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Research article

Impact of three phosphate solubilizing species of *Penicillium* on growth of *Piper longum* L. under inoculated condition

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Abstract: Efficient phosphate (P) solubilising strains of *Penicillium* such as *Penicillium oxalicum*, *Penicillium glabrum* and *Penicillium* sp. isolated from endophytic sources was applied as biofertiliser and it enhanced the productivity of *Piper longum*, a RET Medicinal plant which is one of the endangered species of Odisha state. Periodical study of the growth performance of *Piper longum* plants inoculated with different *Penicillium* sp. under pot culture conditions showed outstanding impact of the fungal strains on plant growth. Maximum height was recorded in plants treated with *Penicillium oxalicum* strains (76.75±22.9) cm after 120 days as compared to control plants with (57.4±16.3) cm. In control plants, spike development was observed after 180 days. Maturation of the fruits occurred in *Penicillium glabrum* and *Penicillium* sp. at 150 days and all the spikes obtained were of the male type and maturation of female spikes occurred after 180 days. The Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) were higher in plants inoculated with *Penicillium* sp. and recorded as 12.3 $mg.g^{-1}.day^{-1}$ and 4.49 $g.cm^{-2}.day^{-1}$ respectively.

Keywords: *Piper longum* - Pikovskaya's medium - P solubilising fungi - Growth parameters.

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INTRODUCTION

Phosphate is an essential irreplaceable element which is building block of all life forms. Globally, phosphate needs are currently met from geochemical sedimentary rock formations which are available only in selected areas of the world. These are finite resources that will last for half a century, depending upon the total resources that exist or currently accessible sources of P in the world. Hence, phosphate rock deposits are non-renewable and availability of high-grade P ores is finite. Since India suffers from lack of geological reserves of high-grade P it is dependent on imports to meet up to 90% of its domestic P requirement and it also witnessed a steep increase in the prices of P fertilizers in the recent years. Therefore, efficient usage of P to minimize wastage /loss remains the only option. However, Phosphate represents an interesting case of resource management, a key component in the pillar of information (DNA) and energy (ATP) molecules of the cell and is non-substitutable for all living organisms. In Indian soils, inorganic P contributes 54–84% of total P whereas the share of organic P varies from 16–46% in different states (Tomar 2000). However, since soils of more than 90% of the districts belonged to low to medium fertility categories, P fertilization is necessary to produce optimum crop yields. On the basis of accessibility and extractability, soil exists in 4 different pools such as Soil solution P, Surface adsorbed P, strongly bonded or absorbed P and inaccessible or precipitated P (Syers *et al.* 2008). Soil P solution concentration is affected by dissolution-precipitation (mineral equilibria), sorption-desorption, mineralization-immobilization. Hence P in soils is present in two main insoluble forms- mineral forms such as apatite, hydroxyapatite, oxyapatite and organic forms such as inositol phosphate (soil phytate), phosphomonoesters, phosphodiesters, phosphotriesters etc.

Natural solubilisation of mineral phosphates is a characteristic exhibited by most of the Phosphate Solubilising Microbes or PSM (Perez *et al.* 2007). In rhizosphere soil, these microbes play an ecophysiological role by mobilizing the insoluble inorganic phosphates so that they can be absorbed by plant roots and thus PSM

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can be used for biofertilization to improve crop yields (Richardson 2001, Khan *et al.* 2007, Wafula *et al.* 2016, Nayak *et al.* 2017). Soil inoculation with phosphate solubilising organisms improves solubilisation of fixed soil P and applied phosphates so that P is available to the plant for growth and development. Plant growth and development is also related to its access to minerals such as P (Santos *et al.* 2010, Ghosh *et al.* 2017). *Aspergillus* and *Penicillium* among fungi are the most common P-solubilising fungi (Seshadri *et al.* 2004, Wakelin *et al.* 2004, Sahoo & Gupta 2016, Nayak *et al.* 2017, Panigrahi *et al.* 2017). Different species of *Penicillium* obtained from Agricultural soil, hill soil, mine soil and rhizosphere of plants were reported to have P solubilising activity by various investigators (Singh & Reddy 2011, Morales *at al.* 2011, Mahamuni *et al.* 2012). Various species of *Penicillium* with P solubilizing activity were tested under pot trial to evaluate their growth promoting abilities with crops such as cereals (maize, corn, wheat); Vegetable crops (Lettuce); oilseed crops (Groundnut); Forest trees (*Dalbergia sisso*) etc. (Kassim & Al-Zandinary 2011, Singh & Reddy 2011, Malviya *et al.* 2011, Morales *at al.* 2011, Patil *et al.* 2012, Dash *et al.* 2013). Endophytic microbes associated with plant also promote its growth and increases productivity due to their capacity of solubilising P mostly Ca and Fe phosphates (Vitorino *et al.* 2012).

Piper longum L. is a slender aromatic climber having several medicinal properties due to the dry spikes of female types which is economically important. This is the reason for its overexploitation and more than 100 metric tons/year of the dry weight of long pepper is being traded, as a result of which it is disappearing rapidly. Therefore, large-scale cultivation should be promoted to enhance its population in the wild. However, several problems such as lack of quality planting material, mortality in the field and poor growth are associated with large-scale cultivation (Singh & Gogoi 2010). Field trial of *P. longum* with P solubilising fungi was not reported earlier. The present study emphasizes on the application of efficient P solubilising strains of *Penicillium* such as *Penicillium oxalicum* Currie & Thom, *Penicillium glabrum* (Wehmer) Westling and *Penicillium* sp. as biofertiliser in order to enhance the productivity of *P. longum* which is one of the endangered species of Odisha state.

MATERIALS AND METHODS

A pot culture experiment was designed to test the effectiveness of P solubilising fungal strains on growth and development of *Piper longum* L. under the greenhouse conditions. 3 Phosphate solubilising fungi belonging to *Penicillium* genera were selected for the experimental trial. Polybags of 10" × 10" size containing red laterite soil was used. All the polypots of 5 kg capacity were filled with sterile soil before transplanting the rooted stem cuttings of *Piper longum* plant. This was done in 10 replications for each treatment. The experiment was done in completely randomized block design with 3 treatments, F1, F8 and F11 inoculated with *Penicillium* sp., *Penicillium oxalicum* and *Penicillium glabrum* respectively in 10 replications.

The characteristic features of the soil used for the experiment was acidic with pH 6.06, electrical conductivity 0.2 dSm^{-1}, Organic carbon 0.9% with available N, P, K as 232, 25 and 99 $kg.ha^{-1}$ respectively, Ca and Na with 6.6 and 0.174 meq/100g respectively. However, the concentration of micronutrients such as Fe, Mn, Cu and Zn was found to be 32, 11, 1 and 0.3 $mg.kg^{-1}$ respectively. The pots were inoculated with fungal cultures at regular intervals and were maintained in the greenhouse at an adequate temperature with daily water supply to maintain the soil moisture level and without any application of fertilizer. The plant growth parameters especially observation of morphological details was done. The parameters such as leaf number, branch number, shoot height and fresh weight, root length and fresh weight, leaf area and fresh weight were recorded periodically. The dry weight of plant biomass such as shoot, root and leaf samples were determined after drying at 60ºC in the hot air oven for 48 hours. The observations recorded after 30, 60 and 90 days were compared with the control setup. The RGR, NAR and LAR were also calculated for all the treatments. Fruiting was observed after 120 days of fungal inoculation into the plants. The male and female spike development, their maturation and spike length was also recorded after 150 and 180 days.

RESULTS AND DISCUSSION

Periodical study of the growth performance of *Piper longum* L. plants inoculated with different *Penicillium* sp. under pot culture conditions revealed that *Penicillium oxalicum* and *Penicillium glabrum* showed a higher number of branches (11.25±2.5) and (11.25±4.03) respectively as compared to control plants (8.4±2.77) at the end of 120 days as shown in table 1. However, no significant increase is observed in number of branches for *Penicillium* sp. inoculated plant throughout the growth period. Leaf development in plants treated with different

strains of *Penicillium* showed a good increase in number at the end of 120 days. *Penicillium oxalicum* treated plants showed (38.25±8.54) number of leaves as compared to other plants. All the strains showed higher number of leaves from control plants. The plant height also increased after treatment with strains of *Penicillium* as compared to control. Maximum height was recorded in plants treated with *Penicillium oxalicum* strains (76.75±22.9) cm after 120 days. After fungal treatment at regular intervals, the plants showed a great increase in their height. All the three strains improved the Growth of *Piper longum* plant. *P. oxalicum* also increased the root length of plants and maximum of (33.75±2.47) cm is recorded after 90 days. The increment in root length of these plants is almost the double of control plants. However, all the three strains showed an increase in root length as compared with control. *P. oxalicum* also influenced the leaf development as higher leaf area (386.5±17.7) cm^2 is obtained for the same.

Table 1. Growth parameters recorded for *Piper longum* L. after regular interval inoculated with different species of *Penicillium.*

A. 30 days			SHOOT			ROOT			LEAF		
Treatment	**No. of branches**	**No. of leaves**	**Height**	**Fresh weight**	**Dry weight**	**Length**	**Fresh weight**	**Dry weight**	**Area**	**Fresh weight**	**Dry weight**
Control	5.5± 0.71	7± 1.41	36.5± 12.02	2.133± 0.89	0.27± 0.115	9.25± 0.35	1.01± 0.13	0.138± 0.018	261.63± 23.16	3.615± 0.61	0.497± 0.038
F1	3.5± 2.12	4.5± 2.12	26.35± 17.89	1.11± 0.37	0.18± 0.094	20± 12.73	0.735± 0.53	0.124± 0.082	301.88± 273.12	1.94± 1.14	0.33± 0.19
F8	5.5± 0.71	6.5± 0.71	29.25± 12.37	1.187± 0.56	0.173± 0.082	19.7± 0.71	0.89± 0.28	0.151± 0.046	240.33± 21.8	2.81± 0.5	0.36± 0.08
F11	6± 0	7± 0	41.25± 7.42	1.695± 0.8	0.203± 0.045	23.5± 2.12	1.052± 0.142	0.142± 0.034	210.878± 37.13	3.113± 0.99	0.385± 0.11

B. 60 days			SHOOT			ROOT			LEAF		
Treatment	**No. of branches**	**No. of leaves**	**Height**	**Fresh weight**	**Dry weight**	**Length**	**Fresh weight**	**Dry weight**	**Area**	**Fresh weight**	**Dry weight**
Control	5± 2.83	4± 2.83	43.45± 3.18	2.857± 0.62	0.343± 0.15	9± 1.41	1.15± 0.5	0.317± 0.14	253± 171	2.775± 1.3	0.44± 0.23
F1	4.5± 2.12	6± 1.41	32.2± 17.8	1.504± 0.47	0.228± 0.07	10.7± 1.63	0.678± 0.28	0.149± 0.06	173.88± 98.8	1.971± 1.26	0.313± 0.21
F8	8± 1.41	9.5± 2.12	55± 0.63	2.352± 0.24	0.417± 0.17	7.8± 1.41	0.482± 0.06	0.108± 0.013	353.63± 54.62	3.63± 0.03	0.683± 0.06
F11	6.5± 2.12	8± 1.41	53.25± 6.72	2.74± 0.22	0.344± 0.02	8.2± 1.34	0.769± 0.47	0.144± 0.09	273.38± 11.14	2.94± 0.29	0.6± 0.05

C. 90 days			SHOOT			ROOT			LEAF		
Treatment	**No. of branches**	**No. of leaves**	**Height**	**Fresh weight**	**Dry weight**	**Length**	**Fresh weight**	**Dry weight**	**Area**	**Fresh weight**	**Dry weight**
Control	6.5± 3.54	8± 4.24	41.25± 5.3	2.672± 0.54	0.43± 0.04	17.5± 3.53	0.47± 0.27	0.101± 0.059	270.4± 214.4	3.17± 2.32	0.49± 0.32
F1	6± 2.83	8.5± 4.95	41.75± 18.74	2.26± 1.66	0.32± 0.27	22± 2.82	0.91± 0.68	0.20± 0.16	292.13± 257.2	2.71± 2.04	0.56± 0.53
F8	8.5± 0.71	12± 2.82	65.7± 8.7	3.11± 0.35	0.325± 0.07	33.75± 2.47	1.1± 0.31	0.21± 0.06	386.5± 17.7	4.77± 0.11	0.69± 0.008
F11	7± 2.83	11± 4.24	50.25± 23.69	2.41± 1.49	0.299± 0.16	30.75± 0.35	1.082± 0.013	0.286± 0.056	260.25± 29.34	4.07± 2.84	0.68± 0.33

D. 120 days

Treatment	**No. of leaves**	**No. of branches**	**Shoot height**
Control	27.63±9.43	8.4±2.77	57.4±16.3
F1	29.25±16.52	7.75±3.3	64.75±17
F8	38.25±8.54	11.25±2.5	76.75±22.9
F11	36.75±4.99	11.25±4.03	62±6.7

Observations recorded for plant biomass studies highlight that *P. oxalicum* showed higher fresh shoot weight (3.11±0.35) g at 90 days as compared to plants inoculated with other strains and control plants whereas the dry biomass of shoot is not much significant and less in treated plants than that of control plants. No effect of the

strains was observed on the biomass of the roots of the plants. As after 60 days, the fresh and dry biomass of root was highest in control and recorded as (1.15±0.5) g and (0.317±0.14) g respectively. Higher fresh and dry leaf biomass of (4.77±0.11) g and (0.69±0.008) g respectively is observed in *P. oxalicum* inoculated plants after 90 days. Hence, it is clear that among the 3 strains of *Penicillium*, *Penicillium oxalicum* can be used as efficient P solubiliser for biofertilization studies as it enhanced the values of the parameters related to plant growth and development.

Table 2. Fruit development recorded in *Piper longum* L.

Treatment	120 days	150 days	180 days
Control	0	0	0
F1	4	4	5
F8	6	7	8
F11	6	2	2

The fruit development was observed in plants treated with fungal strain but it is absent in control plants even after 180 days as depicted in table 2. However, in control plants, spike development was observed after 180 days. A maximum number of fruits was recorded in *P. oxalicum* treated plants (6 in 120 days, 7 in 150 days and 8 in 180 days, respectively). However, maturation of the fruits occurred in *Penicillium glabrum* and *Penicillium* sp. at 150 days and all the spikes obtained were of the male type. The maximum average length of the male spike was found to be (6±1.3) cm which is highest in *Penicillium* sp. However, maturation of female spikes occurred after 180 days. The maximum average length of the male spike was found to be (5.8±0.42) cm and the female spike was (4.6±0) at 180 days as reflected in table 3.

Table 3. Harvesting of spikes of *Piper longum* L.

A. 150 days				
Treatment	**Number of male spike harvested**	**Number of female spike harvested**	**Length of Male spike (in cm)**	**Length of female spike (in cm)**
Control	0	0	0	0
F1	2	0	6±1.3	0
F8	0	0	0	0
F11	4	0	5.6±1.14	0
B. 180 days				
Treatment	**Number of male spike harvested**	**Number of female spike harvested**	**Length of Male spike (in cm)**	**Length of female spike (in cm)**
Control	0	0	0	0
F1	2	1	5.8±0.42	3.4±0
F8	2	1	4.9±0.71	4.6±0
F11	1	1	5.4±0	3.6±0

The Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) were higher in plants inoculated with *Penicillium* sp. and recorded as 12.3 $mg.g^{-1}.day^{-1}$ and 4.49 $g.cm^{-2}.day^{-1}$ respectively (Table 4). However, Leaf Area Ratio (LAR) was highest in *P. oxalicum* treated plants and found to be 662 $cm^{-2}.g^{-1}$. Hence, all the parameters recorded were mostly higher in treated plants as compare to the control plants.

Table 4. Relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) recorded in *Piper longum* L.

Treatment	RGR ($mg.g^{-1}.d^{-1}$)	NAR ($g.cm^{-2}.d^{-1}$)	LAR ($cm^2.g^{-1}$)
Control	3.25	0.68	616.25
F1	12.3	4.49	517.5
F8	6.42	0.9	662
F11	6.98	0.31	385.7

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