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# *In vitro* screening of bioagents from rhizosphere soil against *Macrophomina phaseolina* and seedling health of jute

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Jute seeds were primed with two bacteria and one fungal isolates using charcoal and soil based bioformulation with fungicide like carbendazim (Bavistin), growth regulator (IAA 25 ppm) to evaluate the plant growth and over all biomass of the crop. The bioagent as PGPB ($S_3$), one PGPR ($S_7$) and fungal antagonist ($S_{12}$) were used to see the potentialities in terms of improvement in per cent germination of seeds, vigour index and seedling biomass of jute.

Present study also aimed to see the antagonistic potential in dual cultures, formation of volatile components (HCN), siderophore and indole production of selected isolates. The results suggested that irrespective of nature of biocontrol agents (may be bacterial or fungal), an enhancement in the seed germination, vigour index and seedling biomass was noticed in pot culture of jute after challenge inoculation with crop pathogen *Macrophomina phaseolina.* Among the forms of inoculants, the activated charcoal based seed dressing with respective bioagent ($S_7$) singly followed by Carboxy Methyl Cellulose (CMC) based soil formulation 15 days after sowing in and around the root zone/collar region gave better responses with respect to enhanced germination of seeds and increasing seed vigour index.

The results on the biopriming revealed that the isolate $S_7$ is the most efficient isolate with respect to its potentiality in enhancing the percent germination, vigour index and seedling biomass of the crop. The isolate $S_3$ was found intermediate effect with respect to their potential in different parameter, where as $S_{12}$ are equally effective in inducing the seedling vigour and inferior in respect to biomass or seedling germination. All isolates of bio-agents significantly suppressed the incidence of seedling disease or damping off even after challenge inoculation with the pathogen ($S_{15}$).

**Key words:** Carboxy Methyl Cellulose, HCN, jute, *Macrophomina phaseolina,* plant growth promoting bacteria, rhizobacteria, vigour

## INTRODUCTION

Seed treatment with bioagents for protection of seeds and control of seed borne pathogens or diseases offers the growers/farmers an alternative means of chemical fungicides. The biological seed treatment can be highly effective, it must be recognized that they differ from chemical seed treatment by their utilization of living microorganisms. In the scenario of modern agriculture indiscriminate use of chemicals imparts hazardous effect on soil-microbe-ecological balance, lysis of beneficial organism, resurgence of pesticide resistant mutant of pathogens and also the environmental pollution. Some biocontrol agents applied as seed treatment are capable of colonizing the rhizosphere potentiality providing benefits to the plants beyond the emergence stage of the seedlings (Callan *et*

*al.*, 1997). Therefore, natural control through conservation and manipulation of biological agents is by far more important in the niche of biodiversity and inclusion of all these components in the integrated disease management (IDM) is ultimate practical approach for cost effective sustainable agriculture. Use of superior strains with high antagonistic potential to stem/root rot pathogen and lesion spread on foliage is a vital step for devising effective biological control strategies and thus several researchers have reported the biological seed treatments for protection of seed quality, enhancement of crop biomass and evaluate the parameters by seedling vigour under green house and field level conditions (Bhagat and Pan, 2010; Bandopadhyay *et al.*, 2006).

*Macrophomina phaseolina* (Tassi.) Goid is soil borne and a root infecting fungus, which is an important pathogen of jute (*C. olitorius* L. and *C. capsularis* L.) including 500 wide hosts range (Sarkar and Bhattacharyya, 2008). It is very difficult to eradicate the pathogen because it produces resting structure like sclerotia, chlamydospores for its survival for a longer period of time under adverse environmental conditions (Baker and Cook, 1974). Infection of *M. phaseolina* on jute seed results poor seedling germination and reduces plant vigour which results to less biomass production cum and deteriorates the fiber quality. Its destructive nature has been well documented (Beckman, 1987). This necrotic fungus attacks different stages of jute growing condition from seedling till harvest (Bandopadhyay *et al*., 2004).

In the present study, suitable plant growth promoting bacteria (PGPB), rhizobacteria (PGPR) and fungi (PGPF) were screened *in-vitro* on the basis of siderophore production in CAS medium, HCN formation, biochemical natures, indole 3 acetic acid (IAA) and biocontrol efficiency by dual culture method and both; where as *in-vivo* plant growth promoting potential has been evaluated by seed health status based on germinability, biomass and seedling vigour index under compatible condition.

## MATERIALS AND METHODS

### *Pathogenicity test of different isolates*

Twelve isolates of *Macrophomina phaseolina* was collected from infected jute plant in various parts of jute growing tracts and screened for pathogenicity. Selected fungal isolate (pathogen) was screened for virulence on jute seedling (c.v JRO 524) in moist chamber on sterilized blotting paper circles in 90 mm size Petri plates *in vitro*. The metabolite produced in broth culture was then primarily filtered with sterilized muslin cloth and then by bacteriological grade G-5 sintered glass filter (Bandopadhyay et al., 2004) and collected aseptically. The pathogens were isolated by surface serilized the infected plant bits by 0.1% $HgCl_2$ following repeated washing with sterile distilled water and then placed in Petri plates supplied with 20 ml Potato Dextrose Agar (PDA) medium, after one week (7 day's) growth in BOD incubator at 28±2 °C it was purified by repeated subculturing and finally maintained in PDA slants. Isolate $S_{15}$ namely $JMP_3$ selected as virulent pathotypes for conducting the experiment which was isolated from diseased jute stem part (*C. olitorius* L.) of farmer's field.

Pathogenicity test was carried out under glass house condition. The extracted metabolite of selected pathogenic isolate ($S_{15}$) mix in 250 ml conical flasks having 50 g sterilized jute seeds supplemented with 2% dextrose as substrate for 15days to form inocula. The inocula then mixed with sterilized soil kept in pots @1.5 g per 3 kg (w/v) prior to sowing the treated seeds with bioagent for evaluation under challenged inoculation.

### *Preparation of inocula cum sick soil*

Jute seeds were surface sterilized with 0.1% $HgCl_2$, dried and soaked in selected pathogenic culture metabolites for 24 h in sterilized Petri plates. Then seeds were allowed to germinate on sterilized blotting paper thrice in moistened with sterile distilled water. Seeds soaked in sterile distilled water alone served as control. Germination of seed and growth rate of seedling varied with the isolates/pathovars and screened as most virulent path type.

For pathogenicity test in pot culture, the selected pathogenic isolate grown in 250 ml conical flasks with sterilized 50 g jute seeds supplemented with 2% dextrose as substrate for 15 days to form inocula. The inocula then mixed with sterilized soil kept in pots @1.5 g per 3 kg (w/v) prior to sowing the treated seeds with bioagent for evaluation under challenged inoculation.

### *Screening and selection of micro bioagent*

Out of 75 bioagents isolated from jute rhizosphere,

rhizoplane and retting water source of diverse origin seven (7) were screened initially against virulent pathotype ($S_{15}$) of *Macrophomina phaseolina* by dual culture method technique (Bell *et al.*, 1982; Dhingra and Sinclair 1985; Bandopadhyay and Bandopadhyay, 2004) for their antagonistic potential and by production of phytohormone viz. IAA (Suslow and Schroth, 1982), siderophore (Hihgly specific $Fe^{3+}$ chelating) compound by producing orange-yellow hallow zone, volatile organic compound like HCN for their plant growth promoting potential by colonizing the root zone and improving plant growth (Kloepper *et al.*, 1980; Weller, 1988). Finally 3 were used in pot culture in the form of seed dressed and soil based under challenge inoculation which was maintained in NA (bacteria) and PDA (fungi) media.

### *Seed priming with bioagent*

The surface sterilized seeds of test crop were thoroughly washed with sterile water, air dried and finally dipped into the suspension of micro bioagent containing $10^7$ cells/ml of respective treatment prepared singly and in possible compatible combinations for few minutes and stirred to ensure uniform coverage of seeds with those suspension. The treated seeds were then mixed with activated charcoal dust@ 10 g/kg of seed as sticker and spreaded on a cleaned blotter and allowed to shade dry.

### *Evaluation for seedling vigour and seed health status*

The treated seeds were evaluated by the standard Roll Towel method (ISTA; Denmark, 1996) and incubated in seed germinator (INDOSAW) for one week with a temperature 30 ± 1 $^0$C and relative humidity around 80%. The germination of seeds was observed periodically and the root length, shoot length, roots and shoot weight under wet and dry conditions was measured. The vigour index of respective crop seedlings to evaluate the effect of treatments was calculated based on following formulae as described by Abdul-Baki and Anderson (1973):

Vigour Index of Seedlings = [Root length (cm) + Shoot length (cm)] × Germination %

### *Green house test*

*In vivo* efficacy of selected bioagent singly and in combination was evaluated against damping off, collar rot and stem rot of jute under green house condition. The sclerotia of *M. phaseolina* in the form of seed inoculum were buried 2-3 c.m depth into 8 inch. (20 cm) earthen pot duly filled with a mixture of well rotten FYM and sterilized soil (1:2) with CRD design having three (3) replications. The isolate was grown in Nutrient broth and Czapeck Dox broth (Hi media) for 15 days before sowing of treated seeds.

The details of treatments in the green house test were as follows:

T1: ST with S3 + SA with S3 15 DAS
T2: ST with S7 + SA with S7 15 DAS
T3: ST with S12 + SA with S12 15 DAS.
T4: ST with Bavistin (Carbendazim) @ 2g/kg of seed.
T5: ST with Bavistin (Carbendazim) @ 2g/kg of seed + SA with S3 & S7 (1:1)
T6: ST with Bavistin (Carbendazim) @ 2g/kg of seed + SA with S3 & S12 (1:1)
T7: ST with Bavistin(Carbendazim) @ 2g/kg of seed + SA with S7 & S12 (1:1)
T8: SS with IAA@ 25 ppm + SA with S3+S7+S12 (1:1:1) 21 DAS
T9: T4 +T8 + Targa super (Quizalofop ethyl) sprays @1.5 ml/l. 30 DAS.
T10: ST with S3+S7+S12 (1:1:1) + SA with S3+S7+S12 (1:1:1) 21 DAS.
T11: Control (Inoculated/Diseased)
T12: Control (Uninoculated/Healthy).

ST= Seed Treatment, SS= Seed Soaking, SA= Soil Application, DAS= Day's After Sowing.

## RESULTS AND DISCUSSION

### *Biochemical parameters for plant growth promoters*

Efficient isolates were screened and finally selected before seed priming on the basis of their response in siderophore production (orange-yellow halo zone) in CAS blue agar (Fig.1b), IAA (Brick *et al.*, 1991), HCN positive (Bakker and Schipper, 1987). Amongst all the isolates $S_7$ showed highest intensity to response against siderophore, IAA and HCN tests. Whereas $S_3$, $S_4$ were IAA negative but HCN positive and $S_5$ unable to show any impacts in HCN test (Table 1). Besides two fungi $S_{10}$ and $S_{12}$ also showed better response against different biochemical tests.

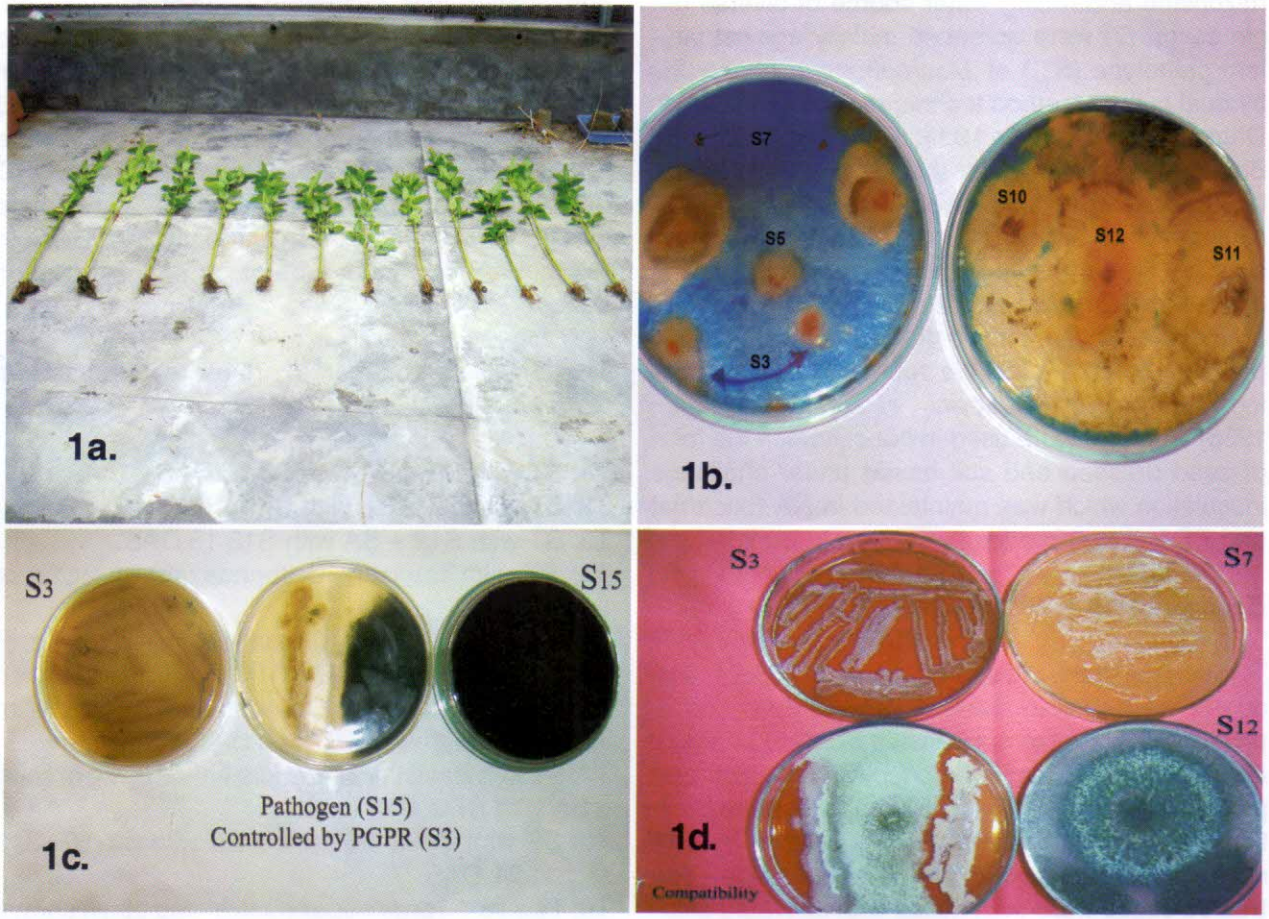


**Fig. 1 :** Treated Plants (1.a) from Left – Right (T1 – T12). Different mechanisms for screening of isolates – 1b. Siderophore, 1c. Dual culture and 1d. Compatibility.

***Study of antagonism in vitro***

The antagonistic study against *M. phaseolina* was carried out by direct antagonism i.e. dual culture technique (Fig.1c). The dual culture study revealed that all the antagonists i.e. five (5) bacterial isolates and two (2) fungal isolates had the ability to inhibit the pathogen growth significantly. The inhibition ranged from 68.88% to 75.55%. The maximum inhibition i.e. 75.55% was shown by $S_3$ (*Alcaligens faecalis*), whereas 74.26%, 72.33% and 68.88% inhibition shown by $S_7$ (*Bacillus amyloliquefaciens*), $S_{12}$ (*Trichoderma aureoviridae*) and $S_{10}$ respectively (Table 2).

The results as on green house test after biopriming of seeds suggested that the isolate, $S_7$ singly showed the best effects in all the parameters studied (Table 3). It was the most efficient isolates regardless of crop seeds and form of inocula used even when it was applied with other bioagents ($S_3$ and $S_{12}$), agrochemicals (Carbendazim) and herbicides (Quizalofop ethyl) for post emergence effect in an integrated manner. The bacterial antagonist, $S_3$ and fungal bio agent $S_{12}$ found to have intermediate effect with respect to their potential in enhancing the pe rcent seed germination, seedling biomass on dry weight basis and seedling vigour on possible extent of the crop seeds tested. Among two forms of formulation the charcoal based seed treatment with respective bioagents singly or in compatible combination along with seed treating chemicals gave better responses with respect to enhanced germination of seed. Whereas Carboxy methyl cellulose (CMC) based Soil formulation gave better response in increasing seed vigour index either in terms of calculated vigour index or estimated biomass on dry weight basis. The growth regulator IAA used for seed soaking and gave better performance in stimulat-

**Table 1 :** Biochemical parameters for plant growth promoters

| Sample no. | Siderophore Positive/ Negative | Radius of Halo zone (mm) | IAA Positive/ Negative | HCN positive/ Negative |
|---|---|---|---|---|
| $S_2$ | ++ | 5.0 | + | ++ |
| $S_3$ | + | 4.5 | - | ++ |
| $S_4$ | + | 2.1 | - | + |
| $S_5$ | ++ | 5.0 | ++ | - |
| $S_7$ | +++ | 15.3 | +++ | +++ |
| $S_{10}$ | +++ | 17.5 | + | + |
| $S_{12}$ | +++ | 18.0 | + | +++ |

+ = Mild/Scanty positive, ++ = Moderately positive, +++ = Highly positive, - = Negetive.

**Table 2 :** Dual culture studies amongst bioagents and pathogens

| Sample | Colony Diameter/ Radial growth (mm) | | Inhibition Zone (mm) | % Inhibition |
|---|---|---|---|---|
| | Pathogen | Antagonist | | |
| $S_2$ | 25.0 | 61.0 | 3.5 | 72.22 |
| $S_3$ | 22.0 | 64.0 | 3.5 | 75.55 |
| $S_4$ | 24.0 | 64.0 | 2.0 | 73.33 |
| $S_5$ | 23.0 | 61.0 | 5.0 | 74.20 |
| $S_7$ | 22.0 | 63.0 | 4.8 | 74.26 |
| $S_{10}$ | 28.0 | 60.0 | 1.7 | 68.88 |
| $S_{12}$ | 25.0 | 64.9 | 0.1* | 72.33 |
| $S_{15}$ | 90.0 | - | - | - |
| C. D. (p = 0.05) | 0.687 | 0.868 | 0.581 | 1.652 |

*= Almost over growth, - = Nil

**Table 3 :** Effects of Seed treatment & Seedling vigour Index

| Treatments | Germination (%) | Root Length (cm) | Shoot Length (cm) | Fresh Weight (g) | Dry Weight (g) | Vigour Index (VI) |
|---|---|---|---|---|---|---|
| T1 | $90.93^{ef}$ | $3.45^{ab}$ | $11.64^{bc}$ | $3.26^{de}$ | $0.45^{cd}$ | $1370.815^{d}$ |
| T2 | $93.51^{f}$ | $3.80^{bc}$ | $12.81^{f}$ | $3.95^{fg}$ | $0.55^{e}$ | $1552.619^{e}$ |
| T3 | $89^{def}$ | $3.40^{ab}$ | $12.40^{def}$ | $3.87^{f}$ | $0.47^{d}$ | $1404.87^{d}$ |
| T4 | $86.93^{cde}$ | $3.40^{ab}$ | $12.58^{ef}$ | $3.48^{e}$ | $0.41^{bcd}$ | $1385.088^{d}$ |
| T5 | $85.02^{bcd}$ | $3.29^{a}$ | $11.58^{bc}$ | $2.85^{b}$ | $0.36^{ab}$ | $1263.941^{bc}$ |
| T6 | $83.33^{bc}$ | $3.30^{a}$ | $12.32^{de}$ | $2.83^{b}$ | $0.35^{ab}$ | $1301.613^{c}$ |
| T7 | $85.06^{bcd}$ | $3.49^{ab}$ | $11.46^{b}$ | $3.12^{cd}$ | $0.33^{ab}$ | $1270.834^{bc}$ |
| T8 | $90.29^{ef}$ | $3.96^{c}$ | $12.85^{f}$ | $4.33^{h}$ | $0.37^{ab}$ | $1517.692^{e}$ |
| T9 | $90.6^{ef}$ | $4.51^{d}$ | $12.87^{f}$ | $4.16^{gh}$ | $0.38^{abc}$ | $1574.52^{e}$ |
| T10 | $84^{bc}$ | $3.37^{ab}$ | $12.00^{cd}$ | $3.31^{de}$ | $0.34^{ab}$ | $1289.687^{c}$ |
| T11(Diseased Control) | $73.33^{a}$ | $3.28^{a}$ | $10.39^{a}$ | $2.91^{bc}$ | $0.30^{a}$ | $1002.419^{a}$ |
| T12 (Healthy Control) | $81.73^{b}$ | $3.36^{ab}$ | $11.46^{b}$ | $2.22^{a}$ | $0.33^{a}$ | $1210.275^{b}$ |

Results based on Duncan's Multiple Range of Test (DMRT) Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

ing germination, root elongation and shoot length over of some treatments using bioagents or chemicals (Fig.1a).

The results also suggested that these three micro bioagents having with the synergistic/mutuality effects with each other's (Fig.1d) and potential even when applied with different agrochemicals which revealed their use in an integrated way.

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