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Study on the preparation and evaluation of Insecticidal/pesticidal activity of Entomopathogenic fungi (Beauveria bassiana) metabolites fused Silver Nanoparticles

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Article History

Abstract

The green revolution strategy, which promotes the use of synthetic agricultural chemicals like pesticides and fertilizers, the adoption of high-yielding, nutrient-responsive crop varieties, increased irrigation potential exploitation, etc., has, for the most part, increased production output. However, it will also cause a decline in the productivity and production of various crops as well as harm to the environments and soil health. Plant Growth Promoting Rhizobacteria (PGPRs) have special functions in the soil that improve the health and productivity of plants. PGPR produce phytohormones, fix atmospheric nitrogen, colonize the rhizosphere, aid in the production of secondary metabolites, shield plants from pathogens, produce siderophores, and aid in the uptake of nutrients by solubilizing phosphate. They also produce biologically active substances that have an impact on the development and growth of plants. In the present investigation, the fungicidal activities of Streptomyces sp., the significant PGPRs were screened for antifungal activities while an Entomopathogenic fungi (Baeuveria bassiana) was utilized for production of crude metabolites which were utilized to prepare Silver fused nanoparticles, the nanoparticles were found to have significant insecticidal/pesticidal properties against larvae invading Cauliflower crops.

Keywords: Plant growth promoting Rhizobacteria (PGPRs), Entomopathogenic fungi, Baeuveria bassiana, metabolites, silver based nanoparticles, insecticidal and pesticidal properties

1. INTRODUCTION

The creation of nanostructures is frequently a crucial first step in the development of numerous significant goals, such as nanomedicines, nanosensors, nanodevices, and nanocatalysts and nanosystems as well. There are numerous kinds of techniques for the production of many nanostructures in the shape of thin nanorods, nanotubes, and nanoparticles nanoporous materials and films. To obtain new materials, some of the previously

established classical processes for the synthesis of various kinds of nanomaterials are enhanced. Since nanoscience is an interdisciplinary field, producing nanostructures can be done using a variety of top-down, bottom-up, and hybrid techniques. The material's shape, textural characteristics (pore size, particle size, and surface area), chemical and thermal stability, and kind of nanomaterial all influence the process that should be used. Recent years have seen the rise of the bio-based economy and the use of renewable biomass as a raw material as viable solutions to the issues relating to regional and global pollution. A growing amount of interest has been drawn to agricultural waste biomass, particularly lignocellulosic ones (such as rice husk and walnut shell), as a low-cost renewable resource for the manufacture of fuels[1-3] and, more recently, chemicals and materials[4]. The goal of green synthesis is to advance cutting-edge chemical technologies that minimize or completely do away with the need for dangerous materials in the creation, production, and usage of chemical products. This entails using hazardous or polluting materials in the production of products, cutting down on the amount of pollution generated during the synthesis processes, and minimizing, if at all feasible, the pollution that results from these operations. To create nanoparticles, two methods can be applied. Active biomolecules found in fungi, such as proteins or enzymes, contribute to the creation of nanoparticles and increase their stability and yields[5-7]. Certain fungal species can use extracellular amino acids to create nanoparticles. For instance, the reductase enzyme in the cytoplasm of fungus or the glutamic and aspartic acids on the surface of yeast both reduce metal ions to create nanoparticles. The mycelium's hydroxyl groups, which give the metal ion electrons and reduce it to produce nanoparticles, make this possible. Certain proteins or aliphatic and aromatic amines function as coating agents to stabilize them[8-11].

2. MATERIALS AND METHODS

Study area

Uttarakhand State is diverse with agro-climatic endowments, the plains and hills which are describing different scenarios for agriculture and is practiced in the plains. The hills practice mixed cropping while plains in given season, single crops are grown mostly. Irrigated lands are freely available in the plains, with over 87 percent land being irrigated as against a mere of 10 percent in the hills. The government of Uttarakhand is promoting the cultivation of some selected crops like rice (basmati), aromatic and medicinal plants, vegetables cultivation, flower cultivation, litchi production and milk production. Major crops grown in Uttarakhand are mango, guava, apricot, litchi, potato, tomato, green pea, cauliflower and capsicum.

Sample collection and their preparation

Systematic random soil samples from rhizospheric region were collected from the fields of Uttarakhand regions having plantations of different crops. A total of 15-20 soil samples were collected. The soil samples weighing about 0.5 kg each were brought to the laboratory in properly sealed polythene bags. The visible plant debris and fauna were removed and stored at 4 °C until they are transferred for residual analysis. In soils, the concentration was calculated on air-dry basis for which samples were air-dried at room temperature. A portion of the samples were air-dried, passed through a 2 mm sieve, and used for physicochemical analysis.

Isolation of plant growth promoting rhizobacteria (PGPRs)

Microbial isolates were isolated from the rhizospheric soil samples by serial dilution technique on nutrient agar (NA) and PDA plates, incubated at 28±2 ^{0}C for 72 hours. After incubation period, NA plates were observed for morphological appearances and number of bacterial and fungal colonies[12-14].

Antifungal activity of screened PGPR isolates against fungal phyto-pathogens

The antifungal activity of broth culture of PGPR isolates was determined against fungal phytopathogens by well diffusion method[15-16].

Preparation of entomopathogenic PGPR isolates based nanoparticles to determine insecticidal activity against insects/pests

The entomopathogenic fungi metabolites were extracted and purified in fermentation growth medium, further the metabolites were extracted and purified by solvent extraction. The 5 days growth of the culture was extracted with solvent (Methanol: Chloroform- 2:1) to collect organic layer which was further dried to obtain the crude extract. The crude extract is further dissolved in DMSO for the preparation of silver based nanoparticles using 10 mM silver nitrate. The change in color of silver nitrate solution results in preparation of nanoparticles. Further the nanoparticles were screened for insecticidal/pesticidal activities.

3. RESULTS

The rhizospheric soil samples from different crops (rice, wheat, pulses and local vegetables) of different fields were collected from districts of Chamoli and Pauri Garhwal regions. In the present study, total 25 soil samples were collected. Amongst these samples, total of 56 microbes were isolated; amongst which 14 microbial strains were contributed by 12 dominant genera and 18 species of PGPRs. The positive PGPR strains were found to be in the form of *Bacillus subtilis*, *Bacillus cereus*, Bacillus mycoides, *Pseudomonas fluorescens*, *Glomus sp.*, *Gigaspora sp.*, *Streptomyces AM101*, *Streptomyces AM102*, *Streptomyces AM103*, *Rhizobium sp.*, *Trichoderma viride*, *Streptomyces AM104*, *Beauveria bassiana* and *Trichoderma harzianum* **(Table 1; Table 2; Figures 1; Figure 2).** The antifungal activities of selected PGPR isolates (Streptomyces sp.) was determined. The results showed significant antifungal potential against the fungal phytopathogens as studied **(Table 3; Figure 3).** In the present investigation, the metabolites of some of the microbial cultures were produced in the liquid broth. The biological nanoparticles were prepared using AgNO3 as an oxidizing agent and metabolites of the PGPR strains as reducing agent. In the present study, *Beauveria bassiana* metabolites fused silver nanoparticles were also prepared. These nanoparticles were tested for insecticidal/pesticidal properties and results were found to be promising **(Table 4; Figure 4).**

Table 1: Percent diversity of PGPR isolates

Soil samples	Total number of microbes isolated	PGPR isolates	Diversity of PGPR genera	Diversity of PGPR species
25	56	14	12.0	18.0

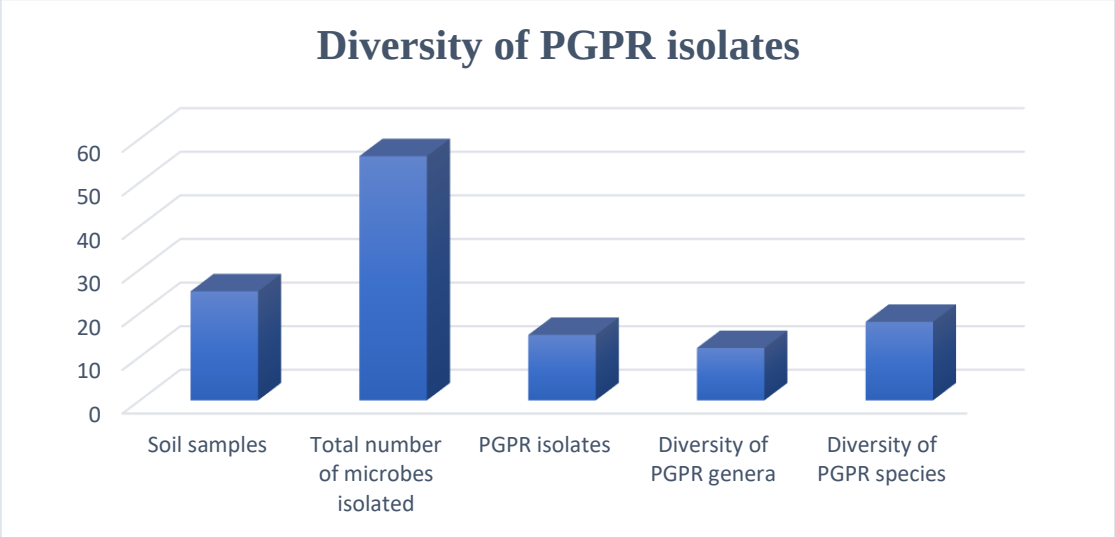
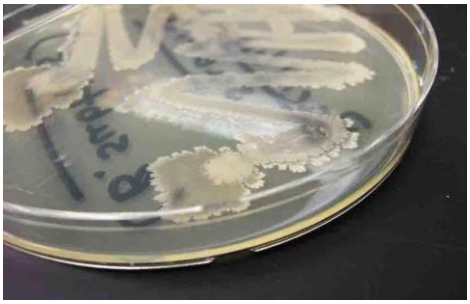


Figure 1: Graphical representation of percent diversity of PGPR isolates

Table 2: Isolated PGPR cultures – bacterial and fungal cultures

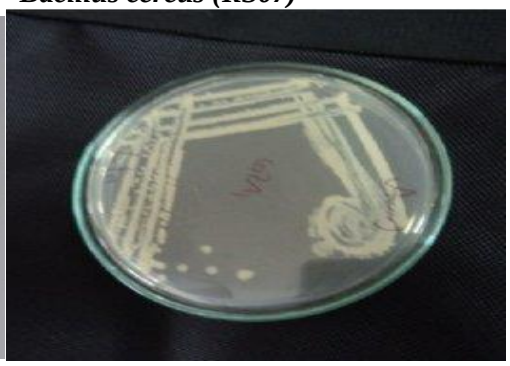
S.No.	Strain code	PGPR isolates	
		Strain Name	**Type of strain (Bacterial/Fungal)**
1.	RS03	*Bacillus subtilis*	Bacterial strain
2.	RS07	*Bacillus cereus*	Bacterial strain
3.	RS21	*Bacillus mycoides*	Bacterial strain
4.	RS25	*Pseudomonas fluorescens*	Bacterial strain
5.	RS28	*Glomus sp.*	Fungal strain/Mycorrhiza
6.	RS31	*Gigaspora sp.*	Fungal strain/Mycorrhiza
7.	RS34	*Streptomyces AM 101*	Fungal strain/Actinomycetes
8.	RS35	*Streptomyces AM 102*	Fungal strain/Actinomycetes
9.	RS37	*Streptomyces AM103*	Fungal strain/Actinomycetes
10.	RS39	*Rhizobium sp.*	Bacterial strain
11.	RS42	*Trichoderma viride*	Fungal strain/Biocontrol
12.	RS43	*Streptomyces AM104*	Fungal strain/Actinomycetes
13.	RS47	*Beauveria bassiana*	Fungal strain/Entomopathogenic
14.	RS52	*Trichoderma harzianum*	Fungal strain/Biocontrol



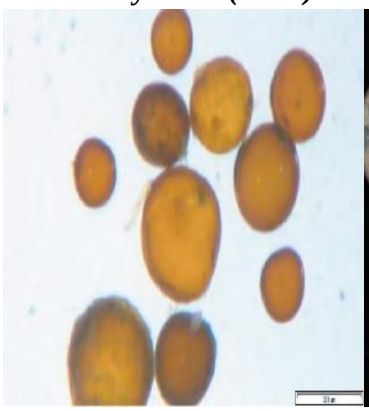
Bacillus subtilis (RS03)

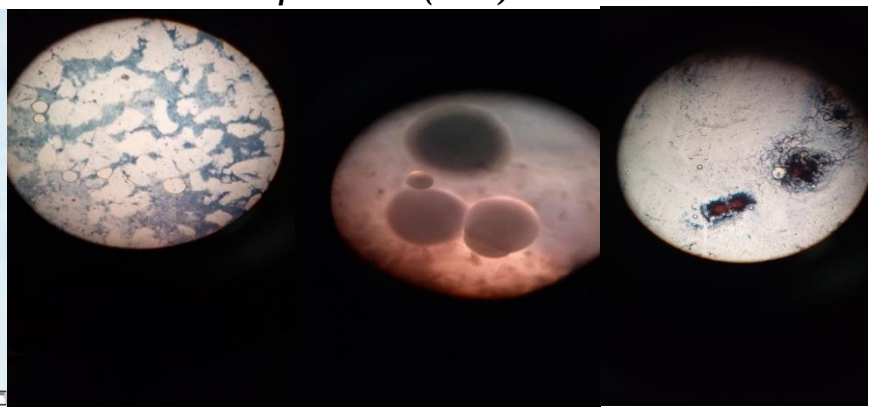
Bacillus cereus (RS07)

Bacillus mycoides (RS21)



Pseudomonas fluorescens (RS25)





Spores (a-n)of Glomus sp. (RS28); Gigaspora sp. (RS31

Streptomyces sp AM 101 (RS34)

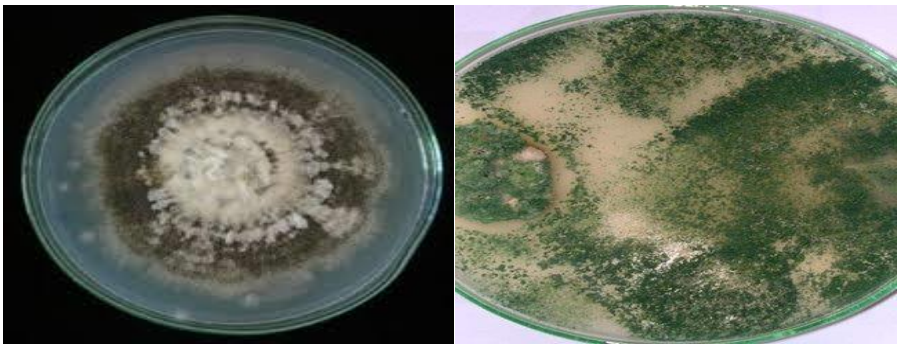
Streptomyces AM 102 (RS35)

Streptomyces AM 103 (RS37

Rhizobium sp. (RS39)

Trichoderma viride (RS42)

Streptomyces AM104 (RS43)



Beauveria bassiana (RS47) ***Trichodema harzianum (RS52)***

Figure 2: PGPR- Bacterial and Fungal-PGPR isolates

Table 3: Antifungal activity of selected PGPRs against fungal phyto-pathogens

Antifungal activity of PGPR extracts via well diffusion method - Diameter of zone of inhibition (mm)						
S.No.	**Strain code**	**Strain Name/Positive Control**	***Fusarium oxysporum***	***Sclerotium rolfsii***	***Colletotrichum sp.***	***Rhizoctonia solani***
1.	RS34	*Streptomyces AM 101*	41.0±0.020	32.0±0.065	45.0±0.0015	26.0±0.025
2.	RS35	*Streptomyces AM 102*	32.0±0.054	25.0±0.056	41.0±0.022	22.0±0.047
3.	RS37	*Streptomyces AM103*	33.0±0.067	26.0±0.048	27.0±0.023	26.0±0.053
4.	RS43	*Streptomyces AM104*	28.0±0.020	35.0±0.020	22.0±0.018	28.0±0.035
5.	-	Fluconazole/ Positive Control (1 mg/ml)	45.0±0.025	40.0±0.020	25.0±0.020	27.0±0.018

***P<0.05, level of significance**

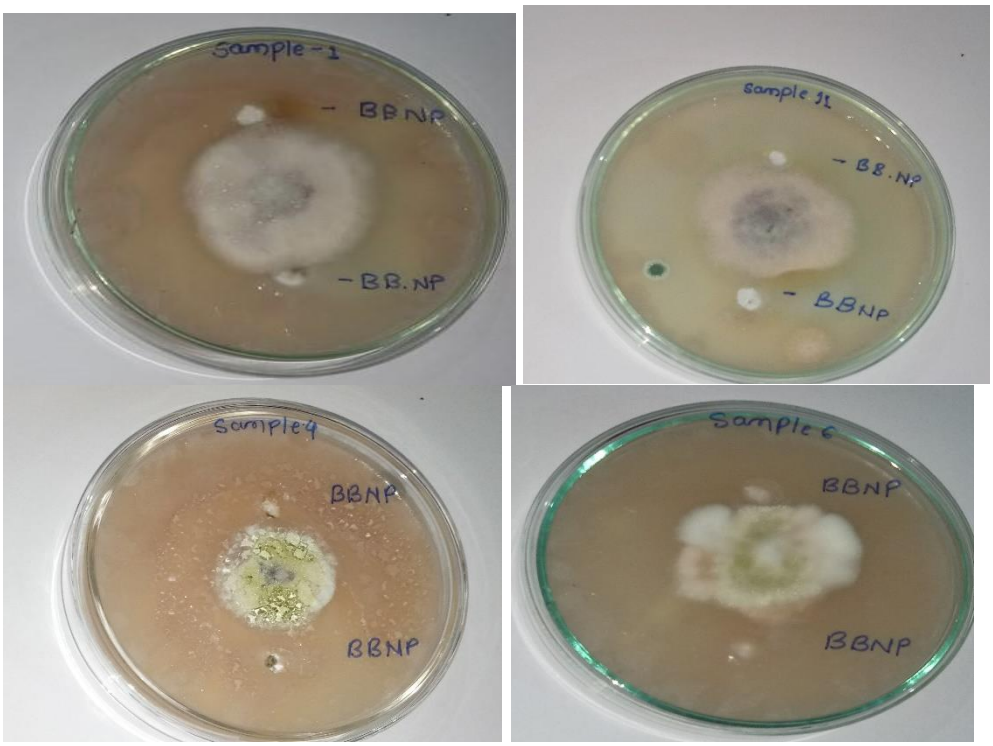


Figure 3: Antifungal activity of selected PGPR isolates against fungal phytopathogens (Reduction in radial axis-centre)

Table 4: Insecticidal activity of *Beauveria bassiana** metabolites fused silver nanoparticles (*Entomopathogenic fungi)

***Beauveria bassiana* metabolites fused Ag Nanoparticle**	**Insecticidal activity against Larvae (Percent reduction in population)**				
Doses (ppm)	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**
10	2	5	10	15	20
20	5	18	27	38	52

Figure 4: Preparation of Beauveria bassiana metabolites fused Silver nanoparticles

4. CONCLUSION

The present study strengthens on beneficial microbes viz. PGPRs- Growth promotion agents, Fungicidal agents, Biocontrol agents (Insecticidal and Pesticidal) and also their metabolites for unique product formulations. The study illustrates the preparation of biological nanoparticles that can be utilized in much more effective manner in comparison to conventional fertilizers in terms of less dosages and long term sustainable productivity. The biological synthesis of nanoparticles viz. *Beauveria bassiana* fused silver nanoparticles for insecticidal activity as validated in the study justify the objectives of the study and thus prove the hypothesis.

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