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Screening of Plant Growth Promoting Fungi (PGPF) for Sustainable Cultivation of Tulaipanji, an Endemic Aromatic Rice Variety of Uttar Dinajpur, West Bengal, India

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ABSTRACT

Background: Rice is one of the major staple crops of human population. Worldwide escalating human populace exerts pressure to increase rice production. To fulfill the demand, the injudicious application of chemical fertilizers is posing grave threat to the environment together with the entire living population. A suitable replacement of these harmful fertilizers is needed in modern agriculture to keep the biodiversity undisturbed. The main objective of the study is to screen and explore indigenous plant growth promoting fungi which can replace the conventional fertilizers.

Methods: Rhizospheric soil of different rice cultivating fields were collected in the year 2018. Different *in vitro* assays of the fungal isolates obtained from the soil were performed to check their plant growth promotion capabilities.

Result: 20 different indigenous fungal isolates were studied to evaluate their ability as PGPF for organic cultivation of Tulaipanji, an endemic aromatic rice variety cultivated in Uttar Dinajpur, West Bengal, India. *Trichoderma harzianum* TaK12 and *Trichoderma aureoviride* TaN16 were found to enhance maximum shoot and root length of rice plant. These two isolates also showed efficacy in phosphate solubilization. *Trichoderma aureoviride* TaN16 together with *Penicillium citrinum* PcK10 and *Aspergillus niger* AnK1 produced high amount of IAA. The results obtained from the study clearly indicate that these plant growth promoting fungi (PGPF) possess the ability to boost up plant growth.

Key words: Indole acetic acid, Phosphate solubilization, Plant growth promoting fungi, Tulaipanji, Vigor index.

INTRODUCTION

Rice is the main staple crop that contributes to fulfill the food needs of St rikeout half of the human population across the globe. The increasing population of the earth puts pressure to escalate the rice production (Mahajan 2020). Excess fertilizers are applied in rice cultivation fields to encounter the escalating pressure of high production (Polthanee 2021). This results in negative impact on both the environment and on all living beings (Habibah 2011). In this perspective, an alternative to traditional fertilizers is urgent requisite to strengthen agricultural production in organic approaches. Plant growth promoting fungi (PGPF) due to their eco-friendly nature can be used in organic agriculture practice in place of these traditional inorganic fertilizers. The rhizosphere is inhabited by a large number of microbes that succor in plant growth and development and sustain soil fertility (Mendes, Garbeva *et al*. 2013). They may be free-living in the soil, epiphytic when residing on the root surface or endophytic dwelling inside the roots (Hossain, *et al*. 2017). PGPFs are reported to synthesize plant hormones like IAA, solubilize soil phosphorus, produce low molecular weight volatile compounds called microbial volatile organic compounds (mVOCs), thus assisting in plant growth and development (Jogaiah, *et al*. 2013). The PGPF also help in seed germination and seedling vigor, root and shoot growth, augmenting photosynthetic efficacy and improving crop-yield (Contreras-Cornejo, *et al*. 2011).

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PGPF is a heterogenous group of non-pathogenic, non-symbiotic, saprotrophic fungi that mainly belong to the phylum Ascomycota (*Aspergillus*, *Fusarium*, *Penicillium*, *Phoma*, *Talaromyces*, *Trichoderma*), Basidiomycota (*Limonomyces*, *Rhizoctonia*) and Zygomycota (*Mucor*, *Rhizopus*) (Muslim, Hyakumachi *et al*. 2019). The property of PGPF for enhancing the growth of plants make them suitable for organic based agriculture and can be a new innovative approach in organic agriculture with less dependence on traditional inorganic fertilizers. In the present

study, this approach is considered for organic cultivation of Tulaipanji, a renowned endemic aromatic rice variety of Uttar Dinajpur, West Bengal, India.

MATERIALS AND METHODS

Collection of rhizospheric soil

Rhizospheric soil adhering to root of rice plants was collected in sterile polythene bags from different rice cultivating fields of Uttar Dinajpur, West Bengal in the year 2018. The samples were brought to Mycology and Plant Pathology Laboratory, Raiganj University.

Isolation of the fungal isolates

Isolation of indigenous soil borne fungi were executed through serial dilution method as demonstrated by Johnson and Curl (1972). In this method, 1 gm soil was dissolved in 10 ml of sterile double distilled water aseptically to make soil concentration 0.1 g/ml (10^{-1}). A serial dilution in the range of 10^{-3} to 10^{-5} was prepared with sterilized double distilled water. 20 ml of potato dextrose agar (PDA) amended with Monocef antibiotic (2.5 mg/ml) was poured aseptically in each sterile Petridish and allowed to solidify for 1 hr. Then 1.0 ml of soil suspension from dilution 10^{-4} and 10^{-5} were poured into the Petridishes separately under aseptic condition and spread uniformly, followed by sealing the Petridishes with parafilm. The Petridishes were incubated at 28°C for 7 days. Different fungal colonies started to appear into the Petridishes after 3-7 days of incubation period. These colonies were picked up carefully and transferred on freshly prepared PDA slants and PDA plates, marked with proper labelling and incubated at 28±2°C for 7 days. The PDA slants were then stored at -4°C for future use.

Preparation of fungal inoculum

Spore suspension

Fungal isolates were multiplied in 250 ml Erlenmeyer flask containing 200 ml Potato dextrose broth (PDB) at 23±2°C for 10 days. Then the culture broth was centrifuged at 10,000 rpm for 10 mins. The pellets were suspended in sterile distilled water and washed repeatedly for 3-4 times. The washed fungal pellet was made into a turbid solution with sterile distilled water. The OD of the solution was adjusted to 0.45 at 610 nm to obtain 1×10^{8} cfu ml^{-1} (Niranjana, Umesha *et al*. 2006).

Maize granules inocula (MGI)

One kilogram of maize grains was crushed into granules using mixer grinder for 2-3 minutes. Then the granules were semi-boiled for 5 minutes and air dried. The air-dried maize granules were amended with calcium carbonate (2%) and calcium sulphate (1%) at pH 6.48. Then mixed granules were filled ($1/4^{th}$) in 500 ml Erlenmeyer flask, plugged with non-absorbent cotton, wrapped with paper and autoclaved. The autoclaved flasks were inoculated with mycelia discs (5 mm) obtained from the actively growing margins of 7 days-old culture of fungal isolates and incubated at 25°C. After 12-15 days of incubation period, completely colonized maize granules were used as maize granules inocula (MGI) and stored at 4°C until further studies.

Priming of seed with mycoflora isolates

Rice seeds were surface sterilized with 0.1% mercuric chloride for 2-3 minutes and then rinsed 3-4 times with sterile distilled water. Then average 50 seeds were treated with 10 ml of conidial suspension (10^{8} cfu ml^{-1}) of fungal isolates. Three replicates were maintained for each treatment. In another set of experiment, surface sterilized 50 rice seeds were coated with MGI of different fungal isolates in sterile potting soil in the ratio of 5:95 (5%), 10:90 (10%) and 20:80 (20%). Treated seeds were kept at normal temperature for germination and growth observation. Seeds treated with sterile distilled water St rikeout served as untreated control. After 10 days, germination percentage root length and shoot length were recorded and vigor index was calculated following the method of Abdul-Baki and Anderson (1973).

Vigor index (VI) = Seed germination (%) × [Mean of root length + Mean of shoot length]

In vitro assay of indole acetic acid (IAA) production

Indole acetic acid (IAA) production was measured following standard methods with some modification (Bric, *et al*. 1991). Fungal isolates were cultured on PDA media and incubated at 37°C for 7 days. After 7 days of incubation, fungal discs (5mm) from culture media of each isolate were transferred to PDA broth amended with 500µg/ml tryptophan as the precursor of IAA production and incubated in a shaker incubator at 28±2°C with rotation of 120 rpm for 3-5 days. Control set was maintained without tryptophan. Fully grown cultures were centrifuged at 10,000 rpm for 10 minutes. Then 1 ml of the supernatant was mixed with 4 ml of the Salkowski reagent (50 ml of 35% of $HClO_4$, 1 ml of 0.5 m $FeCl_3$ solution) followed by addition of 2-3 drops of 10mM orthophosphoric acid and kept in dark for colour formation. Three replicates for each isolate were maintained. Appearance of pink color in test tubes indicated the production of IAA.

IAA production was expressed by + and – sign.

In vitro assay of phosphate solubilization

The phosphate (P) solubilizing activity of fungal isolates was performed on Pikovskaya's solid agar medium amended with Rose Bengal at 10 ml/L as described by Johnson (1959) with some modifications. After incubation for seven days at 28°C, the formation of clear zone around the fungal hyphae indicated the ability of the fungus to solubilize inorganic phosphorous. Each treatment was replicated three times. The performance of each fungus was marked by assigning - and + sign. The - sign indicates no P solubilisation; + sign indicates small amount of P dissolved; ++ denotes medium amount of P dissolved; +++ indicates high amount of P dissolved. Percent solubilization efficiency and phosphate solubilization index was calculated as:

$$\text{Solubilization index (SI)} = \frac{(R+Z)}{R}$$

$$\text{Solubilization efficiency (SE)} = \frac{(R+Z)}{R} \times 100$$

Where, SI = Solubilization Index, SE= Solubilization Efficiency, Z = Clear zone diameter (mm), R = Colony diameter (mm).

RESULTS AND DISCUSSION

Identification of fungal isolates

Based on the morphological study, the fungal isolates were identified from ITCC and NFCCI, India (Table 1).

Promotion of growth of rice plant

Influence on growth of rice seedlings following treatment with different fungal isolates

Two isolates *Trichoderma harzianum* TaK12 and *Trichoderma aureoviride* TaN16 showed maximum shoot length and root length after 15 days of treatment with 20% maize grain inocula (MGI). In addition, they both showed maximum percent of seed germination after 15 days of treatment with 20% MGI. Maximum vigor index together with growth of rice seedling was found with the treatment of spore suspension of *Trichoderma harzianum* TaK12 after 15 days with 20% of MGI (Table 2 and 3).

Earlier reports support the findings of the current study. Doni *et al.*, 2014 reported rice seeds treated with *Trichoderma* spp. SL2 improved germination rate along with

Table 1: Identification of fungal isolates from Indian Type Culture Collection (ITCC), New Delhi and National Fungal Culture Collection of India (NFCCI), Pune, India.

Fungal isolate	ITCC/NFCCI Accession no.	Identification remarks	Family
AnK1	NFCCI 4966	*Aspergillus niger*	*Aspergillaceae*
AnR2	11,205.19	*Aspergillus niger*	*Aspergillaceae*
AnH3	NFCCI 4967	*Aspergillus niger*	*Aspergillaceae*
AnK4	11,204.19	*Aspergillus niger*	*Aspergillaceae*
AnD5	11,211.19	*Aspergillus niger*	*Aspergillaceae*
AnD6	11,204.19	*Aspergillus niger*	*Aspergillaceae*
TaK12	11,203.19	*Trichoderma harzianum*	*Hypocreaceae*
TaL13	11,209.19	*Trichoderma asperellum*	*Hypocreaceae*
TaM14	11,210.19	*Trichoderma asperellum*	*Hypocreaceae*
TaN15	11,212.19	*Trichoderma asperellum*	*Hypocreaceae*
TaN16	NFCCI 4962	*Trichoderma aureoviride*	*Hypocreaceae*
TaN17	NFCCI 4964	*Trichoderma yunnanense*	*Hypocreaceae*
TpG11	NFCCI 4965	*Talaromyces purpureogenus*	*Aspergillaceae*
PcK7	11,201.19	*Penicillium pinophilum*	*Aspergillaceae*
PcR8	11,207.19	*Penicillium purpurogenum*	*Aspergillaceae*
PcK9	NFCCI 4959	*Penicillium citrinum*	*Aspergillaceae*
PcK10	NFCCI 4960	*Penicillium citrinum*	*Aspergillaceae*
PcK11	NFCCI 4961	*Penicillium citrinum*	*Aspergillaceae*
Fm	11,208.19	*Fusarium moniliforme*	*Nectriaceae*
Fp	NFCCI 4963	*Fusarium pseudoanthophilum*	*Nectriaceae*

Table 2: Effect on shoot length and root length of rice seeding following treatment with spore suspension ($1x10^8$ cfu ml^{-1}) and maize granules inocula (MGI) of different fungal isolates.

Treatments	Shoot length (cm)				Root length (cm)			
	(1×10^8 cfu ml^{-1})	5%	10%	20%	(1×10^8 cfu ml^{-1})	5%	10%	20%
Control	2.7±0.01^a	2.8±0.03^a	3.8±0.02^a	2.6±0.02^a	2.4±0.03^a	2.9±0.03a	2.2±0.02^a	2.1±0.02^a
Aspergillus niger AnH3	4.3±0.02^b	3.7±0.04^b	4.3±0.03^b	4.5±0.01^b	4.7±0.01^b	3.9±0.02^b	4.8±0.03^b	4.3±0.03^b
Penicillium citrinum PcK10	3.9±0.03^b	3.2±0.01^b	3.3±0.02^b	4.2±0.02^b	4.4±0.02^b	3.3±0.02^b	3.7±0.02^b	4.6±0.02^b
Talaromyces purpureogenus TpG11	6.7±0.04^b	4.4±0.02^b	5.4±0.01^b	6.8±0.03^b	8.4±0.01^b	5.3±0.01^b	6.4±0.02^b	8.8±0.02^b
Trichoderma aureoviride TaN16	7.8±0.02^b	5.8±0.03^b	6.8±0.03^b	8.1±0.01^b	8.3±0.03^b	5.9±0.03^b	6.1±0.01^b	8.1±0.01^b
Trichoderma harzianum TaK12	8.9±0.03^b	6.3±0.02^b	7.3±0.02^b	9.5±0.02^b	12.5±0.01^b	8.9±0.03^b	10.3±0.02^b	12.8±0.02^b

Note: Average of three replicates; ± = SE; Difference between control and treated significant at p =0.05 (t test) where superscript of control and treated different, where superscript same, difference insignificant at p =0.05.

Table 3: Effect on seed germination and vigour index of rice seedling following treatment with spore suspension (1×10^8 cfu ml^{-1}) and maize granules inocula (MGI) of different fungal isolates.

Treatments with	% Seed Germination				Vigor Index			
isolates	(1×10^8 cfu ml^{-1})	5%	10%	20%	($1x10^8$ cfu ml^{-1})	5%	10%	20%
Control	82±1.4^a	82±1.5^a	83±1.4^a	82±1.7^a	418.2±1.7^a	467.4±1.4^a	498.0±1.5^a	385.4±1.6^a
Aspergillus niger AnH3	89±1.6^b	86±1.3^b	91±1.5^b	92±1.2^b	801.0±1.4^b	653.6±1.6^b	828.1±2.3^b	809.6±1.7^b
Penicillium pinophilum PcK7	90±1.3^b	82±1.3^a	84±1.1^b	92±1.3^b	720.0±1.8^b	549.4±1.1^b	621.6±1.6^b	864.8±2.1^b
Penicillium citrinum PcK10	91±1.4^b	82±1.6^a	87±1.4^b	90±1.7^b	755.3±1.3^b	533.0±1.3^b	609.0±1.5^b	792.0±2.2^b
Talaromyces purpureogenus TpG11	92±1.1^b	88±1.3^b	90±1.2^b	93±1.8^b	1389.2±1.4^b	853.6±1.8^b	1062.0±2.1^b	1450.8±1.8^b
Trichoderma aureoviride TaN16	93±1.3^b	84±1.6^b	88±2.1^b	94±1.2^b	1497.3±1.5^b	982.8±1.5^b	1135.2±2.3^b	1525.6±1.4^b
Trichoderma harzianum TaK12	96±1.4^b	84±1.1^b	92±1.5^b	97±1.5^b	2054.4±1.8^b	1233.1±1.2^b	1619.2±1.5^b	2163.1±2.3^b

Note: Vigor Index = Seed Germination (%) × [Mean of Root Length + Mean of Shoot Length];
Average of three replicates; ± = SE; Difference between control and treated significant at p =0.05 (t test) where superscript of control and treated different, where superscript same, difference insignificant at p =0.05.

seedling vigor (Doni, Isahak *et al*. 2014). Co-inoculation of *Trichoderma asperellum* and *Pseudomonas fluorescens* were reported to promote fresh weight, shoot height, tiller numbers and dry biomass of rice and was found to be more effective as compared to single inoculation (Singh, *et al*. 2020).

Indole acetic acid (IAA) production efficacy

Penicillium citrinum PcK10, *Aspergillus niger* AnK1 and *Trichoderma aureoviride* TaN16 exhibited high amount of IAA production. *Trichoderma harzianum* TaK12 showed medium amount of IAA production. Small amount of IAA production was shown by *Talaromyces purpureogenus* TpG11 (Fig 1, Table 4).

There are several studies that support the production of IAA by *Trichoderma, Aspergillus,* (Pedrero-Méndez, Insuasti *et al*. 2021, Tiru 2021).

Table 4: *In vitro* Indole acetic acid (IAA) production by different fungal isolates of rice rhizosphere of Uttar Dinajpur, West Bengal.

Fungi	Isolate	Degree of IAA production
Trichoderma aureoviride	TaN16	+ + +
Trichoderma harzianum	TaK12	++
Penicillium citrinum	PcK10	+ + +
Penicillium pinophilum	PcK7	+ +
Talaromyces purpureogenus	PcR8	+ +
Aspergillus niger	AnK1	+ ++
Talaromyces purpureogenus	TpG11	+
Control		-

(–) indicates no IAA production; (+) indicates small amount of IAA production; (++) indicates medium amount of IAA production; (+++) indicates high amount of IAA production.

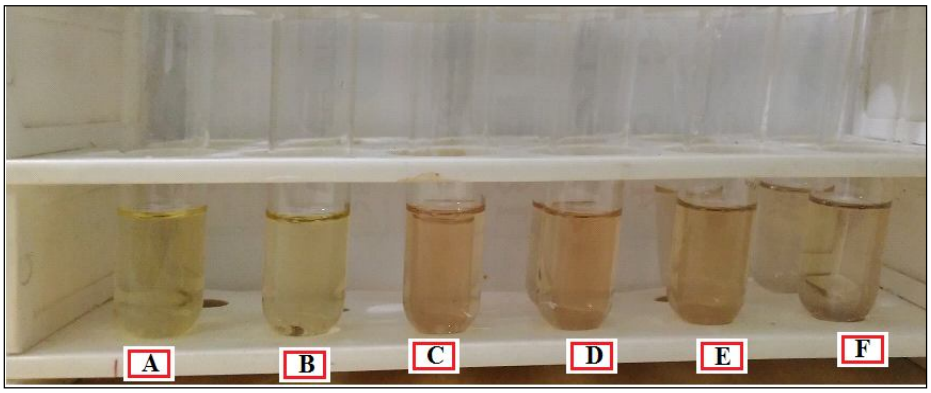

Fig 1: Production of Indole acetic acid (IAA) by fungal isolates collected from rhizosphere of rice of Uttar Dinajpur, West Bengal. (A = Control, B = T*alaromyces purpureogenus* TpG11*;* C = *Aspergillus niger* AnK1, D =*Penicillium citrinum* PcK10, E = *Trichoderma aureoviride* TaN16, F =*Trichoderma harzianum* TaK12).

Phosphate solubilisation efficacy

Trichoderma aureoviride TaN16 and *Trichoderma harzianum* TaK12 showed highest efficiency in phosphate solubilization with solubilization index (SI) 1.3 and 1.27 respectively. The isolates of *Aspergillus* species exhibited moderate response in terms of phosphate solubilization activity (Fig 2, Table 5). Filamentous fungi such as *Aspergillus niger*, *Penicillium* and *Talaromyces* were reported to be highly efficient in rock phosphate solubilization (Yin, Shi *et al*. 2015, Nelofer, Syed *et al*. 2016). *Trichoderma viride, Trichoderma longibrachiatum, Trichoderma asperellum* and *Trichoderma harzianum* were found with phosphate solubilization capability (Hewedy, Abdel Lateif *et al*. 2020). These reports are in conformity with the findings of our present study.

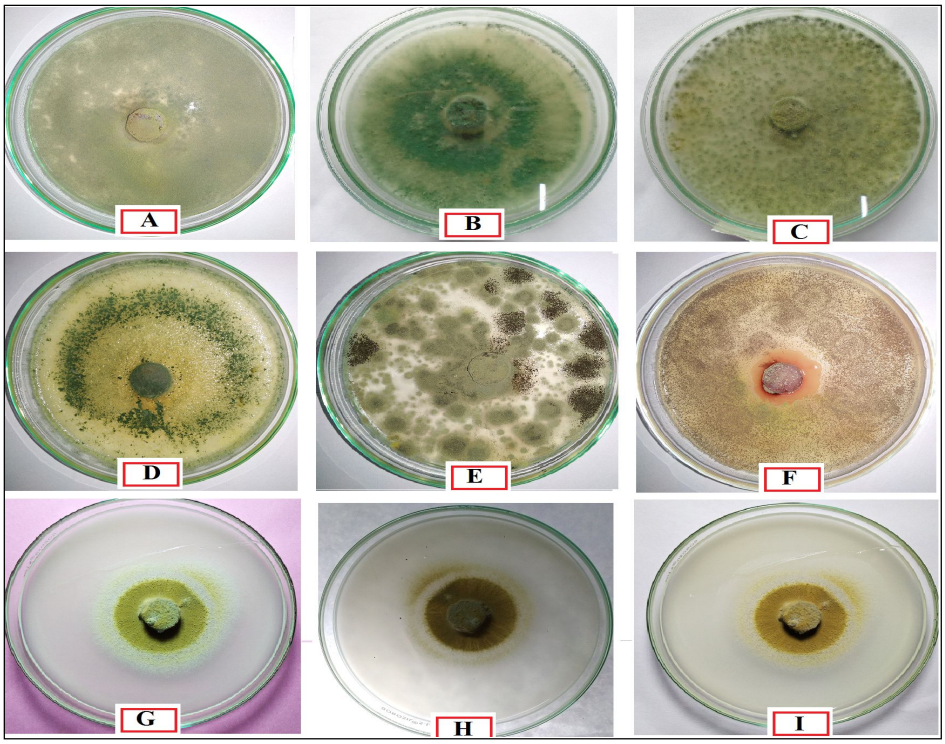

Fig 2: In vitro phosphate solubilisation ability of fungal isolates collected from rhizosphere of different rice cultivated fields of Uttar Dinajpur, West Bengal. A =*Penicillium citrinum* PcK9; B =*Trichoderma aureoviride* TaN16; C =*Penicillium pinophilum* PcK7; D =*Trichoderma harzianum* TaK12; E =*Penicillium purpurogenum* PcR8; F =*Talaromyces purpureogenus* TpG11; G = *Aspergillus niger* AnK1; H = *Aspergillus niger* AnR2; I = *Aspergillus niger* AnK4.

Table 5: Phosphate solubilization efficiency of different fungal isolates.

Fungal isolates	Clear zone diameter (mm)	Colony diameter (mm)	Solubilization Index (SI)	Solubilization efficiency (SE)
Penicillium citrinum PcK9	-	-		- -
Penicillium pinophilum PcK7	-	-	-	-
Aspergillus niger AnK1	4±0.04	45±0.5	1.08	108.88±4
Aspergillus niger AnR2	5±0.05	42±0.3	1.11	111.90±5
Aspergillus niger AnK4	6±0.02	47±0.7	1.12	112.76±7
Aspergillus niger AnH3	-	-	-	-
Trichoderma aureoviride TaN16	27±0.08	90±0.7	1.3	130.00±4
Trichoderma harzianum TaK12	25±0.34	90±0.5	1.27	127.77±6
Penicillium purpurogenum PcR8	-	-	-	-
Talaromyces purpureogenus TpG11	12±0.14	87±0.5	1.13	113.79±4

± - SE, Three replica of each treatment is followed.

CONCLUSION

Rhizosphere represents a complex ecosystem on earth (Sharma, *et al*. 2021). Interaction of plant with rhizospheric fungi play a key role not only on boosting plant productivity rather influence the overall soil fertility (Htwe, *et al*. 2019). To minimize the usage of traditional fertilizers, the exploration of the beneficial rhizospheric root colonizing fungi can be advantageous in sustainable management of rice. Intense study of these eco-friendly fungi of the rhizosphere is needed to improve agricultural productivity in sustainable ways.

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Conflict of interest: None.

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