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In Vitro Effects of Alachlor and Haloxyfop Herbicides on Wheat Straw Degradation by Some Fungal Species

S A Omar,* S K Hemida and A Y Abdel-Mallek

Department of Botany, Faculty of Science, University of Assiut, Assiut, Egypt

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Using pure culture experiments, the effect of alachlor and haloxyfop at 100µg active ingredient ml^{-1} on wheat straw degradation by six fungal species were investigated. The following parameters were examined: the weight loss, CO_2 evolution, C- and N-mineralization. The used rates of both herbicides inhibited wheat straw breakdown by five (out of six) fungal species. However, the decay of wheat straw inoculated with *Aspergillus niger* was accelerated by haloxyfop (at the two concentrations used) and alachlor (at 100 µg ml^{-1} only).

Key words: Herbicides, Wheat straw, Cellulose decomposition, Fungi.

Introduction

It is often anticipated that, herbicide treatment may inhibit the degradation of plant residues in soil (wain wright 1978) Since cellulose is one of the major components of plant litter, the effect of herbicides on cellulose degradation in soil has been investigated extensively (Grossbard and Wingfield 1975; Abdel Mallek 1987; Katayama and Kuwatsuka 1991; Katayana *et al*1992 Hemida *et al*1993). Because of the importance of fungi in soil systems, chemicals which interfere with the growth and activity of these organism may influence several fungal processes (Rosas and De Storani 1987; Edwards 1989). However, the response of fungi to herbicides in pure culture may not accurately reflect their response to the same chemicals in the field conditions (Anon 1987; Groaves 1987; Wardle and Parkisson 1990). Investigations concerning the effect of herbicides on the degradation of plant litters in pure culture are still limited (Grossbard and Harris 1977). Therefore the purpose of the present study was to determine the effect of two herbicides, alachlor and haloxyfop at two concentrations on the decomposition of wheat straw in pure cultures of six fungal species.

Materials and Methods

Herbicides. The herbicides tested were: Alachlor as "Lasso": active ingredient 48% wv^{-1} 2-chloro-2', 6' diethyl-N-methyoxy methylacetanilide.Haloxyfop as "Gallant": active ingredient 12% wv^{-1} (Rs)-2-[4-(3-chloro-5-trifluoromethyl-2-pyridyloxy) phenoxy] propionic acid.

Fungal isolates. Six fungal species isolated from herbicide-free wheat straw were used in this investigation: *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A niger* van Tieghem, *Penicillium funiculosum* Thom, *Rhizopus stolonifer* Lindt and *Trichoderma harzianum* Rifai. For each species, pure cultures were prepared on 2% potato dextrose agar.

Effect of herbicides on wheat straw decay. Aliquots of 25 ml of a mineral salts medium (Dalton and Postgate 1969) amended with 0.1% (wv^{-1}) NH_4Cl were added to 100g of washed sand in 250 ml Erlenmeyer flasks and autoclaved at 121°C for 15 min. Two (in dry basis) wheat straw segments (approx. 50 mm length) in nylon mesh bag was sterilized separately and added to sand-mineral medium mixture. Herbicide suspension was incorporated into the medium to obtain concentrations of 0, 100 and 1000 µg a.i ml^{-1}. Three flasks were prepared per herbicide concentration for each fungal species tested. The medium was seeded with 1 ml spore suspension (approx. 10^6 spores) of the tested fungus and each flask fitted with rubber stopper equipped with two glass tubes. One of the tubes fitted to a glass bottle containing 40 ml of 0.5 N NaOH to trap CO_2, and the second is connected at intervals to CO_2-free air generator to provide good aeration. Blanks were prepared in the same way to correct values obtained for all measured parameters. Cultures were incubated at 28 ± 2°C and after time intervals alkali was replaced by fresh NaOH to estimate evolved CO_2. To avoid error due to contaimination with atmospheric CO_2 inherent during titration method, CO_2 evaluation was measured by a conductivity method (Wollum and Gomez 1970). At the end of the experiment (8 wks) loss in weight of straw and the total water soluble carbon and nitrogen were determined as follow:

Dry weight loss. The residual straw in each mesh bag was removed, washed gently with distilled water, dried at 80°C for 24 h and weighed.

C- and N-mineralization. Culture content of each flask was extracted with 100 ml deionized water by shaking for h.

*Author for correspondence

Flasks were left standing at 4°C before filteration to allow sedimentation of suspended particles. Total soluble C was estimated by the method of Malik *et al(1979)* Total soluble N was estimated following the digestion method (Banerjee and Banerjee 1987). After cooling, 1 ml of digest was mixed with 1 ml of a mixture of 10% Na_2SIO_3 + 10% NaOH (1:1) and 5 ml of Nessler's reagent. The amount of amonia was read from a calibration curve prepared with $(NH_4)_2SO_4$.

The results were analyzed statistically by Duncan's multiple range test.

Results and Discussion

The results in Fig. 1 illustrate the effects of alachlor and haloxyfop at 100 and 1000 µg a. i ml^{-1} on the loss in weight of wheat straw inoculated with selected fungal species after 8 weeks of incubation at 28±2°C.Maximum weight loss in wheat straw was recorded at control treatment with *Aspergillus flavus* followed by *Penicillium funiculosum* and *Trichoderma harzianum.*

Both herbicides exerted an inhibitory effect on wheat straw decomposition by five (out of six) fungal species. the greatest inhibition occured at 1000 µg a. i ml^{-1} of alachlor with *Rhizopus stolonifer.* In that case the rate of degradation was about 9.0% only comparable with the control treatment. On the other hand, the degradation of wheat straw by *A. niger* was significantly increased by 100 and 1000 µg a.i ml^{-1} of alachlor and haloxyfop, respectively.

The result in Table 1 show the effects of alachlor and haloxyfop on CO_2 evaluation and C- and N-solubilization from wheat straw inoculated with tested fungi. Both herbicides at 100 and 1000 µg a. i. ml^{-1} exerted inhibitory effect on the amount of CO_2 evolved from wheat straw after 8 weeks incubation period with all tested fungi. These effects were significant at most treatments. The capabilities of *Alternaria alternata, Aspergillus flavus, Penicillium funiculosum, Rhizopus stolonifer and Trichoderma harzianum* to solubilize C and N from wheat straw were reduced when the cultures were incorporated with 100 and 1000 µg a. i ml^{-1} of either alachlor or haloxyfis, On the other side, the amount of soluble carbon extracted from wheat straw inoculated with *Aspergillus niger* was significantly increased by the two concentrations of haloxyfop and by the low concentration only of alachlor. At 1000 µg a. i ml^{-1} alachlor, carbon solubilization by *A. niger* was significantly retarded. N-mineralization by *A. niger* was also significantly estimulated by the two doses used of both herbicides.

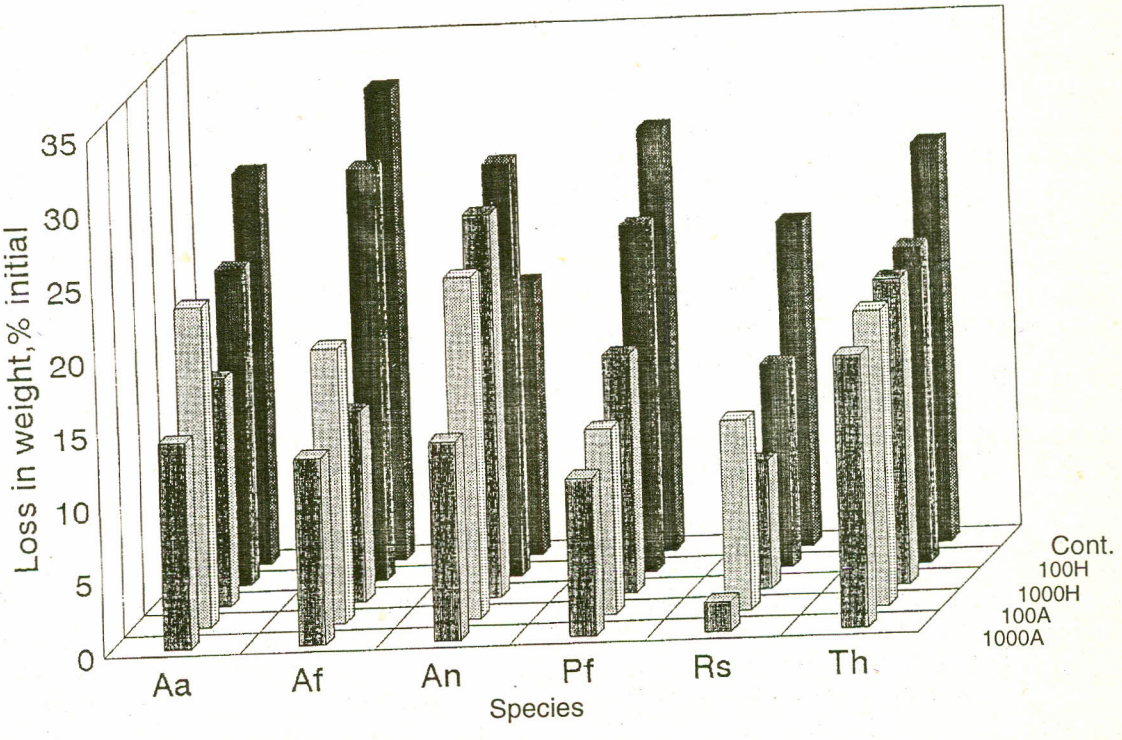

Fig. 1. Effect of alaclor (A) and haloxyfop (H) on the weight loss of wheat straw by selected fungal species in pure cultures incubated for 8 weeks new line Aa, *Alternaria alternata*; Af, *Aspergillus flavus;* An, *A. niger;* Pf, *Penicillium funiculosum;* Rs, *RHizopus stolonifer;* Th, *Trichoderma harzianum.*

Table 1

Effect of alachlor and haloxyfop on wheat straw degradation by some fungi (each value is the mean of three replicates)

Herbicide dose (µgml^{-1})	CO_2 evolution*					Soluble carbon*					Soluble nitrogen*				
		Alachlor		Haloxyfop			Alachlor		Haloxyfop			Alachlor		Fungus	
	0	100	1000	100	1000	0	100	1000	100	1000	0	100	1000	100	1000
Alternaria alternata	44.4^{a}	37.7^{b}	32.5^{c}	38.5^{b}	28.1^{c}	98.4^{a}	82.0^{b}	76.0^{b}	75.0^{b}	72.3^{b}	0.75^{a}	0.65^{b}	0.60^{b}	0.67^{b}	0.40^{c}
Aspergillus flavus	41.1^{a}	39.0^{a}	38.5^{a}	39.7^{a}	37.2^{a}	99.2^{a}	68.0^{c}	64.0^{d}	85.6^{b}	72.0^{c}	1.29^{a}	0.72^{d}	0.75^{d}	0.90^{b}	0.86^{c}
A. niger	40.0^{a}	32.2^{c}	33.3^{c}	39.4^{a}	36.9^{b}	96.8^{b}	130.0^{a}	70.0^{c}	130.0^{a}	125.0^{a}	0.65^{c}	1.00^{a}	0.80^{b}	1.20^{a}	1.10^{a}
Penicillium funiculosum	43.3^{a}	28.0^{c}	27.0^{c}	37.7^{b}	35.0^{b}	192.0^{a}	130.0^{d}	128.0^{d}	160.0^{b}	145.0^{c}	0.95^{a}	0.58^{d}	0.67^{c}	0.82^{a}	0.70^{b}
Rhizopus stolonifer	40.8^{a}	36.2^{b}	29.5^{c}	28.3^{c}	25.5^{c}	127.2^{a}	80.0^{b}	35.0^{d}	85.0^{b}	73.0^{c}	0.72^{a}	0.63^{a}	0.40^{b}	0.65^{a}	0.50^{b}
Trichoderma harzianum	41.2^{a}	35.0^{c}	28.0^{d}	38.7^{a}	34.1^{b}	144.0^{a}	115.0^{c}	103.0^{d}	120.0^{b}	105.0^{d}	1.21^{a}	0.85^{c}	0.65^{d}	0.95^{b}	1.02^{b}

*Calculated as mg^{-1}g wheat straw; Values followed by the same letter are not significantly different at 0.05 level.

The results of this study illustrate the effects on the *in vitro* wheat straw decay by the selected six fungal species of the two herbicides under test. The role of the tested fungi in cellulose decomposition, especially in straw, is well documented (Moubasher *et al 1982*; Omar, 1993).

The results of the present investigation show that both herbicides exerted inhibitory effects on wheat straw weight loss, CO_2 evolution and C- and N-solubilization by all tested fungi except *Aspergillus niger.* In case of cultures inoculated with *A. niger,* haloxyfop (at 100 and 1000 µg a. i ml^{-1}) and alachlor (at 100 µg a. i ml^{-1}) significantly increased the rate of wheat straw degradation and the amount of soluble carbon and nitrogen. Also, alachlor at 1000 µg a. i ml^{-1} stimulated N-mineralization of wheat straw by *A.niger.* The possible explanations of the inhibitory effects of the two used herbicides on wheat straw decay are to : a) the reduction in fungal growth, and b) the interference with the synthesis and activities of the wheat straw degrading enzymes. In this respect, the inhibitory effect of atrazine on cellulases activity of some pathogenic fungi was recorded by Zaki *et al.* (1979). Also, Abdel-Mallek (1984) reported that both diquat (at 20.5 and 41.0 µg a.i ml^{-1}) and simazine (at 81.0 µg a. i ml^{-1}) significantly inhibited cellulase production by *Chaetomium globosum*. However, in contrast, Hemida *et al.* (1993) found that Primextra (a mixture of acetanilide and triazine) induced a significant decrease in activities of C_1 and C_x produced by *A. niger.*

The present study indicates that, wheat straw decay by *A. niger* was significantly accelerated at least by 100 µg ml^{-1} of both herbicides used. Nepomilluev and Kuzyakina (1972) found that, growth of *A. niger* was increased by atrazine and simazine at 4,10 and 100 µg ml^{-1}. Also, Abdel-Mallek (1984) reported that the growth of *A. niger* was stimulated by simazine at 8 µg ml^{-1}.

The inhibitory effects of herbicides on the decay of Plant litters in soil were previously investigated (Grossbard and cooper 1974; Grossbard and Harris 1979; Pollard 1979). Reports on the effect of herbicides on plant residues degradation by a single fungal inoculum are still limited. In this respect, Grossbard and Harris (1977) reported that, no decomposition of milled barley leaves took place when cultures of *C. globosum* incorporated with 80 µg a. i ml^{-1}. "Gramaxone W" and a marked reduction occurred at 40 µg a. i ml^{-1}. However, fungal response to herbicides in pure culture has a different physiological basis from those expressed in soil (Wordle and Parkirson 1990), and this is consistent with the conclusions of Greaves (1987) that pure culture studies do not effectively represent ecological reality.

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