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EFFECTS OF HERBICIDES AND MICROBIAL ANTAGONISTS ON ROOT PATHOGENIC FUNGI AND GROWTH OF CROP PLANTS

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Abstract

The aim of present study was to examine the effects of herbicides and microbial antagonists combinedly on growth and root rot diseases of crop plants. For this bromacil and bromoxynil herbicides and antagonists like *Bacillus thuringiensis* and *Rhizobium meliloti* were used. Pot experiment was conducted in which herbicides and antagonists were used separately and combinedly as seed dressing and soil drenching. Results indicated that combination between *B. thuringiensis* soil drenching with bromacil seed treatment improved growth of cowpea and mung bean plants. Root infecting pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., were significantly reduced or completely controlled as a result of combined applications of both bacterial antagonists with herbicides.

KEYWORDS: Bromacil and bromoxynil, Cowpea, Mung bean, Root infecting fungi, Soil drenching, Seed treatment.

INTRODUCTION

Herbicides are the chemicals used to control unwanted plants, also referred as "weed killers". Main purpose of removing the weed from field is to reduce the competition as weeds appear faster and grow rapidly than crop using the resources like nutrients, moisture, sunlight and space during vegetative and early reproductive stages (Isik *et al*., 2009). The application of herbicide in field depends largely on its effectiveness and economics like some microorganisms are degraded due to herbicide, while some others were harmfully affected by it (Zain *et al*., 2013; Sebiomo *et al*., 2011). Bromacil (5-Bromo-3sec-butyl-6-methyluracil) is an effective herbicide used to control perennial, annual, and broadleaf grasses and also used as inhibitor of photosynthesis (Gardiner *et al*., 1969). Some of the fungi, however, utilize the bromacil molecule or parts of the molecule as a carbon and energy source (Wolf *et al*., 1975). Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile; $C_7H_3Br_2NO$), is a nitride herbicide. This herbicide is used for the control of weeds. The weeds around crops like cereal, corn, sorghum, onions, flax, mint, turf, were controlled due to use of bromoxynil by inhibiting photosynthesis in the target plants (Cupples *et al*., 2005).

The effect of herbicides may be positive as well as negative on soil microorganisms. Microorganisms enhance the soil quality due to their function (Scholter *et al*., 2003). Applied herbicides are deleterious for soil microorganisms (El-Ghamry *et al*., 2000; Pampulha and Oliveira, 2006). Bromoxynil herbicide reported to reduce population of nitrifying bacteria in soil (Ratnayak and Audus, 1987).

The biocontrol agent (BCA) is referred to the organism which suppresses the pest or pathogen e.g., *Pseudomonas* known to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) which can aggressively colonize roots and suppresses pathogen activity in the wheat rhizosphere through competition for organic nutrients (Raaijmakers *et al.,* 1999). In contrast, to chemical used, mostly researchers used beneficial organisms to improve the growth of plant with improvement in soil productivity and also environmental health (O'Connell, 1992; Esitken *et al*., 2005). The number of bacterial species associated with rhizosphere such as *Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Beijerinckia, Burkholdria, Enterobacter, Erwinia, Flavobacterium, Rhizobium* and *Serrotia* species, are found to be beneficial for plant growth, yield and crop quality (Bashan and de-Bashan, 2005). Keeping in view the benefits of BCA, an experiment was carried out to evaluate the effects of herbicides in combination with biocontrol agents on the growth and in the control of root infecting fungi of leguminous plants.

MATERIALS AND METHODS

Herbicides and microbial antagonists' collection: Herbicides like Bromacil and Bromoxynil were purchased from Agrochemical market of Karachi. These concentrated liquid were diluted by adding sterile distilled water to make different concentrations (0.1, 1 and 2% v/v). 2% concentration gave better result in the improvement of crop growth so, this concentration was further used (Zehra, 2016). However, microbial antagonists includes *Bacillus thuringiensis* and *Rhizobium meliloti* were obtained from Karachi University Culture Collection (KUCC). These antagonists were grown on nutrient broth medium and yeast extract mannitol agar (YEMA), respectively, incubated at 28-30°C for 24-96 h depending upon population growth. Bacterial colonies (*B. thuringiensis* and *R. meliloti*) growing on Petri plates were counted and multiplied by the dilution factor which gave CFU/mL of bacteria (Yadav *et al.,* 2010).

Experimental setup: Seeds of mung bean and cowpea were surface sterilized using sodium hypochlorite (1%) for three minutes, rinsed thoroughly in running tap water and dried aseptically. After which seeds were coated with *B. thuringiensis* and *R. meliloti* separately by using 1% gum Arabic solution. Seeds were air dried, sown in pots containing 300 g soil. Herbicides (bromoxynil and Bromacil at the rate of 2%v/v) were drenched in soil and the plants allowed growing upto 30 days after germination. After thirty days plants were removed from pots and record data for growth of crops along with the colonization % of root rot fungi. For this, roots were washed in running tap water, surface sterilized with 1% sodium hypochlorite and then placed on PDA poured plates (five root pieces). Plates were incubated for 5 days at room temperature after which colonization of roots by pathogenic fungi were recorded.

In another experiment soil was drenched with microbial antagonists (10 mL/pot) and seeds of tested crops were treated with 2% herbicide. Similar procedure was adopted as mentioned as above Data obtained were analyzed using COSTAT software.

RESULTS AND DISCUSSION

Combination of herbicide and microbial antagonists showed effective results on cowpea growth. Significant ($p<0.05$) increment in shoot length, root length and root weight were recorded when *B. thuringiensis* soil drenching used in combination with bromacil seed treatment. However, shoot weight was maximum when *R. meliloti* soil drenching combined with seed treatment with bromacil. Similar results were found by Rafi and Dawar (2014) where bio priming of leguminous and non-leguminous seeds at different intervals with fungal and bacterial antagonists such as *P. variotii, T. harzianum, R. meliloti* and *B. subtilis* enhanced growth parameters and reduced the incidence of root rot fungi. Decrease in frequency of colonization % of root rot fungi were recorded by combined effect of microbial antagonists with herbicides. Maximum reduction in colonization of *Fusarium* spp., was recorded when combination of *B. thuringiensis* soil drenching with bromacil seed treatment was used. Furthermore, complete reduction of *R. solani* was recorded when *B. thuringiensis* soil drenching with bromoxynil seed treatment and *R. meliloti* soil drenching with bromoxynil seed treatment were used in combination. However, *R. meliloti* soil drenching with bromoxynil seed treatment, *B. thuringiensis* seed treatment with bromoxynil soil drenching were effective in complete inhibition of *M. phaseolina* colonization ($p<0.05$; Table 1).

In case of mung bean, all growth parameters (shoot length, shoot weight, root length and weight) were significantly enhanced ($p<0.05$) by combination of *B. thuringiensis* soil drenching with bromacil seed treatment. Both herbicides used as seed treatment in combination with *B. thuringiensis* soil drenching reduced colonization % of *Fusarium* spp. However, *R. solani* colonization % was reduced by application of *B. thuringensis* seed treatment with bromoxynil soil drenching, *B. thuringiensis* seed treatment with bromacil soil drenching, *R. meliloti* soil drenching with bromacil seed treatment, *R. meliloti* seed treatment with bromacil soil drenching, *B. thuringiensis* soil drenching with bromacil seed treatment and *B. thuringiensis* soil drenching with bromoxynil seed treatment (Table 2). Complete reduction of *M. phaseolina* was observed by the application of *R. meliloti* soil drenching with bromacil seed treatment.

Bacteria produced different kinds of metabolites including antibiotics and toxins which inhibit the growth of pathogenic organisms (Laura *et al*., 1998). Treatment of cotton seeds with *Gliocladium virens* and *Bacillus* spp. reduced the colonization of roots by *F. oxysporum* and reduced *Fusarium* wilt incidence and severity (Zhang *et al*., 1996).

Herbicides produce changes in the physiology and development of crop plants. The life of bromoxynil in soil is few days with minimum contamination in soil and food materials and its safer to use for wheat during spring fall season (Reddy *et al.,* 2012; Grewal and Sharma, 2017). Changes like plant exudates modification and associated microorganism stimulation or inhibition is reported by Mussa and Russel (1977). Steven (1969) reported that total soluble protein of shoots was increased by Barban and Bromacil. According to Davison (1983), by using herbicides, trees and other ornamental plants growth parameters increased. The studies on root system showed increased in root length and weight when herbicides in combination with antagonists were applied (Schnelle *et al*., 1989).

It is necessary to assess the positive effect of herbicides on target plants. Plant growth regulators have been recognized to stimulate growth and development of plants under optimal conditions and tolerance of plants to biotic and abiotic stresses (Ahammed *et al.,* 2013; Bhajbhuje, 2015). Application of some herbicides resulted in increased lipid peroxidation and proline accumulation (Langaro *et al.,* 2016). Experiments have shown that microbes may use herbicides as a source of carbon. It increased the microbial populations with time period (Radosevich *et al.,* 1995).

Table 1. Effect of microbial antagonists in combination with herbicides on growth and colonization % of root infecting fungi of cowpea.

Antagonists + Herbicides	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	*F. oxysporum*	*R. solani*	*M. phaseolina*
Control	18.2	1.03	7	0.226	80	60	53.33
R. meliloti (S.T)	21.33	0.89	7.66	0.29	26.66	20	20
B. thuringiensis (S.T)	19.1	0.623	8.66	0.366	13.33	13.33	13.33
Bromacil (S.D)	21.16	0.93	9.66	0.306	40	20	13.33
Bromoxynil (S.D)	20	1.2	7.56	0.39	33.33	13.33	20
R.meliloti (S.D)	20.83	1.156	10.33	0.413	26.66	13.33	6.66
B. thuringiensis (S.D)	22	0.95	8	0.41	33.33	6.66	13.33
Bromacil (S.T)	23.6	0.433	13.33	0.367	26.66	20	13.33
Bromoxynil (S.T)	21.5	0.75	7.44	0.176	33.33	33.33	26.66
R. meliloti (S.T) + Bromacil (S.D)	22.3	1.2	10.33	0.29	20	13.33	20
R .meliloti (S.T) + Bromoxynil (S.D)	22.1	1.15	9.66	0.306	26.66	13.33	13.33
R.meliloti (S.D) + Bromacil (S.T)	23	1.2	8.66	0.413	13.33	6.66	0
R. meliloti (S.D)+ Bromoxynil (S.T)	22.1	0.95	7.56	0.413	20	0	13.33
B. thuringiensis (S.T)+ Bromacil (S.D)	21.5	1.15	8.33	0.24	20	6.66	6.66
B .thuringiensis (S.T)+ Bromoxynil (S.D)	22	0.51	10.43	0.173	13.33	13.33	0
B. thuringiensis (S.D)+ Bromacil (S.T)	23.8	1.03	15.66	0.58	6.66	0	6.66
B. thuringiensis (S.D) + Bromoxynil (S.T)	22.33	0.47	9.33	0.293	20	0	6.66
$LSD_{0.05}$	**5.119**	**0.422**	**3.087**	**0.187**	**1.089**	**2.902**	**1.064**

S.T = Seed treatment and S.D = Soil drenching methods

Table 2. Effect of microbial antagonists in combination with herbicides on growth and colonization % of root infecting fungi of mung bean.

Antagonists + Herbicides	Growth parameters				Colonization % of root rot fungi		
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	*F. oxysporum*	*R. solani*	*M. phaseolina*
Control	16.7	0.52	6	0.156	53.33	60	53.33
R. meliloti (S.T)	21.43	0.60	10.33	0.206	46.66	20	13.33
B. thuringiensis (S.T)	19.3	0.59	7.44	0.203	26.66	20	13.33
Bromacil (S.D)	22.2	0.57	11.7	0.352	26.66	13.33	20
Bromoxynil (S.D)	20.5	0.35	7.2	0.162	26.6	20	13.33
R.meliloti (S.D)	16.93	0.67	7.33	0.306	40	26.66	26.66
B. thuringiensis (S.D)	16.2	0.69	6.16	0.251	46.66	40	26.66
Bromacil (S.T)	21.73	0.67	13.33	0.367	13.33	13.33	13.33
Bromoxynil (S.T)	21	0.75	7.44	0.176	26.6	13.33	13.33
R. meliloti (S.T) + Bromacil (S.D)	19.5	0.56	10.42	0.33	26.6	6.66	13.33
R .meliloti (S.T) + Bromoxynil (S.D)	21.83	0.583	9.72	0.25	20	13.33	6.66
R.meliloti (S.D) + Bromacil (S.T)	19.3	0.49	7.44	0.203	40	6.66	0
R. meliloti (S.D) + Bromoxynil (S.T)	20.61	0.45	7.22	0.176	20	13.33	6.66
B. thuringiensis (S.T) + Bromacil (S.D)	17.8	0.58	7.2	0.156	26.66	6.66	13.33
B .thuringiensis (S.T) + Bromoxynil (S.D)	18.3	0.46	7.33	0.223	33.33	6.66	6.66
B. thuringiensis (S.D) + Bromacil (S.T)	23.2	1.55	14.33	0.553	6.66	6.66	6.66
B. thuringiensis (S.D) + Bromoxynil (S.T)	20.3	0.54	6.33	0.32	6.66	6.66	13.33
$LSD_{0.05}$	**1.839**	**0.239**	**3.923**	**0.1757**	**1.309**	**1.207**	**1.469**

S.T = Seed treatment and S.D = Soil drenching methods

We can conclude that application of herbicides in combination with antagonists showed better results in improvement of growth of crop plants with reduction of colonization of root infecting fungi. Therefore, seed treatment and soil drenching with bromoxynil and bromacil with antagonists should be applied in field to obtain better growth of crop plant.

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REFERENCES

Ahammed, G.J., Y.H. Zhou, X.J. Xia, W.H. Mao and K. Shi. (2013). Brass in osteroids regulates secondary metabolism in tomato towards enhanced tolerance to phenanthrene. *Biol. Plant,* 57: 154-158.

Bashan, Y. and L.E. de-Bashan. (2005). Bacteria/plant growth-promotion. In: Hillel, D. (Ed.), *Encyclopedia of Soils in the Environment*. Elsevier, Oxford, pp. 103-115.

Bhajbhuje, M.N. (2015). *In vitro* fungitoxic effect of some plant growth regulators on spore germination and germ tube emergence of *Alternaria solani*. *Int. J. Life Sci.,* 3: 125-130.

Cupples, A.M., R.A. Sanford and G.K. Sims. (2005). Dehalogenation of the herbicides bromoxynil (3, 5-dibromo-4-hydroxybenzonitrile) and ioxynil (3,5-diiodino-4-hydroxybenzonitrile) by desulfitobacterium chlororespirans. *Appl. Environ. Microbiol*., 71(7): 3741-3746.

Davison, J.G. (1983). Weed control in newly planted amenity trees. *Proc. Symp. on 'Tree Establishment'.* Bath University, pp. 78.

El-Ghamry, A.M., H. Chang-Yong and X. Jian-Ming. (2000). Influence of chlorsulfuran herbicide on size of microbial biomass in soil. *J. Environ Sci*., 12(2): 138-143.

Esitken, A., S. Ercisli, H. Karlidag and F. Sahin. (2005). Potential use of plant growth promoting rhizobacteria (PGPR) in organic apricot production. In: *Proceedings of the International Scientific Conference of Environmentally Friendly Fruit Growing.* (Eds.): Libek, A., E. Kaufmane & A. Sasnauskas. pp. 90-97.

Gardiner J. A., R.C.Rhodes, J.B. Adams and E.J. Soboczenski. (1969). Synthesis and studies with 2-C14-labeled bromacil and terbacil. *J. Agr. Food Chem*., 17: 980-986.

Grewal, R.P.S. and G. Sharma. (2017). A comparison between different properties of bromoxynil and its derivatves by DFT method. *Orient J. Chem.,* 33(5): 2668-2672.

Isik, D., E. Kaya, M.N. Gouajio and H. Mennan. (2009). Weed suppression in organic pepper (*Capsicum annum* L.) with winter cover crops. *Crop Protect*, 28: 356-363.

Langaro, A.C., D. Agostinetto, C. Oliveira, J.D.G. Silva and M.S. Bruno. (2016). Biochemical and physiological changes in rice plants due to application of herbicides. *Planta Daninha,* 34(2): 277-289.

Laura, A.S., E.V. Stabb, S.J. Raffel and J. Handelsman. (1998). Target range of zwittermycin A, an Aminopoyol Antibiotic from *Bacillus cereus. Current Microbiology,* 37: 6-11. 436-444.

Mussa, A. and P. Russell. (1977). The influence of pesticides and herbicides on the growth and virulence of *Fusarium solani* f.sp. *phaseoli. J. Agr. Sci., 88*: 705-709.

O'Connell, P.F. (1992). Sustainable agriculture-a valid alternative. *Outlook Agric*., 21: 5-12.

Pampulha, M.E. and A. Oliveira. (2006). Impact of an herbicide combination of bromoxynil and prosulfuron on soil microorganisms. *Current Microbiol*., 53: 238-243.

Raaijmakers, J.M., R.F. Bonsall and D.M. Weller. (1999). Effect of cell density of *Pseudomonas fluorescens* on the production of 2,4-diacetylphloroglucinol in the rhizosphere of wheat. *Phytopathol*., 89: 470-475.

Radosevich, M., S.J. Traina, Y.I. Hai and O.H. Touvinen. (1995). Degradation and mineralization of atrazine by a soil bacterial isolate. *Appl. Environ. Microbiol*., 61: 297-302.

Rafi, H. and S. Dawar. (2014). Effects of biopriming of leguminous and non-leguminous crop seeds in the management of root rot fungi and growth of crop plants. *Int. J. Biol. Biotech.,* 11(2-3): 375-382.

Ratnayake, M. and L.J. Audus. (1978). Studies on the effects of herbicides on soil nitrification. II. *Pest Biochem. Physiol*., 8: 170-185.

Reddy, S.S., P.W. Stahlman, P.W. Geier, D.E. Peterson. (2012). Broadleaf weed control and crop safety with premixed pyrasulfotole and bromoxynil in winter wheat. *American Journal of Plant Sciences,* 3: 1613-1618.

Schloter, M., O. Dilly and J.C. Munch. (2003). Indicators for evaluating soil quality. *Agric. Ecosyst. Environ*., 98: 255-262.

Schnelle, M.A., J.R. Feucht and J.E. Klett. (1989). Root systems-facts and fallacies. *Journal of Arboriculture*, 15(9): 201-204.

Sebiomo, A., V.W. Ogundero and S.A. Bankole. (2011). Effects of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *Afr. J. Biotech.,* 10(5): 770-778.

Steven, J.W. (1970). *Stimulation of plant growth by sub-lethal concentrations of herbicides*. Ph.D. thesis, Oregon State University, pp. 94.

Wolf, D.C., D.I. Bakalivanov and J.P. Martin. (1975). Reaction of bromacil in soil and fungus cultures. University of California, Riverside, USA. Roczniki Gleboznawcze T.XXVI, Z. 2, WARSZAWA

Yadav, R.K.P., K. Karamanoli and D. Vokou. (2010). Estimating bacterial population on the phyllosphere by serial dilution plating and leaf imprint methods. *Ecoprint*, 17: 47-52.

Zain, N.M.M., R.B. Mohamad, K. Sijam, M.M. Morshed and Y. Awang. (2013). Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment. *African Journal of Microbiology Research*, 7(5): 367-374.

Zehra, S.L. (2016). *Effect of herbicides for stimulation of plant growth and in the control of root rot fungi*. M.Sc. thesis, Department of Botany, University of Karachi.

Zhang, J., C.R. Howell and J.L. Starr. (1996). Suppression of *Fusarium* colonization of cotton roots and *Fusarium* wilt by seed treatments with *Gliocladium virens* and *Bacillus subtilis*. *Biocontrol Sci. Technol*., 6: 175-187.

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