998) [29]. The surface sterilized groundnut seeds were inoculated on half-strength hormone-free MS media (Murashige and Skoog, 1962) [17].

for seed germination (Fig.1). The proper root growth was achieved by adding 1.0 mg/l naphthalene acetic acid (NAA) (Adu-Dapaah, *et al*., 2004) [1] along with controlled conditions at 25±1 ºC with a light intensity of 80 l E/m^2/s and 16 h light/8 h dark cycle (Fig.2). The four weeks old culture disc (4 mm diameter) with actively growing hyphae along with newly formed conidia (Verma, *et al*., 1998) [28] and twelve days old *in vitro* plants were sub-cultured to PNM media (Johnson, *et al*., 2011) [11]. Another set of seeds without the inoculation of fungus was also maintained under similar conditions.

Transplanting of the *in vitro* plants

The forty five days old, fully grown, healthy groundnut seedlings form the both controlled and treated seeds were selected for the transplantation. The colonization of fungus to the plant roots is confirmed by PCR assay (Buetehorn *et al*. 2000) [5] and microscopic observation (Dickson, *et al*., 1998) [6] before transplanting. The pot mixture of sand, peat and perlite at ratio of 2:1:1 was prepared and sterilized in autoclave at 15 psi for 20 minutes and further fumigated with 7% formaldehyde. The sterile pot mixture was filled in 25 cm diameter pots provided a hole plugged with glass wool at bottom. Initially plant were covered with plastic film to maintain the moisture of the plants (Hisajima, *et al*., 1989) (Fig.3) [9] and hardened over 10 days by gradual removal of the film. The regular irrigation was done with tap water and fully acclimatized plantlets were maintained for harvesting the seeds (Maina, *et al*., 2010) [16]. The experiment was carried out in paired T-test with 2 treatments (*P. indica* treated and controlled). Plants grown in pots were analyzed after 120 days.

Growth parameters

Plant height

The plant height was measured by using centimeter scale and recorded.

Primary branches plant^{-1}

The number branches arise from the main branch (axis) were counted and recorded.

Secondary branches^{-1}

The nodes on the primary branches bear more branches were counted as secondary branches.

Nodulation (nodule/plant)

Five plants from each treatment with intact roots were removed with the help of digging fork. The root with root nodules were carefully separated from the soil and washed thoroughly and the number of nodules per plant were counted and recorded.

Total leaf area (Kalra and Dhiman, 1977) [12] five plant samples were collected at various sampling days and the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemp's formula:

Total Leaf area = L B K

Where L = Length, B = Breadth and K = Kemp's constant (for dicot 0.66)

Yield parameters

Number pods per plant

The pods were harvested from the mature plant, counted and expressed in number of pods per plant.

Number of seeds per plant

The seeds were obtained from the pod, counted and expressed in number of seeds per plant.

Hundred seed weight (g/seed)

100 matured seeds were collected from test plants the dry weight of the seeds was noted by an electrical single pan balance.

Shelling percentage

To calculate shelling %, 1000 gm pods were taken from each replication, shells were removed from seed and then converted into % by following formula:

Shelling % = Kernels weight (gm)/1000 (gm) x 100

Pod yield (g plant^{-1})

The weight of the harvested pods of plant was recorded using electrical single pan balance.

Statistical analysis

SAS 6.12 was used to statistically analyze the recorded data. Paired T test used to determine significant differences between treatments. Means and standard deviations were calculated from three replicates. The growth and yield parameters were measured in five plants in each replication.

Result and discussion

In vitro* cultivation of *P. indica* and *A. hypogaea

The 81% seeds were germinated on half-strength hormone-free MS media and the well grown roots were observed with 1.0 mg/l naphthalene acetic acid (NAA). The *P. indica* spore persistence in the plant roots was confirmed by Microscopic observation and PCR assay.

Growth parameters

The growth parameters under observation were plant height, number of primary branches plant^{-1}, secondary branches plant^{-1}, nodulation and total leaf area. The growth parameters of *P. indica* treated viz. plant height and total leaf area were found significantly superior over controlled plant. The plant height observed 32.53±0.7 cm and 28.2±1.1 cm in treated and controlled plants respectively. Moreover the *P. indica* inoculation also affect the total leaf treated plants (5.92±0.37 cm^2/plant) compared to controlled plants (4.12±0.31 cm^2/plant). The rest growth parameters under present investigation i.e. primary branches plant^{-1}, secondary branches plant^{-1}, and nodulation found non-significant after co cultivation with the fungus.

It has been observed that the Sebacinales fungus. *P. indica* inoculation contributed in growth and development of many crop plants viz. plant height, root length, plant and root dry weight, and essential oil yield in previous studies (Sahay & Varma 1999, Kumari, *et al*., 2003 and Peskan-Berghofer, *et al*., 2004) [21] [13] [18], the similar observation recorded the present investigation.

Yield parameters

All the yield parameters analyzed under study were found with increased proliferation in *P. indica* treated plants. T-test analysis showed that differences between treatment with *P. indica* inoculation and control were significant in terms of yield parameters viz. number pods plant^{-1} (17.31±2.3),

number of seeds plant^{-1} (37.36±2.4), hundred seed weight (69.16±7.9 g/seed), shelling percentage (67.33±3.6 %) and pod yield (19.4±2.2 g plant^{-1}).

The enhanced amount in the growth and yield parameters of the *P. indica* treated groundnut plant might be due to production of phytohormones (Singh, *et al*., 2000 and Varma, *et al*., 2001) [22] [27]. It has been reported in earlier studies that P. indica produces small amounts of auxins and relatively large amounts of cytokinins in the host plants (Vadassery, *et al*., 2008) [24]. The increased amount of auxin can cause root proliferation in the plant (Sirrenberg, *et al*., 2007) [23]. The proliferated roots of the groundnut plant may indirectly affect the pod production related activities. Moreover *P. indica* is characterized by its greater absorption capacity for water and mineral nutrients (Varma, *et al*., 1999) [25] which ultimately due results in significant increase in growth parameters. The *P. indica* affect production of flowers and seeds in host plant (Rai, *et. al*., 2001) [19] such relevance was also observed in the current study. It can be concluded that the *P. indica* can be used as a promising mycofertilizer to increase the production in important agricultural crops.

Table 1: Effect of *P. indica* on the growth parameters of *A. hypogaea* plants in pot culture experiment

Parameter	Controlled Mean ±SD	Treated Mean ±SD	T-test
Growth Parameter			
Plant height	28.2±1.1	32.53±0.7	S
Primary branches plant-1	5.6±0.7	5.9±0.4	NS
Secondary branches -1	5.9±0.72	6.1±0.3	NS
Nodulation	56.26±6.2	64.63±5.4	NS
Total leaf area (cm2/plant)	4.12±0.31	5.92±0.37	S
Yield Parameter			
Number pods plant-1	12.73±1.5	17.31±2.3	S
Number of seeds plant -1	28.03±3.3	37.36±2.4	S
Hundred seed weight (g/seed)	55.83±6.4	69.16±7.9	S
Shelling (%)	59.16±2.7	67.33±3.6	S
Pod yield (g plant-1)	14.06±2.1	19.4±2.2	S

T-test: $P \leq 0.05$; S significant; NS not significant

Fig 1: Seed germination on 1/2 MS media

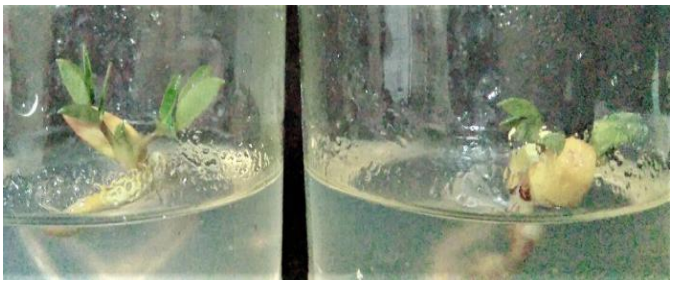

Fig 2: MS media, 1.0 mg/l naphthalene acetic acid (NAA)

Fig 3: Transplantation of *in vitro* plants

Fig 4: Pot culture experiment

Conclusion

Overall, to summarize it can be concluded that co-cultivation of *P. indica* and *A. hypogaea* promotes growth and development of therefore, *P. indica* treated plants would be helpful for increase growth rate, productivity. The findings of this investigation, in pot culture experiment, can be extended by establishing field trials. The exact mechanism of the active ingredients responsible for triggering plant growth promotion needs to be further studied.

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