http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (5): 731-734, 2000

Combined Effects of Chlorsulfuron and Bensulfuron-Methyl Herbicides on the Size of Microbial Biomass in a loamy sand soil

A.M. El-ghamry[j], Huang Changyong[k], Xu Jianming[k] , Xie Zhengmiao[k] and Abid Subhani[l]
[k]College of Natural Resources and Environmental Sciences, Zhejiang University, Hangzhou 310029 (China)
[j]Soil Science Department, Faculty of Agriculture, El-Mansoura University, (Egypt)
[l]Agriculture Department (Research wing) Lahore, (Pakistan)

Abstract: A laboratory incubation experiment was conducted to examine the combined effects of chlorsulfuron and bensulfuron-methyl herbicides on the soil microbial biomass C (C_{mic}), N (N_{mic}) and their ratio in a loamy sand soil. The herbicides were added at four levels that were (C0B0) control, (C1B1) 0.01& 0.01, (C1B2) 0.01& 0.1 and (C1B3) 0.01&1.0 µg g^{-1} soil of chlorsulfuron and bensulfuron-methyl, respectively. The soil was then incubated in the dark at 25 ± 1°C. Determinations of C_{mic} and N_{mic} were carried out at 1, 3, 5, 7, 10, 15, 25 and 45 days after herbicide application. The results showed that combined applications of herbicides have significant effect on soil microbial biomass C and N, particularly during the first 15 days of incubation, which declined after 25th day of incubation with C1B1 application rate. At 45th day of incubation, the effect of herbicides decreased significantly in C1B2 and it become non-significant as compared with the control. While, C1B3 was still found to be significant as compared to the control and C1B1 but it was at par with C1B2. The results also revealed that with C1B1 treatment the biomass C/N ratio was significantly higher within first 3 days of incubation as compared with the control. But with C1B2, the ratio was significantly different within first 5 days of incubation. While with C1B3 the ratio was found significantly higher within first 25 days of incubation as compared with the control. Increasing the application rate resulted in increased inhibitory effect on the soil microbial biomass and also combined addition of herbicides caused negatively more effect than their single application.

Key words: Chlorsulfuron, bensulfuron-methyl, C_{mic}, N_{mic}, biomass C/N ratio

Introduction

The behavior of herbicides in the soil has been studied now for several decades. When herbicides are applied to soil, they may exert certain side effects on non-target organisms. Therefore, there has been considerable interest on the influence of herbicides on the soil microflora and microbially mediated processes. The effects of these chemicals on certain variables are associated with microbial biomass and their activity (Wardle and Parkinson, 1991) .

There is only a superficial knowledge concerning the combined effect of herbicides on soil microbial activities. Concern has been expressed in recent years that exposure of non-target species to more than one pesticide, including herbicides, in a short period of time may result in unpredicted toxic effects (Johnston *et al.*, 1994 a, b, c). When two pesticides are applied simultaneously, one can inhibit the microorganisms responsible for the degradation of the other or modify the physicochemical conditions in a way that reduces the degradation of both. In a model ecosystem, the combination of the insecticide methyl parathion with atrazin substantially increased the persistence of both pesticides in water and soil phases of the system (Au, 1980). On the other hand, soil incorporation of thiobencarb with simetryn or propanil had no significant effect on degradation rate of any of these pesticides (Nakamura *et al.*, 1977).

In a laboratory study, cellulose decomposing activity and microbial biomass in a sandy loam soil were stimulated by low or normal rates of herbicides, insecticides and fungicides used typically in winter wheat and in orchard fruit crops. Higher rates or the use of combinations or sequences of several compounds, however, could lead to reduced microbial activity and populations (Nowak *et al.*, 1990).

It is generally recognized that the microbial biomass is the eye of the needle through which all organic matters that enter the soil must pass (Jenkinson, 1988). Although the soil microbial biomass represents only a small fraction of the total amount of soil C, N, P and S, it has a relatively rapid turnover (Amato and Ladd, 1980). The maintenance of soil fertility depends on the size and the activity of soil microbial biomass (Alexander, 1977). Changes in microbial number and activities in two non-flooded soils, treated with bensulfuron-methyl at 16 and 160 µg/kg were studied after 1 and 4 weeks of incubation under laboratory conditions. The data suggests that concentrations greater than those present under normal agricultural practice can alter some aspects of the structure and activity of the soil microbial community (Gigliotti *et al.*, 1998). Tu (1994) reported that the pesticide effects had no consistent pattern with length of incubation. No inhibition was observed after 3 weeks incubations. He suggested that the nitrifying organisms recover after 3 weeks and nitrification proceeds as normal.

Nowak and Michalcewicz (1992) determined the soil microbial biomass, in a sandy loam soil under laboratory conditions after 7, 14 and 21 d and found that it was stimulated by the lower concentration of Gramoxone alone and in combination with Afalon, while Afalon by itself was inhibitory. Increasing doses increased the inhibitory effects of the herbicides.

The sulfonylurea herbicides have emerged as a major new class of herbicides, which are very effective at low rates of application (Rahman and James, 1995). These compounds degrade in the soil both by biological and chemical processes and they are not environmentally persistent (Brown, 1990). Chlorsulfuron and bensulfuron-methyl, sulfonylurea herbicides, are characterized as a broad-spectrum herbicides that are effective for controlling broad-leaved weeds at very low use rates in wheat and rice fields, respectively (Takeda *et al.*, 1985; Blair and Martin, 1988).

The objective of this study was to observe the dynamic responses of chlorsulfuron and bensulfuron-methyl herbicides, applied in combined form, on the size of soil microbial biomass C, N and the ratios of C_{mic}/N_{mic} in a loamy sand soil under controlled conditions.

Materials and Methods

Sampling and preparation of soil sample: A laboratory incubation experiment was conducted using a loamy sand soil collected from the surface layer (0-20 cm) from Hangzhou, Zhejiang province, China. The fresh soil collected from the field was brought to the laboratory immediately, hand picked to remove discrete plant residues and large soil animals (earthworms, etc.), passed through

Table 1: Some physical and chemical properties of the soil used

Coarse sand (g kg^{-1})	90	Soil water (% at 33 kPa)	22.40
Fine sand (g kg^{-1})	730	pH	6.27
Silt (g kg^{-1})	100	CEC (c mol kg^{-1})	10.50
Clay (g kg^{-1})	80	Total organic carbon (g kg^{-1})	17.60
Soil texture	Loamy sand	Total nitrogen (g kg^{-1})	1.58

a 2 mm sieve and homogenized thoroughly. A sub-sample of the soil was taken, air-dried, ground and part of it was analyzed for some physical and chemical properties listed in Table 1.

Herbicide treatment: After sampling and preparation of soil, the soil sample was subdivided into four sub-samples. One sub-sample was used as a control and the others were treated with chlorsulfuron and bensulfuron-methyl herbicides, applied at various concentrations.
Methanol solutions of bensulfuron-methyl were prepared at three different concentrations: 1, 10 and 100 µg ml^{-1} and a chlorsulfuron solution of 1 µg ml^{-1} concentration, respectively.
The herbicides were incorporated into the soil sub-samples as follows: twenty-four ml of the bensulfuron-methyl methanolic solution was taken from each of the three concentrations of bensulfuron-methyl and was added to 120 g of air-dried sub-samples soil. Twenty-four ml methanol solution of chlorsulfuron, prepared at one concentration of 1 µg ml^{-1}, was also added to the same three sub-samples having bensulfuron-methyl at three different concentrations. Forty-eight ml of methanol was added to 120 g of air-dried soil for the control soil.
After complete removal of the methanol by evaporation at room temperature, each of the 120 g soil was again divided into 24 portions of 5 g each and transferred them into the beakers containing 95 g fresh soil (oven dry basis) and homogenized.
With this procedure four application rates corresponding to 0.0+0.0 (C0B0), 0.01+0.01(C1B1), 0.01+0.10 (C1B2) and 0.01+1.00 (C1B3) µg g^{-1} soil (chlorsulfuron+bensulfuron-methyl, respectively) were obtained.
Soil moisture was adjusted to 60% water content at 33 kPa and incubated in the dark at 25±1°C. The beakers were removed from the incubator every day and brought to the original weight by adding the required amount of distilled water. Three beakers each for control and treated soils were removed and submitted to analysis for soil microbial biomass C and N at 1, 3, 5, 7, 10, 15, 25 and 45 days after combined additions of bensulfuron-methyl and chlorsulfuron.

Microbial biomass determination: Soil samples for the determination of microbial biomass C were extracted by a fumigation-extraction (FE) method (Vance *et al*., 1987) and the organic carbon in the soil extracts was measured using an automated total organic carbon analyzer (Wu *et al*., 1990). Soil samples for the estimation of microbial biomass N were extracted by a fumigation-extraction (FE) method (Brookes *et al*., 1985b) and the total nitrogen in the soil extracts was measured after Kjeldahl digestion (Brookes *et al*., 1985a).

Soil analysis: Water contents at an applied pressure of 33kPa (0.33 bar) were determined using a pressure membrane system similar to that described by Reining (1963). The pH (in water, 1:2.5) of the soils was measured with a pH meter. Total N was determined by Kjeldahl method and total organic carbon by Walkley-Black procedure (Jackson, 1958).

Statistical analysis: Data were examined by analysis of variance completely randomized and Duncan's multiple range tests using statistix software (CoStat Statistical Software, 1990).

Results

Combined effect of chlorsulfuron and bensulfuron-methyl on C_{mic}: The effects of combined addition of chlorsulfuron and bensulfuron-methyl herbicides are shown in Table 2. The results indicated that all combined applications of herbicides have significant effect on soil microbial biomass C, particularly during the first 15 days of incubation, which declined after 25th day of incubation with C1B1 application rate. At 45th day of incubation the effect of herbicides decreased significantly in C1B2 and it become non-significant as compared with the control. While, C1B3 was still found to be significant as compared to the control and C1B1 but it was at par with C1B2.
The maximum reduction (38.1%) in C_{mic} was observed at the 1st day of incubation with C1B3 dose while the minimum decline (3.2%) was recorded at 45th day of incubation with C1B1 addition as compared with control. The data further revealed that the reduction in C_{mic} with C1B1 addition was 18.9, 18.0, 18.7, 16.5, 10.4, 8.3, 5.2 and 3.2% at 1, 3, 5, 7, 10, 15, 25 and 45th day of incubation, respectively, as compared with the control. This reduction was improved to 25.5, 24.5, 22.3, 23.3, 20.1, 12.3, 9.5 and 7.7% at the same incubation period with C1B2 and it was further promoted to 38.1, 32.8, 28.6, 30.2, 27.0, 20.8, 14.9 and 12.4% at the same incubation period with C1B3 application as against the control.

Combined effect of chlorsulfuron and bensulfuron-methyl on N_{mic}: The influence of application of two herbicides (chlorsulfuron and bensulfuron-methyl), in combined form, on N_{mic} is exhibited in Table 3. The data showed that all the herbicide treatments have significantly influenced the N_{mic}, particularly during the first 15 days of incubation, which declined to non-significant with C1B1 after 25th day of incubation. At 45th day of incubation the effect of herbicides decreased significantly in C1B2 and it become non-significant as compared with the control and C1B1. While, C1B3 was still found to be significant as compared to the control but it was non-significant as compared to C1B1 and C1B2 application rates. The maximum reduction (63.1%) in N_{mic} was observed at the 3rd day of incubation with C1B3 while the minimum decline (12.2%) was recorded at 45th day of incubation with C1B1 addition as against the control.
The data further show that the decline in N_{mic} with C1B1 addition was 32.3, 29.7, 25.7, 30.5, 23.2, 19.0, 14.1 and 12.2% at 1, 3, 5, 7, 10, 15, 25 and 45th days of incubation, respectively as relevant to the control. This reduction was increased to 45.1, 45.2, 39.4, 37.1, 29.0, 28.3, 17.2 and 15.1% at the same incubation period with C1B2 and it was further enhanced to 59.0, 63.1, 53.1, 56.5, 43.8, 38.0, 25.8 and 22.0% at the same incubation period with C1B3 addition as against the control.

Combined effect of chlorsulfuron and bensulfuron-methyl on C_{mic}: N_{mic} ratio: The combined effect of chlorsulfuron and bensulfuron-methyl on C_{mic}:N_{mic} ratio is expressed in Figure 1. The results show that all the treatments are significant at first day of incubation and it become non-significant at 45th day of incubation as compared to the control. The result shows that with C1B1 treatment the ratio was found non-significant from 3rd up to 45th day of incubation as compared with the control. But with C1B2 the ratio was significant within first 5 days of incubation then it become non-significant as compared with the control. While with C1B3 the ratio was found significant within first 25 days of incubation and it became non-significant at 45th day of incubation as compared with the control.
At 1st day of incubation the ratio of C_{mic}: N_{mic} increased from 5.4 with C0B0 to 6.47, 7.32, 8.15 with C1B1, C1B2, C1B3, respectively, at the 3rd day of incubation the ratio was increased

Table 2: Combined effect of chlorsulfuron and bensulfuron-methyl herbicides on microbial biomass C (µg g^{-1} soil) in a loamy sand soil

Incubation period (d)	Herbicide treatment (µg g^{-1}soil)				Reduction in C_{mic} (%)			LSD (0.05)
	C0B0**	C1B1	C1B2	C1B3	C1B1	C1B2	C1B3	
	µg g^{-1}							
1	243.98a*	197.83b	181.86c	150.97 d	18.92	25.46	38.12	13.76
3	236.04a	193.63b	178.29c	158.65 d	17.97	24.47	32.79	11.38
5	217.04a	176.49b	168.60b	154.90 c	18.68	22.32	28.63	9.47
7	224.80a	187.67b	172.47c	156.84 d	16.52	23.28	30.23	8.36
10	220.56a	197.67b	176.20c	160.98 d	10.38	20.11	27.01	7.07
15	213.34a	195.73b	187.12b	169.01 c	8.25	12.29	20.78	16.18
25	219.26a	207.92ab	198.46bc	186.59 c	5.17	9.49	14.90	18.71
45	223.88a	216.69a	206.68ab	196.05 b	3.21	7.68	12.43	
17.10*								

*Means with different letters ,within rows, differ significantly according to LSD (p = 0.05)
**C0B0, C1B1, C1B2 and C1B3 = control, 0.01+0.01, 0.01+0.1, 0.01+1.0 µg g^{-1} soil of chlorsulfuron and bensulfuron-methyl, respectively

Table 3: Combined effect of chlorsulfuron and bensulfuron-methyl herbicides on microbial biomass N (µg g^{-1} soil) in a loamy sand soil

Incubation period (d)	Herbicide treatment (µg g^{-1}soil)				Reduction in C_{mic} (%)			LSD (0.05)
	C0B0**	C1B1	C1B2	C1B3	C1B1	C1B2	C1B3	
	µg g^{-1}							
1	45.19a*	30.60b	24.83c	18.52d	32.29	45.05	59.02	4.49
3	37.92a	26.66b	20.80c	14.00d	29.69	45.15	63.08	3.61
5	29.85a	22.18b	18.09c	14.01d	25.70	39.40	53.07	2.55
7	28.04a	19.50b	17.64b	12.20c	30.46	37.09	56.49	2.49
10	29.80a	22.88b	21.15b	16.75c	23.22	29.03	43.79	3.09
15	33.49a	27.13b	24.01bc	20.76c	18.99	28.31	38.01	3.35
25	34.11a	29.29ab	28.24b	25.32b	14.13	17.21	25.77	5.29
45	33.33a	29.26ab	28.31ab	26.00b	12.21	15.06	21.99	6.14

*Means with different letters ,within rows, differ significantly according to LSD (p = 0.05)
**C0B0, C1B1, C1B2 and C1B3 = control, 0.01+0.01, 0.01+0.1, 0.01+1.0 µg g^{-1} soil of chlorsulfuron and bensulfuron-methyl, respectively

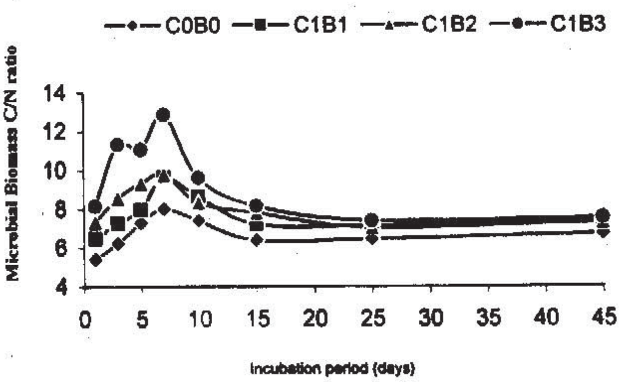

Fig. 1: Combined effect of chlorsulfuron and bensulfuron-methyl herbicides on microbial biomass C/N ratio in a sandy loam soil

to 7.26, 8.57, 11.33 as compared with the control (6.22), at 5th day of incubation the ratio increased to 7.96, 9.32, 11.06 against to C0B0 (7.27), at the 7th day of incubation the ratio was further increased to 9.62, 9.78 and 12.86 as compared with C0B0 (8.02), at the 10th day of incubation the data of this ratio showed that all treatments increased the ratio compared to the control from 7.4 (control) to 8.64, 8.33 and 9.61, also at 15th day of incubation the ratio increased from 6.37 with control to 7.21, 7.79 and 8.14, at the 25th day of incubation the data showed that the ratio increased from 6.43 (with C0B0) to 7.10, 7.03 and 7.37 and at the 45th day of incubation the ratio was further increased to 7.41, 7.30 and 7.54 with C1B1, C1B2 and C1B3,respectively, against the control (6.72, C0B0).

Discussion

Herbicides affect various soil microbial processes (Johnen, 1977; Ross, 1974) including N-mineralization (Domsch and Paul, 1974), inhibit decomposition (Pugh and Williams, 1971; Grossbard and Wingfield, 1978) and depending upon type and rate of application, can alter the biomass quantitatively and qualitatively in both the short and long-term (Anderson *et al.*, 1981). Anderson *et al.* (1992) concluded that, when used as directed, none of the pesticide tested should have negative or ecological unacceptable effects on C or N mineralization in soils. Our results revealed that the combined application of herbicides (Chlorsulfuron and bensulfuron-methyl), at different concentrations, had significant effect on microbial biomass. The reason for the decrease of microbial biomass may be related to the toxicity effect of the herbicides because these herbicides are toxic even at low rates. Other investigators, in different incubation experiments, have also reported the same pattern with different types of herbicides (Ismail *et al.*, 1996). They observed a decrease in microbial biomass with an increase in herbicide concentration on day 1 and decreased bacterial population as the concentration increased in sandy loam soil during first 9 days. Other scientists (Perucci and Scarponi, 1996), who experimented with remsulfuron, found a decrease in microbial biomass-C levels at 10 field rate and 100 time of field rate within the first 10 d. Elsewhere, it has been shown that repeated paraquat applications significantly lowered soil microbial biomass (Duah-Yentumi and Johnson, 1986). For this reason, the decrease of microbial biomass content was negatively correlated with dosage of herbicides. So it can be inferred that a portion of the soil microflora was killed by chlorsulfuron herbicide.

The noted increase of C_{mic}/N_{mic} ratio was related to the decrease of microbial biomass C and N and due to increase in the levels of chlorsulfuron and bensulfuron-methyl combination and subsequent increase in toxicity caused greater decline in N_{mic} in treated soil than in the control. Decline in C_{mic} was relatively lower than N_{mic}. The increase in biomass C/N ratio can give a highlight of changes in microbial populations.

Our study further showed that the toxic effect of combined herbicides application is higher than the single addition of these herbicides to the soil. This may be due to increase in the application dose of herbicides, as compared to single application of these herbicides. When two pesticides are applied simultaneously, one can inhibit the microorganisms responsible for the degradation of the other or modify the physicochemical conditions in a way that reduces the degradation of both. Also, the cumulative effect of these two herbicides, when applied in combination was stronger than their single application, our results are in full agreement with these findings.

References

Alexander, M., 1977. Introduction to Soil Microbiology. 2nd Edn., John Wiley and Sons Inc., New York, USA., ISBN: 9780471021797, pp: 115-380.

Amato, M. and J.N. Ladd, 1980. Studies of nitrogen immobilization and mineralization in calcareous soils-V. Formation and distribution of isotope-labelled biomass during decomposition of ^{14}C- and ^{15}N-labelled plant material. Soil Biol. Biochem., 12: 405-411.

Anderson, J.P.E., D.J. Arnold, F. Lewis and L. Torstensson, 1992. Side-effects of pesticides on carbon and nitrogen transformations in soil. Proceedings of the International Symposium on Environmental Aspects of Pesticide Microbiology, August 17-21, 1992, Sigtuna, Sweden, pp: 61-67.

Anderson, J.P.E., R.A. Armstrong and S.N. Smith, 1981. Methods to evaluate pesticide damage to the biomass of the soil microflora. Soil Biol. Biochem., 13: 149-153.

Au, L.A., 1980. Pesticide interactions in laboratory rice paddy model ecosystem. Diss. Abstr. Int. B, 40: 3567-3568.

Blair, A.M. and T.D. Martin, 1988. A review of the activity, fate and mode of action of sulfonylurea herbicides. Pest Manage. Sci., 22: 195-219.

Brookes, P.C., J.F. Kragt, D.S. Powlson and D.S. Jenkinson, 1985a. Chloroform fumigation and the release of soil nitrogen: The effects of fumigation time and temperature. Soil Biol. Biochem., 17: 831-835.

Brookes, P.C., A. Landman, G. Pruden and D.S. Jenkinson, 1985b. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem., 6: 837-842.

Brown, H.M., 1990. Mode of action, crop selectivity and soil relations of the sulfonylurea herbicides. Pest Manage. Sci., 29: 263-281.

CoStat Statistical Software, 1990. CoStat Manual Revision 4.2. CoStat Statistical Software Inc., New York, Pages: 271.

Domsch, K.H. and W. Paul, 1974. Simulation and experimental analysis of the influence of herbicides on soil nitrification. Arch. Microbiol., 97: 283-301.

Duah-Yentumi, S. and D.B. Johnson, 1986. Changes in soil microflora in response to repeated applications of some pesticides. Soil Biol. Biochem., 18: 629-635.

Gigliotti, C., L. Allievi, C. Salardi, F. Ferrari and A. Farini, 1998. Microbial ecotoxicity and persistence in soil of the herbicide bensulfuron-methyl. J. Environ. Sci. Health, 33: 381-398.

Grossbard, E. and G.I. Wingfield, 1978. Effects of paraquat, aminotriazole and glyphosate on cellulose decomposition. Weed Res., 18: 347-353.

Ismail, B.S., K.M. Goh and J. Kader, 1996. Effects of metsulfuron-methyl on microbial biomass and populations in soils. J. Environ. Sci. Health Part B: Pestic. Food Contamin. Agric. Wastes, 31: 987-999.

Jackson, M.L., 1958. Nitrogen Determinations for Soils and Plant Tissue. In: Soil Chemical Analysis, Jackson, M.L. (Ed.). Constable and Co., London.

Jenkinson, D.S., 1988. The Determination of Microbial Biomass Carbon and Nitrogen in Soil. In: Advances in Nitrogen Cycling in Agricultural Ecosystems, Wilson, J.R. (Ed.). CAB International, Wallingford, UK., pp: 368-386.

Johnen, B.G., 1977. Ecological effects of pesticides on soil microorganisms. Soil Sci., 123: 319-324.

Johnston, G., C.H. Walker and A. Dawson, 1994a. Interactive effects of prochloraz and malathion in pigeon, starling and hybrid red-legged partridge. Environ. Toxicol. Chem., 13: 115-120.

Johnston, G., C.H. Walker and A. Dawson, 1994b. Interactive effects between EBI fungicides (prochloraz, propiconazole and penconazole) and OP insecticides (dimethoate, chlorpyrifos, diazinon and malathion) in the hybrid red-legged partridge. Environ. Toxicol. Chem., 13: 615-620.

Johnston, G., C.H. Walker and A. Dawson, 1994c. Potentiation of carbaryl toxicity to the hybrid red-legged partridge following exposure to malathion. Pesticide Biochem. Physiol., 49: 198-208.

Nakamura, Y., K. Ishikawa and S. Kuwatsuka, 1977. Degradation of benthiocarb in soils as affected by soil conditions. J. Pesticide Sci., 2: 7-16.

Nowak, A. and W. Michalcewicz, 1992. Studies on the activity of Afalon and Gramoxone on the microbial biomass in the soil in relation to temperature and moisture. Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz, 13: 387-395.

Nowak, A., W. Michalcewicz and K. Hurle, 1990. Effect of pesticide combinations on the cellulose activity and microbial biomass in the soil. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 12: 373-378.

Perucci, P. and L. Scarponi, 1996. Side effects of rimsulfuron on the microbial biomass of a clay-loam soil. J. Environ. Qual., 25: 610-613.

Pugh, G.J.F. and J.I. Williams, 1971. Effect of an organo-mercury fungicide on saprophytic fungi and on litter decomposition. Trans. Br. Mycol. Soc., 57: 164-166.

Rahman, A. and T.K. James, 1995. Improving performance and reliability of soil applied herbicides in New Zealand soils. Proceedings of the 15th Asian-Pacific Weed Science Society Conference, July 24-28, 1995, Tsukuba, Japan, pp: 627-635.

Reining, B., 1963. A pressure membrane apparatus. J. Agric. Eng. Res., 8: 48-49.

Ross, D.J., 1974. Influence of four pesticide formulations on microbial processes in a New Zealand pasture soil: II. Nitrogen mineralisation. N. Z. J. Agric. Res., 17: 9-17.

Takeda, S., T. Yuyama, R.C. Ackerson, R.C. Weigel and R.F. Sauers *et al*., 1985. Herbicidal activities and selectivity of a new rice herbicide DPX-F5384. J. Weed Sci. Technol., 30: 284-289.

Tu, C.M., 1994. Effects of herbicides and fumigants on microbial activities in soil. Bull. Environ. Contam. Toxicol., 53: 12-17.

Vance, E.D., P.C. Brookes and D.S. Jenkinson, 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem., 19: 703-707.

Wardle, D.A. and D. Parkinson, 1991. Relative importance of the effect of 2,4-D, glyphosate, and environmental variables on the soil microbial biomass. Plant Soil, 134: 209-219.

Wu, J., R.G. Joergensen, B. Pommerening, R. Chaussod and P.C. Brookes, 1990. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. Soil Biol. Biochem., 22: 1167-1169.