Effect of application methods of *Pseudomonas fluorescens* for the late leaf spot of groundnut management

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ABSTRACT

Late leaf spot incited by *Cercosporidium personatum* is the serious disease in groundnut. The effect of different methods of application of *Pseudomonas fluorescens* (Pf1) in managing late leaf spot of groundnut was assessed under greenhouse condition. Among the different methods tested, seed treatment coupled with foliar application of Pf1 formulation was found to be the most effective in managing late leaf spot disease. The boosting effect of Pf1 formulation on plant growth was evidenced by the fact that the plant height, leaf area index, root length, nodules per plant and dry matter production were the maximum in *P. fluorescens* treated plants. Pod yield was also found to be increased by using Pf1. Seed treatment followed by foliar application of Pf1 formulation gave the maximum pod yield.

Key words: Biocontrol, groundnut, late leaf spot**,** *Pseudomonas fluorescens*

INTRODUCTION

Late leaf spot caused by *Cercosporidium personatum* is the destructive foliar disease in groundnut. The most obvious effect of this disease is the loss of photosynthetic tissue, which leads to premature defoliation. Late leaf spot is almost co-existent with the crop and contributes to significant loss in yield throughout the world (Smith *et al.,* 1992). The control of leaf spot disease is becoming difficult as most of the cultivars are susceptible and no variety is absolutely resistant to the disease. Biological control of plant diseases using antagonistic bacteria is now considered as a promising alternative to the use of hazardous chemical fungicides.

Fluorescent pseudomonads have emerged as the largest and potentially the most promising group of plant growth promoting rhizobacteria (PGPR) for biocontrol of plant diseases (Liu *et al.,* 1995). Several fluorescent Pseudomonads were known to control soil borne fungal pathogens like *Pythium, Fusarium, Rhizoctonia* in a wide range of crops (Vidhyasekaran *et al.,* 1997a, 1997b). Fluorescent pseudomonads have received particular attention throughout the global science because of their catabolic versatility, excellent root-colonizing abilities and their capacity to produce a wide range of antifungal metabolites. Biopesticides are cheaper, ecofriendly and do not pose risk of the pathogen developing resistance. Plant growth promotion and yield increase are the twin additional benefits from PGPR. Hence, the present study was undertaken to test the efficacy of the developed *P. fluorescens* Pf1 formulation as seed treatment and foliar spray for the management of late leaf spot disease of groundnut.

MATERIALS AND METHODS

To determine the effect of different methods of application and their combination on the management of late leaf spot of groundnut, a trial under greenhouse condition was conducted in Randomized Block Design. The pathogen, *Cercosporidium personatum* was isolated from infected leaves using Potato Dextrose Agar Medium. A talc-based powder formulation of *P. fluorescens* Pf1 was developed as described by Vidhyasekaran and Muthamilan, 1995. Pf1 was grown in King's B broth (King *et al*., 1954) for 48 h at 26 ± 2ºC. Bacterial suspension (400 mL) containing $9x10^8$ cfu mL^{-1} was added to 1 kg of the talc substrate and mixed well under sterile

conditions. Seed treatment with *P. fluorescens* was done by using freshly prepared talc-based culture at the rate of 10 g kg^{-1} seed and sown in pots. In another set of experiment, plants were sprayed with Pf1 formulation at the rate of 5 g per litre of water (0.5%) on 30, 45, 60, 75 and 90 days after sowing. The combination of seed treatment and foliar spray with Pf1 formulation was also done. As a check, seeds were treated with carbendazim at the rate of 2 g kg^{-1} of seeds and sprayed with mancozeb at the rate of 0.2 per cent on 30, 45, 60, 75 and 90 days after sowing.

Conidial suspensions of *C. personatum* were obtained from 3-week old culture by flooding Petriplates with sterile distilled water and scrapping them with a glass rod. At forty five days after sowing, the plants from treated and untreated seeds were inoculated with spore suspension of *C. personatum* (5 x 10^4 spores per mL of water). The pots were placed in a glasshouse maintained at 22-28^{o}C and 60-90% relative humidity. Disease intensity was assessed at 90 days after sowing using a modified 9-point scale (Subrahmanyam *et al.,* 1995). The number of nodules per plant was recorded at 45 days after sowing. The plant height and leaf area index (LAI) were recorded at 60 DAS. Root length per plant, dry matter production and pod yield were also recorded. Leaf area index was worked out as suggested by Padalia and Patel (1980) by using the formula

LAI = Total leaf area (cm^2)/Land area occupied by the plant

For the estimation of dry matter production (DMP), plants were initially air-dried in shade and then oven dried at 65±5^{o}C till the samples attained a constant weight. The data were statistically analyzed using Duncan's Multiple Range Test (DMRT, P=0.05).

RESULTS AND DISCUSSION

Seed treatment, foliar spray and the combination of seed treatment and foliar spray with *P. fluorescens* Pf1 formulation were tested for the management of late leaf spot disease. Among the different methods tested, seed treatment coupled with foliar application of Pf1 formulation was found to be the most effective in managing the late leaf spot disease. There was a 40 per cent reduction in the disease due to the combination of seed treatment and foliar spray of Pf1 formulation (Table 2). Gnanamanickam *et al.* (1992) observed that seed treatment followed by two sprayings with *P. fluorescens* effectively controlled rice sheath blight disease. Though seed treatment with

Table 1. Effect of different methods of *P. fluorescens* (Pf1) formulations on plant growth promotion

Treatment	Plant height (cm) (60 DAS)	Leaf Area Index (60 DAS)	Root length plant^{-1} (cm) (at harvest)	Nodules plant^{-1} (45 DAS)
T_1 – ST (Pf1) @ 10 g kg^{-1} seed	38.4^{ab}	3.9^{a}	34.2^{ab}	142^{b}
T_2 - FS (Pf1) @ 0.5% on 30, 45, 60, 75 & 90 DAS	35.6^{c}	3.4^{b}	31.1^{bc}	125^{b}
T_3 – ST + FS (Pf1) on 30, 45, 60, 75 & 90 DAS	40.2^{a}	4.1^{a}	36.4^{a}	166^{a}
T_4 – ST (Carbendazim 2 g kg^{-1} seed)	36.7^{b}	3.5^{b}	31.4^{b}	111^{c}
T_5 - FS (Mancozeb 0.2%) on 30, 45, 60, 75 & 90 DAS	32.4^{d}	3.6^{ab}	29.2^{c}	114^{c}
T_6 – ST (Carbendazim 2 g kg^{-1} seed) + FS (Mancozeb 0.2%) on 30, 45, 60, 75 & 90 DAS	36.1^{bc}	3.8^{a}	30.1^{c}	121^{bc}
T_7 - Control – Pathogen inoculated	29.4^{c}	2.8^{c}	21.2^{b}	77^{d}
T_8 - Control – Pathogen uninoculated	32.2^{d}	3.1^{bc}	25.6^{c}	104^{c}

Data followed by the same letter in a column are not significantly different (p=0.05) by DMRT

Table 2. Effect of different methods of *P. fluorescens* Pf1 formulation on late leaf spot management

Treatment	Disease intensity (grade)	Pod yield plant^{-1} (g)	Dry matter production plant^{-1} (g)
T_1 – ST (Pf1) @ 10 g kg^{-1} seed	4.9^{b}	5.4^{a}	46.4^{a}
T_2 - FS (Pf1) @ 0.5% on 30, 45, 60, 75 & 90 DAS	4.6^{b}	5.2^{ab}	47.2^{a}
T_3 – ST + FS (Pf1) on 30, 45, 60, 75 & 90 DAS	4.2^{a}	5.9^{a}	48.1^{a}
T_4 – ST (Carbendazim 2 g kg^{-1} seed)	5.2^{b}	4.4^{bc}	40.2cd
T_5 - FS (Mancozeb 0.2%) on 30, 45, 60, 75 & 90 DAS	4.8^{b}	4.6^{b}	41.3^{bc}
T_6 – ST (Carbendazim 2 g kg^{-1} seed) + FS (Mancozeb 0.2%) on 30, 45, 60, 75 & 90 DAS	4.2^{a}	5.2^{ab}	42.6^{b}
T_7 - Control – Pathogen inoculated	7.1^{c}	4.2^{c}	34.7^{f}
T_8 - Control – Pathogen uninoculated	5.2^{b}	4.6^{b}	38.2^{de}

Data followed by the same letter in a column are not significantly different (p=0.05) by DMRT

Pf1 formulation alone reduced the disease intensity, when it was followed by foliar application of Pf1 formulation, the disease was effectively controlled (Table 2). All the treatments were significantly superior to control. The boosting effect of Pf1 formulation on plant growth was evidenced by the fact that the plant height, leaf area index, root length, nodules per plant and dry matter production were the maximum in the combination of seed treatment and foliar application of Pf1 formulation (Table 1). It was even better than a carbendazim treatment. The beneficial effects of these bacteria, in most cases, have been related to their ability to produce plant growth hormones and or antimicrobial substances and to protect growing roots from deleterious root microbes present in the rhizosphere (Harish *et al.*, 2008). Paul and Sharma (2006) reported the production of two antibiotics – pyoluteorin and pyrrolnitrin by *P. fluorescens*, which inhibits the growth of *Phytophthora capsici*, the pathogen on black pepper. Sreenivasulu *et al.* (2006) reported that the volatile metabolite of *P. fluorescens* completely inhibited the pathogen of basal stem rot of coconut, *Ganoderma lucidum.*

Pf1 treatment also increased the pod yield per plant when compared to carbendazim treatment. Seed treatment followed by foliar application of Pf1 formulation gave the maximum pod yield per plant (Table 2). Significant increase in yield of several crops due to *P. fluorescens* had been reported (Saravanakumar *et al*., 2007). Recently reports on mechanism of biological control revealed that several microbial strains protect plants from various pests, diseases and phyto-nematodes in several crops by activating defense genes encoding chitinase, glucanase, peroxidase and synthesis of phytoalexins and inducing physiological changes (Kavino *et al*., 2007; Rajendran *et al*., 2007; Saravanakumar *et al*., 2007; Harish *et al.,* 2008).

The present studies indicated the usefulness of talc based powder formulation of Pf1 isolate for the control of late leaf spot of groundnut. Studies conducted so far have thus reinforced the prospects of using this biocontrol agent on a commercial scale as a successful alternative for chemical control of foliar diseases. Hence, this approach can be exploited as it is a natural, safe, effective, persistent and durable alternative to chemical pesticides for controlling plant diseases.

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