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EVALUTION OF BIOCONTROL AGENT *BACILLUS SUBTILIS B5* FOR CONTROL OF *PHYTOPHTHORA INFESTANS* ON POTATO LEAVES

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ABSTRACT: Foliar infection during five days period of incubation at 18°C and humidity above 90% revealed no appearance of *Phytophthora infestans* lesions till 96 h of incubation where five leaflets in each set were abaxially sprayed with 10^6 cfu/ml suspension of biocontrol agent *Bacillus subtilis* B5 and 0.2% concentration of a mancozeb + metalaxyl formulation. The leaflets in control set exhibited average infection severity index of 1.8 and 3.8 after 72 and 96 h of incubation, respectively. After 120 h of incubation, the average infection severity index of 0.6 and 0.2 was recorded in bca B5 and fungicide formulation sprayed leaves compared to control where infection severity index of 4.0 to 5.0 was recorded in foliar tissues of all the five leaflets of a set resulting in an average infection severity index of 4.8.

Late blight of potato caused by *Phytophthora infestans* is an aggressive disease affecting yields under favourable environment conditions. Resistance alone has never effectively controlled late blight, therefore control of *P. infestans* is traditionally attained by cultural controls and crop protection strategies that rely on applications of foliar fungicides which are costly and add pesticide to the environment (Bruck *et al*., 1980, Kirk *et al*., 1999). The other possibility of control is through some ideal biocontrol agent (bca). The present research summarizes the potential of bca *Bacillus subtilis* B5 in managing *P. infestans* because this bca has shown broad-spectrum antifungal and antibacterial activity and was found to be highly effective in controlling many soil and tuber borne pathogens of potato (Ajay and Sunaina, 2005).

In vitro evaluation of bca B5 for control of *P. infestans* was assessed by detached leaf assay method (Deahl *et al*., 1993) at the Central Potato Research Institute Campus, Modipuram, Meerut in 2006. Three sets of detached leaves, each consisting of one terminal and four lateral leaflets were washed in sterile distilled water and blotted on sterile paper towel. Top three leaflets of each of the three sets were inoculated with a drop of zoospore suspension of *P. infestans* containing 4 x 10^4 zoospores/ml. Of the three sets, abaxial surfaces of all the five leaflets of set one were sprayed with bca *B. subtilis* B5 suspension containing 10^6 cfu/ml and set second was sprayed with 0.2% suspension of mancozeb + metalaxyl formulation and labeled as treated while the set three which was inoculated with zoospores of *P. infestans* alone was labeled as control. The two treated and one control sets were placed in 150 mm x 50 mm Petri-dishes and incubated at 18°C and humidity above 90% for 5 days. Infection severity (Deahl *et al*., 1993) was recorded after 72, 96 and 120 h of incubation using a scale of 0, 1, 2, 3, 4 and 5 representing, 0, 0-20, 20-40, 40-60, 60-80, 80-100%, respectively, of the leaf surface covered by *P. infestans.* The potential of bca B5 for control of *P. infestans* was evaluated by visually observing the leaf area covered by sporulating lesions of the pathogen in each of the five leaflets of a set and categorizing them under infection severity category on the bases of 0 to 5 scale and by measuring the greatest width and length of individual lesion over different periods of incubation (Bruck *et al*., 1980). Average infection severity index in each set was calculated by adding infection severity of each of the five leaflets per set divided by five. The experiment was repeated five times. Analysis of variance was carried out on square root transformed values and means compared at $P \leq 0.05$.

During five days of incubation, disease did not appear where the leaflets were abaxially sprayed with 10^6 cfu/ml suspension of bca B5 and 0.2% of the fungicide formulation although leaflets in control set exhibited average infection severity index of 1.8 and 3.8 after 72 and 96 h of incubation, respectively (**Table 1**). After 120 h of incubation, average infection severity index was 1.0 on terminal leaflet alone leaving remaining four lateral leaflets infection free leading to an average infection severity index of 0.2 in the fungicide treated leaflets while in bca B5 treated set, infection severity index was 1.0 on terminal and two lateral leaflets (next to terminal leaflet) and the remaining two lateral leaflets still remained infection free leading to an average infection severity index of 0.6 compared with the control value of 4.8. Both bca B5 and the fungicide inhibited the penetration of *P. infestans* into the uninoculated lower lateral leaves in contrast to control where average infection severity index of 4.8 was recorded (**Table 1, Fig. 1**). The fungicide retarded lesion expansion more than what B5 did. Both bca B5 and the fungicide were equally effective in restricting lesion expression on inoculated leaflets till 96 h of incubation and retarded the lesion size by 61.49 and 82.33% after 72 h and 96 h of incubation whereas in control, average lesion size expanded to 2.8 cm after 72 h and 15.2 cm after 96 h of incubation, respectively. The overall lesion reduction was brought down by bca B5 and ridomil to 65.42 and 79.60%, respectively, over control after 120 h of incubation (**Fig. 1**).

Table 1. Infection severity index and leaf area affected by *P. infestans* in various treatments

Treatment	Average infection severity index* (0-5 scale) and area affected after incubation					
	72 h		96 h		120 h	
	Infection severity index	Area affected (cm)	Infection severity index	Area affected (cm)	Infection severity index	Area affected (cm)
Leaves treated with *B. subitilis* B5	0.00 (0.707)	0.00 (0.62)	0.00 (0.70)	0.00 (0.62)	0.60 (1.04)	1.97 (1.39)
Leaves treated with Ridomil	0.00 (0.707)	0.00 (0.62)	0.00 (0.70)	0.00 (0.62)	0.20 (0.83)	0.36 (0.82)
Control	1.80 (1.42)	2.80 (1.61)	3.80 (2.07)	15.20 (3.51)	4.80 (2.30)	20.15 (4.02)
CD (P≤0.05)	0.473	0.748	0.628	0.628	0.574	0.677

*0= No infection; 1= 0-20% infection; 2= 20-40% infection; 3= 40-60% infection; 4=60-80% infection; 5= 80-100% infection, () = square root transformed values

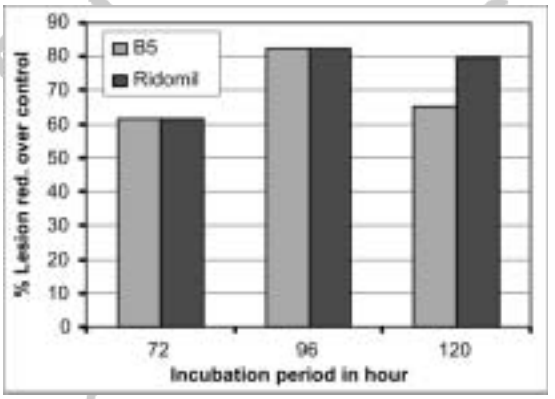

Fig. 1. Percent lesion reduction of *P. infestans* by *B. subtilis* B5 and mancozeb + metalaxyl formulation

The bca B5 and the fungicide had their greatest effect in reducing average infection severity index and lesion expansion on inoculated leaflets. The set of detached leaves, abaxially sprayed with bca B5 alone prior to *P. infestans* inoculation also suggests that the biocontrol agent might have helped in reducing the vulnerability of leaflets to infection by *P. infestans* which was specifically true for two lateral leaflets near the base of the set but not with the upper terminal and two adjoining lateral leaflets where no protection from late blight infection was observed. However bca B5 is not as effective as the fungicide in checking leakage of *P. infestans* mycelium in leaf tissues but can be tried when disease pressure is not high. These observations confirm the earlier findings (Ajay and Sunaina, 2005, Stephen *et al.*, 2005) that product Serenade, prepared from *B. subtilis* and bca B5 gave significantly controlled the infection by *P. infestans*.

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