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Side Effect of Three Herbicides on Soil Microorganism

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ABSTRACT

Field experiment was carried out to study the side effect of 3 herbicides, namely; atrazine, ametryn and paraquat, on population dynamics of beneficial and non-target soil microorganism in sugarcane field at Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaengsaen Campus. Soil samples were collected before and at 0, 7, 14, 30, 60 and 90 days after application of herbicides. The soil samples were air dried, sifted and divided into two parts. One was used for microorganism isolation on selective media, the rest was used for toxic residues analysis by gas chromatograph. Forty isolates of bacteria and forty-six isolates of fungi were obtained. It was found that the total population of microorganisms decreased during the first week after application. Accordingly, the amount of residues detected in soil reached the maximum quantity (1774.08 ppb and 112.3 ppb of detected Atrazine and Ametryn, respectively). Half life of atrazine and ametryn in these soil samples were 35 and 6 days, respectively. The isolated soil microorganism that showed antagonistic effects to *Fusarium moniliforme,* the causal agent of root and foot rot of sugarcane were also studied for residual toxicity under laboratory condition. The result showed that atrazine and ametryn gave higher inhibitory effect on mycelial growth than paraquat.

INTRODUCTION

Herbicides are widely used today for the control of annual weeds in the field of corn and sugarcane. The most common herbicides are atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-1,3,5,-triazine ; ametryn (2-ethylamino-4-isopropyl-amino-6-methylthio-1,3,5-triazine) and paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride). Atrazine and ametryn are effective inhibitors of the Hill reaction in photosynthesis and are known to reduce the rate of CO_2 fixation in plants. Using herbicide is still inevitable and certainly affects the environment. The application of herbicides can affect to the phyllosphere microorganisms (Korpraditskul, 1981). It has been reported that the residues in soil of these chemicals resulted in the fluctuation of soil microorganism population (Audus, 1964; Bollen, 1961; Fletcher, 1960; Johnen, 1978). Soil microorganism play an important role on soil fertility and soil properties. Microbial degradation of herbicides is one route by which toxicants are lost from the soil. It is therefore important to study the degree of degradation of herbicide in sugarcane cultivated soil as well as the impact of these herbicide on soil ecosystem. Thus, the knowledge of herbicide residues accumlated in food chain or the occurrence resulting from the impact of herbicide upon benificial and/or pathogenic soil microorganisms should be very useful.

The objective of this research work is to investigate the side effect of the mentioned herbi-

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cides on the population dynamics of soil microorganisms in the field and also under laboratory conditions. Therefore, the toxic residue of these three herbicides in the field have been analysed. In addition, the effect of herbicide to plant pathogen, *Fusarium moniliforme,* the causal agent of root and foot rot of sugarcane and its antagonists were investigated under laboratory condition.

MATERIALS AND METHODS

1. Field plots: Sugarcane experimented plot were located at Central Laboratory and Greenhouse Complex experimental field. The experiment was set for 4 treatments and 4 replications through Randomized Complete Block Design (RCB). The plot was set as shown in Figure 1. Each replication has 4 rows plot, 5 × 6 m. Sugarcane variety U-Thong 1 was planted 50 × 150 cm, 10 settlings in each row.

Soil characters from experimental plot are as follows:-Kamphaengsaen series, pH (KCl 0.1 N) = 7.78, Conductivity = 0.15 ms, Maximum Water Holding Capacity (MWHC) = 43.69%. The amount of rainfall recorded monthly during the experimental work (August 1987-November 1987) were 80.1, 136.9, 270.9 and 213.8 mm, respectively.

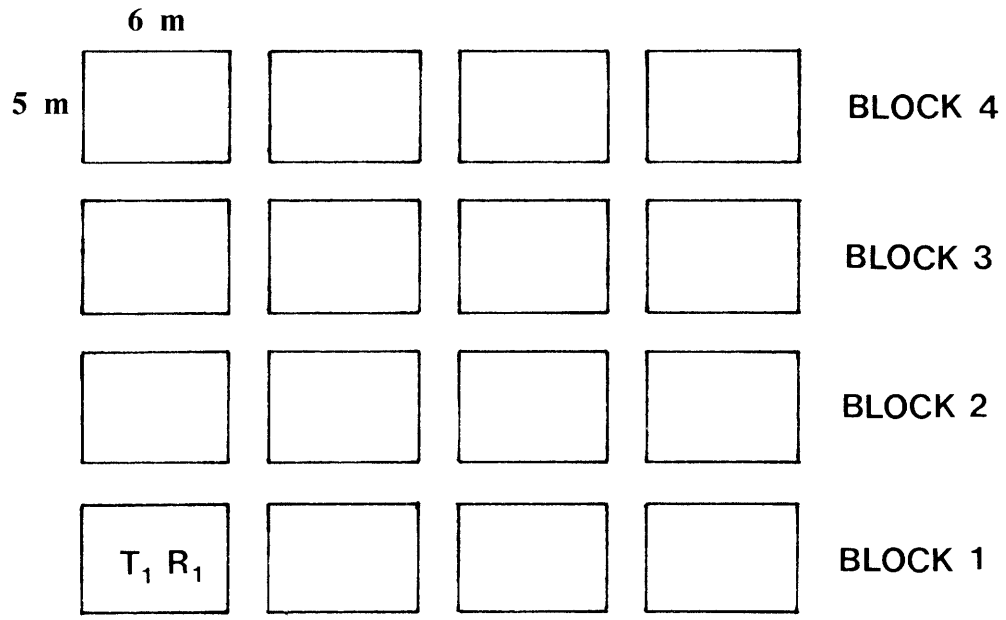

Figure 1 Lay out of experimental plot

2. Herbicide application: Three herbicides, atrazine, ametryn and paraquat, were applied for weed control in sugarcane field. Atrazine was used as pre-emergence herbicide, where as ametryn and paraquat were used as post-emergence herbicide. The common name, trade name, chemical name and recommended rate of each herbicide was shown in Table 1.

3. Soil sampling: Soil samples were taken from each plot before herbicidal application and other samples were taken intervally at 7, 14, 30, 60 and 90 days after application. Soil samples were taken from sub-soil surface, 6-12 inches deep, 5 spots from each replication. Soil sample ca. 300 g each were put into plastic bag and transferred to laboratory immediately for preparation.

Table 1 The technical data of three herbicides which applied in sugarcane field at Kamphaengsaen experimental field.

Common name	Trade name	Chemical name	Recommended rate
atrazine	Atrazine 80 WP. Gesaprim	2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine	480-960 g/rai
ametryn	Ametryn 80 WP. Gesapax	2-methylthio-4-ethylamino-6-isopropylamino-1,3,5-triazine	480-960 g/rai
paraquat	Gramoxone	1,1'-dimethyl -4,4' -bipyridilium dichloride	60-80 ml/20l

4. Soil preparation:

4.1 Residue analysis: Soil samples from each plot was mixed homogeneously, then air-dried for one day and sieved through 200 mesh soil sifter until became one kilogram of soil. Two hundred gm of soil for each herbicide was put into plastic bag. Toxic residue of atrazine and ametryn were extracted by diethyl ether, the analytical method was shown in Figure 2. For paraquat analysis, the soil was refluxed in sulphuric acid. The extract was then passed through a cation exchange resin which absorbed paraquat, then eluted with saturated ammonium chloride solution. The eluent was detected by UV-VIS spectrophotometer.

4.2 Isolation of microorganisms: Soil microorganisms, especially bacteria and fungi were isolated through dilution plate method modified from Dhingra and Sinclair (1986). Placed 10 g of soil (dry basis) in a sterile Erlenmeyer flask with 90 ml sterile water and shaked with rotary shaker for 30 min. Transfered 10 ml of soil suspension to 90 ml sterile water and shaked vigorously by Voltex mixer for 1 min, repeated the process until the suitable dilution was obtained; 10^{-5} for bacteria and 10^{-3} for fungi. Potato Dextrose Agar (PDA) and Nutrient Agar (NA) were used as a selective media for isolating fungi and bacteria, respectively. Soil suspension, 0.1 ml was dropped on agar surface that placed on turntable, then the suspension was spreaded with a flamed "L-shaped glass rod". Plates were incubated at room temperature ca. 25-30°C for 2-4 days for colony counts and isolation to obtain pure culture and for further investigation in laboratory.

5. Effects of herbicides on antagonistic soil fungi of *Fusarium moniliforme.*

5.1 Selection of antagonists: Forty six isolates of soil fungi obtained from sugarcane field was tested for antagonistic effect against *F. moniliforme,* a root and foot rot pathogenic fungus of sugarcane, under laboratory condition. One piece of 5 mm diameter agar block of seven-day-old pathogen and one piece of soil fungi were inoculated on PDA plate, 5 cm apart from each other. Plates were incubated at room temperature for 5 days. Fungal isolates that showed the antagonistic effect were selected for further investigation.

5.2 Effects of herbicide on antagonistic fungi and plant pathogen: Antagonistic fungi which were selected from 5.1 in the same way as *F. moniliforme* was tested by poison medium method with 5 concentrations ie. 2.0, 1.0, 0.5, 0.25 and 0.125 NRR of atrazine, ametryn and paraquat. They were incubated at room tempera-

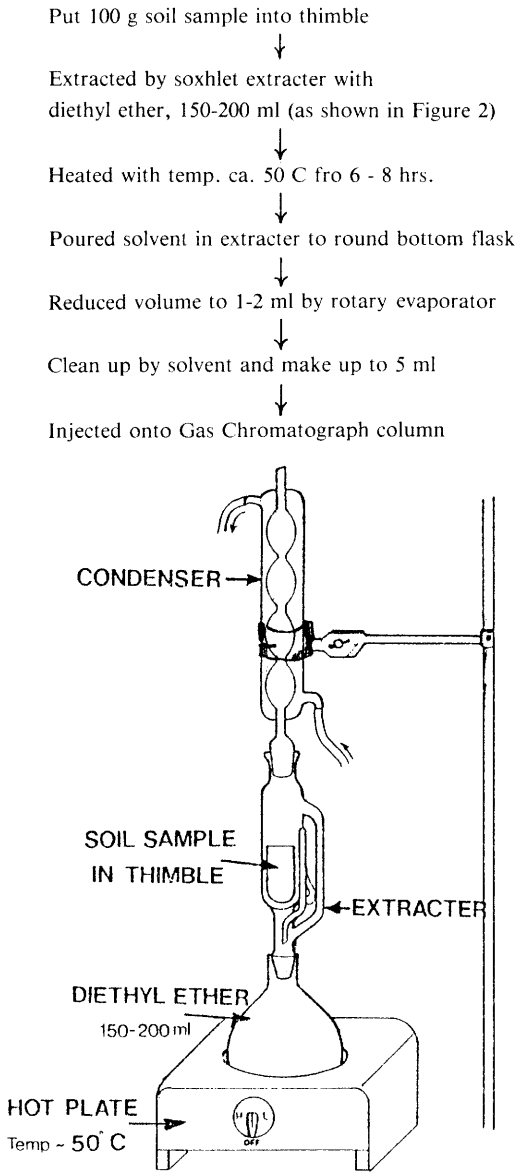

Figure 2 Method for residue analysis of atrazine and ametryn

ture and the vegetative growth of those fungi was recorded every two days after inoculation.

RESULTS AND DISCUSSIONS

1. Soil microorganism in sugarcane field:

Forty isolates of soil bacteria and forty-six isolates of soil fungi were isolated from sugarcane field. *Aspergillus* spp. was dominant in both treated and untreated soil, the rest was *Penicillium* sp., *Scopulariopsis* sp., *Cephalosporium* sp., *Paecilomyces* sp., *Curvularia* spp., *Helminthosporium* spp. *Gliomastrix* sp. etc. The number of each isolates was given as listed in Table 2. The morphological and cultural characteristics of isolated bacteria was shown in Table 3.

Table 2 Soil fungi isolated from sugarcane field.

Isolate number	Genus/Species	Isolate number	Genus/Species
A-1	Aspergillus fumigatus gr.	A-24	A. ochraceus gr.
A-2	A. teres	A-25	Penicillium sp.
A-3	A. auricomus gr.	A-26	Aspergillus sp.
A-4	A. flavus	0-1	Scopulariopsis sp.
A-5	A. auricomus gr.	0-2	Cephalosporium sp.
A-6	Penicillium sp.	0-3	Paecilomyces sp.
A-7	A. candidus	0-4	Curvularia sp.
A-8	A. niger gr.	0-5	Trichophyton sp.
A-9	A. fumigatus gr.	0-6	unidentified
A-10	A. teres	0-7	Nematogonium sp.
A-11	A. candidus	0-8	Curvularia sp.
A-12	Aspergillus sp.	0-9	unidentified
A-13	A. glaucus gr.	0-10	unidentified
A-14	Penicillium sp.	0-11	unidentified
A-15	A. fumigatus gr.	0-12	Helminthosporium sp.
A-16	A. fumigatus gr.	0-13	unidentified
A-17	A. ochraceus	0-14	Helminthosporium sp.
A-18	Aspergillus sp.	0-15	unidentified
A-19	Aspergillus sp.	0-16	Aspergillus sp.
A-20	Aspergillus sp.	0-17	Gliomastrix sp.
A-21	A. ochraceus gr.	0-18	Fusarium sp.
A-22	Aspergillus sp.	0-19	unidentified
A-23	Penicillium sp.	0-20	Aspergillus sp.

Table 3 Morphological and cultural characteristics of soil bacteria isolated from sugarcane field.

Isolate no.	Description of colonies on Nutrient Agar (NA)	Cell morphology	Gram reaction
1	Circular, convex, creamish white, smooth edged	long rod	–
2	Circular, convex, creamish white, smooth edged	short rod	+
3	Circular, convex, creamish white, smooth edged	long rod	+
4	Circular, convex, creamish white, smooth edged	short rod	–
5	Circular, convex, creamish white, smooth edged	long rod	+
6	Circular, convex, creamish white, smooth edged	long rod	+
7	Circular, convex, creamish white, smooth edged	long rod	+

Table 3 (Cont.)

Isolate no.	Description of colonies on Nutrient Agar (NA)	Cell morphology	Gram reaction
8	Tiny spot, convex, creamish white, smooth edged	long rod	+
9	Tiny spot, convex, creamish white, smooth edged	long rod	+
10	Tiny spot, convex, creamish white, smooth edged	long rod	+
11	Circular, raised, creamish white, smooth edged	long rod	–
12	Circular, raised, creamish white, smooth edged	short rod	–
13	Circular, raised, creamish white, smooth edged	filamentous	–
14	Circular, raised, creamish white, smooth edged	short rod	–
15	Circular, umbonate, creamish white, smooth edged	short rod	–
16	Circular, umbonate, creamish white, smooth edged	comma	–
17	Circular, umbonate, creamish white, smooth edged	short rod	–
18	Circular, raised, yellow to cream, smooth edged	filamentous	–
19	Circular, convex, orange, smooth edged	comma	+
20	Circular, raised, creamish white, smooth edged	long rod	+
21	Circular, raised, creamish white, smooth edged	long rod	–
22	Circular, flat, pink, smooth edged	comma	–
23	Irregular, raised, creamish white, irregular edged	long rod	–
24	Irregular, raised, creamish white, irregular edged	long rod	–
25	Circular, convex, white, entired	short rod	–
26	Circular, convex, white, entired	short rod	–
27	Irregular, raised, creamish white, irregular edged	comma	+
28	Irregular, pulvinated, yellow to cream, undulated	long rod	–
29	Irregular, flat, yellow, undulated	long rod	–

Table 3 (Cont.)

Isolate no.	Description of colonies on Nutrient Agar (NA)	Cell morphology	Gram reaction
30	Rhizoid, umbonated, creamish white, lobated	long rod	–
31	Rhizoid, umbonated, creamish white, lobated	long rod	–
32	Filamentous, flat, yellow, irregular edged	rod	+
33	Circular, umbonated, yellow, entired	long rod	–
34	Irregular, pulvinated, hyaline, irregular edged	short rod	–
35	Irregular, raised, creamish white, undulated	short rod	–
36	Circular, convex, yellow, entired	short rod	+
37	Circular, raised, yellow, entired	short rod	–
38	Filamentous, raised, white, lobated	short rod	+
39	Circular, raised, white, irregular edged	long rod	+
40	Punctiform, flat, yellow, entired	short rod	–

2. Population dynamics of soil microorganisms:

2.1 Bacterial counts: The colony counts from atrazine treated soil on nutrient agar was shown in Figure 3. The total population was drastically decreased in 7 days after treatment, whereas at 14, 30, 60 and 90 days, the total population was not obviously fluctuated. The effect of ametryn and paraquat on bacteria showed similar pattern of population dynamics as shown in Figure 4. It is noteworthy that even the bacterial population fluctuated after being treated by these herbicides, the populations never came by as high as before treated ones.

2.2 Fungal counts: The number of colony forming unit (cfu) of the soil fungi when treated with atrazine, ametryn and paraquat were shown in Figure 5. and Figure 6. respectively. Atrazine showed no effect to population number of fungi under field conditions, whereas ametryn and paraquat seemed to decrease the population of fungi in the first two weeks after application.

Although there may be doubts about the validity of the field trial, the fact that similar results were obtained when counting on fungi and bacteria of the three herbicides treated soil, provided substatial evidence that the herbicides, more or less, decreased the total population of soil microorganisms.

The influence of herbicides on changing the population of soil bacteria was reported by many scientists. Anderson (1978) reported that the population changes of soil bacteria after applied with herbicides depended on the tolerance to toxic residues in soil and soil fertility. The effect of herbicide on soil bacteria was studied on 2,4-D and trifluralin by Breazeale and Camper

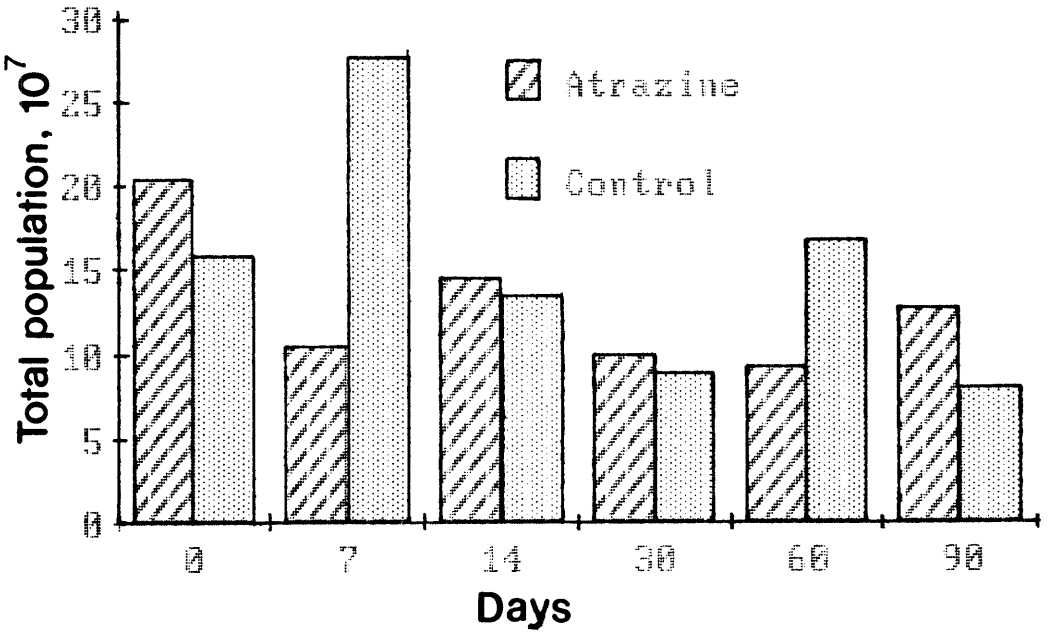

Figure 3 Total colony forming unit (cfu) of bacteria ($\times 10^7$) per g dry soil weight isolated from atrazine treated soil in sugarcane field.

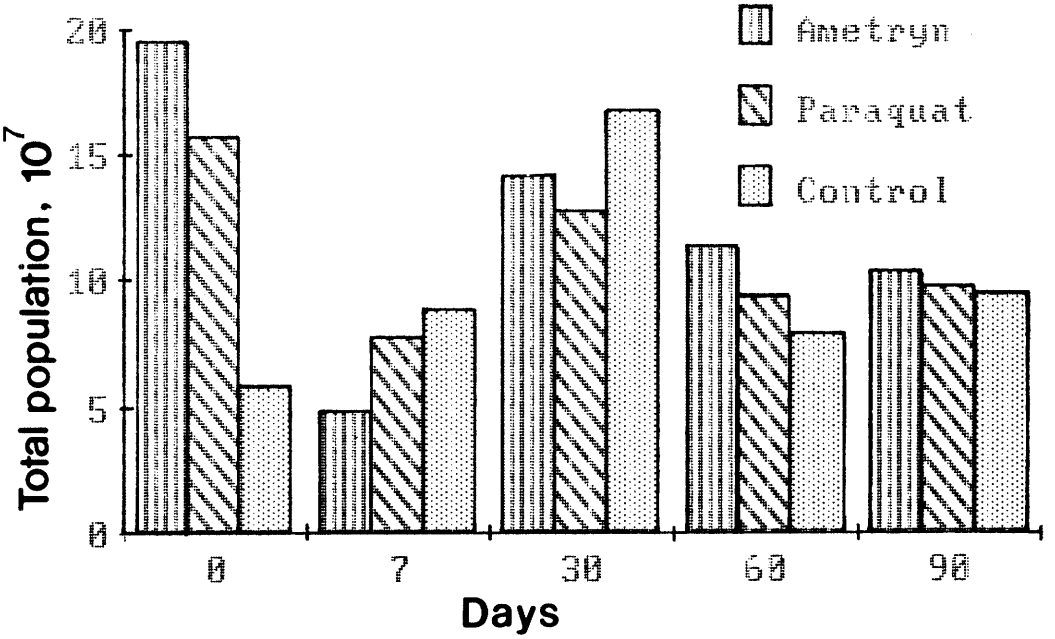

Figure 4 Total colony forming unit (cfu) of bacteria ($\times 10^7$) per g dry soil weight isolated from ametryn and paraquat treated soil in sugarcane field.

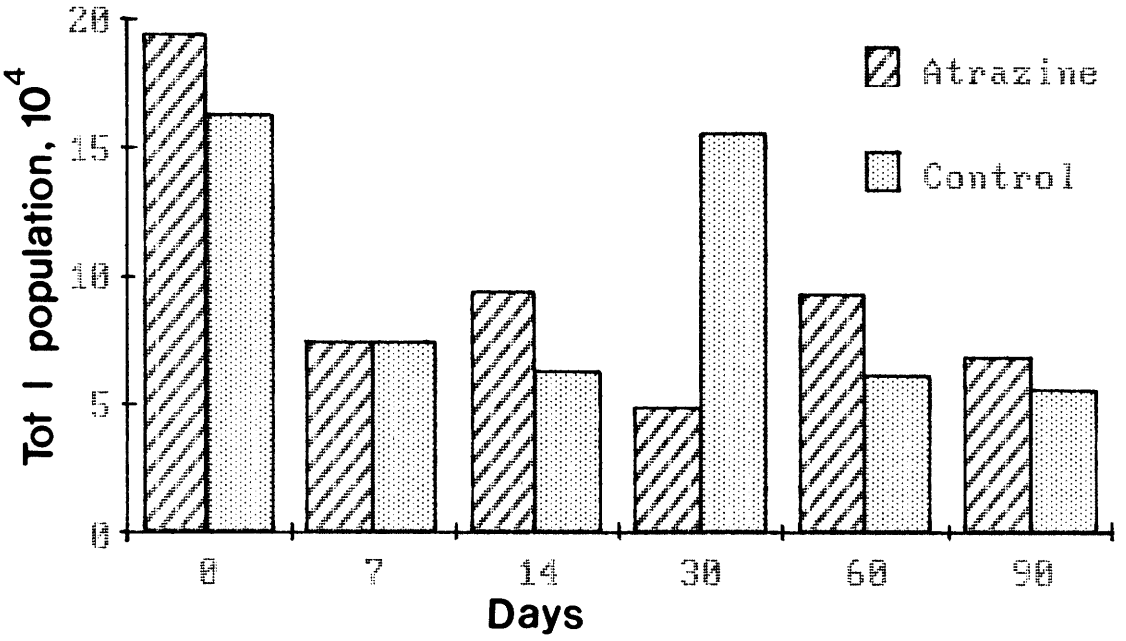

Figure 5 Total colony forming unit (cfu) of fungi ($\times 10^4$) per g dry soil weight isolated from atrazine treated soil in sugarcane field.

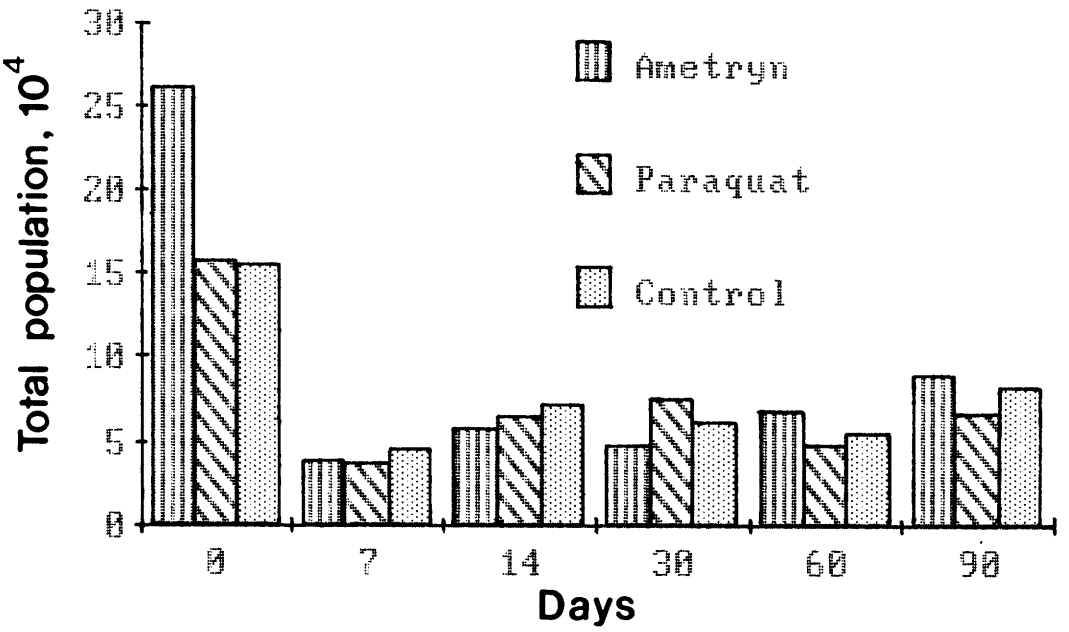

Figure 6 Total colony forming unit (cfu) of fungi ($\times 10^4$) per g dry soil weight isolated from ametryn and paraquat treated soil in sugarcane field.

(1970). They found that the population of bacteria decreased 50 and 46% compared to the control when applied with 2,4-D and trifluralin to the soil. Atrazine at concentration 0.001 and 1.0% affected the amino acid metabolism of the microorganisms (Balicks and Pantera, 1964). Breazeale and Camper (1970) reported that the population of actinomycetes increased whereas the population of the fungus decreased in 2,4-D treated soil. The number of soil bacteria was decreased when applied with atrazine, 4 kg/ha, to sugarcane field whereas the population of fungi was decreased in ametryn treated soil (Yengle *et. al,* 1978). In this study the population of soil bacteria was decreased after treated with atrazine, ametryn and paraquat, while they has no effect on the population changes of soil fungi. The application of herbicides to the soil can affected the population density and the activities of soil microorganisms for a period of time.

3. Quantitative analysis of toxic residues in soil:

Toxic residues of atrazine and ametryn was analysed by GC from soil samples before and after application of herbicides (7, 14, 30, 60 and 90 days, respectively). As shown in Figure 7. atrazine was highest at 7 days after application and decreased gradually. Only 80.31 ppb were detected at 90 days after application. Ametryn was also highest at 7 days after treatment and decreased to 31.91 ppb in 90 days as shown in Figure 8. It was noted that half life of ametryn was 6 days, whereas half life of atrazine was 35 days. From the point of view of microbial ecology and the breakdown of atrazine, ametryn and paraquat degradation in nature, the interested species which was remarkably influenced in both positive and negative results will be further investigated.

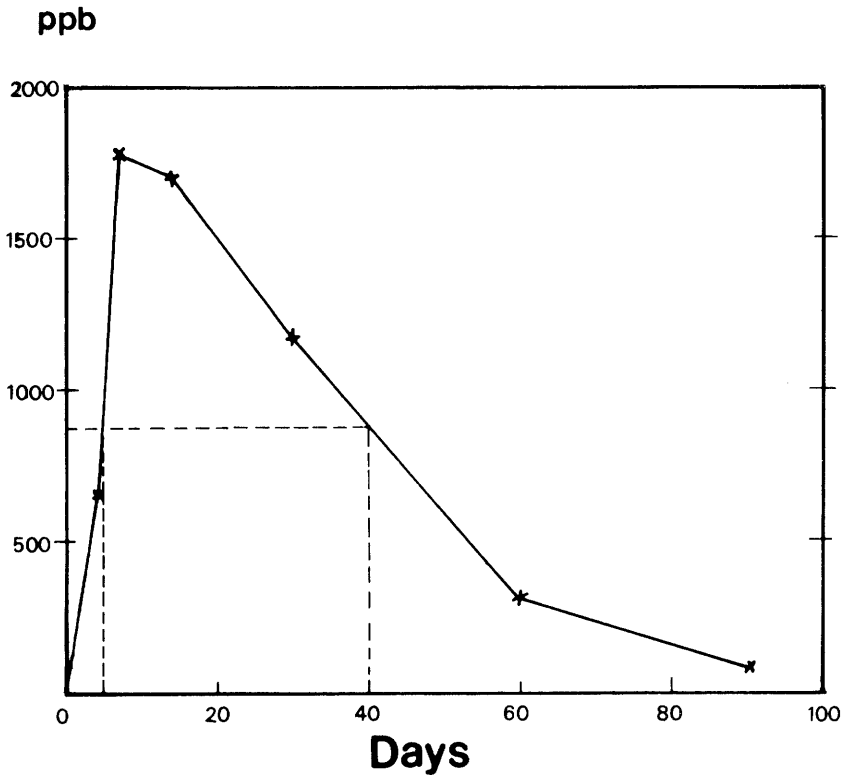

Figure 7 Residue analysis of atrazine in sugarcane field.

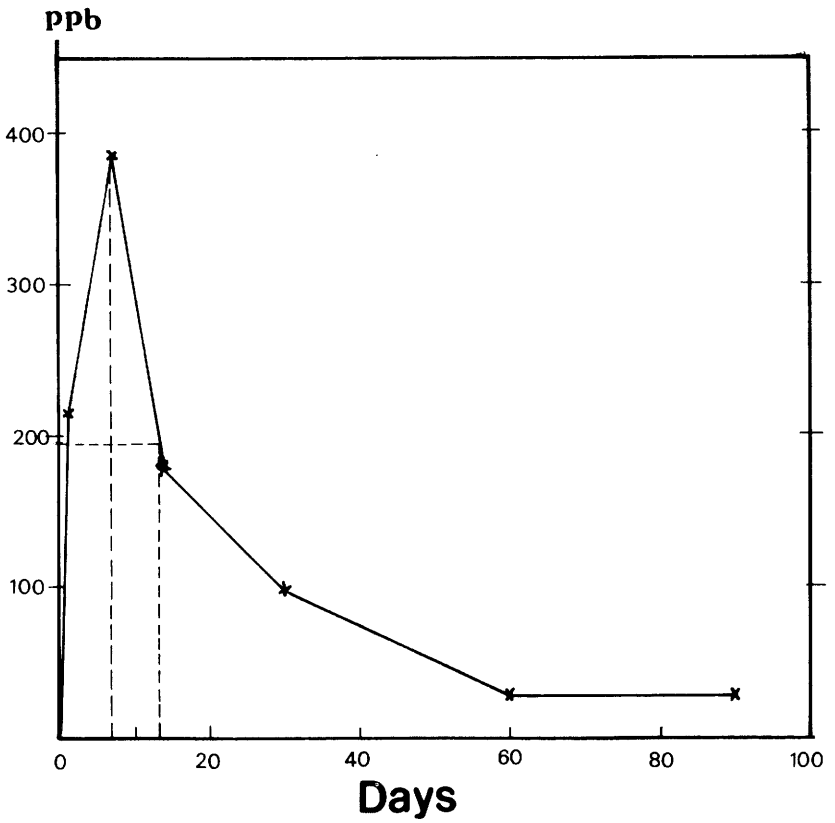

Figure 8 Residue analysis of ametryn in sugarcane field.

4. Antagonistic soil fungi to *Fusarium moniliforme* :

Forty-six isolates of soil fungi obtained from sugarcane field was tested for antagonistic effect against plant pathogenic fungi, *F. moniliforme,* under laboratory condition. Eight isolates which showed the inhibitory effect to vegetative growth of the pathogen in laboratory were selected for further study with herbicides. They were *Penicillium* sp. (A-4), *Aspergillus niger* gr. (A-8), *A, fumigatus* gr. (A-9), *Aspergillus* spp. (A-12, A-8, A-26) and two isolates of *Curvularia* spp. (0-4, 0-8).

5. Effects of herbicides on antagonistic fungi and *Fusarium moniliforme:*

Atrazine and ametryn had more inhibitory effect to vegetative growth of both antagonistic and pathogenic fungi as shown in Table 5. At the highest concentration of atrazine, 2.0 and 1.0 NRR, were completely inhibited the vegetative growth of antagonists, whereas at 1.0 NRR it had 76% inhibition to *F. moniliforme.* The very sensitive antagonist to atrazine (A-9) could not grow at any concentration, however it could grow on ametryn or paraquat containing media.

The most tolerant strain of antagonostic fungi to atrazine was the isolates number 0-4, 0-8 and A-8, while isolate number A-8 and A-18 was tolerant to ametryn. Four isolates, namely A-6, A-12, A-18 and A-26 was tolerated to Paraquat. *Fusarium moniliforme* was more sensitive to atrazine and ametryn than paraquat. The highest concentration of paraquat could inhibit the vegetative growth of *F. moniliforme* only

Table 5 Inhibitory percentage of three herbicides at 5 concentrations to antagonistic fungi and *Fusarium moniliforme*

Herbicide	conc.	Inhibitory percentage									
		Antagonistic fungi									F. moniliforme
		(NRR)	A-6	A-8	A-9	A-12	A-18	A-26	0-4	0-8	
Atrazine	2.0	100	100	100	100	100	100	100	100	100	100
	1.0	100	100	100	100	100	100	100	100	100	76.2
	0.5	100	78.2	100	100	100	100	88.5	77.0	20.8	
	0.25	58.4	65.5	100	46.9	100	100	73.1	55.1	2.9	
	0.125	32.6	53.8	100	18.3	48.5	32.0	53.8	34.9	17.3	
Ametryn	2.0	75.1	73.0	88.5	72.8	71.7	69.2	88.9	91.7	83.4	
	1.0	69.4	72.5	86.1	65.0	64.0	61.9	86.8	89.9	78.0	
	0.5	60.4	58.2	81.2	64.0	52.5	54.4	84.7	87.5	78.5	
	0.25	55.4	41.7	76.0	58.1	50.6	54.2	81.7	86.5	73.3	
	0.125	49.5	37.5	73.2	53.8	44.4	52.5	78.6	84.3	70.6	
Paraquat	2.0	24.2	33.5	67.2	46.7	22.6	9.3	82.8	77.8	57.0	
	1.0	16.3	15.8	45.6	17.7	9.9	0.0	79.5	60.4	53.1	
	0.5	8.5	0.0	44.7	20.4	2.2	0.0	71.9	35.4	23.9	
	0.25	8.4	19.7	27.6	8.1	2.0	1.3	60.3	14.9	5.9	
	0.125	2.2	30.3	17.5	8.7	0.6	0.0	45.6	9.0	3.4	

1 = NRR : Normal Recommended Rate (kg/ha or l/ha)

2 = A-6 : *Penicillim* sp. A-18 : *Aspergillus* sp.
A-8 : *Aspergillus nige* gr. A-26 : *Aspergillus* sp.
A-9 : *A. fumigatus* gr. 0-4 : *Curvularia* sp.
A-12 : *Aspergillus* sp. 0-5 : *Curvularia* sp.

57%, whereas ametryn and atrazine inhibited at 83 and 100%, respectively.

The effect of herbicides to plant pathogens and antagonist has been reported in various pathogen and herbicides. Bozarth and Tweendy (1971) reported that atrazine and other herbicides affected mycelial growth and sclerotial production of *Sclerotium rolfsii*. Atrazine and paraquat inhibited mycelial growth of *Rhizoctonia solani* more than diuron and EPTC (Kabana *et. al,* 1966). In 1972, Kaufman reported that four herbicides namely diuron, linuron, atrazine and simazine could stimulated the antagonistic fungi of *Fusarium* sp. and other soil-borne pathogens. In this study, three herbicides at high concentration showed inhibitory effected both antagonistic fungi and *Fusarium moniliforme*. In the same way, Richardson (1970) reported that the appication of herbicides to the soil could affect both saprophytic and pathogenic microorganisms.

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