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EFFECT OF SOIL AMENDMENT WITH *LAUNAEA NUDICAULIS* L. ON BIOCONTROL POTENTIAL OF ENDOPHYTIC FLUORESCENT *PSEUDOMONAS* AGAINST ROOT ROT DISEASE OF SUNFLOWER

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ABSTRACT

Application of endophytic *Pseudomonas aeruginosa, P. putida* and *P. monteilii,* in soil amended with *Launaea nudicaulis,* a medicinal plant showed a significant suppression of *Macrophomina phaseolina* on sunflower in screen house trials as compared to bacteria applied in un-amended soil. Treatment of *P. aeruginosa* alone or with combination of *L. nudicaulis* showed a complete reduction of *Rhizoctonia solani*. *F. solani* significantly ($p<0.05$) control by the use of *P. aeruginosa* with *L. nudicaulis.* Maximum fresh shoot weight was noticed after the inoculation of *P. aeruginosa* individually and with *L. nudicaulis.*

KEYWORDS: Biocontrol potential, *Pseudomonas aeruginosa,* Root rots, Sunflower, *Launaea nudicaulis,* Soil amendment.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) belongs to family Asteraceae is one of the most edible seed crops (Khan *et al.,* 2009) grown worldwide over 22 million hectares with 26 million tones production (Skoric *et al.,* 2007). It is considered as premium oil seed crop and most important cash crop in all countries including Pakistan because of unsaturated fatty, light colour and lack of linolenic acid (Mukhtar, 2009). Sunflower crop severely attacked by number of pathogens especially root rotting fungi. *Macrophomina phaseolina,* causal agent of charcoal rot disease causes severe looses in Pakistan (Ijaz *et al*., 2013).

There is a growing awareness that fertilizers and pesticides damage the human health and environment (Perkins and Patterson, 1997). Presently, there is a development to discover various techniques to reduce the application of fungicides (Yiridoe *et al.,* 2005). The biocontrol agents and different management strategies (e.g., cultural practices, cover crops and organic amendments) are supposed to be less damaging than straight fungicides and possibly remarkable in controlling plant infections (Elad and Shtienberg, 1996). By the increasing attention towards biocontrol agents in the rhizosphere, it grows to be particular significance to distinguish the efficient biocontrol agents in diverse situation. Due to adverse environmental conditions and competition among the biocontrol microbes become ineffective and mixtures of technique, or combination offer more defense against pathogens (Izhar *et al*., 1995; Ehteshamul-Haque *et al*., 1995; Lazarovits and Nowak, 1997).

Management and handling of natural communities of hostile microbes with biofertilizers received less attention, instead of their effectiveness (Hoitink and Boehm, 1999; Rahman *et al.,* 2016; Urooj *et al.,* 2018). Soil alteration with organic matters have the imminent to control plant diseases through a multiplicity of mechanisms, together with chemical, such as producing antimicrobial compound during decaying (Tenuta and Lazarovits, 2002; Sarfaraz *et al.,* 2017) and biochemical (Farhat *et al.* 2017; Mazzola, 2004).

Now a day's scientists giving attention towards ecofriendly release of strategies for infection control instead of application of synthetic chemicals which have hazardous effects. *Launaea nudicaulis* L., a familiar medicinal plant belongs to family Asteraceae. It is one of the ordinary weeds of field crops with characteristic allelopathic property, but its effect on root infecting organism received little attention (Mansoor *et al.,* 2007). Current research report illustrates the outcome of soil amendment with *L. nudicaulis* on the effectiveness of endophytic *Pseudomonas aeruginosa, P. putida* and *P. monteilii* in decreasing the disease intensity caused by root-infecting fungi on sunflower.

MATERIALS AND METHODS

Bacterial culture and plant material: Cultures of endophytic isolates of *Pseudomonas aeruginosa*, *P. montelii* and *P. putida* applied in this research were formerly isolated from *Salvadora* spp. and identification was reported somewhere else (Korejo *et al.,* 2019).

Screen house experiment: In sandy loam soil (pH 8.0) dry powder of *L. nudicaulis* was mixed at 1.0% w/w. The infestation of soil with *Rhizoctonia solani* (2-6% colonization) was check by the method of Wilhelm, (1955). Seven sclerotia per gram soil of *Macrophomina phaseolina* determined by Sheikh and Ghaffar (1975) method of wet sieving and dilution plating and mixed population of *Fusarium oxysporum* and *F. solani* (3000 cfu g^{-1} of soil) was observed through soil dilution technique of Nash and Snyder (1962). Plastic pots having 8 cm diameter were used, containing 300 g amended soil. Pots were reserved at 50% water holding capacity by daily watering (Keen and Raczkowiski, 1921). Following three weeks of *L. nudicaulis* decomposition, 25 mL per pot aqueous suspensions of fluorescent *Pseudomonas* (3.8 $x10^8$cfu/mL) full-grown on KB broth were drenched on top of amended and non-amended soil in pots. Similarly, 25 mL per pot aqueous suspension (100 ppm) of carbendazim (fungicide) was also drenched for control treatment. Six seeds of sunflower (*Helianthus annuus*) were sown in each pot. After germination four seedlings were kept per pot and excess were removed and discarded. Pots without amendment/antagonists or fungicides served as controls. Each treatment was replicated four times and pots were placed in a screen house in a randomized complete block design.

Determination of fungal infection and growth parameter: To assess the potential of fluorescent *Pseudomonas* against root disease, plants were harvested after 45 days. To evaluate the infection of root rot fungi, method used by Habiba *et al*. (2016) were chosen.

Plant physical growth parameters such as plant height and weight, root length and root weight were also recorded.

RESULTS AND DISCUSSION

The management of root-infecting fungi by using biocontrol agents and phytochemicals offers alternative method to the use of synthetic inorganic pesticides. A large number of plant material has been found effective against parasitic fungi related with crop plants (Ehteshamul-Haque *et al.*, 1995; 1996; Rahman *et al.*, 2016, Urooj *et al.*, 2018). In the present study application of *L. nudicaulis* significantly suppressed the infection by soil borne plant pathogen on sunflower. *L. nudicaulis* is a medicinal plant and its latex used in constipation, leaves give relieve in fever, provide relief in itching, cuts, ulcer and eczema (Rashid *et al.,* 2000). Similarly, antifungal and antibacterial activity of *L. nudicaulis* has been reported (Rashid *et al.*, 2000).

In this study applications of endophytic *Pseudomonas aeruginosa, P. putida* and *P. monteilii,* in soil amended with *L. nudicaulis* significantly suppressed *R. solani, F. solani* and *M. phaseolina* (Tables 1 and 2). Plant growth promoting bacteria (PGPR) have been used to enhance plant growth either by direct stimulation or by inhibition of phytopathogens (Noreen *et al.*, 2015, 2018; Weller *et al*., 2002). Among different rhizospheric bacteria, fluorescent pseudomonades are aggressively colonize on different plant crops and have large antagonistic potential against the phytopathogens (Weller *et al.,* 2002; Rahman *et al.,* 2016; 2017). Similar application of *P. aeruginosa* alone or in soil amended with *L. nudicaulis* significantly increased plant height and fresh weight of shoot (Tables 3 and 4).

In this study application of endophytic fluorescent *Pseudomonas* in soil amended with *L. nudicaulis* significantly increased their suppressive potential against root rotting fungi. In avocado and citrus, biocontrol agent with agricultural and urban waste were effective to suppressing root pathogen (Casale *et al.*, 1995). In the same way use of some compost to soil increased PGPR population in tomato rhizosphere having inhibitory effects towards *Fusarium, radicis-lycopersici, Pyrenocheta lycopersici, Pythium ultimum* and *Rhizoctonia solani* (Alvarez *et al.,* 1995). Application of microbial antagonists in soil amended with neem cake or seaweed have been reported to increase their biocontrol potential via inducing systemic resistance in plants against root rotting fungi (Shafique *et al.*, 2015; Rahman *et al.*, 2017). Mansoor *et al.* (2007) have reported enhancement of biocontrol potential of microbial antagonists by the *L. nudicaulis.* Application of PGPR with common medicinal weeds *L. nudicaulis* holds promise for the control of root diseases of sunflower.

Table 1. Effect of endophytic fluorescent *Pseudomonas* on the infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* and *F. oxysporum* on sunflower in natural soil (un-amended with *L. nudicaulis*).

Treatments	*F. Solani*	*R. solani*	*M. phaseolina*
		Infection %	
Control	31.2	25	6.2
Carbendazim	0	6.2	6.2
P. montelli	0	12.5	12.5
P. aeruginosa	18.7	0	0
P. aeruginosa	6.2	0	12.5
P. aeruginosa	18.7	0	6.2
P. putida	18.7	0	18.7
$LSD_{0.05}$	**Treatment = 2.81; Pathogens = 2.13**		

[1]Mean values in column showing differences greater than LSD values are significantly different at $p< 0.05$

[2]Mean values in rows showing differences greater than LSD values are significantly different at $p<0.05$

Table 2. Effect of endophytic fluorescent *Pseudomonas* on the infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* and *F. oxysporum* on sunflower in soil amended with *L. nudicaulis.*

Treatments	*F. Solani*	*R. solani*	*M. phaseolina*
	Infection %		
Control (*L. nudicaulis* at 1% w/w)	31.2	0	25
L. nudicaulis at 1% w/w + Carbendazim	25	0	25
L. nudicaulis at 1% w/w + *P. montelli*	18.7	0	25
L. nudicaulis at 1% w/w + *P. aeruginosa*	25	0	18.7
L. nudicaulis at 1% w/w + *P. aeruginosa*	0	0	25
L. nudicaulis at 1% w/w + *P. aeruginosa*	25	0	25
L. nudicaulis at 1% w/w + *P. putida*	25	0	18.7
$LSD_{0.05}$	**Treatment = 5.2; Pathogens = 3.9**		

[1]Mean values in column showing differences greater than LSD values are significantly different at p< 0.05
[2]Mean values in rows showing differences greater than LSD values are significantly different at p< 0.05

Table 4. Effects of endophytic fluorescent *Pseudomonas* in soil amended with *Launaea nudicaulis* on the growth of sunflower.

Treatments	**Shoot length (cm)**	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**
Control + *L. nudicaulis* at 1% w/w	30.5	4.0	4.3	0.39
L. nudicaulis at 1% w/w + Carbendazim	40.7	5.4	7.8	0.8
L. nudicaulis at 1% w/w + *P. monteilli*	35.7	4.7	5.6	0.8
L. nudicaulis at 1% w/w *P. aeruginosa*	32.6	4.1	5.6	0.8
L. nudicaulis at 1% w/w + *P. aeruginosa*	41.9	6.3	18.6	2.2
L. nudicaulis at 1% w/w+ *P. aeruginosa*	35.3	5.8	2.3	0.8
L. nudicaulis at 1% w/w + *P. putida*	35.3	3.3	5.2	0.3
$LSD_{0.05}$	**12.04**	**3.02**	**2.6**	**0.59**

Table 3. Effects of endophytic fluorescent *Pseudomonas* on the growth of sunflower plants in natural soil (un-amended).

Treatments	**Shoot length (cm)**	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**
Control	30.35	2.3	10.8	0.43
Carbendazim	33.02	2.34	13.0	0.51
P. monteillii	33.05	2.13	8.01	0.38
P. aeruginosa	42.7	3.56	11.0	0.55
P. aeruginosa	38.85	2.7	12.7	0.74
P. aeruginosa	39.25	3.05	9.5	0.59
P. putida	33.1	2.34	9.62	0.42
$LSD_{0.05}$	**7.04**	**0.84**	**3.24**	**0.29**

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