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Research article

BIOPESTICIDE FORMULATION OF *PSEUDOMONAS* FOR GROWTH OF TOMATO PLANT

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ABSTRACT

Pseudomonas was applied as biopesticide formulation containing magnesium silicate and sodium alginate for growing tomato plants. The results obtained suggested that there was synergism in promoting seed germination and plant growth.

Key words: Biopesticide, Pseudomonas, tomato plant, biocontrol.

INTRODUCTION

Pseudomonas species is widely present in agricultural soils and has many characters that make it well suited as Plant Growth Promoting Rhizobacteria (PGPR) (Pandey *et al*., 2013). Most of the *Pseudomonas* produces water soluble, yellow green pigment. *Pseudomonas fluorescens* and *Pseudomonas putida* are well studied species of *Pseudomonas* as a PGPR and is reported to produce siderophore; IAA, phosphate solubilization and some of the species of *Pseudomonas* can also fix atmospheric nitrogen (Caron *et al*., 1995; Tripathi *et al*., 2005; Shabayev, 2010).

Biopesticides are the formulated form of active ingredients based on microorganisms such as bacteria, viruses, fungi, nematodes or naturally occurring substances including plant extracts (Burges, 1998; Knowles, 2005, 2006). Microbial biopesticides constitute the largest group of broad-spectrum biopesticides. There are at least 1500 naturally occurring insect-specific microorganisms, 100 of which are insecticidal (Khachatourians, 2009) and over 200 microbial biopesticides are available in 30 countries affiliated to the Organization for Economic Co-operation and Development (Kabaluk and Gazdik, 2005). Pseudomonas induced resistance is associated with accumulation of PR protein and synthesis of phenolics. *Fusarium* wilt of tomato caused by *Fusarium oxysporum* is a soil-borne disease in tomato and seed treatment with *Pseudomonas* species prevented the entry of *Fusarium* wilt pathogen in the vascular tissue by strengthening cell wall structures and accumulation of phenolics substances and chitinases. *Pseudomonas* biopesticide application results in production of peroxidase (PO), polyphonol oxidase (PPO) that catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and

phenolics synthesis and other enzymes include pathogenesis related proteins (PRs) such as β-1, 3-glucanases (PR-2 family) and chitinases (PR-3 family) which degrade the fungal cell wall and cause lysis of fungal cell (Frindlender *et al*., 1993). Thaumatin-like protein (TLP) belongs to PR-5 family showing antifungal activity and enhancing resistance to pathogen infection (Van LC, 1997; Datta *et al*., 1999).

METHODS AND MATERIALS

Isolation of *Pseudomonas*

Pseudomonas was isolated from soil samples using King's B medium and Tryptone broth.

Biopesticide inoculum preparation

Rhizobacterial inoculum for seed bacterization was prepared by growing the Pseudomonas species on King's B broth for 36 h on a rotary shaker at 150 rpm. Bacterial cells pellet from the log phase were obtained by centrifugation at 8000 rpm for 10 min and concentration was adjusted to 1 x 10^8 cfu/ ml spectrophotometrically.

Preparation of biopesticide using carrier

Talcum powder i.e. magnesium silicate [$Mg_3Si_4O_4(OH)_2$] and sodium alginate were used as carrier. One hundred gram of talcum powder was placed in a metal tray under aseptic condition and pH adjusted to 7 by adding $CaCO_3$ at the rate of 15 g/kg. The mixture was autoclaved at 15 psi for 20 min and then 400 ml of bacterial suspension (1 X 10^8 cfu/ml) was added to the carrier (1kg) followed by mixing. It was allowed to dry and then ground to powder. Similarly sodium alginate based biopesticide was prepared.

Seed treatment

Tomato seeds were surface-sterilized using 1% sodium hypochloride for 30 sec, washed in distilled water, blot dried and then air dried. Just before sowing, seeds were coated with formulation at the rate of 5g/kg and 15g/kg of seeds. Sand, soil and farmyard manure was mixed in the ratio of 2:1:1. Potting soil was autoclaved and was placed in earthen pots; untreated and treated seeds of tomato in different groups were sown 2 cm deep in pot.

RESULTS AND DISCUSSION

Isolation methods allowed direct isolation of *Pseudomonas* sp. on its selective medium i.e. King's B medium from the collected soil sample. All the members of genus Pseudomonas produced large, opaque, flat colonies with irregular margins and distinctively fruity odor colonies. The isolates from soil sample and water produce small round colonies.

Seeds treated with biopesticide formulations showed positive growth responses among all parameters recorded in pot culture compared with non bacterized control. The highest plant height was resulted from seeds treated with fresh bacterial suspension i.e. 20.60 cm followed by Talc based biopesticide formulation at 15 g/kg of seeds i.e. 16.75 cm and at 5 g/kg of seeds i.e. 16.43 cm compared with untreated control i.e. 12.8 cm after 35 days.

Similarly seeds treated with powdered formulation of sodium alginate showed positive growth responses among all parameters. The highest plant height resulted from seeds treated with sodium alginate based biopesticide at 15 g/kg of seeds i.e. 26.0 cm compared with formulation at 5 g/kg of seeds i.e. 18.0 cm after 35 days. Among different formulations test, the highest seed germination was observed in seeds treated with fresh bacterial suspension that is

63.41%, followed by Talc based formulation at 15 gm/kg and 5 gm/kg of seeds that is 42.68% and 26.82% as compared to control that showed 17.07% seed germination. While seeds treated with sodium alginate based biopesticide at 15 gm/kg of seed showed seed germination of 34.15 % as compared to at 5 gm/kg that is 30.48 % (Table 1-3).

Table 1: Growth in plants (cm) treated with Talc based biopesticide.

Talc based formulation	**Control**	**5 g/kg of seeds**	**15 g/kg of seeds**	**Seeds treated with bacteria**
After 5 days	2.1	2.2	3.4	2.7
After 10 days	3.2	3.2	3.6	4.2
After 15 days	5.1	5.2	7.6	7.6
After 20 days	5.4	5.6	7.8	7.9
After 25 days	7.9	8.1	8.2	8.5
After 30 days	9.1	12.1	12.8	15.7
After 35 days	12.8	16.43	16.75	20.6

Table 2: Growth in plants (cm) treated with sodium alginate based biopesticide.

Sodium alginate based formulation	**5 g/kg of seeds**	**15 gm/kg of seeds**
After 5 days	3.2	3.3
After 10 days	4.3	5.4
After 15 days	5.8	7.8
After 20 days	13.9	14.6
After 25 days	14.5	18.7
After 30 days	19.2	27.6
After 35 days	20.1	30.5

Table 3: Seed germination in talc and sodium alginate based biopesticides.

	Talc based	**Sodium alginate based**
Control	17.07%	
5 g/kg of seeds	26.82%	30.48 %
15 g/kg of seeds	42.68%	34.15 %
Seeds treated with bacteria	63.41 %	

Pseudomonas species is most common bacteria found in agricultural soil and it posses many characters that make it as PGPR. Inoculation with plant growth promoting rhizobacteria has been attributed to production of plant growth regulator at root surface interface, which stimulates root development and result in better absorption of water and nutrients from soil.

In our experiments, the fresh culture and formulations of selected Pseudomonas species significantly increased seed germination and seedling vigor over the control which is in agreement with the findings of Ramamoorthy *et al*. (2002). Probability of root colonization is higher for fresh cultures of biocontrol agents rather than formulations which were evident from pot culture performed. In addition, PGPR increased plant growth directly by mediating the production of secondary metabolites and phytohormones such as auxins, cytokinins or gibberellic acid (Verma *et al.*, 2010). Root colonization by rhizobacteria appears to be an important factor in plant growth promotion and biological control. The colonization of roots by inoculated bacteria is an important step in the interactions between beneficial bacteria and host plant to reduce the attack of deleterious pathogen. The result of the present investigation suggests Pseudomonas species exhibited synergism in promoting seed germination and plant growth. Hence, the Talc and Sodium alginate formulation of Pseudomonas species can be recommended to the farmers as one of the crop protection strategies for the management of yield of tomato plant and this practice may also be extended to other groups.

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