Pakistan J. Agric. Res. Vol. 26 No. 4, 2013

INTERACTION OF HERBICIDES AND BIO-INOCULANTS WITH AGRICULTURAL CROPS AND WEEDS

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ABSTRACT:- Bioinoculants contribute to soil environment through their versatile role. Whereas weeds are a limitation and herbicide carryover can also be injurious to subsequent crops. The residual/carryover herbicides may influence sowing decisions for the next crop due to reduced herbicide breakdown. The present study aimed at exploring the contribution of bio-fertilizer microbes (*Azospirillum*) to mitigate the inhibitory effect of herbicide residue in soil, and their inhibitory effect on weed seeds. The crops studied under laboratory conditions were *Brassica* spp. and *Zea mays* while *Phalaris minor* was the test weed. In first experiment, *Brassica* and maize, grown in soils having residues of either Triasulfuron+Terbutryn (Logran) or Sulfosulfuron (Leader), were found to significantly mitigate (% increase in root length, shoot length, fresh weight and dry weight) the inhibitory effect of residues , when inoculated with *Azospirillum* A4. In second experiment two *Azospirillum* (B2 and B3) and two *Azorhizobium* (3.6ksk and srsn2k4) isolates reduced seed germination of *Phalaris minor* by 95%. The results imply that biofertilizer microbes can mitigate the carryover effect of the two sulfonylureas; they can also inhibit *Phalaris minor*. Therefore, the use of bio-inoculants, weed seed germination and residual effect of weedicide can be manipulated for the betterment of the crops and these microbes can be used for a positive interaction of maintaining a threshold of weeds in a crop field.

Key Words: Brassica; Zea mays; Phalaris minor; Azospirillum; Azorhizobium; Herbicides; Soil Environment; Pakistan.

INTRODUCTION

Though herbicides are susceptible to breakdown by microbes, plants and animals, yet carryover/residual effect tend to occur under conditions where they can cause damage. The fate and persistence of the herbicide in the soil and its potential to contaminate surface and ground water are of key importance in relation to phytotoxicity to following crop and quality of water.

Herbicides of diverse nature are decomposed by microorganisms and the carbon fragments are used as food. This action of soil microbes on herbicides determines to a large extent the persistence of herbicides in the soil.

Some herbicides are responsible for inhibiting the growth of microorganisms directly or indirectly. Herbicide activity (persistence and mobility) are affected by environmental factors agronomic factors, soil type, the kind of organisms and sometimes the amount of organic matter in the

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soil (Mercado, 1979). Field studies must be carried out with great care to take maximum benefit and reduce risks associated with these chemicals.

The sulfonylureas and imidazolinones are potent commercial herbicides. They are among the most popular choices for farmers worldwide, because they are nontoxic to animals and highly selective (McCourt et al., 2006). Sulfonylurea residues inhibit the growth of some legume crops and pastures in seasons following application. Negative effects of these herbicides on symbiotic nitrogen fixation by legume crops and pastures have been demonstrated.

Many sulfonylureas degrade in soils through multiple mechanisms, which are affected by pH, soil temperature, and soil moisture. Sulfonylurea herbicides degrade at different rates in soils and this is exhibited differently in each field situation, thus generalizations cannot be made over the entire class. Strek (2005) is of the opinion that rotational crops can be injured by low concentrations of sulfonylurea herbicides only when used carelessly. In studies conducted by Rajvir et al. (2002) growth of sorghum after wheat was significantly affected by the residues of chlorsulfuron at 30 gha^{-1} during second year of study, however it requires further experimentation under different climatic conditions. In studies conducted by He et al. (2006) indicate that fungi might become dominant microbial type in the soil with sulfonylurea residues.

Safety of Non-target Flora

Herbicides entering the aquatic environment by spray drift, run-off and leaching to field drains may cause adverse effects on non-target aquatic vegetation. The potential for such effects has typically been evaluated from tests with floating, monocotyledonous *Lemna* sp. This study has also demonstrated that *Glyceria maxima* (Hartm) Holmb, *Lagarosiphon major* (Ridl) Moss and *Myriophyllum spicatum* L. grown in small outdoor tanks can be used successfully to assess the effects of crop protection products on non-target aquatic flora (Davies et al., 2003).

Reduction in nitrogen fixation may result from direct effect of the herbicide on rhizobial growth and/or an indirect effect on plant growth (Anderson et al., 2004).

Application of the herbicide glufosinate-ammonium altered the community level physiological profile of the microbial community, altered soil microbial community structure measured by ester linked fatty acids, reduced soil basal respiration and the abundance of protozoa (Griffiths et al., 2008).

Time for Degradation

The phytotoxicity and sensitivity of succeeding crops to the new sulfonylurea, sulfosulfuron, have been reported; growth chamber bioassays were conducted to detect the presence of residues in soil samples previously treated with sulfosulfuron at the recommended and double rates (20 and 40 g (a.i)ha^{-1}) that could affect the succeeding crop. Sulfosulfuron residues did not affect barley and common vetch, but inhibited shoot length, root length and root dry weight of sunflower sown into soils treated with the 2x rate (40 g (a.i)ha^{-1}) nine months earlier (Alonso et al., 2002)

Conditions for Degradation

The difference in the rates of photodegradation of bensulfuron-methyl on soil surface depends upon the soil texture and organic matter content (Si et al., 2004).

Flooding and constant high temperature have been reported to enhance mineralization of cinosulfuron in soil, indicating the possibility of chemical hydrolysis and microbial degradation of the compound in the flooded soil (Lee et al., 2002).

Amount of Residue

Sulfonylurea (SU), imidazolinone (IMI), and sulfonamide (SA) affect both weed and crop species at low dosages and must be carefully used. On non-crop plant species, a concentration of 100 ngl^{-1} in water has been proposed as the threshold for possible plant toxicity for most of these herbicides (Furlong et al., 2000). In studies conducted by Hernandez (2001), sulfonylurea herbicide residues in soil can affect rotational crops even at low concentrations. Therefore a growth chamber bioassay using sunflower was developed to detect sulfosulfuron and triasulfuron residues in two different soils.

Choice of Test Plants

The residual herbicides are an important tool in fallow and in-crop weed control. However herbicide carryover problems are likely to be worse during drought years therefore checking for indicator plants or conducting a pot bioassay is a quick help to determine any risk of herbicide carryover. The use of residual herbicides prior to drought may influence sowing decisions for the next crop due to reduced herbicide breakdown (McMullen et al., 2004).

A sigmoid equation can describe plant root length response to herbicide concentration using sunflower as an indicator plant (Hernandez, 2001). The nonlinear regression can establish a range of I50 values from 0.9 to 2.9 ppb (a.i) for both sulfonylureas.

A bioassay on the basis of the injury index to vegetable seedlings showed that both field mustard (*Brassica campestris*) and Chinese mustard (*Brassica rapa* [*B. campestris*]) were more sensitive to bensulfuron, imazosulfuron and pyrazosulfuron than lettuce (*Lactuca sativa*) and cabbage (*Brassica oleracea* var. capitata) in winter and mustard was the most sensitive species among six summer vegetables (Li and Wang, 2005).

Compared to mung bean and cotton, maize was more sensitive to chlorsulfuron. Sulfosulfuron @25 gha^{-1} and pendimethalin @1500 gha^{-1} applied in wheat also caused residual toxicity to maize but not to mung bean and cotton.

Bioremediation is the productive use of microorganisms to remove or detoxify pollutants. The success of bioremediation depends upon the physical and chemical characteristics of the substrate, such as nutrients status and pH, and is influenced by environmental factors such as temperature and biotic factors such as inoculum density (Jilani et al., 2005).

The use of naturally occurring soil microbe selection for specificity is important. While using the microbes the nutrient status of the soil needs to be considered. The basic concept used is to bring the active microbes, and the chemicals to be detoxified into close contact. This should be done maintaining the environment

and nutritional conditions of the soil at an optimum level with respect to the needs of the active organisms responsible for breaking down the toxic herbicides.

Weed Seeds in Soil

The emergence of annual weed species depends on the number of seeds present, their physiological state and the biotic and abiotic conditions directly surrounding these seeds. Seed-bank densities and proportional emergence from seed-banks also represent variables that are crucial to success of bio-economic weed management models. Refining methods for sampling and analyzing seed-banks and determining factors that govern seedling emergence from seed-banks are important topics for weed science research in the future.

For any weed management method to be effective the number of weed seeds germinating at the time of intervention has to be considered. The indigenous microflora can also be effective in weed seed decay or enhancement of seed coat decay for effective germination. There is the possibility of microbial decay of weed seeds or microbial decay of the seed coat for effective germination of hard seeds which subsequently can be controlled by integrated weed management or by one application of a herbicide (Boyd and Acker,2004)

The bioherbicide potential of these isolates can be of immense use in productivity enhancement. The aim is the effective weed management together with high yield obtained by the application of one isolate or a combination of different isolates. The weed control potential of these microbes needs to be determined.

Long Term Weed Dynamic

The main process in the life cycle of annual weeds include i) germination and emergence of seedlings from seeds in the seed bank in the soil; ii) establishment and growth of weed plants; iii) seed production; iv) seed shedding; v) dispersal; vi) predation and seed mortality in the soil.

There is possibility that germination and emergence of seedlings from seeds in the seed bank in the soil and predation and seed mortality in the soil are influenced by the microflora in the soil.

Predicting Weed Population

To prepare the weed predictive index one must know: i) weed seed population in the soil; ii) dormancy period; iii) germination capacity; iv) response to environmental, cultural methods and v) weed control practices.

The environmental factor like the microbial degradation or germination enhancement plays the role.

MATERIALS AND METHOD

Two experiments were carried out under laboratory conditions following the procedures as of Fujii et al. (2005).

Mitigation of Sulfonylureas's Effect on *Brassica* and *Zea mays* by *Azospirillum*

The soil (silty clay loam, pH= 7.5, organic matter= 0.9%) was collected from wheat field at NARC with no chemical treatment during the previous year. Two different weedicides used were Triasulfuron+

Terbutryn (Logran) 200gha^{-1} and Sulfosulfuron (Leader) at 27gha^{-1}, as main factor, while three microbes viz., *Azospirillum* A_2, A_4 and B_3, were taken as sub-factor.

In two separate sets, seeds of each *Brassica* and *Zea mays* were sown in soil sprayed with Logran and Leader (Table 1), in petriplates having equal weight of soil. Ten seeds were sown per plate. Each treatment had ten replicates. The seeds were inoculated with three isolates *Azospirillum* A_2, A_4 and B_3, each just as hypocotyls emerged. The plates were incubated at 20±2°C for 10 days. Germination, root and shoot length, shoot fresh and dry weight were recorded for each treatment and data were analyzed by using Statistica software (CRD-2factor design). Weedicides were taken as main factor, while microbes as sub factor, for each crop separately.

Effect of Different Microbes on Germination of *Phalaris minor* Seeds

Phalaris minor seed germination was tested with five *Azospirillum* isolates and four *Azorhizobium* isolates in sterilized petriplates with filter paper. Each petriplate was sown with ten seeds, presoaked in their respective microbe (having cfu 10^7 .ml^{-1}) treatment. The seeds were allowed to germinate for ten days at 20±2°C and were maintained with distilled water.

Data on percent germination was recorded and analysed using statistica 8.1.

RESULTS AND DISCUSSION

Herbicide- microbe Interaction in *Brassica*

Regarding inhibition by sulfonylureas, 4-8% reduction in germination, 40-42% reduction in root length, 22-28% in shoot length and 13-26% in fresh weight was recorded in the presence of the test weedicides (sulfonylureas). Weedicides affected shoot length (F= 23.18, P=0.00), root length (F=33.2, P=0.00), fresh shoot weight (F=11.67, P=0.00) and dry shoot weight (F=5.4, P=0.00) significantly. Weedicide x *Azospirillum* interaction was also significant for both shoot (F= 5.7, P 0.00) and root length (F= 9.9, P=0.00) and fresh weight of shoot (F=3.48, P=0.00) while non-significant for dry weight of shoot.

Table 1. Chemical and trade names of Sulfonylureas

Trade name	Common name (abbreviation used)	Chemical name	Rate (gha^{-1})
Logran 64WG	Triasulfuron + Terbutryn (ST)	1-[2-(2-chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea + 2-(tert-butylamino)-4-(ethylamino) -6-(methylthio) –s-triazine	200
Leader 75 WG	Sulfosulfuron (SS)	N-[[4,6-methoxy-2-pyrimidinyl 1amino]carboxyl]-2-(ethylsulfonyl) imidazo[1,2-a]pyridine-3-sulfonamide]	27

Table 2. Brassica seed germination, root, shoot length and weight in different treatments plus two strains of biofertilizers

Treatment	Germ. %	Root length (cm) (% reduction)			Shoot length (cm)			Fresh weight (g)		
		A_4	B_3	No micr.	A_4	B_3	No micr.	A_4	B_3	No micr.
Control	98	4.33	2.34	2.35	5.81	2.01	1.64	0.40	0.25	0.23
Logran	94	1.55	1.38	1.41 (40%)	3.57	1.49	1.36 (28%)	0.28	0.22	0.20 (13%)
Leader	90	1.31	1.49	1.34 (42%)	3.82	1.44	1.42 (22%)	0.28	0.18	0.17 (26%)

Inoculation by *Azospirillum* mitigated this effect to different extent on different parameter of plant growth. It was observed that *Azospirillum* generally had mitigatory effect on shoot length of *Brassica* (Table 2). *Azospirillum* A_4 promoted root and shoot length significantly in *Brassica* in the presence of both sulphonylureas. However negative effect of Triasulfuron+ Terbutryn (ST) on shoot dry weight of *Brassica* was totally compensated / mitigated due to the addition of *Azospirillum* B_3 (Figure 1). The combinations of these microbes can be considered for further development of these as biofertilizers and mitigation of herbicide residue.

Herbicide-microbe Interaction in *Zea mays*

In maize, the test weedicides caused 4-8% reduction in seed germination, 67-78% in root length and 58-62% in shoot length (Table 3). Weedicides significantly affected root length (F=9.5,P=0.00), shoot length(F=44.59, P=0.00), root fresh weight (F=22.2, P=0.00), root dry weight (F=11.4, P=0.00), shoot fresh weight (F=23.4,P=0.00), shoot dry weight (F=3.7,P=0.03). Weedicide x *Azospirillum* interaction was significant on root length (F= 6.9, P=0.00), shoot length (F=17.4, P=0.00), root dry weight (F=14.7, P=0.00). Though generally *Azospirillum* mitigated the inhibitory effect of the test weedicides, inoculation by *Azospirillum* A_4 best increased the root and shoot length both for Triasulfuron+ Terbutryn (ST) and Sulfosulfuron,

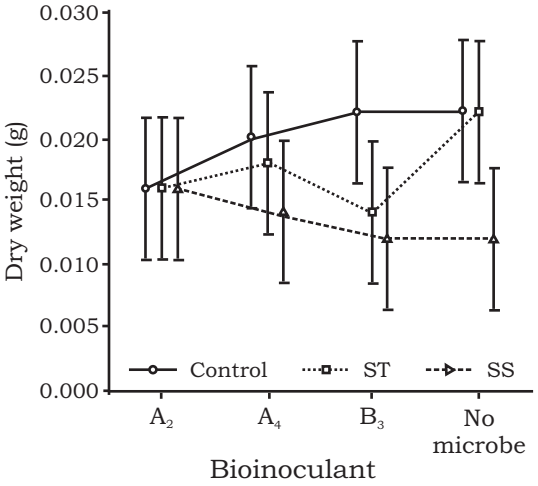

Figure 1. Dry shoot weight of *Brassica* as affected by different combinations of sulphonylureas and *Azospirillum* isolates

Table 3. Maize germination, root, shoot length and weight in different treatments plus two strains of biofertilizers

Treatment	Germ. %	Root length (cm) (% reduction)			Shoot length (cm)			Fresh weight (g)		
		A_4	B_3	No micr.	A_4	B_3	No micr.	A_4	B_3	No micr.
Control	90	11.34	10.42	9.50	8.26	6.50	7.64	2.20	1.59	2.20
Logran	85	7.54	3.01	3.10 (67%)	4.65	2.56	2.84 (62%)	0.92	0.60	0.91 (58%)
Leader	78	6.82	2.52	2.20 (78%)	4.50	2.82	3.15 (58%)	0.82	0.39	0.68 (69%)

(SS) treated soil. When compared with the treatment without microbes (Table 3) the % increase is 16.2, 58.9 and 67.7 in control (without weedicides), Triasulfuron+ Terbutryn and Sulfosulfuron respectively. The *Azospirillum* B_3 showed different response for root and shoot length requiring further investigation (Figures 2 and 3). In the presence of ST shoot dry weight was produced at par with when no weedicide was added. There is a possibility that the application of biofertilizer having microbe A_4 will mitigate the residual effect for both the weedicides.

Residual effect of other sulphonylureas has previously been reported on *Brassica* and maize and has also been used for bioassay of such weedicides (Li and Wang, 2005). However no such studies involving mitigatory effect of inoculants have been reported. It seems *Azospirillum* was not only capable of surviving but also remains active in the presence of both the weedicides used.

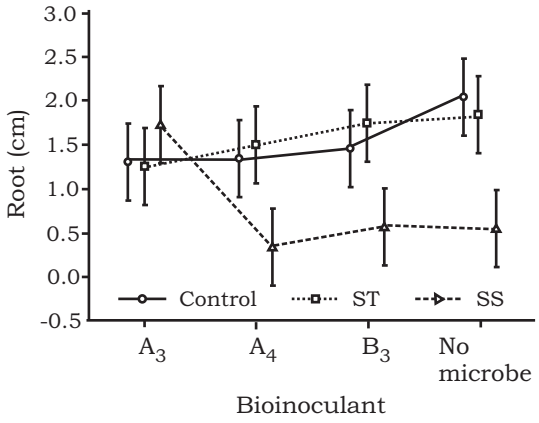

Figure 2. Root length in maize

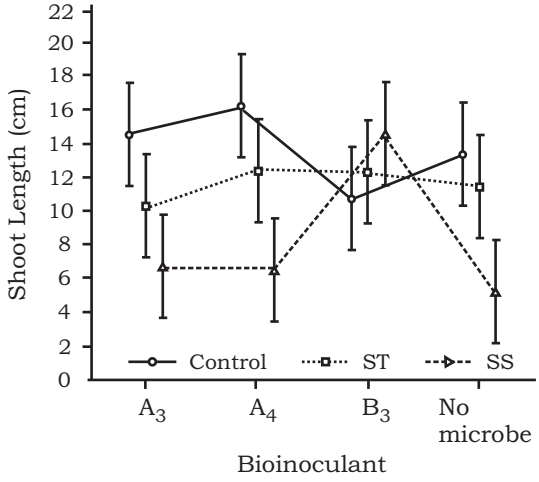

Figure 3. Shoot length in maize

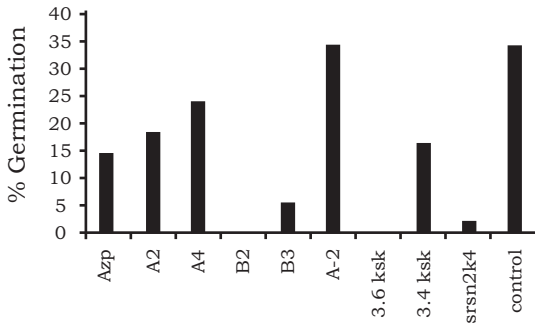

Figure 4. Effect of different microbes on percent seed germination of *Phalaris minor*

Effect of Different Microbes on Seed Germination of *Phalaris minor*

Phalaris minor seed germination was strongly inhibited by the *Azospirillum* B_2, B_3 and *Azorhizobium* 3.6ksk and srsn2K4 in this study (Figure 4). Both the group of microbes have been reported to produce bioactive compounds (hormones, rhizobitoxine), in addition to their nitrogen fixing ability in association with plant roots (Kloepper et al.,1988; Weyens et al., 2009). The inhibition in germination observed by the addition of the microbes in this study can be used to reduce the weed seed bank in the soil and at the same time be effective as biofertilizer.

Many bacteria, fungi and actinomycetes degrade pesticides. Herbicides may affect the soil microflora in different ways. In previous studies population of diazotrophs has been reported to be least affected by pendimethalin (Khalid et al., 2001). Therefore studies need to be conducted in different soils and cropping patterns.

Sulfonylureas are persistent in neutral or mild alkali soils having clay minerals other than montmorilonites. Soil used, was a sandy loam having kaolinite clays. So adsorption or breakdown is not expected.

Maize and *Brassica* were adversely affected by sulfonylurea herbicide residue in terms of root, shoot lengths and weight (Annonymous, 2008). In an attempt to explore the role of biofertilizer microbes in counteracting this effect, it was found that *Azospirillium* had mitigatory effect on shoot length of both the plants. There is a need to look into its ability to utilize these herbicides as a carbon source.

The microorganism mediated degradation of pesticides in soil is affected by many factors, including the chemical structure, the concentration of the pesticides, the bioavailability and the bioactivity of the soil. Present findings may contribute in designing a combined bioremediation (plant-microorganism, chemical-microorganism and plant-chemical-microorganism) protocol. Such approach has been recommended by Guo et al. (2005).

it is thus concluded that study on the weed seed bank requires a thorough knowledge of the soil microflora. The microbes may inhibit germination or decay the seed coat for better germination. Their ability to counter inhibitory effect of weedicides on follow-up crops and at the same time the biofertilizer properties of these microbes need to be explored to obtain sustainable food production.

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