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EVALUATION OF CONCURRENT TREATMENT OF RHIZOBIA AND NODULE ASSOCIATED FLUORESCENT *PSEUDOMONAS* ALONG WITH CHEMICAL FERTILIZERS ON ROOT ROTTING FUNGI AND ROOT KNOT NEMATODE OF CHICKPEA

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ABSTRACT

The influence of rhizobia and fluorescent *Pseudomonas* associated with root nodule and chemical fertilizers on the growth and root rotting fungi and nematode has been determined in this study. Sixteen treatments were laid in randomized complete block design in screen house experiment. Treatments of inorganic fertilizers such as NPK or urea along with concurrent treatments of rhizobia and fluorescent *Pseudomonas* had adversarial effect on root rotting fungi such as *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum.* Penetration of root knot nematode *Meloidogyne javanica* was decreased maximum in combined application of nodule associated fluorescent *Pseudomonas* and rhizobia than untreated control which eventually improved chickpea growth.

KEYWORDS: Biological control, Fertilizer, Root infecting fungi, Root knot nematode, Chickpea.

INTRODUCTION

Chickpea (*Cicer arietinum* L.), the 3rd most important leguminous crop is rich in carbohydrates, protein, minerals (P, Zn, Ca, Fe and Mg) and carotene with better quality protein than other legumes (Jukanti *et al.*, 2012; Siddique *et al.*, 2012). Leguminous plants are characterized to form symbiotic relationship with nodule inhabiting bacteria which in turn increase soil fertility and fixation of nitrogen (Mahmood and Athar, 2008; Mandal *et al.*, 2009; Noreen *et al.*, 2018) but when nitrogenous fertilizer are applied as initial dose, it increases yield and growth of crops (Leghari *et al.*, 2016).

Inorganic fertilizers are applied to rise the produce, nourishing qualities and appealing significance of crops. An alternate source of fertilization in the form of biofertilizers has been developed by scientists which produces greater yield and friendly to the environment (Baset Mia *et al.*, 2010). Minerals have greater effect on defense mechanism of plant (Waraich *et al*., 2011). Biofertilizers increase the production of crops by increasing fixation of nitrogen present in atmosphere and increasing the accessibility of nitrogen and phosphorus to plant (Selvakumar *et al.*, 2012). Biofertilizers diminishes the expanse of phosphate fertilizer and improve nutrient absorbance to increase crop yield

(Welley *et al.,* 2005). Various agricultural systems has been developed in the world to use of biofertilizers as an eco-friendly and cost effective procedure to increase crop productivity which limits the use of non-renewable assets (Caliskan *et al.,* 2013). Phosphorus makes up 0.2% plant dry weight and its involved in seed formation and is present in abundance in seed and fruits. It is not only involved in improving the quality of fruits, vegetables and forage but is also involved in improving plant growth by increasing root formation and increasing resistance in plant (Dordas, 2008).

In general, higher yield of plants are obtained by excessive use of inorganic fertilizers which in turn increase soil acidity, unbalancing nutrient content in plant, inhibiting plant growth (Stewart *et al.,* 2005). Chemical fertilizers are deposited to form a sink source which are released during vegetative growth. Increment of plant growth was observed when chemical fertilizers are applied in combination which makes increased absorption of N, P and K (Sifola and Barbieri, 2006; Shams, 2012). The chances of some diseases may decrease by the uptake of some nutrients (Bhaduri *et al*., 2014). Combined application of two biocontrol agents along with mineral fertilizer increased plant growth via suppression of root rotting fungi and root knot nematode (Parveen *et al*., 2008). NPK utilization reduces population of *M. javanica* (Olowe, 2012). Many diseases can also be reduced by the utilization of different nitrogen fertilizers (Ihejirika *et al*., 2006). Significant enhancement of growth was observed by application of chemical and biofertilizers (Salah Uddin *et al.,* 2009). The aim of present study is to evaluate the combined effect of root nodule associated rhizobia and fluorescent *Pseudomonas* with chemical fertilizers in suppressing the root diseases of chickpea.

MATERIALS AND METHODS

Bacterial cultures: Bacterial cultures of rhizobia and fluorescent *Pseudomonas* were obtained from nodules of mungbean (*Vigna radiata* L.) plant which has been previously reported for their biocontrol potential and molecular identification of fluorescent *Pseudomonas* (Noreen *et al.,* 2015) and rhizobia (Noreen *et al.,* 2019) has been reported).

Role of *Pseudomonas* and rhizobia along with chemical fertilizers in screen house experiment: Biocontrol agents such as *Rhizobium vignae* {NFB-103 (4.7×10^8 cfu/mL)} and fluorescent *Pseudomonas* NAFP-19 (2.3×10^8 cfu/mL) along with urea (N_2H_4CO) and NPK (chemical fertilizers) individually or in combination were used in this experiment. Chickpea (*Cicer arietinum* L.) seeds (8) were sown in clay pots each containing 1 Kg soil. After germination four seedling were kept after sprouting. The soil had 3-6 sclerotia/g of *Macrophomina phaseolina* (Sheikh and Ghaffar, 1975), 5-10% colonization of *Rhizoctonia solani* on sorghum seeds (bait) (Wilhelm, 1955) and 3000 cfu/g infestation of *Fusarium solani* and *F. oxysporum* (Nash and Snyder, 1962). NPK was amended in pots at 0.1 g/ kg soil or urea 0.15 g/1 kg or mixture of both urea and NPK. At two leaves stage of seedling pots were inoculated with 2000 egg/female of *Meloidogyne javanica*. Randomized complete block design was applied for experiment and each treatment was replicated four times with 50% water holding capacity were retained in green house. After 45 days of nematode inoculation, experiment was terminated, growth parameters and infection by root rotting fungi and root knot nematode were determined. Infection of root rot fungi was determined by using method of Noreen *et al*., (2015), while nematode's penetration in roots was examined by using the method described by Siddiqui *et al*. (2000).

RESULTS

Concurrent treatment of rhizobia and fluorescent *Pseudomonas* along with chemical fertilizers on chickpea: Greater plant height was seen in Urea along with NPK and NAFP-19 treatment whereas shoot weight amplified considerably in NPK along with NAFP-19 and *R. vignae* (NFB-103) treatment. Even though root length improved considerably in NAFP-19 treatment whereas treatment with urea and NPK increased root weight in contrast to control (Table 1). Individual and combined treatment of NPK and urea with biocontrol agents significantly controlled infection of root rot fungi. Infection of *M. phaseolina* completely suppressed by NPK(0%) followed by Urea + NPK + NAFP-19, Urea + NPK + *R. vignae* (NFB-103) and Urea + NPK + NAFP-19 + *R. vignae* (NFB-103) (6.2%) than control (100%) while all treatments suppressed infection of *R. solani* and *F. oxysporum* than control plants. Infection of *F. solani* significantly controlled by NAFP-19, NPK + *R. vignae* (NFB-103) and NPK + NAFP-19 + *R.vignae* (NFB-103) (31.2%) than control (100%) (Table 2). Significant decline of nematode population was detected in NPK with NAFP-19 and *R. vignae* (NFB-103) treatment. While, incidence of nematode's population in roots was decreased maximum in NPK + NAFP-19 + *R. vignae* (NFB-103) followed by NPK than untreated control plants (Table 2).

DISCUSSION

Plant resistance against disease can be increased by treatment with mineral fertilizers which thickens cell wall and cuticle which ultimately hinder the penetration of pathogen (Huber and Haneklaus, 2007). Protein can be synthesized by absorbed nitrogen and potassium, while the process of photosynthesis, reproduction and water maintenance, phosphorylation, thickness of cell wall increased by K level, declining nitrates, construction of new tissues and amino acid assembly are done by association of K in necessarily all cellular functions (Huber, 1980a,b).

During this work, individual or collective application of rhizobia and fluorescent *Pseudomonas* along with NPK 0.10 g/ kg and urea 0.15 g/kg of urea in soil helped in increasing the development of chickpea and repressed the growth of phytopthogenic organism which are similar to the results described by Parveen *et al.* (2008). Rhizobia along with fluorescent *Pseudomonas* in addition with NPK and urea significantly reduced infection of *M. phaseolina* in chickpea. Previous studies also showed that treatment of biological agents along with fertilizers suppressed the growth of *R. solani* and *F. oxysporum* along with *Meloidogyne* spp. (Ashoub and Amara, 2010).

Nitrogen concentration around roots and dry weight of plant is enhanced by inoculation of plant growth promoting rhizobacteria (Baset Mia *et al.,* 2010). Treatment with nitrogen fertilizers enhanced the release of root exudates which enhance the number of antagonists in the vicinity of roots (Marschner, 1995). Root knot nematode is reported to be significantly reduced by the treatment of ammonium based fertilizers (Wachira *et al.,* 2009). Concurrent treatment of mineral fertilizers, compost along with biological inoculant enhanced the wheat's productivity (Akhtar *et al.,* 2009) while *P. aeruginosa* (LES4) and NPK treatment enhanced the growth of seasame (*Sesamum indicum* L.) (Kumar *et al.,* 2009).

Table 1. Effect of chemical fertilizers with isolate of fluorescent *Pseudomonas* and rhizobia used as soil drench on the growth of chickpea plants.

Treatments	Growth parameter			
	Shoot length (cm)	**Shoot weight (gm)**	**Root length (cm)**	**Root weight (gm)**
Control	39.66	3.17	17.83	1.05
Urea	38.87	3.76	15.25	0.88
NPK	37	3.46	20.46	0.90
Urea + NPK	38.31	4.49	19.87	1.48
NAFP-19	36.56	2.86	20.52	0.66
Urea + NAFP-19	41.72	2.94	17.32	0.69
NPK + NAFP-19	38.45	3.35	15.39	0.73
Urea + NPK + NAFP-19	44.54	4.51	14.08	0.67
R. vignae (NFB-103)	41.61	3.62	16.53	1.10
Urea + *R. vignae* (NFB-103)	36.67	3.14	11.82	0.69
NPK + *R. vignae* (NFB-103)	37.56	3.24	13.06	0.58
Urea + NPK + *R. vignae* (NFB-103)	39.46	3.85	15.06	0.62
NAFP-19 + *R. vignae* (NFB-103)	41.12	3.60	17.16	0.70
Urea + NAFP-19 + *R. vignae* (NFB-103)	41.45	4.12	14.91	0.61
NPK + NAFP-19 + *R. vignae* (NFB-103)	43.43	5.18	12.84	0.80
Urea + NPK + NAFP-19 + *R. vignae* (NFB-103)	34.25	2.89	13.68	0.52
$LSD_{0.05}$	**5.97**[1]	**1.60**[1]	**5.19**[1]	**0.52**[1]

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

Table 2. Effect of chemical fertilizers with isolate of fluorescent *Pseudomonas* and rhizobia used as soil drench on root infection by *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani, F. oxysporum* and root-knot nematode on chickpea plants.

Treatments	Infection %				
	M. phaseolina	***R. solani***	***F. solani***	***F. oxysporum***	**Juveniles & female/ g root**
Control	100	43.7	100	25	16
Urea	37.5	0	56.2	6.2	10
NPK	0	0	50	0	5
Urea + NPK	50	0	50	0	5.2
NAFP-19	25	0	31.2	0	8.5
Urea + NAFP-19	37.5	0	68.7	0	5.2
NPK + NAFP-19	25	0	43.7	0	5.7
Urea + NPK + NAFP-19	6.2	0	50	0	4.7
R. vignae (NFB-103)	18.7	0	37.5	0	5.2
Urea + *R. vignae* (NFB-103)	37.5	0	50	6.2	5.5
NPK + *R. vignae* (NFB-103)	18.7	0	31.2	0	6
Urea + NPK + *R. vignae* (NFB-103)	6.2	0	37.5	0	7
NAFP-19 + *R. vignae* (NFB-103)	31.2	0	87.5	6.2	5.5
Urea + NAFP-19 + *R. vignae* (NFB-103)	18.7	0	43.7	0	5.5
NPK + NAFP-19 + *R. vignae* (NFB-103)	31.2	0	31.2	0	4
Urea + NPK + NAFP-19 + *R. vignae* (NFB-103)	6.2	0	68.7	6.2	5.7
$LSD_{0.05}$					**1.72[1]**
	Treatments = 12.58[1]		**Pathogens = 6.29[2]**		

[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 $p<0.05$

CONCLUSION

This work concluded that utilization of endo-nodule rhizobia and fluorescent *Pseudomonas* accompanied by chemical fertilizers has great impact on chickpea growth and also caused repression of root rot and root knot infection.

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