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APPRAISAL OF TWO *PSEUDOMONAS* SPECIES AS A BIOFERTIZER FOR SUN-FLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

The inoculation of sunflower (*Helianthus annuus* L.) var SF-107 was carried out with two species of *Pseudomonas* which resulted in significant plant growth promotion when cultivated in pots at 28 ± 2°C. Statistical analysis demonstrated that different species of *Pseudomonas* showed varying effects on vegetative growth of sunflower in comparison to recommended dose of NPK fertilizer. *Pseudomonas fluorescence* (BCCP # 083) significantly increased number of leaves and roots. Similarly maximum shoot growth in term of shoot height was observed by its inoculation. *Pseudomonas maltophilia* (BCCP # 097) significantly increased fresh biomass of plant.

Key words: *Pseudomonas,* chemical fertilizer, sunflower, inoculation, plant growth.

INTRODUCTION

Plant growth-promoting rhizobacteria colonize plant roots and enhance plant growth by a wide variety of mechanisms (Nelson, 2004). The use of PGPR in sustainable agriculture is gradually increasing and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. PGPR are found to be useful for various crops to enhance growth, seed emergence, crop yield, and disease control, and some have been commercialized. *Pseudomonas fluorescens* is a plant growth-promoting rhizobacterium. It significantly promotes the growth important crops and some other plants under greenhouse and field conditions. It was previously isolated from the roots of graminaceous plants, colonized the roots of various plants and produces an antibacterial compound effective against certain plant root pathogens (Choi *et al*., 2008).

Pseudomonads are common inhabitants of the rhizospheric soil. These beneficial microorganisms are significant component of management practices to achieve the attainable yield, which has been defined as crop yield limited only by the natural physical environment of the crop and its innate genetic potential (Cook, 2002).

The use of multi-strain or mono-strain inocula of PGPR with known functions is of interest as these formulations may increase consistency in the field. They offer the potential to address multiple modes of action, multiple pathogens, and temporal or spatial variability (Siddiqui and Shaukat, 2002).The concept of engineering or managing the rhizosphere to enhance PGPR function by manipulation of the host plant, substrates for PGPR, or through agronomic practices, is gaining increasing attention (Mansouri *et al*., 2002).Therefore, more recently there has been a recurrence of interest in environmental friendly bacteria for sustainable and organic agricultural practices (Esitken *et al*., 2005).

The sunflower (*Helianthus annuus*) is one of the four major oilseed crops (soybean, peanut, rapeseed and sunflower) grown for edible oil in the world. It is cultivated on about 23.31 million hectares all over the world with a production of 29.90 million tones. In Pakistan, sunflower is planted on about 0.363 million hectares in the four provinces. Medicinally, seeds are diuretic, expectorant, and used for colds, coughs, throat, and lung ailments. According to Hartwell 1967-1971, the flowers and seeds are used in folk remedies for cancer in Venezuela, often incorporated in white wine. The present work was carried out to evaluate the effect of mono inoculation of three Pseudomonas species on plant growth promotion of sunflower.

MATERIALS AND METHODS

Certified seeds of sunflower were obtained from Department of Agriculture, Punjab. Healthy seeds were sorted out; surface sterilized with 0.1% HgCl2 for four minutes; after thorough washing placed on moist filter paper in sterilized 9 cm Petri plates and percentage of germination was recorded after one weak. Plastic pots of 5 × 7″were filled with soil which was sieved by mesh size 40 and sterilized at 80 ºC for 12 hrs soil. Surface sterilized seeds were sowed in pots and placed in growth chamber at 28 ± 3°C.

The pot experiment was arranged in a randomized complete block design with five replications and five treatments. After emergence, the plants were thinned down to one plant per pot. One set of pots was inoculated with

P. flourescence (treatment P-1), second set of pots with *P. maltophilia* (treatment P-2) and third set with full NPK. Control pots received only distilled sterilized water.

Pure cultures of three selected species of Pseudomonas being identified and preserved in conservatory under accession numbers (BCCP # 083 and BCCP# 097) were grown on LGI semi solid medium and Luria Bertani (LB) solid and broth medium. The logarithmic growth phase of cultures was used for inoculation. The cells were harvested by centrifugation at 5,000 rpm at 4°C for 20 min. The supernatant was discarded and the pellet was washed two times with 0 .5% saline solutions and re-suspended in saline at a concentration of 108 colony forming units (CFU) per ml. 10 ml of the bacterial culture was inoculated to each plant in a pot.

The plants were grown under glasshouse conditions (28 ± 3°C day / 20 ± 2°C night) and were harvested at the end of 45 days. The shoot height of all the plants was measured from transition part to the tip of the plant after 15, 30 and 45 days of sowing. Five plants were recorded for each treatment and the data were statistically analyzed. The dry weights of shoot and root were estimated at the end of 45 days by drying the plant material at 60°C for 72 h in a hot air oven. Data was subjected to analysis of variance and means of samples were compared by Duncan's Multiple Range (DMR) test (Duncan, 1955)

RESULTS AND DISCUSSION

Statistical analysis through Duncan's Multiple Range (DMR) test (Steel and Torrie, 1955) at ($P < 0.05$) revealed that three tested species of *Pseudomonas* resulted in enhanced growth of sunflower (*Helianthus annuus* L.) var SF-107. Inoculation of *Pseudomonas fluorescence* significantly increased shoot height on successive days of sampling. *P. maltophilia* inoculation caused significant increase of plant biomass.

Effect of Pseudomonads inoculation on shoot height

A variety of rhizospheric microbes including *Pseudomonas* and *Bacillus* species, have been found to be beneficial for plant growth (Weller, 1988; Bakker and Schippers, 1991).As a result of inoculation by three different species of *pseudomonas* effects on the shoot height is presented in the (Table 1). All the treatments stimulated the plant growth over control at the different stages. *Pseudomonas fluorescence* (P-1) led to the significance increase 8.9, 11.2 and 13.4 inches in shoot height after 15, 30 and 45 days respectively. *P. maltophilia* (P-2) also resulted in increased 5.7, 9.3 and 11.6 inches shoot height after 15, 30 and 45 days respectively, the increase of P-2 was not as pronounced as in case of P-1 as well as it is not significantly different for both treatments (Table 1). Treatment with NPK gave an increase of 7.75 and 19.5 cm respectively. All treatments resulted in increased shoot height over the control irrespective of sampling days.

Table 1. Effect of Pseudomonads inoculation on shoot height of sunflower (*Helianthus annuus*)

Days after sowing	Shoot height (cm)			
	Control	**P-1**	**P-2**	**NPK**
15	17.5k	22.25f	14.27i	7.75j
30	15.5h	28c	23.25e	19.5g
45	21f	33.5a	29b	60d

Abbreviation
Control = control; **P-1**=*P. fluorescence*; **P-2** = *P. maltophilia;* **NPK=**Chemical fertilizer
Each value followed by the different letter in the columns are significantly different ($P < 0.05$), according to DMR test.

Effect of Pseudomonads inoculation on plant biomass

In this study all the species increased plant fresh as well as dry biomass over control as in (Table 2). Some variable effect of inoculation were observed as *P. maltophilia* (P-3) showed maximum increase in plant fresh (0.621gm) and dry biomass (0.526 gm) significantly over control i.e. fresh biomass (0.476 gm) and dry biomass (0.382 gm) *P. fluorescence* (P-1) and NPK showed minimum increase in plant biomass i.e. fresh biomass (0.56 gm) and dry weight (0.411 gm), fresh biomass (0.51 gm) and dry weight (0.412 gm) over control (Table 2). These variations in plant-specific growth stimulation could be attributed to differential behavior of the *Pseudomonas* species to composition of root exudates, temperature variation or to their interaction with rhizosphere microflora predominant in the particular crop (Miller *et al*.,1989; Seong *et al*.,1991and Sindhu *et al*.,1999).

Table 2. Effect of Pseudomonads inoculation on biomass of sunflower (*Helianthus annuus*)

Treatments	weight(g)	
	Fresh biomass	Dry biomass
Control	0.47d	0.38f
P-1	0.56b	0.41e
P-2	0.62a	0.52c
NPK	0.51c	0.42e

For each value followed by the different letters in each column is significantly different ($P < 0.05$) according to DMR test.

Effect of Pseudomonads inoculation on number of leaves and roots

Number of roots and leaves increased by inoculation over control (Table 3). *P. fluorescence* (P-1) inoculation increased maximum number of leaves (5.6) and roots (7.6) non significantly. *P. maltophilia* (P-2) was observed to be less effective on number of leaves (4.9) and roots (6.6) similar to the NPK treatment showed number of leaves(4.9) and number of root (6.8) as compared to control (Table 3). *Pseudomonas fluorescens* was reported to enhance the plant growth by action as a plant growth-promoting bacterium as well biocontrol agent against plant pathogens (Chunxia *et al*., 2004).

Table 3. Effect of Pseudomonads inoculation on number of leaves and roots of sunflower (*Helianthus annuus*)

Treatments	Number	
	Leaves	**Roots**
Control	3.8c	5.4b
P-1	5.6b	7.6a
P-2	4.9bc	6.6ab
NPK	4.9bc	6.8ab

For each value followed by the different letters in each column is significantly different ($P < 0.05$) according to DMR test.

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