Effect of Herbicides Fenoxaprop-P-ethyl and 2,4-D Ethyl-ester on Soil Mycoflora Including VAM Fungi in Wheat Crop

Anil Gupta, Ashok Aggarwal, Chhavi Mangla, Aditya Kumar and Anju Tanwar Department of Botany Kurukshetra University, Kurukshetra (Haryana), India

ABSTRACT

Wheat (Triticum aestivum L.) is the major staple food of India and its increased production is essential for food security. Weeds constitute one of the biggest problems in agriculture that not only reduce the yield and quality of wheat crop but also utilize essential nutrients. Hence, weed control is essential for increasing wheat production. Despite of its control on weeds, herbicides also affect beneficial non-targeted soil microbes including VAM fungi. Fenoxaprop-P-ethyl and 2,4-D ethyl-ester are two most widely used herbicides in northern India to control monocot and dicot weeds, respectively. However, their effects on mycorrhizal fungi are seldom highlighted. Therefore, the present investigation was focused on the effect of these herbicides on soil fungi of wheat crop alongwith special emphasis on mycorrhizal fungi. Three doses of each herbicide i. e. fenoxaprop and 2,4-D (recommended dose 0.1 kg/ ha; 0.5 kg/ha, half of the recommended dose 0.05 kg/ha; 0.25 kg/ha and double of the recommended dose 0.2 kg/ha; 1.0 kg/ha), respectively, were applied and their effect on soil fungi was studied at 30th, 60th, 90th and 120th day of treatment. Warcup's soil plate method, wet sieving and decanting technique and rapid clearing and staining techniques were used for qualitative study, isolation of mycorrhizal spores and root colonization, respectively. Our results indicate that both herbicides had significant deleterious effects on soil fungi, mycorrhizal spore numbers and percentage root colonization and this effect increased with herbicide concentration. In our chemical warfare against weeds, it is necessary to avoid serious injuries to the beneficial soil microbes. Therefore, use of herbicides in high doses should be resorted to carefully and judiciously.

Key words : Wheat, mycorrhizal fungi, herbicides, deleterious effect

INTRODUCTION

Wheat (Triticum aestivum L.) is the basic component of human diet and meets the major dietary requirements (Bibi et al., 2008). An undeniable and expensive consequence of agricultural practices is the adaptation of weeds to agricultural systems and recognized as a major constraint to food production. It has been reported that with production of each kilogram of weed, one kilogram wheat grains are reduced (Chaudhary et al., 2008). Herbicides are one of the major groups of pesticides which contribute to the increased and economical production of plant and minimize human toil in agriculture production (Subhani et al., 2000). Earlier in India, isoprutron was the most effective and economical for controlling grassy weeds in wheat (Yadav et al., 1984), with the passage of time its continuous use resulted in the evolution of resistant biotypes (Malik and Singh, 1995). To control resistant population of grassy weeds, alternate herbicides such

as sulfosulfuron, clodinafop and fenoxaprop have been recommended (Walia *et al.*, 1999).

The effect of herbicides on wheat soil microbial populations is likely to involve both its direct and indirect effects. The direct effect would involve reduction in numbers and indirect effects would be via reduction in rhizosphere exudates. Ayansina and Oso (2006) reported that herbicides atrazine and metolachlor treatments at both the recommended and above recommended rates resulted in decreased microbial counts. Herbicides also influence VA mycorrhizal association. Rachel et al. (1996) reported that application of 2,4-D had adverse effect on mycorrhizal association. At present, fenoxaprop and 2,4-D are among the most popular herbicides used in northern part of India to control monocot and dicot wheat weeds, respectively, whether these compounds affect mycorrhizae is unknown. Therefore, the present study was planned to see the effect of these herbicides on wheat soil mycoflora.

MATERIALS AND METHODS

Soil samples were collected from Botanical Garden of Department of Botany, Kurukshetra University, Kurukshetra, District Kurukshetra. It lies between latitude 29°-52' to 30°- 12' and longitude 76°-26' to 77°- 04' in the north-eastern part of Haryana. The soil of the study site is sandy loam in texture and relatively calcareous, alkaline in nature, pH varies from 8.0-8.4. Soil samples were air-dried for two days, then crushed, freed from coarse roots and plant debris and sieved prior to further experiment.

Two kg of soil was taken in each pot (size $30 \times$ 25 cm). Soil was treated with two herbicides i. e. fenoxaprop-P-ethyl and 2, 4-D. Fenoxaprop was added at recommended dose (0.1 kg/ha), half of the recommended dose (0.05 kg/ha) and double of the recommended dose (0.2 kg/ha) and similarly 2.4-D ethylester was added at recommended dose (0.5 kg/ha), half of the recommended dose (0.25 kg/ha) and double of the recommended dose (1.0 kg/ha). Approximately 10-12 healthy seeds of wheat variety WH 711 were sown in each pot. To maintain the moisture for germination, the pots were regularly watered. Soil samples were taken out for mycoflora studies after 30, 60, 90 and 120th days of sowing. Control samples were kept without any treatment. Three replicates were taken for each treatment and control.

Warcup's soil plate method (1950) was used for qualitative analysis of soil mycoflora. Wet sieving and decanting method (Gerdemann and Nicolson, 1963) was used for isolation of VAM spores. Rapid clearing and staining technique of Phillips and Hayman (1970) was used for estimation of root colonization. The data were statistically analyzed by 2-way analysis of variance.

RESULTS AND DISCUSSION

Effect of Herbicides on Soil Mycoflora

It is evident from Table 1 that fenoxaprop and 2,4-D had significant inhibitory effect on mycorrhizal spore number. The number of spores inversely proportion to the concentration of herbicides, as the concentration increased the number of spores decreased. The lowest numbers of spores were observed at 2 x dose as compared with all concentrations and time intervals. 2,4-D produced more deleterious effect on mycorrhizal spore number in comparison to fenoxaprop. Table 2 shows the mycorrhizal root colonization at different concentrations of fenoxaprop and 2,4-D at different time intervals. The mycorrhizal infection was inversely proportional to the concentration of both the herbicides. The least root colonization was in 2 x dose at 120th day of treatment. 2,4-D produced more deleterious effect on mycorrhizal root colonization in comparison to fenoxaprop.

Biodiversity and natural occurrence of VAM spores were higher in control soil than in treated soil. Table 3 shows that *Acaulospora flavis*, *Glomus fasciculatum*, *G. macrocarpum* and *G. mosseae* were resistant to fenoxaprop application as they were observed at higher concentrations. *Acaulospora* sp., *G. versiforme*, *G. multicaulis* and *G. pallidum* were present at the lower concentrations and absent at higher concentrations. *Gigaspora margarita*, *Acaulospora lacunosa*, *Glomus scientilens* and *Sclerocystis coremioides* were completely inhibited by fenoxaprop application even at the lower concentration. Table 4 shows that *Acaulospora* sp., *Gigaspora gigantea*, *Glomus geosporum*, *G. aggregatum*, *G. intraradices*, *G. fasciculatum*, *G. macrocarpum*, *G.*

Table 1. Effect of fenoxaprop and 2,4-D on mycorrhizal spore number/50 g of wheat soil at different concentrations and at different time intervals (Wet sieving and decanting technique)

Treatment	30th	day	60th	day	90th	day	120th	n day
	Fenoxaprop	2,4-D	Fenoxaprop	2,4-D	Fenoxaprop	2,4-D	Fenoxaprop	2,4-D
Control	89.67±3.84	89.67±3.84	92.33±4.7	92.33±4.7	98.00±3.46	98.00±3.46	76.33±3.28	76.33±3.28
HRE	85.00±2.52	85.00 ± 4.04	93.67±2.96	92.00±4.16	100.33±1.76	96±3.21	75.67±3.18	73.67±2.19
RE	77.00±3.79	79.33±3.84	87.33±4.84	83.67±4.26	86.33±1.2	84.67±2.91	68.67±1.45	66.67±0.88
DRE	59.00±2.51	58.00±1.15	65.00±2.08	63.00±1.53	69.67±0.88	66.33±1.76	54.33±1.85	51.33±0.88

Mean values of three replicates±Standard error.

HRE-Half of the recommended dose, RE-Recommended dose, DRE-Double of the recommended dose (2 x).

Treatment	30th	day	60th	day	90th	day	120th day			
	Fenoxaprop	2,4-D	Fenoxaprop	2,4-D	Fenoxaprop	2,4-D	Fenoxaprop	2,4 - D		
Control	60.67±0.67	60.67±0.67	69.00±1.15	69.00±1.15	73.00±1.73	73.00±1.73	85.67±1.2	85.67±1.2		
HRE	55.67±1.2	54.33±1.33	61.33±0.88	60.67±0.33	64.67±0.33	62.33±0.33	65.67±1.86	64.67±0.33		
RE	49.67±0.88	48.33±0.88	52.67±0.33	52.33±0.33	55.33±1.76	55.33±0.33	56.33±1.45	56.67±0.67		
DRE	46.00±1.73	43.00±1.15	50.33±1.2	46.00±0.58	53.00±1.55	49.67±0.33	56.67±0.33	53.00±0.58		

Table 2. Effect of fenoxaprop and 2,4-D on per cent mycorrhizal root colonization of wheat crop at different concentrations and at different time intervals

Treatment details are given in Table 1.

mosseae, Sclerocystis coremioides and S. sinuosa were more resistant to herbicide 2,4-D as they were observed at higher concentrations, whereas A. lacunosa, A. nicolsana, G. margarita, G. multicaulis, G. pallidum, G. scientilans, G. constrictum and Scutellospora sp. were not observed at higher concentration as these are sensitive to 2,4-D application.

Table 5 shows that application of fenoxaprop reduced soil fungi qualitatively in comparison to control at all concentrations, especially 2 x rate. Aspergillus ochraceus appeared after 90 days of treatment otherwise it was absent in early half of the experiment. Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Alternaria alternate, Penicillium chrysogenum, Trichoderma viride and Mucor racemosus were found to be resistant to fenoxaprop application and were present in all concentrations. A. ruber, Aureobasidium sp., Humicola sp. and Trichosporium sp. were very sensitive to fenoxaprop application and were not observed. Table 6 shows that application of herbicide 2,4-D also adversely affected the soil fungi. Reduction in fungal species appeared to be directly related with herbicidal concentrations. Decreased number of fungal species were observed in all concentrations of 2,4-D in the soil in comparison with control. A. flavus, A. fumigatus, A. niger, A. terreus, A. alternate, C. lunata, F. oxysporum, P. chrysogenum and T. viride were found to be resistant to 2,4-D application and were present in all concentrations. The fungal species Aspergillus candidus was present upto 60 days of treatment after that it did not appear. A. rubber and Aureobasidium sp. were found very sensitive to herbicide application as these were absent even at the lowest herbicide concentration. Cladosporium oxysporum was susceptible to herbicide upto 60th day after treatment but after that it was present in nearly all concentrations.

Thus from the results, it is clear that both the

herbicides had deleterious effects on mycorrhizal root colonization as well as on mycorrhizal spore number. Double of the recommended dose produced maximum deleterious effect as compared to recommended and half of the recommended dose. It would be most desirable for a herbicide to act upon the targeted weeds alone, leaving the soil mycoflora undisturbed. In the present investigation, none of the herbicides appeared to possess such ideal behaviour.

Heavy treatment of soil with herbicides causes populations of beneficial soil microorganisms to decline. Overuse of herbicides has effects on the soil organisms that are similar to human overuse of antibiotics. Indiscriminate use of chemicals might work for a few years, but after a while, there aren't enough beneficial soil organisms to hold onto the nutrients (Savonen, 1997). The result of the present investigation that both herbicides fenoxaprop and 2,4-D produced negative effect on soil microflora including VAM are in agreement with those of Gorlach-Lira et al. (1997) who reported that glyphosate reduced fungal populations in forest soil. Abdel-Mallek and Moharram (1986) also reported that herbicide ametryn exerted a depressive effect on the total count of almost all fungal genera and species at the three doses (25, 125 and 250 ppm). Saini et al. (2009) reported that herbicide clodinafop decreased the microbial count in wheat. On the other hand, there are some contradictory reports of increased populations of soil actinomycetes and fungi. Devi et al. (2008) reported that application of herbicide 2,4-D benefited soil fungi in a unique lowland ecosystems and major rice production area of Kole lands of Thrissur district of Kerala (India).

Nemec and Tucker (1983) reported that herbicide simazine decreased VAM formation in *Citrus* sp. Askif (2004) reported that herbicide alachol at higher concentrations had adverse effect on mycorrhizal

S. No	. Name of fungi								Treatn	nent							
			30th day				60th day				90tł	n day	120th day				
		С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE
1.	Acaulospora sp.	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
2.	A. lacunosa	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
3.	A. laevis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	A. nicolsana	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
5.	G. gigantea	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	-
6.	G. margarita	+	-	-	-	+	+	-	-	+	-	+	-	+	-	+	-
7.	G. aggregatum	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
8.	G. geosporum	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
9.	G. intraradices	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
10.	G. fasciculatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11.	G. macrocarpum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12.	G. mosseae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13.	G. multicaulis	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
14.	G. pallidum	+	+	+-	-	+	+	-	-	+	-	-	-	+	-	-	-
15.	G. scientilans	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
16.	G. reticulatum	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
17.	G. versiforme	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
18.	S. coremioides	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-
19.	S. sinuosa	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
20.	Scutellospora sp.	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	-

Table 3. Effect of fenoxaprop on VAM fungi (spore) of wheat crop at different concentrations and at different time intervals

S. No	. Name of fungi	Treatment															
		30th day				60th day					90tł	n day		120th day			
		С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE
1.	Acaulospora sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	A. lacunosa	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
3.	A. laevis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
4.	A. nicolsana	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
5.	A. scrobiculata	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	G. gigantea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7.	G. margarita	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
8.	G. aggregatum	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+
9.	G. geosporum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.	G. intraradices	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11.	G. fasciculatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12.	G. macrocarpum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13.	G. mosseae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14.	G. multicaulis	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
15.	G. pallidum	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
16.	G. scientilans	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
17.	G. reticulatum	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
8.	G. constrictum	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
19.	S. coremioides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20.	S. sinuosa	+	+	+	-	+	+	+	-	+	+	+	-	-	-	+	+
21.	Scutellospora sp.	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-

Table 4. Effect of 2,4-D on VAM fungi (spore) of wheat crop at different concentrations and at different time intervals

S. No	b. Name of fungi		Treatment														
		30th day				60th day					90tl	n day			120th	n day	
		С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE
1.	A. candidus	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
2.	A. flavus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	A. fumigatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	A. luchuensis	-	-	-	-	+	+	-	-	+	+	+	-	-	-	-	-
5.	A. niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.	A. ruber	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
7.	A. terreus	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-
8.	A. ochraceus	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+
9.	A. flavipes	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
10.	A. alternata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11.	Aureobasidium sp.	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
12.	C. cladosporioides	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
13.	C. lunata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14.	F. oxysporum	+	+	+	+	+	+	-	-	+	+	+	-	+	+	-	-
15.	F. equiseti	+	+	+	+	+	+	+	-	+	+	+	+-	+	+	+	+
16.	P. chrysogenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17.	Torula sp.	+	+	+	-	+	-	-	-	+	-	-	-	+	-	-	-
18.	Trichosporium sp.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19.	T. viride	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20.	M. racemosus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21.	Humicola sp.	-	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-

Table 5. Effect of fenoxaprop on soil fungi of wheat crop at different concentrations and at different time intervals (Warcup's soil plate method)

S. No. Na	ame of fungi		Treatment															
		30th day					60th day				90tł	n day		120th day				
		С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE	С	HRE	RE	DRE	
1. A. ca	andidus	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	
2. A. fl	avus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
3. A. fla	avipes	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
4. <i>A. fu</i>	ımigatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5. A. lu	ichuensis	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	
6. A. ri	uber	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
7. A. te	erreus	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	
8. A. ni	iger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
9. A. oc	chraceus	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	
10. A. al	lternata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	eobasidium sp.	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
	ladosporioides	+	+	-	-	+	+	-	-	-	-	-	-	-	-			
13. <i>C.ox</i>	xysporum	-	+	-	-	-	-	+	-	-	+	+	-	-	+	+	+	
14. <i>C. lu</i>	unata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
15. <i>F. ox</i>	xysporum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
16. <i>F. eq</i>	quiseti	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	
17. <i>P. ch</i>	hrysogenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	vanicum	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	
	<i>ıla</i> sp.	+	+	+		+	+	+	-	+	+	+	-	+	+	+	-	
20. T. vi	iride	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
21. M. r	racemosus	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	
22. Hum	<i>nicola</i> sp.	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	

Table 6. Effect of 2,4-D on soil fungi of wheat crop at different concentrations and at different time intervals

association. One study found that oryzalin and trifluralin both inhibited the growth of certain species of mycorrhizal fungi (Kelley and South, 1978). Triclopyr was also found to be toxic to several species of mycorrhizal fungi (Chakravarty and Sidhu, 1987). The depressive effect of herbicides on mycorrhizae may be due to many reasons, they are known to repress cell division as a consequence of their disturbing nucleic acid metabolism and protein synthesis (Audus, 1976), but an inhibition of photosynthetic activity by herbicides has also been reported (Diaz et al., 1989). It is known that the carbon requirements of mycorrhizal fungi must be supplied by the host photosynthetate (Smith, 1980). Any factor which modifies the photosynthetic products available for distribution might affect VA mycorrhizal development (Daft and El-Giahmi, 1978). Thus, it is to be expected that herbicides could affect VA mycorrhizal infection through their effect on plant .photosynthesis. These results in the present investigation suggest that the inhibitory effect of herbicides on VA mycorrhiza may be due to the influence on plant metabolism and growth.

Herbicides are often considered a quick and easy solution for controlling weeds in agriculture. They have contaminated almost every part of our environment and their residues are found in soil and air, and in surface and ground water across the countries. Herbicide contamination poses significant risks to the environment and non-target organisms ranging from beneficial soil microorganisms to plants. In fact, weed killers can be especially problematic because of their continuous use. In addition to direct effects, each chemical and biological change may cause secondary, tertiary and other changes until the entire management program becomes improved or hindered by the use of certain herbicides. It can be theorized that frequent use of certain herbicides does alter the long-term costs of such management procedures as controlling pests and soil acidity. The best way to reduce pesticide contamination (and the harm it causes) in our environment is for all of us to do our part to use safer, non-chemical pest control (including weed control) methods. To sum up, the non target effects of herbicides need greater attention in the original decision-making process. Although product costs, application costs, technical services provided, immediate availability of a product and personal preferences are very important considerations. It is also important for scientists to provide additional facts on which to base herbicide-use decisions. There is thus every reason to develop education packages based on knowledge, aptitude and

practices and to disseminate them within the community in order to minimize non target organisms exposure to herbicides.

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