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Management of grey blight disease of Som plants using value added vermicompost with *Glomus constrictum* and *Bacillus altitudinis*

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Abstract

Grey blight disease caused by *Pestalotiopsis disseminata*, is one of the major foliar fungal diseases that constantly affects *Persea bombycina* Kost, a primary host plant of muga silkworm. Under nursery condition, grey blight disease was recorded mostly in S5 and S6 morphotypes of som plants. Vermicompost, PGPR and AMF, alone and in combination were applied for the improvement of the growth of eight morphotypes of som plant as well as to reduce the disease incidence. Growth in terms of height (cm), no. of leaves and no. of branches were studied. Analysis of some major defense related enzymes such as POX, PAL, CHT and GLU was also carried out to check induction of resistance after treatment. Artificial inoculation of som plants under nursery condition with spore suspension of *P. disseminata* was performed and disease progression noted for 7, 14, 21 and 28 days. It was clearly seen that disease progression was slow and less in treated inoculated plants. The results emphasize the fact that application of bioinoculants can be studied in larger scale for the upliftment of the health status of muga host plants.

Keywords: *Persea bombycina*, Vermicompost, PGPR, AMF, foliar fungal diseases.

Introduction

Persea bombycina, commonly called as 'Som' plant is an evergreen tree that belongs to the family Lauraceae. Som is the primary host plant of the silkworm *Antharea assamensis* that produces the golden yellow silk famously called as Muga silk. Cultivation of muga silk is an all year round practice in North Eastern India, mainly Assam. Som plants are mostly grown in the wilds. Hence these plants received very little attention of the scientific community and very less is known about their biochemical and genetic composition. High demand of muga silk has led to the domestication of som plants and rearing of silkworms in closed area. These plants are now-a-days grown significantly in West Bengal, mainly in Coochbehar disrict. Since leaf quality has significant impact on quantity and quality of the silk fiber, for sustaining muga culture it is important to ensure availability of adequate quantity of qualitatively superior leaves.

A major problem in cultivation of healthy som plants that reduces the quantity and quality of the leaves are the various foliar fungal diseases of this plant. One of the major foliar fungal diseases of som plant is grey blight caused by *Pestalotiopsis disseminata* (Das *et al.*, 2010). These diseases are usually controlled through application of various fungicides. But application of fungicides and pesticides cause decline in the quality of the leaves. Use of bioinoculants along with vermicompost have caused decline in the disease incidence as well as improvement in the growth of plants in several cases that has been reported earlier by many researchers in the field (Sahni *et al.*, 2008, Theunissen *et al.*, 2010., Asciutto *et al.*, 2006). Vermicomposting is the simple biotechnological process by which organic material is consumed by earth worms and in the process of their digestion it enhances the process of degradation of the material and converts it into a nutrient-rich end product, called vermicompost. Vermicompost contains most nutrients in plant available forms such as nitrates, phosphates and exchangeable calcium and soluble potassium (Bhattacharjee *et al*, 2015). These help the plant to easily assimilate the required nutrition for its growth and development. Plant growth promoting bacteria (PGPR) can act as an added value. It is considered that as PGPR are soil borne bacteria they can easily mix well with the vermicompost and enhance the effect of the compost. Arbuscular mycorhizal fungi (AMF) are soil microbes forming symbiotic association with plant root system of all most all plant species. Chakraborty *et al* (2013) reported the presence of various AMF spores associated with the different morphotypes of som plants and effect of these

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spores of improvement of plant health. Keeping these findings in mind the following study was undertaken to understand the effect of vermicompost, PGPR as well as AMF, singly as well as jointly on the growth as well as disease establishment of the muga host plant.

Materials and methods

Plant sample

Eight different morphotypes of som plants (S1–S8) were collected from Boko, Assam and maintained under net house condition in Immuno-Phytopathology laboratory, Dept. of Botany, University of North Bengal.

Assessment of disease severity

The disease severity on the leaves was recorded on the basis of a 0-5 rating scale and calculated following the method of Chakraborty *et al* (2014).

Isolation of pathogen and morphological identification

The causative agent of grey blight disease was isolated from infected leaves on PDA slant after surface sterilization with 0.01% $HgCl_2$. The slants were incubated at 28°C for 5-7days till development of black acervulus. The spores were then observed under light microscope for identification of the pathogen. The fungal culture was further maintained in PDA slants.

Preparation and application of bioinoculants

Vermicompost was prepared in plastic beds using organic waste materials collected from the local area. 15-20 cm layer of this waste was covered with another 2-3 cm of dried aquatic plants. *Eisenia foetida*, the earthworm used for vermicomposting was added on the top. The final top layer was made of dried cow dung and the vermin bed was sealed with plastic cover. This set was kept undisturbed for 15-20 days, after which the bed was stirred and shaken to release the organic gas produced during vermicomposting process and for proper mixing of the materials. The compost was ready after 40-45 days when it turned into black light weight powder with no odour. After its completion the earthworms are separated from the final product and the manure was dried and sieved for further use.

The selected PGPR strain (BRHS/ P 73) of *B. altitudinus* was grown in nutrient broth for 48h and then centrifuged at 15000 rpm for 15min. The pellet obtained was re-suspended in sterile distilled water. The optical density of this suspension was measured using a UV-VIS Spectrophotometer at 600nm, to obtain a final density of 1×10^6 cfu/ml. For the preparation of bio-formulation, 10gm of Carboxy methyl cellulose (CMC) was added to 1 kg of talc and the pH was adjusted to 7 by adding calcium carbonate. They were sterilized by autoclaving at 15lbs.p.s.i for 30 mins at 120°C twice. 100ml of bacterial suspension was added to the talc formulation in a mass mixture and mixed for 5 mins. The resultant mixture is packed in polythene bags, labelled and kept at room temperature for further use.

The arbuscular mycorrhizal fungi (AMF) spores obtained from the rhizosphere of Som plants were mass multiplied in maize plant. The spores were washed several times with distilled water. They were then inoculated in roots of 7-10 d-old maize seedlings which were grown in Petri plates. After inoculation they were transferred to black plastic pots (30-cm) having autoclaved soil to eliminate other fungal propagules. The presence of AM spores was confirmed 45 d after inoculation. The maize roots were cut into small pieces and these shredded roots along with soil was added to the rhizosphere of the som plants for treatment.

The selected som plants for study were first treated with 200 g of vermicompost @per pot followed by a mixture of shredded roots and soil containing AMF. 200 g of talc based formulation of PGPR was then added to the treated pots. Aqueous suspension of selected PGPR was also sprayed on the leaves of som plant for three times with an interval of three days after each spray.

Inoculum preparation and application of pathogen

The grey blight pathogen *P. disseminata* was grown in 100ml PDA media in 250 ml flask for 10 days till black acervulus is formed. The spores are then scrapped off the surface of the medium with the help of inoculating media and collected in sterile distilled water. This suspension was filtered through muslin cloth and the filtrate containing $2X10^6$ spores/ml was used further as inoculum source.

This suspension was mixed with few drops of Tween 20 and sprayed onto the leaves of bioinoculant treated as well as untreated potted plants. These plants were kept covered with moist polythene bags for 48hrs to provide adequate temperature for the spores to germinate and establish disease.

Evaluation of Growth

Growth promotion in terms of height, no. of leaves and no. of lateral branches was recorded after every 15 days in both treated and control plants.

Biochemical analyses of leaves

Determination of total soluble protein

Total soluble protein was extracted from the leaves using phosphate buffer (pH 7.2) and was estimated following the method of Lowry *et al.*, 1951.

Estimation of total phenol content of the leaf

Phenol was extracted from the leaves of som plants by boiling in 100% alcohol and crushing in 70% alcohol and filtered. The filtrate is used for estimation of total phenol content following the method of Mahadevan and Sridhar (1978).

Extraction and estimation of defense enzymes

Four major defense enzymes were estimated in the leaves of som plants following the treatments. Phenylalanine ammonia lyase (PAL) enzyme was extracted from the leaves using sodium borate buffer (pH 8.8) and Peroxidase (POX) was extracted from the leaves using phosphate buffer (pH 6.8). Both enzyme extracts were estimated following the method of Chakraborty *et al* (1993). Chitinase and β-1,3-Glucanase both were extracted from leaves using acetate buffer (pH 5). Chitinase was assayed from the enzyme extract following the method of Boller and Mauch (1988) and Glucanase was assayed following the method of Pan *et al* (1991).

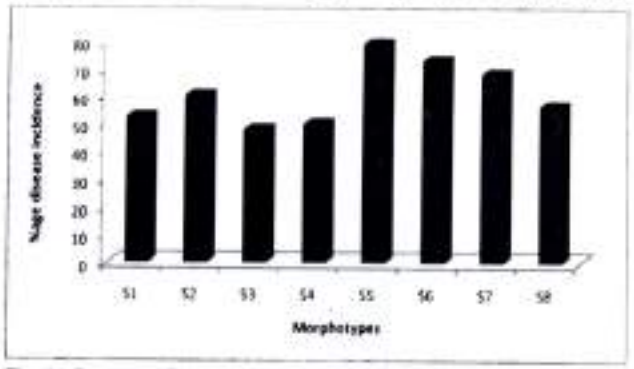

Fig 1: Percent disease incidence in leaves of som plants under nursery condition

Result

Analysis of disease occurrence in nursery condition

Under nursery condition presence of grey blight disease was recorded and percentage disease incidence (PDI) was calculated accordingly. It was recorded that establishment of disease was highest in S5 morphotype and lowest in S3 morphotype (Fig 1). So it can be assumed that morphotype S5 is susceptible to this disease.

Identification of the causal organism

The causal organism of the disease was isolated from the infected leaves in PDA slants. After proper growth, morphological examination showed the growth of white mycelia and presence of black acervulus, typical characters of *Pestalotiopsis* sp. Spores were examined under light microscope and based on the spore characters the organism was identified as *Pestalotiopsis disseminata* (Fig. 2).

Fig 2: (A) Som plant showing symptom of Grey blight; (B-D) *Pestalotiopsis disseminata* - grown in PDA (B), Spores (C) and Germinated spores (D)

Effect of bioinoculants on growth

Application of different bioinoculants was carried out accordingly as outlined in materials and methods. Growth promotion in terms of height, no. of leaves and no. of lateral branches was recorded after 45 days of treatment. In all morphotypes, growth was significantly increased in treated plants in comparison to their respective control plants. Growth was observed to be highest in case of triple treatment of bioinoculants (Table 1, Fig. 3 & 4).

Quantification of different biochemical components of leaf

Total soluble protein was quantified in leaves of control and treated som plants where it was noticed that protein content increased in leaves following the treatments. However the content was more in case of dual treatment of Vermi and PGPR as well as triple treatment when compared to control and single treatment. Total phenol content also increased in leaves following treatment and it was recorded to be highest in S3 and S4 morphotypes. (Fig. 5).

Fig 3: Growth promotion of som plants in glass house conditions after 45days of treatment with *Bacillus altitudinus*

Fig 4: Growth promotion in som plants after 45 days of treatments with bioinoculants (Row 1 Vermi+*B. altitudinus*+ *G. constrictum*, Row 2- Vermi+*B. altitudinus*, Row 3- Vermi, Row 4- *B. altitudinus*, Row 5- *G. constrictum* and Row 6- control)

Table 1: Growth promotion in som plants after 45 days of treatment with bioinoculants

Morphotype	Treatment	Height (cm)	No. of leaves	No. of Branches
S1	Control	29	22	3
	Vermi	54	60	5
	G. constrictum	66	52	6
	B.altitudinus	64	35	5
	B.altitudinus+Vermi	97	35	4
	Vermi+*G.constrictum*+*B.altitudinus*	99	63	8
S2	Control	28	10	1
	Vermi	59	52	4
	G. constrictum	31	42	5
	B.altitudinus	31	43	6
	B.altitudinus+Vermi	69	32	4
	Vermi+*G.constrictum*+*B.altitudinus*	59	33	6
S3	Control	28	25	3
	Vermi	67	77	8
	G. constrictum	69	51	6
	B.altitudinus	67	55	8
	B.altitudinus+Vermi	94	62	6
	Vermi+*G.constrictum*+*B.altitudinus*	67	63	7
S4	Control	26	15	2
	Vermi	50	76	5
	G. constrictum	50	63	5
	B.altitudinus	49	62	4
	B.altitudinus+Vermi	95	65	8
	Vermi+*G.constrictum*+*B.altitudinus*	54	64	9
S5	Control	31	32	3
	Vermi	72	46	5
	G. constrictum	71	57	7
	B.altitudinus	70	55	6
	B.altitudinus+Vermi	93	50	5
	Vermi+*G.constrictum*+*B.altitudinus*	73	53	6
S6	Control	14	16	0
	Vermi	48	55	4
	G. constrictum	55	58	4
	B.altitudinus	56	59	3
	B.altitudinus+Vermi	71	52	4
	Vermi+*G.constrictum*+*B.altitudinus*	60	54	5
S7	Control	30	18	2
	Vermi	48	31	3
	G. constrictum	40	40	4
	B.altitudinus	41	43	5
	B.altitudinus+Vermi	82	55	8
	Vermi+*G.constrictum*+*B.altitudinus*	49	56	9
S8	Control	25	7	0
	Vermi	64	54	4
	G. constrictum	61	60	8
	B.altitudinus	62	63	6
	B.altitudinus+Vermi	68	53	3
	Vermi+*G.constrictum*+*B.altitudinus*	63	55	5
CD (P=0.05)	Treatment	9.308	8.890	1.395
	Morphotype	10.748	10.265	1.611

Assay of different defense enzymes

Four major defense enzymes were studied in leaves of som plants following different treatments. It was observed that PAL, POX, CHT as well as GLU increased in all treatments than in control sets. Highest increased was seen in dual and triple treatment irrespective of morphotypes. (Fig 6)

Artificial inoculation of the pathogen and disease establishment

As disease incidence under nursery condition was highest in S5 followed by S6 morphotype, these two particular morphotypes were taken for further study. After treatment with bioinoculants, treated as well as healthy plants were inoculated with spore suspension of the pathogen and percent disease incidence (PDI) was recorded after 7,14,21 and 28 days of inoculation. It was observed that disease incidence was much less in treated inoculated plants in comparison to untreated inoculated (UI) plants (Table 2). Among the various treatments a consistent decrease in disease incidence was observed in plants treated with PGPR followed by Vermi + PGPR treatment when compared to the untreated inoculated plants. It was seen that disease progression in treated plants were very slow when compared with untreated plants.

Discussion

In the present study growth of all the eight morphotypes was noted following treatment but combined application of vermicompost, PGPR and AMF showed the best result. In an earlier study by Bhattacharjee *et al* (2015) it was found that maximum enhancement of growth and yield was

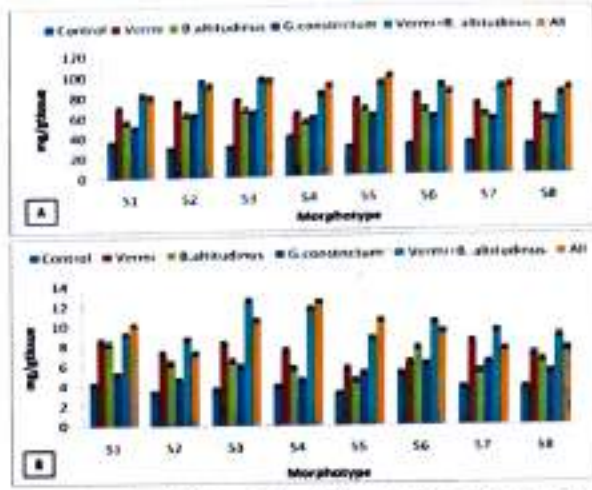

Fig. 5: Total soluble protein (A) and Total phenol (B) contents in som plants following different treatments

observed in tomato plants treated with vermicompost alone followed by vermicompost along with all microorganisms and then vermicompost with plant growth promoting bacteria (PGPR). An integrated approach by using vermicompost and a strain of *Pseudomonas syringae* (PUR46) containing plant growth promoting traits was adopted under green house condition by Sahni *et al* (2008) where it was recorded that 25% vermicompost and seed bacterization of *Cicer arietinum* resulted in an increased plant growth and also reduced plant mortality against Collar rot disease of chickpea. Pathak *et al* (2003) reported that height of guava plant was stimulated by different bioinoculants (PGPR, VAM, Azotobacter) in combination with farm yard manure as well as vermicompost. VAM inoculation with vermicompost also positively affected number of leaves per plant.

Table 2: Percent Disease Incidence (PDI) in S5 and S6 morphotype after artificial inoculation with *P. disseminata*

Treatments	S5 morphotype				S6 morphotype			
	7d	14d	21d	28d	7d	14d	21d	28d
Untreated Inoculated	25.3±0.82	35.6±0.65	68.2±0.42	85.2±0.25	21.2±0.12	29.6±0.22	55.2±0.21	72.5±0.32
Vermicompost	16.2±0.42	25.6±0.62	36.5±0.22	44.2±0.29	14.2±0.11	22.6±0.23	30.5±0.36	39.2±0.35
B. altitudinus	12.5±0.45	20.3±0.35	28.6±0.26	30.5±0.32	17.5±0.24	14.3±0.12	22.6±0.24	28.5±0.36
G. constrictum	18.5±0.84	30.2±0.25	39.6±0.45	48.2±0.25	20.5±0.32	18.2±0.19	29.6±0.24	35.2±0.34
Vermi+*B.altitudinus*	10.3±0.65	15.3±0.28	20.2±0.48	30.5±0.31	12.3±0.15	15.3±0.18	21.2±0.25	25.5±0.24
Vermi+*B.altitudinus*+ *G.constrictum*	15.2±0.52	19.5±0.56	25.5±0.52	29.5±0.36	11.2±0.16	17.5±0.16	22.5±0.32	27.5±0.48

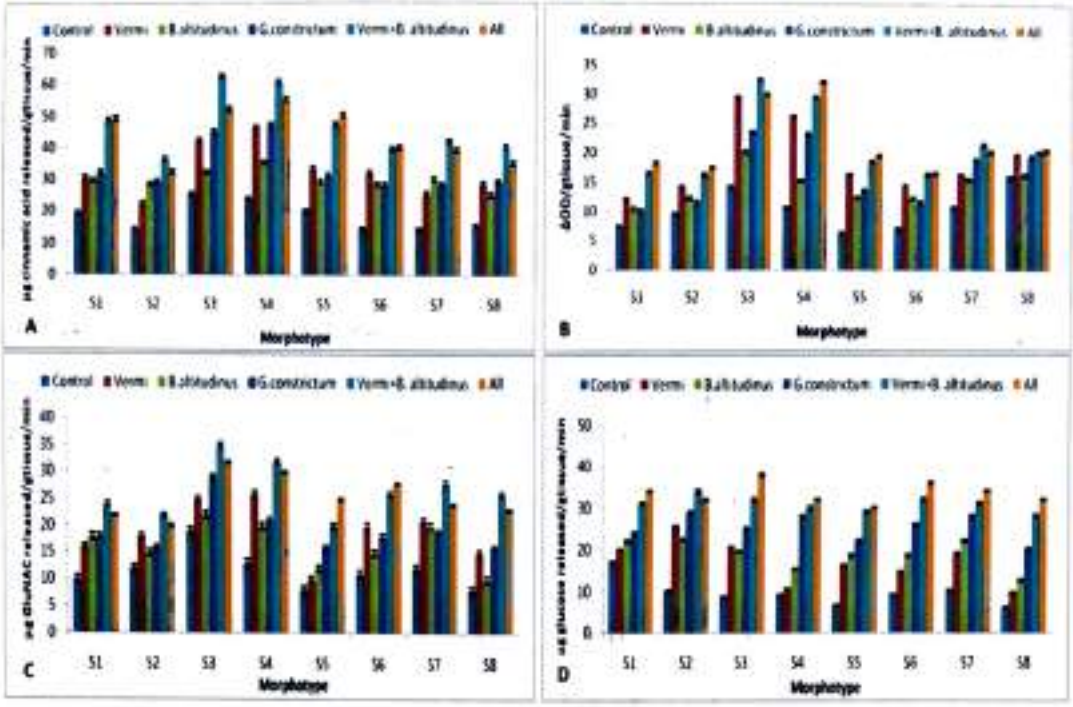

Fig. 6: Activities of defense enzymes (A) PAL, (B) POX, (C) CHT and (D) GLU in leaves of som plants following treatment

Patil (2010) reported that a combined treatment of biofertilizer and chemical fertilizer increased chlorophyll, growth, carbohydrates and proteins content in *Stevia rebaudiana* Var Bertoni compared to control. Similarly, in our study it was observed that treatment with vermicompost and other biofertilizers increased the protein and phenol content in treated plants than in control. However no treatment with chemical fertilizers was carried out.

In an earlier study by Chakraborty *et al*, 2014, it was recorded that dual application of AMF and PGPR increased phenolics as well as other defence enzymes in som plants. Treatment with these bioinoculants also decreased disease incidence in som plants artificially inoculated with *Colletotrichum gleoesporioides*, the causal agent of leaf blight disease of the plant. Similar results were also observed in our study were different defense enzymes were increased following treatment with vermicompost and other bioinoculants. Decrease of disease incidence of grey blight in som plants was also noted in the present study following treatment and artificial inoculation. Therefore it can be concluded that the treatment of som plants with value added vermicompost can lead to sustainable agriculture of such plants related to sericulture.

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