J. Soils and Crops 29 (2) 243-250, December 2019

ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)
NAAS Rating - 4.46
Scientific Journal Impact Factor - 7.375

# BIOCONTROL AND GROWTH PROMOTING POTENTIAL OF EIGHT PGPFS ON JUTE AND SUNNHEMP

Anuradha Bandopadhyay[1], Saurindra Kishore Bhattacharya[2] and Nirmalendu Das[3]

## ABSTRACT

**Jute and sunnhemp, two important bast fibre crops in India suffer from diseases like stem and root rot caused by *Macrophomina phaseolina* and wilt caused by *Fusarium udum*, respectively which are economically imperative. Eight native fungal bioagents were screened for disease management and plant growth promoting ability in jute and sunnhemp. The fungal bioagents were assayed for antagonistic and growth promoting traits by standard protocols and finally evaluated for disease control and plant growth promotion *in vivo* under field condition. Biochemical characterization revealed that the bioagents were efficient in production of cell wall degrading enzymes (CWDE), siderophore, plant growth hormone and phosphorus solubilization. Five fungal isolates produced siderophores in varied amount differing in chemical nature. *Aspergillus* isolates didn't produce IAA except AN27. Field evaluation of PGPFs for three years (2009-2012) on jute exposed that stem and root rot disease incidence in jute was significantly reduced (p=0.05) by A26 and increased plant biomass. Highest fibre yield was envisaged by JPG4. In sunnhemp, *A. niger* AN27 co-inoculated with *Rhizobium* Rjc2 significantly (p=0.05) controlled *Fusarium* wilt incidence, increased root nodulation, plant biomass and fibre yield. Therefore, the selected PGPFs can be utilized for pathogen suppression and growth promotion in jute and sunnhemp as well as their siderophore producing traits can be exploited in soil bioremediation for removal of toxic metals like arsenic in the affected adjoining areas of North 24 Parganas in West Bengal.**

(**Key words: Biocontrol agent, disease management, growth promotion, PGPF**)

## INTRODUCTION

*Macrophomina phaseolina* (Tassi.) Goid. is an omnipresent fungus initiating a number of diseases in a wide range of host. The pathogen is versatile with three different forms namely *Rhizoctonia bataticola* as sclerotial stage, *Orbilia obscura* as teleomorph and *Macrophomina phaseolina* as pycnidial stage (De and Ghorai, 2017). Jute, the golden bast fibre crop is cultivated in the pre *kharif* season mainly in the eastern India in West Bengal, Bihar and Assam contributing 77, 17.1 and 5.5% of National production respectively (Anonymous, 2013). In jute, seed and soil borne pathogen *Macrophomina phaseolina* incites several diseases which are wide spread in nature. Disease is spread secondarily through pycnospores which are air-borne in nature. The facultative parasite attacks the host causing seedling blight, damping off, collar rot, stem rot and root rot at any part of the plant during any stage of growth from seedling till harvest (Roy *et al.,* 2008). However, stem and root rot are economically most important diseases of jute affecting both yield and quality of fibre and seed in both cultivated species, namely, *Corchorus olitorius* (L.) and *C. capsularis* (L.) in all the jute growing areas. Under the influence of conducive predisposing factors the mortality of plant may rise from 60-80% even with the root rot disease (Anonymous, 2006).

India is the largest producer of another bast fibre crop Sunnhemp (*Crotalaria juncea* L.), which is around 18800 tons annum$^{-1}$ followed by Bangladesh and Brazil. Sunnhemp is grown for fibre in almost all the Indian states while its cultivation for green manuring is largely confined to Andhra Pradesh, Gujarat, Maharashtra, Tamil Nadu, Madhya Pradesh, Rajasthan, Uttar Pradesh and for fodder in states of Andhra Pradesh and Maharashtra. The fibre is used for various purposes like making ropes, twines, floor mat, fishing nets, hand made paper, etc. in cottage industry. Apart from industrial values, the plant as a legume is advantageous to grow on poor, fallow or freshly reclaimed soil playing role as soil builder or renovator adding 50-60 kg N ha$^{-1}$ in the form of root nodules at about 85-90 days of crop age as well as deterrent to nematode. The apical part of the plant, after harvest, can also be incorporated in the soil,

1. Faculty, P.G. Dept. of Botany, Barasat Govt. College, Barasat, Kolkata 700124, West Bengal, India
2. Technical Assistant, Central Research Institute for Jute and Allied Fibres (ICAR), Nilganj, Barrackpore, Kolkata 700120, West Bengal, India.
3. Assoc. Professor, P.G. Dept. of Botany, Barasat Govt. College, Barasat, Kolkata 700124, West Bengal, India.

which would substantially improve soil fertility (Sarkar *et al*., 2015). In addition, it also improves the soil physical, microbiological and physico-chemical properties (Kamil *et al*.,2006). However, the crop suffers from serious biotic stresses by several bacterial, viral and fungal pathogen among which wilt disease caused by *Fusarium udum* (Bult) f.sp *crotalaria*e (Kulkarni) is economically most imperative. The crop losses more than 60% under favourable epidemiological condition (Bandopadhyay and Dasgupta, 1999).

Though chemical control is commonly practiced for *Macrophomina phaseolina* and *Fusarium udum* but the process are arduous, uneconomical, and risky for pollution of the environment, bringing about steady decline in soil and human health, productivity and resurgence of pesticide resistant strains of pathogen. In recent years much attention has been given on the antagonistic, hyper-parasitic as well as plant growth promoting fungi (PGPF) and plant growth promoting rhizobacteria (PGPR) for plant disease management and growth promotion finally leading to development of efficient microbial pesticide (Bandopadhy and Das, 2017). The principal mechanisms of plant growth promotion (PGP) by PGPRs and PGPFs include production of plant growth regulators (PGRs), solubilization and mobilization of phosphate, and lowering ethylene levels in root cells through ACC deaminase activity. PGPRs and PGPFs suppress plant pathogen through antibiosis, i.e. production of fungi toxic compounds like volatile or non volatile antibiotics, cell wall degrading enzymes (CWDE) and HCN, competition with pathogenic microorganisms for nutrients through siderophore production or niche exclusion (Hariprasad *et al*., 2014).

PGPRs and PGPFs produce siderophores which play active role in suppressing soil borne fungal pathogen causing iron deficiency in the vicinity whereas plant growth promotion takes place as iron is also supplied for nutrition to the plant. Besides iron, siderophore complex with magnesium, zinc, copper, and cobalt is essential for plant nutrition in macro or micro levels. Fluorescent pseudomonads enhance plant growth and confront deleterious microorganisms in soil producing pyoverdine and pseudobactin type of siderophore which are antibiotic in nature (Hoegy *et al.,* 2014). Siderophores also play role in bioremediation of soil polluted with heavy metals like cadmium, lead, nickel, arsenic (III, V), aluminium, strontium, geranium, valadium and other radionuclides (Gaonkar and Borkar, 2017) .

In the present study eight fungal antagonist or bio-control agents (BCAs) were chosen. They are potent in antagonistic activity against highly virulent strain of *Macrophomina phaseolina* (Tassi.) Goid. (R9) inciting stem and root rot disease complex in jute (both *Corchorus olitorius* L. and *C capsularis* L.) (Bandopadhyay *et al*., 2006) together with seedling and leaf blight in sunnhemp (*Crotalaria juncea* L.). The selected fungal isolates are also antagonistic to *Fusarium udum* Butlar f.sp. *crotalariae* (Kulkarni) Subramanian comb. Nov isolate F5 causing vascular wilt in sunnhemp. These isolates were screened for biocontrol and plant growth promoting trait including siderophore production and chemical characterization as PGPFs. The isolates were finally applied *in vivo* for disease control and growth enhancement in natural environment as well as bioremediation of the environment.

# MATERIALS AND METHODS

**Selection of potential fungal antagonist**

Out of 75 fungal isolates isolated from rhizosphere and rhizoplane of jute at multilocation from cultivated lands, *Trichoderma viride* Pers. Ex S.F. Gray. JPT1 (ITCC-1433) and JPT9 (ITCC-5994.05), *Gliocladium virens* (Miller, 1959) von Arx JPG1 (ITCC-4177), *Gliocladium sp* JPG4, *Aspergillus niger* Van Tieghem. AN15, A26 and AN27, and *Aspergillus fumigatus* Fries. A7 (ITCC-5993.05) were selected on the basis of their antagonistic potentiality against pathogens R9 and F5 *in vitro* by dual culture method, and production of volatile and nonvolatile compounds (Bandopadhyay *et al.*, 2008). *Rhizobium japonicum* kirchnar strain Rjc2 was isolated from fresh root nodule of healthy sunnhemp plant. Different strains of *Macrophomina phaseolina* were screened out for pathogenecity test and strain R9 was found to be highly virulent. The fungal BCAs were identified and indexed by Indian Type Culture Collection Centre, IARI, New Delhi.

**Evaluation of fungal antagonists for bio-control and plant growth promoting traits as PGPFs**

Quantitative assay for production of extracellular enzymes chitinase *(EC 3.2.1.14),* â-1-4- endoglucanase *(EC 3.2.1.4),* â-1, 3-glucanase *(EC 3.2.1.39)* and protease (*EC 3.4.21.40)* was performed for the selected fungal bioagents. Enzymatic hydrolysis of carboxy methyl cellulose (CMC) and laminarin was assayed by dinitrosalicylic acid method (Miller , 1959) with slight modification. Enzymatic hydrolysis of chitin by BCAs was assayed (Ohtakara, 1988). Protease activity was estimated according to Mahadevan and Sridhar (1982) with slight modification. Production of IAA in BCAs was estimated (Gaur *et al.,* 2001). Phosphate solubilization by the fungal isolates were also assayed (Jackson, 1973).

For screening of siderophore production by the PGPFs Czapek Dox medium without iron constituent, was selected as Low Iron Medium (Mondal and Sen, 1999) Screening for siderophore production were done following $FeCl_3$ Test, Chrome azurol sulphonate (CAS) Assay and by CAS Agar Plate Test with slight modifications (Schwyn and Neilands, 1987). The amount of extracellular siderophore produced in the metabolites were calculated as % siderophore (Payne, 1994). Hydroxamate nature of siderophore was detected following the methods of Neiland's spectrophotometric assay and Tetrazolium salt test. Catecholate and carboxylate nature of siderophores were tested following Arnow test and Vogel's chemical test respectively. Absorbance maxima of ferrate hydroxamate

siderophores which is pH dependent was measured to classify ferric complexes of mono, di, and trihydroxamate types. Cell free supernatant of fungal metabolite producing hydroxamate siderophore was examined for binding properties, i.e. number of bonds the ligand formed with metal ions, by observing the stable/unstable nature of color of ferrate siderophores at different pH (Dave *et al.*, 2006).

**Evaluation of potential PGPFs for disease suppression and growth promotion in jute and sunnhemp under field condition**

All the selected siderophore producing PGPFs were evaluated in pot trial for disease suppression and growth promotion of the selected crop plants under controlled green house condition (Bandopadhyay and Das, 2017). Experiment was conducted to evaluate the siderophore producing fungal bioagents singly on jute (c.v. JRO 524) and in combination with *Rhizobium* on sunnhemp (c.v. K12 yellow) in field conditions for three years (2009-2012). Seven and thirteen treatments with 3 replications in each in micro-plots (size 2.5x1.2 m = 3 $m^2$) were designed in RBD with jute and sunnhemp crop respectively. During land preparation urea and single super phosphate @ 40:20 kg $ha^{-1}$ (recommended dose) was applied as basal dose. Fertilizer amended and non-amended plots were considered as standard practice and control plots respectively. Plots were made sick with 200 g of R9 or F5 inoculums prepared in old seed medium. The selected fungal antagonist *Gliocladium* sp. (JPG4), *Aspergillus fumigatus* A7, *Aspergillus niger* (AN15, A26 and AN27) were grown in 90 mm PDA plates for 10 days at 25 ± 1° C. *Rhizobium japonicum* (Rjc-2) were grown in YEM medium for 72 hours at 30 ± 1°C in incubator shaker at 150 rpm. Seed inoculation was done by mixing 100 g seed with the spores of fungal BCA @$10x10^7$ cfu $g^{-1}$ or 50 ml slurry of *Rhizobium* containing $25x10^7$ cfu $ml^{-1}$ inoculum. Seeds were pelleted with 2.5 g each of Carboxy- methyl cellulose sodium salt (CMC) and molasses for better adherence as well as nutrient supplement, air dried in shade before showing in lines. Disease count were taken at 15 days interval till harvest after 100 days and index of disease incidence calculated by using the formula:

Per cent disease incidence = (Number of infected plants incidence / Total number of plants observed) x 100

Number and dry weight of nodule in sunnhemp, plant biomass dry weight, fibre weight of single plants were recorded from 10 plants in each replication (total 30 plants per treatment) including control for both the crops. Total fresh weight and fibre yield were calculated from the total harvested plants per treatment of both the crops.

**Statistical Analysis**

Each data represented were means of three replicates. The data were analysed statistically by ANOVA. Mean values were compared at significance levels of 1% and 5%. Other data were analysed using Microsoft office excel software 2007.

# RESULTS AND DISCUSSION

**Enzymatic activity of PGPFs**

Quantitative assay for production of cell wall degrading enzymes *viz*. chitinase, â-1-4- endoglucanase, â-1, 3-glucanase and protease performed for the selected fungal bioagents revealed higher chitinase activity in *Aspergillus* isolates, highest being in A26 (412.5 CU) followed by AN15 (244.84 CU) and A7 (169.94 CU). *Trichoderma* JPT1 also exhibited 102.3 CU chitinase activity. *Trichoderma* and *Gliocladium* showed higher protease activity. JPT1 released higher aliphatic amino acid (0.920 units) followed by JPG4 (0.777 units), AN27 (0.486 units). JPG1 released higher aromatic amino acids (0.299 units). JPT1 showed highest â-1, 3 glucanase activity (1080.5 GU) followed by JPT9 (923.45 GU). JPT1 also showed highest â-1, 4-endoglucanase activity (45.34 GU) (Table 1). The production of cell wall degrading enzymes by the PGPFs show their antagonistic activity by biochemical breakdown of pathogen cell wall producing elicitor molecules leading to production of phytoalexin and induce systemic resistance in host plants. Chet in 1987 mentioned the production of CWDE by *Trichoderma* and there role as biocontrol agent against soil borne plant pathogen. Srivastava *et al.* (2014) described the production of CWDE by *Trichoderma* and presence of biocotrol genes playing role in processes of biological control against pathogen and other genes providing resistance to several biotic and abiotic stresses.

**Indole Acetic Acid (IAA) production by PGPFs**

Production of IAA is one of the vital tool of PGPF to enhance growth in plants by cell proliferation and elongation. Estimation of IAA production by the selected PGPFs exposed higher production in JPG1 (69.53 ì g $ml^{-1}$) followed by JPT1 (50.52 ì g $ml^{-1}$) and JPG4 (48.02 ì g $ml^{-1}$). *Aspergillus* isolates did not produce IAA except AN27 (2.06 ì g $ml^{-1}$) (Table 2). Likewise, Jogaiah *et al.* (2013) reported that out of seventy-nine plant growth-promoting fungi (PGPFs) isolated from rhizospheric soil, nine revealed production of IAA along with root colonization and plant growth promotion.

**Phosphate solubilization by PGPFs**

Phosphate solubilization capability of the PGPFs were found to be significantly high. The investigation reveals maximum phosphate solubilization from Tri-Calcium Phosphate (TCP) in Czapeck Dox medium to monophosphates was by JPT9 (0.992 mg phosphorus $ml^{-1}$ of medium) followed by A 26 and AN15 with 0.953 and 0. 906 mg phosphorus $ml^{-1}$ of medium. JPG1 did not solubilised TCP (Table 2). This implies the ability of the fungal isolates to reduce phosphate and its potential use as plant growth promoter in agriculture. Similarly, Panchal *et al.* (2015) reported solubilization of Tris minimal rock phosphate (TRP) broth by PGPF *Penicillium expansum* varied from 49.57ì $Mml^{-1}$ to 634.89 ì $Mml^{-1}$ at 48 hrs to 192 hrs incubation respectively with change in pH.

**Siderophore production by PGPFs**

Five isolates viz., JPG4, A7, AN15, A26 and AN27 were identified to produce siderophore as evident from $FeCl_3$ test, CAS Assay and CAS Agar plate Assay. Siderophore from A26 and AN15 were thermally stable up to 121° C. Amount of siderophore production was maximum in AN27 up to 81.81 siderophore units % followed by A26 79.26 units %. (Figure 1). Chemical nature of siderophore was studied. Tests to analyze specific nature of siderophore suggested all the five fungal isolates to produce hydroxamate type of siderophore as evident by positive Tetrazolium salt test and Neiland's spectrophotometric assay. $ë_{max}$ ranged between 422 nm - 438 nm. Isolates JPG4, A26 and AN27 produced catecholate type of siderophore. A26 exhibited maximum absorbance at 510 nm. AN15 and A26 produced carboxylate type of siderophore revealed by positive Vogel's test. With change in pH of growth medium from 4-7, a shift in $ë_{max}$ (3-7 nm) in AN15 and JPG4 indicated trihydroxamate nature of siderophore. While wide shift in $ë_{max}$ (13-33 nm) in A7, A26 and AN27 indicated the presence of dihydroxamate type. Ligand property of hydroxamate siderophores produced by the test fungi were assayed. When number of hydroxamic moieties and ligands were correlated, hydroxamate siderophores irrespective of di or tri-hydroxamate types found to produce hexadentate ligands (Data not shown). In the current study, *Trichoderma* isolates did not produce siderophore although *Trichoderma* is known to produce siderophores as a biocontrol tool (Hariprasad *et al*., 2013). Production of siderophores by the BCAs varied qualitatively as well as quantitatively. *Aspergillus* A26 showed maximum siderophore chelating activity as compared to *Aspergillus niger* AN27 referred as a potent biocontrol agent (Mondal and Sen, 1999). Selected BCAs can be used in controlling the soil borne pathogens due to fungistatic activity of siderophore (Bandopadhyay *et al.*, 2006). The biocontrol activity of siderophore fungistasis is responsive by sequestering iron (III) in iron limiting condition making it unavailable to the pathogen (Flores-Felix *et al.*, 2015). The pathogen apparently lack iron assimilation system for these ferric siderophores from the selected BCA strains. Infested *Macrophomina* or *Fusarium* conducive soil can be converted into pathogen suppressive soil by application of these BCAs. Siderophore have ability to bind a variety of other metals apart from iron thus enhancing plant growth by supplying other macro and micro nutrients viz., Mg, Zn, Cu, Co and other nuclides. Fluorescent pseudomonads, Bacilli and other plant growth promoting Rhizobacteria (PGPR) (Bhattacharyya and Jha, 2012; Verma *et al*., 2016) enhance plant growth as well as confront deleterious microorganisms in soil producing yellow green pyoverdine, pseudobactin and other types of siderophore which are antibiotic in nature.

In soils, the microbial communities colonising mineral surfaces differ from the inhabitants of the surrounding soil (Certini *et al.*, 2004). Microbial attachment to mineral surfaces leads to the formation of a microenvironment protecting the microorganisms against environmental stresses (Ojeda *et al*., 2006). In these microenvironments, mineral nutrients can be chelated directly from the soil minerals or shared amongst the surrounding microorganisms by production of siderophores. Siderophores promote the dissolution of insoluble phases mineral to soluble form (Shirvani and Nourbakhsh, 2010). Siderophores form Fe (III)–siderophore complex at the mineral surface and is then transferred into the surrounding soil solution and becomes available for uptake by microorganisms or plants (Ahmed and Holmström, 2014). The formation of Fe(III)–siderophore complexes are affected by pH because of the competition for the free siderophore ligands between free protons and Fe. In nature, Fe has to compete not only against free protons for the siderophore binding sites but also against other metal ions such as divalent cations, including $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ (Albrecht-Gary and Crumbliss, 1998) trivalent cations, such as $Mn^{3+}$, $Co^{3+}$ and $Al^{3+}$; and actinides, such as $Th^{4+}$, $U^{4+}$ and $Pu^{4+}$ (Peterson *et al*., 2004). There are several studies that have shown that siderophores have an impact on the mobility of these metal ions in the environment (Ahmed and Holmström, 2014). Thus, it can be suggested that phosphate solubilization, production of IAA, and other related compounds like siderophore by the fungal agents will interact with plants as part of its colonization, leading to growth promotion, induced resistance, and modification of basal plant defence mechanisms (Hossain *et al*, 2017).

Property of siderophore for chelating other heavy metals like arsenic may be useful in bioremediation of soil. District of North 24 Pargana of West Bengal, which is a major jute growing belt, is reported to have considerably high arsenic contamination in soil as well as in ground water (Pyne and Santra, 2016). Selected fungal BCAs could be harnessed for decontamination of arsenic and other heavy metals in these agriculturally important areas to obtain a healthy environment for the farmer community. Thus, in this context, the siderophores produced by the selected PGPFs can function as biocontrol, bioremediation and chelation agents, in addition to their important role in enhancing plant growth.

**Evaluation of selected PGPFs for disease suppression and plant growth promotion in jute and sunnhemp under field condition**

Field evaluation for three years of selected siderophore producing PGPFs on jute crop exposed that stem and root rot disease incidence in jute was significantly reduced (*P=0.05*) by *Aspergillus niger* A26 up to 39.5%. *A. fumigatus* A7 controlled disease up to 20.9% only. A26 increased plant biomass by 76.16% and fibre yield increased by 6.27% followed by *A. niger* AN27 47.81% and 2.44% respectively. Among 7 treatments highest fibre yield was envisaged by *Gliocladium* JPG4 31 $qha^{-1}$ (Table 3).

In sunnhemp, the bioagents when applied singly and in combination with *Rhizobium* for three years revealed encouraging results (Table 4). *A. niger* AN27 co-inoculated with *Rhizobium* Rjc2 significantly (*P=0.05*) controlled *Fusarium* wilt incidence by 50.3%, while increased root nodulation and plant biomass by 60.9% and 61.2%

respectively. AN27 and Rjc2+JPG4 also significantly increased plant biomass by 54.1% and 47.9% respectively. Maximum fibre production was envisaged by Rjc2+AN27 combination 8.8 $qha^{-1}$ i.e. 37.5% yield increase.

Application of these siderophore producing BCAs reduced stem and root rot incidence in jute and seedling blight, leaf blight as well as vascular wilt in sunnhemp enhancing plant growth and fibre production in both the crops. The reduction of disease incidence caused by pathogens may be due to ability of the fungal isolates to inhibit growth of the pathogen in soil by direct antagonism, production of mycolytic cell wall degrading enzymes, volatile and non volatile secondary metabolites, and siderophores. The fungal biocontrol agents are found to produce hormone and solubilize phosphorus (TCP) which can be correlated with enhanced plant growth and fibre yield in jute and sunnhemp crops in the fields. Application of biofertilizer *Rhizobium* Rjc2 in combination with PGPFs enhanced growth of leguminous sunnhemp by nitrogen fixation. The PGPFs could be utilized as phosphate solubilizer for remediation of agricultural fields and biofertilizer to the crop thus enhancing plant growth. The fact that certain soil microbes are capable of dissolving relatively insoluble phosphate compounds has made the possibility of inducing microbial solubilisation of phosphates in soil (Ghosh *et al.*, 2016). Some PGPF or PGPR biofertilizers also influence the availability of phosphate by secreting phosphatases for mineralization of organic phosphates. Sufficient densities of these biofertilizing and antagonistic microbes may create a proper rhizosphere for plant growth, convert nutritionally important elements through biological processes thus increasing the availability of N, P, K, as well as inhibiting pathogen growth. Consequently, high availability of N, P and K can enhance soil fertility, improve bio- control effects of antagonist isolates and extend microorganisms' survival rates in the soil (Yang *et al.*, 2011). Thus the PGPFs in the present study have effective growth promoting capacity which could be applied to the rhizospheric soil of crop plant to increase growth and yield. Beside nitrogen fixation, many workers have reported production of plant growth regulating substances (PGR) viz. IAA, cytokinins and ethylene in the rhizosphere by bacterial biofertilizing agents like *Azotobacter, Azospirillum* and *Rhizobium*, and have considered their physiological action in altering plant growth and development. PGPR as well as some PGPFs synthesizes peroxidase, catalase, phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PO) which can induce systemic resistance (ISR) in host plant (Kloepper *et al.*, 2004). The biocontrol and plant growth promoting potential of the organisms tested might have been possible due to simultaneous action of all the components like competition, parasitism, lysis and antibiosis.

Therefore, in the present perspective, it may be concluded that utilization of plant growth promoting fungi like *Trichoderma viride* JPT1, *Gliocladium virens* JPG1, *Aspergillus niger* AN15, A26, AN27 and *Aspergillus fumigatus* A7 along with *Rhizobium* broadens the spectrum of antagonistic and plant growth promoting microbes available for field application in agriculture. These PGPFs can also be harnessed for bioremediation of toxic metals like arsenic in soil , particularly in highly aresenic contaminated areas of North 24 Parganas in West Bengal. The potential BCAs can be utilized in formulation of microbial biopesticide singly and in consortium for use as mixture with chemical pesticide for economically sustainable disease management and plant growth in jute, sunnhemp and allied fibres as also other crops with strategy of an appropriate integrated management system (AIMS).

**Table 1. Estimation of cell wall degrading enzymes (CWDE) produced by fungal BCAs**

| Fungal BCAs | â-1,3 glucanase (GU) | â-1,4-Endo-glucanase (GU)* | Chitinase (CU)* | Protease (mg $min^{-1}$ $mg^{-1}$ of protein) | |
|---|---|---|---|---|---|
| | | | | Aliphatic amino acid | Aromatic amino acid |
| JPT-1 | 1080.52±1.96 | 45.34±5.89 | 102.30±1.80 | 0.920±0.002 | 0.084±0.002 |
| JPT-9 | 923.45±2.22 | 33.53±4.32 | 15.59±3.36 | 0.354±0.002 | 0.127±0.003 |
| JPG-1 | 257.64±2.52 | 13.33±2.76 | 26.23±1.72 | 0.177±0.003 | 0.299±0.002 |
| JPG-4 | 871.23±1.8 | 37.20±1.94 | 7.60±1.20 | 0.777±0.002 | 0.061±0.003 |
| AN-27 | 512.17±1.41 | 32.21±3.85 | 98.50±4.30 | 0.486±0.003 | 0.138±0.002 |
| A-26 | 210.20±1.88 | 29.21±2.04 | 412.49±2.13 | — | 0.123±0.002 |
| AN-15 | 97.41±1.63 | 20.65±5.07 | 244.84±2.71 | 0.128±0.002 | 0.268±0.003 |
| A-7 | 50.10±2.26 | 22.15±2.70 | 169.94±3.69 | 0.153±0.002 | 0.124±0.002 |

*1 GUunit= release of 1 ì mol glucose $ml^{-1}$ of culture filtrate $min^{-1}$

*1 CU unit = release of 1 ì mol N-acetylglucasamine $ml^{-1}$ of culture filtrate $min^{-1}$

Data are represented as mean ± SD

**Table 2. Estimation of IAA production and solubilization of phosphate by fungal BCAs**

| Fungal BCAs | IAA production (ì g $ml^{-1}$) | mg $Pml^{-1}$ of broth culture |
|---|---|---|
| JPT1 | 50.52±2.29 | 0.898±0.302 |
| JPT9 | 30.05±0.93 | 0.992±0.304 |
| JPG1 | 69.53±1.32 | — |
| JPG4 | 48.02±1.09 | 0.821±0.302 |
| AN27 | 2.06±0.06 | 0.871±0.304 |
| A26 | — | 0.953±0.302 |
| AN15 | — | 0.906±0.304 |
| A7 | — | 0.781±0.281 |

Data are represented as mean ± SD

**Table 3. Effect of siderophore producing PGPFs on disease incidence, plant growth and fibre yield in jute under field conditions***

| Bio-agents | Disease incidence (%) | Disease control (%) | Biomass dry wt.$pl^{-1}$ (g) | Biomass increase (%) | Fibre Yield q $ha^{-1}$ | Yield increase (%) |
|---|---|---|---|---|---|---|
| JPG4 | 13.60 | 16.05 | 61.30 | 23.83 | 31.00 | 8.01 |
| A7 | 12.80 | 20.90 | 53.25 | 7.57 | 29.20 | 1.74 |
| AN15 | 14.20 | 12.35 | 64.57 | 30.44 | 29.13 | 1.50 |
| A26 | 9.80 | 39.50 | 87.20 | 76.16 | 30.50 | 6.27 |
| AN27 | 13.00 | 19.75 | 73.17 | 47.81 | 29.40 | 2.44 |
| $N_{40}$ $P_{20}$ $K_0$ | 15.10 | 6.79 | 51.75 | 4.54 | 29.00 | 1.05 |
| Control | 16.20 | — | 49.50 | — | 28.70 | — |
| SE (m)± | 0.638 | | 2.976 | | 0.782 | |
| CD 5% | 1.924 | | 8.915 | | 2.343 | |

*Average data of three years' field trial

**Table 4. Effect of siderophore producing PGPFs on disease incidence, nodulation, plant growth and fibre yield in sunnhemp under field conditions***

| Treatments | Wilt incidence % | Wilt control % | Nodule dry wt mg.pl$^{-1}$ | Nodule increase % | Plant dry wt.pl.$^{-1}$ (g) | Biomass increase % | Fibre yield q ha$^{-1}$ | Yield increase % |
|---|---|---|---|---|---|---|---|---|
| JPG4 | 12.50 | 13.70 | 45.00 | 9.70 | 9.60 | 25.70 | 6.90 | 7.80 |
| A7 | 11.40 | 21.30 | 42.00 | 2.40 | 8.90 | 17.10 | 6.60 | 3.10 |
| AN15 | 12.40 | 14.50 | 43.00 | 4.90 | 8.10 | 6.50 | 6.70 | 4.70 |
| A26 | 10.30 | 28.90 | 44.00 | 7.30 | 9.30 | 22.40 | 6.80 | 6.25 |
| AN27 | 9.60 | 33.70 | 52.00 | 26.80 | 12.80 | 54.10 | 7.80 | 21.80 |
| Rjc2+JPG4 | 10.70 | 27.05 | 58.00 | 41.40 | 11.30 | 47.90 | 8.00 | 25.00 |
| Rjc2+A7 | 12.40 | 14.50 | 46.00 | 4.90 | 9.70 | 27.60 | 7.10 | 10.90 |
| Rjc2+AN15 | 11.60 | 20.00 | 45.00 | 9.70 | 9.60 | 25.60 | 7.20 | 12.50 |
| Rjc2+A26 | 11.40 | 21.30 | 48.00 | 17.00 | 10.10 | 32.90 | 7.40 | 15.60 |
| Rjc2+AN27 | 7.20 | 50.34 | 66.00 | 60.90 | 12.30 | 61.20 | 8.80 | 37.50 |
| Rjc2 | 12.80 | 11.70 | 49.00 | 19.50 | 9.10 | 19.70 | 6.60 | 3.50 |
| NPK | 13.50 | 6.70 | 43.00 | 4.90 | 8.90 | 17.10 | 6.50 | 1.50 |
| Control | 14.50 | — | 41.00 | — | 7.60 | — | 6.40 | — |
| SE (m)± | 0.466 | | 1.301 | | 0.375 | | 0.239 | |
| CD 5% | 1.389 | | 3.893 | | 1.115 | | 0.707 | |

*Average data of three years' field trial

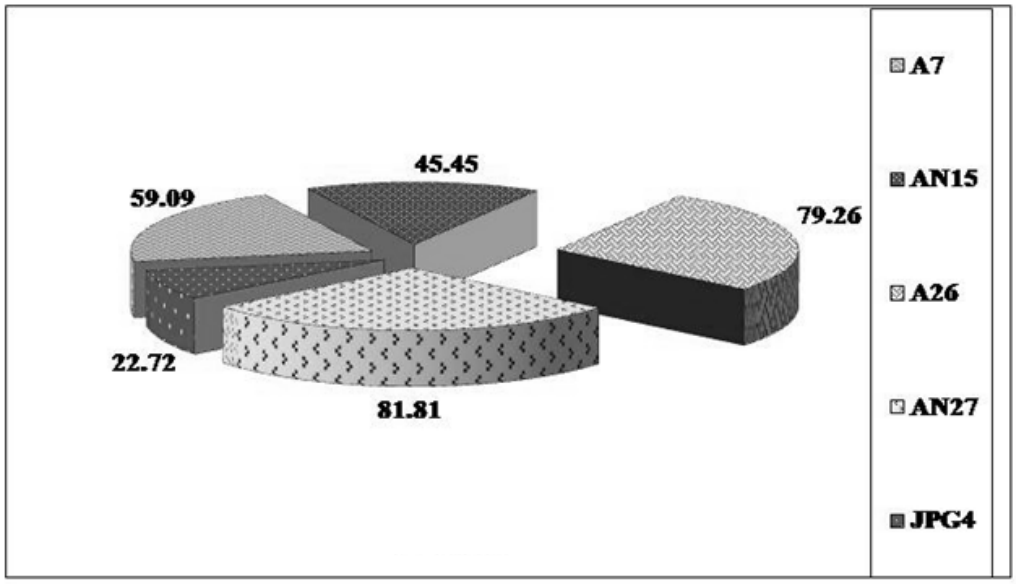


Figure1. Quantification of siderophores in fungal BCAs . Siderophore unit in %.

## REFERENCES


Ahmed, E. and S.J.M. Holmström, 2014. Siderophores in environmental research: roles and applications. Microbial. Biotechnol. **7**(3):196-208 doi:10.1111/1751-7915.121.

Albrecht-Gary, A.M. and A.L. Crumbliss, 1998. Coordination chemistry of siderophores: thermodynamics and kinetics of iron chelation and release. Met. Ions Biol. Syst. **35:**239–327.

Anonymous, 2006. Central Research institute for jute and allied fibres. Annual Reports. pp. 50-51.

Anonymous, 2013. Indian jute. National Jute Board, 3A &3B Park Plaza, 71 Park Street, Ministry of Textiles, Government of India, Kolkata, India. XXV: 2, www.jute.com.

Bandopadhyay, A.K. and M.K. Dasgupta, 1999. Management of vascular wilt disease in sunnhemp by organic amendment. Mode of action of neem cake and strategy of technology developed for wilt control. Proceeding of symposium on: Pest management in changing agriculture situation. PSB, Sriniketan, Viswa Bharati. pp 156-159.

Bandopadhyay, A., A.K. Bandopadhyay, A. Majumdar and N. Samajpati, 2006. Evaluation of antagonistic potential of some rhizosphere fungi and PGPR against

*Macrophomina phaseolina* inciting disease complex in jute. J. Basic Appl. Mycol. **5** (1& II): 82-86.

Bandopadhyay, A., A.K.Bandopadhyay and N. Samajpati, 2008. *In vitro* antifungal activity of volatile and non-volatile components from some biocontrol fungi against jute pathogen *Macrophomina phaseolina*. Ind. Phytopath. **61** (2): 204-211.

Bandopadhyay, A.K., A. Bandopadhyay and A. Majumdar, 2006. Screening and characterization of antagonistic potential of some rhizosphere fungi and PGPR against *Macrophomina phaseolina* in jute. J. Mycopathological Res. **44** (2): 323-330.

Bandopadhyay, A. and N. Das, 2017. Plant growth promoting microbial consortial formulations mediated biological control of stem and root rot disease of Jute caused by *Macrophomina phaseolina* ((Tassi.) Goid. Int. J. Curr. Sci. **20**(1): E1-5.

Bhattacharyya, P.N. and D.K. Jha, 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol. **28**: 1327–1350.

Certini, G., C.D. Campbell and A.C. Edwards, 2004. Rock fragments in soil support a different microbial community from the fine earth. Soil Biol. Biochem. **36**: 1119–1128.

Chet, I. 1987. *Trichoderma*- Application, mode of action, and potential as a biocontrol agent of soil borne pathogenic fungi. In: . I.Chet (ed.) Innovative Approaches to Plant Disease Control. John Wiley & Sons, New York. pp 137-160.

Dave, B.P., K. Anshuman and P. Hajela, 2006. Siderophores of halophilic archaea and their chemical characterization. Ind. J. Exp. Biol. **44**: 340-344.

De, R. and A.K. Ghorai, 2017. Bleaching powder [$Ca(OCl)_2$], a new option for management of stem rot of jute (*Corchorus olitorius* L. and *C. capsularis* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. J. Mycopathol Res. **55**(1):17-22.

Flores-Felix, J.D., L.R. Silva, L.P. Rivera, M. Marcos-García, P. García-Fraile, E. M.Molina, P. F. Mateos, E. Velázquez, P. Andrade and R. Rivas, 2015. Plants probiotics as a tool to produce highly functional fruits: The case of *Phyllobacterium* and vitamin C in strawberries. Plos One. **10** (4): e0122281. doi: 10.1371/journal.pone.0122281

Gaonkar, T. and S. Borkar, 2017. Applications of siderophore producing marine bacteria. In: Naik M and Dubey S (eds), Bioremediation of metals and organic compounds. Marine Pollution and Microbial Remediation, Springer, Singapore https://doi.org/10.1007/978- 981-10-1044-6_11.

Gaur, R., N. Shani, Kawaljeet, B.N. Johri, P. Rossi and M. Aragno, 2004. Diacetylphloroglucinol producing Pseudomonads do not influence AM fungi in wheat rhizosphere. Curr. Sci. **86** (3): 453- 457.

Ghosh, R., S. Barman, R. Mukherjee and N.C. Mandal, 2016. Role of phosphate solubilizing *Burkholderia* spp. for successful colonization and growth promotion of *Lycopodium cernuum* L. (Lycopodiaceae) in lateritic belt of Birbhum district of West Bengal, India. Microbiol. Res. **183**: 80-91.

Hariprasad, P., S. Chandrashekar , S.B. Singh and S.R. Niranjana, 2014. Mechanisms of plant growth promotion and disease suppression by *Pseudomonas aeruginosa* strain 2apa. J. Basic Microbiol. **54**(8):792-801. doi: 10.1002/jobm.201200491.

Hoegy, F., G.L. Mislin and I.J. Schalk, 2014. *Pyoverdine and pyochelin measurements*. Methods Mol. Biol. **1149**: 293–301. doi: 10.1007/978-1-4939-0473-0_24.

Hossain M.M., F. Sultana, F, S. Islam, 2017. Plant Growth-Promoting Fungi (PGPF): Phytostimulation and Induced Systemic Resistance. In: Singh D., Singh H., Prabha R. (eds) Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer, Singapore

Jackson, M.L. 1973. Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi. pp. 134.

Jogaiah,S., M. Abdelrahman, L. P. Tran and I. Shin-ichi, 2013. Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. J. Exp. Bot. **64**(12): 3829–3842. doi:10.1093/jxb/ert212 10.1093/jxb/ert212.

Kamil, D., R. Kumar and A. Sinha, 2009. Effect of green manuring of *Crotalaria juncea* on some soil borne pathogens. Indian Phytopath. **62**(3): 304-309.

Kloepper, J.W., C.M. Ryu and S. Zhang, 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* sp. Phytopathol. **94**: 1259-1266.

Mahadevan ,A. and R. Sridhar, 1982. Measurement of protease activity. Methods in Physiol. Plant Pathol. (Sivakami Publication), **2**: 105.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagents for the determination of reducing sugars. Anal. Chem. **31** (3): 426-428.

Mondal, G. and B. Sen, 1999. Siderophore production by *Aspergillus niger* AN27-A biocontrol agent. Curr. Sci. **77** (3): 337-339.

Ohtakara A. 1988. Chitinase and acetylhexosaminidase from *Pycnoporus cinnabarainus*. Method Enzymol, **168**: 467-468.

Ojeda, L., G. Keller, U. Mühlenhoff, J.C. Rutherford, R. Lill and D. Winge, 2006. Role of glutaredoxin-3 and glutaredoxin-4 in the iron regulation of the Aft1 transcriptional activator in *Saccharomyces cerevisiae*. J. Biol. Chem. **281**: 17661–17669.

Panchal. B. J., S. Patel, Rajkumar, S. Jha, L. Mahatma and D. Singh, 2015. Isolation and identification of phosphate solubilizing *Penicillium expansum* NAUG-B1 and their consequence on growth of brinjal. Eco. Env. & Cons. **21**: S259-S267.

Payne, S.M. 1994. Detection, isolation and characterization of siderophores. Methods in Enzymology (Academic Press, USA.). **235**: 329.

Peterson, R.L., H.B. Massicotte and L.H. Melville, 2004. Mycorrhizas: Anatomy and Cell Biology. Ottawa, Canada: NRC Research Press.

Pyne, S. and S. C. Santra, 2016. Arsenic toxicity through contaminated vegetables in selected blocks of Murshidabad and N-24 Pargana district, West Bengal, India. Advn. Appl. Sci. Res. **7**(4):168-175.

Roy, A., R.K. De and S.K. Ghosh, 2008. Diseases of bast fibre crops and their management. *In* Jute and allied fibres updates, production and technology. Central Research Institute for Jute and Allied Fibres, (Indian Council for Agricultural Research), Barrackpore, Kolkata 700120, India. pp.217-241.

Sarkar, S.K., S.K. Hazra, H.S. Sen, P.G. Karmakar and M.K. Tripathi, 2015. Sunnhemp in India. ICAR-Central Research Institute for Jute and Allied Fibres (ICAR), Barrackpore, West Bengal. pp.140.

Schwyn, B. and J.B. Neilands, 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. **160**: 47–56.

Shirvani, M. and F. Nourbakhsh, 2010. Desferrioxamine-B adsorption to and iron dissolution from palygorskite and sepiolite. Appl. Clay Sci. **48:** 393–397.

Srivastava, M., M. Shahid, S. Pandey, A. Singh, V. Kumar, S. Gupta and M. Maurya, 2014. *Trichoderma* Genome to Genomics: A Review. J. Data Mining Genomics Proteomics. **5**:162 doi: 10.4172/2153-0602.1000162.

Verma, P., A.N. Yadav, K.S. Khannam, S. Kumar, A.K. Saxena and A. Suman, 2016. Molecular diversity and multifarious plant growth promoting attributes of bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. J. Basic Microbiol. **56**: 44–58.

Yang, X., L.Chen, X. Yong and Q. Shen, 2011. Formulations can effect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against *Fusarium* wilt of cucumbers. Naturl. Biol. Fertil. Soils. **47**: 239-248.

**Rec. on 01.07.2019 & Acc. on 20.07.2019**