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Side effects of some herbicides to *Fusarium moniliforme* and its antigonistic microorganisms

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ABSTRACT

Forty six isolates of fungi and forty isolates of bacteria were isolated from sugarcane field soil, Kasetsart University, Kamphaengsaen Campus in 1986-88 by spreading soil suspension on selected media, Marin's medium and Nutrient agar. The dominant isolate of soil microorganisms were *Aspergillus* spp. and gram negative bacteria. Eight isolates of those fungi showed the antagonistic effect to *Fusarium moniliforme* namely *A. niger*, *A. flavus* isolate 1 and 2, *A. fumigatus*, *A. tamarii*, *Curvularia* sp. isolate 1 and 2, and *Trichoderma* sp., where two isolates of gram negative and two isolates of gram positive bacteria were included. The inhibitory effect of herbicides, atrazine, ametryn and paraquat, to *F. moniliforme* and its antagonists was tested by poisoned food technique on PDA and CO_2 production of herbicide treated soil in anaerobic condition. Ametryn showed the best inhibitory effect to *F. moniliforme*. All antagonistic fungi were inhibited by atrazine whereas slightly effect was founded in paraquat and ametryn treatments. Highly inhibitory effect of herbicide to all isolates of antagonistic bacteria was observed in paraquat treatment.

INTRODUCTION

Chemical usage in crop production is an essential part of modern agriculture. Nevertheless a widespread concern exists that the environment may be polluted by these chemicals and the "ecological balance of nature" may be upset in a manner harmful to man. The so called "Side-effect" of herbicides on plant pathogen and soil-microorganisms have been studied intensively (Heitefuss, 1970, Altman and Campbell, 1977). Triazine herbicides, especially atrazine and ametryn, and paraquat have been used as pre-emergence and post-emergence herbicides in sugarcane field instead of hand weeding in most plantation of Thailand.

The function of herbicides is to prevent or retard the growth of weed seedlings or to remove weed and thereby eliminate the competition with the crop. Herbicides, however, may also influence seed germination, plant development and plant growth. Many incidences were pointed out that herbicide residues had side effect on soil microorganisms and plant pathogens (Heitefuss, 1970; Quilty and Geoghegan, 1975; Altman and Campbell, 1977). Altering of disease reaction on many crops after the application with herbicides has been reported by Ross (1965); Romig and Sasser (1972) and Haber *et al.* (1966).

The effects of some herbicides on various plant pathogens under laboratory conditon have been extensively reported. The dry matter of *Fusarium oxysporum* f. sp. *vasinfectum* was significantly decreased when cultivated in mixed culture of Czapek's solution and atrazine herbicide at various concentrations. The CO_2 production of this pathogen in atrazine treated soil was increased at higher concentration compared to low concentration of atrazine (Kabana and Curl, 1970). The mycelial growth and the number of sclerotial production of *Selerotium rolfsii* in atrazine containing medium was also decreased (Bozarth and Tweedy, 1971). In 1974 Deshmukh and Shrikhande reported that the population of soil microorganisms in atrazine treated plot was slightly decreased at 20 days, but there was no evidence at 40 days after the application. The inhibitory effect of paraquat on mycelial growth of pathogens was reported in *Sclerotium rolfsii* (Kabana *et al.*, 1966); *Rhizopus stolonifer* (Wilkin-

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son and Lucus, 1969) and *Septoria nodorum* and *S. tritici* (Jone and Williams, 1971). In airtight container, CO_2 production of *S. rolfsii* was also decreased in every concentration of paraquat treated soil. (Kabana *et al.*, 1966)

The objective of this study was to investigate the side effect of the herbicides commonly used in sugarcane field, namely atrazine, ametryn and paraquat, to *Fusarium moniliforme*, the causal agent of root rot and stem rot of sugarcane. The effect of these herbicides was also observed on antagonistic fungi and bacteria of this pathogen in the laboratory. The results obtained will be considered for recommending the use of herbicide in sugarcane field, which can inhibit the growth of *F. moniliforme* and has no or less effect to its antagonistic soil microorganisms.

MATERIALS AND METHODS

Isolation of *Fusarium moniliforme*

Diseased sugarcane plants naturally infected by root and stem rot pothogen, *F. moniliforme*, were collected from sugarcane field at Samchook District, Suphanburi Provinces. Infected tissue was cut into small pieces ca. 5 mm. then surface was disinfected by clorox 10 % for 5 min. Tissue was transplanted onto water agar plate, then they were incubated at room temperature, 25-30°C. Four days later, the hyphal tip of *F. moniliforme* grown from infected tissue, was transferred to potato dextrose agar (PDA) plates and/ or PDA slants for pure culture isolation and preservation. Pathogenicity studies was carried out in the greenhouse in order to select the servere isolate of *F. moniliforme* for further investigation.

In vitro screening for antagonists of *Fusarium moniliforme*

Fungi

Forty six isolates of soil fungi (Korpraditskul et. at. 1988) and *F. moniliforme* were cultured on PDA plates and incubated at room temperature for 2 days. A mycelial disc of 5 mm diameter was removed from the edge of colonies and placed on one side of PDA plate, where the opposite side, 6 cm. apart from each other, was inoculated with the same size mycelial disc of a test fungus. Four plates were used for each replicate and four replicates/treatment. Cultures were observed daily up to 7 days, the interaction between both isolates and the mycelial growth of the pathogen was recorded. Fungal isolate which showed the best antagonistic interaction was selected for further investigation.

Bacteria

Pure culture of 40 bacterial isolates (Korpraditskul et. at. 1988) were cultivated on PDA plate and incubated at room temperature for 24 hours. A mycelial disc of *F. moniliforme*, as previously explained, was transferred to the center of PDA plate, then inoculated with each agar disc of four bacterial isolates, 3 cm. apart from a mycelial disc of the pathogens. Each replicate consisted of four plates and four replicates were used per treatment. Plates were incubated at room temperature and the antagonistic interaction between both organisms was daily recorded up to 7 days. Bacterial isolate which showed the best antagonistic interaction was selected for further investigation.

Inhibitory effect of herbicides on *Fusarium moniliforme* and its antagonists.

In medium

The effect of herbicides on *F. moniliforme* and eight isolates of its anagonistic fungi was tested by poisoned food technique on PDA plates containing 5 concentrations, ie. 2.0, 1.0, 0.5, 0.25 and 0.125 NRR (NRR = Normal Recommended Rate) of atrazine, ametryn and paraquat. Six plates/ replicate and four replicates/ treatment were used in this study. A mycelial disc of fungal isolate was transferred onto the surface of PDA plate containing different kinds and concentrations of herbicides. Cultures were incubated at room temperature, the mycelial growth of *F. moniliforme* and its antagonists were recorded 3 and 5 days after inoculation compared to the control plate.

Four selected isolates of antagonistic bacteria against *F. moniliforme* were cultured by spread plate on the surface of PDA plates, then incubated at room temperature for 2 hours. Paper discs of 5 mm diameter were dipped into herbicide suspension, then they were transferred onto the surface of agar plate previously spreaded with bacterial culture. Paper disc absorbed with each of five concentrations of a certain herbicide was separately placed on the same plate. Inhibition zone around the paper disc was recorded, 48 hours after treatment.

In the soil

The effect of herbicides on *F. moniliforme* and its antagonistic fungi and bacteria in the soil was observed by quantifying the CO_2 production in an air tight container. Twenty-five grams of sterillized soil

was simultanously mixed with herbicide to obtain 3 conc., 1.0, 0.5 and 0.25 NRR. then soil moisture was adjusted with sterile water to 50% Maximum Water Holding Capacity (MWHC). One millilitre of 5 day-old culture of antagonistic fungi in potato dextrose broth (PDB) or 36 hour old culture of bacteria in nutrient broth (NB) was mixed into soil samples. Five grams of ready mixed soil sample was put into 15 ml test tube and sealed with rubber plug, four replicated tubes/treatment, then they were incubated at room temperature. Two and four days after incubation, one ml of gas sample in each test tube was taken with syringe and it was injected into Gas Chromato-graphy : Shimadzu GC-9A for quantification of CO_2 production from microorganism.

RESULTS AND DISCUSSION

In vitro screening for antagonists of *Fusarium moniliforme*

Fungi

The antagonistic reactions on mycelial growth of 46 isolates of fungi were characterized into 4 types, A, B, C and D as shown in table 1. Only one isolate, *Trichoderma* sp. showed the type-A reaction, whereas reactions of five isolates of *Aspergillus* spp. and two isolates of *Curvularia* spp. were type-B. Five isolates of *Aspergillus* spp. were classified as type-C. Reaction of the 32 remaining isolates of tested fungi were type-D, which included *Aspergillus* spp. (14 isolates), *Penicillium* spp. (3 isolates), *Helminthosporium* spp. (2 isolates) and other genera (13 isolates) as shown in table 1 and Fig 1.

Eight isolates of soil fungi which showed type A and B antagonistic reaction were selected for further investigation on side-effect of herbicides to these antagonists on agar medium and in the soil. These included two isolates of *Aspergillus niger* and *Curvularia* spp. and a single isolate of *A. flavus*, *A. tamarii*, *A. fumigatus* and *Trichoderma* sp.

Bacteria

The antagonistic reactions of 40 isolates of soil bacteria on mycelial growth of *F. moniliforme* was characterized into 3 types, A, B and O (Table 2 and Fig 2).

The results revealed that 15, 2 and 23 isolates of the test bacteria were classified to type A, B and O respectively. Two isolates of each group (gram negative and positive bacteria) were selected for further studied on side effect influenced by herbicide.

Side-effect of herbicides on *Fusarium moniliforme*

Colony diameter of *F. moniliforme* colonies cultured on PDA amended with 3 different concentrations of each herbicide were measured and compared

Table 1. The antagonistic reaction of soil fungi on mycelial growth of *F. moniliforme* on PDA

Fungus	No. of isolate	Type of reaction			
		A	B	C	D
Aspergillus spp.	24	-	5	5	14
Penicillium spp.	4	-	-	1	3
Curvularia spp.	2	-	2	-	-
Helminthosporium spp.	2	-	-	-	2
Trichoderma sp.	1	1	-	-	-
Others	13	-	-	-	13
Total	46	1	7	6	32

Type A: Mycelial growth of *F. moniliforme* was slower than the growth of test fungus.
Type B: The test fungus produced some substances to inhibit mycelial growth of *F. moniliforme*.
Type C: Mycelial growth of the test fungus equaled to the growth of *F. moniliforme*. The test fungus also produced some inhibitory substances to *F. moniliforme*.
Type D: Mycelial growth of the test fungus equaled to the growth of *F. moniliforme* but inhibitory substance was not produced.

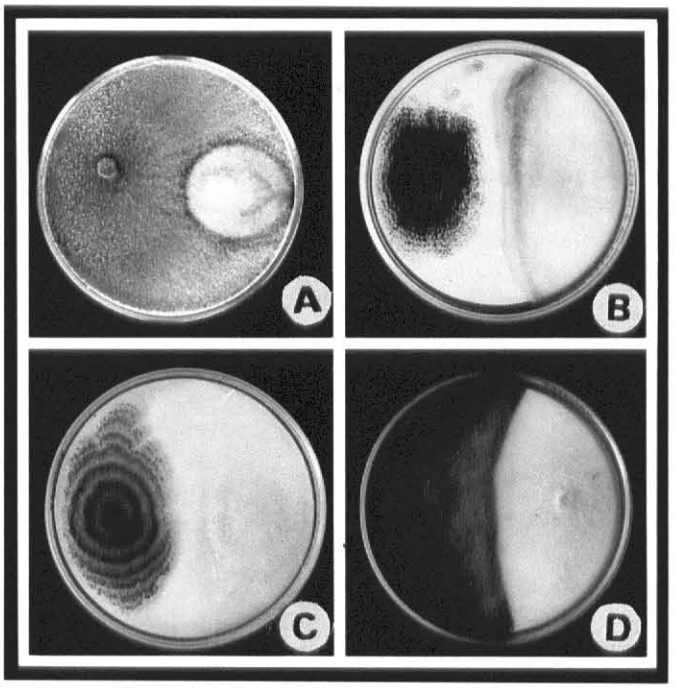

Fig. 1: The types of reactions between *F. moniliforme* and its antagonistic fungi

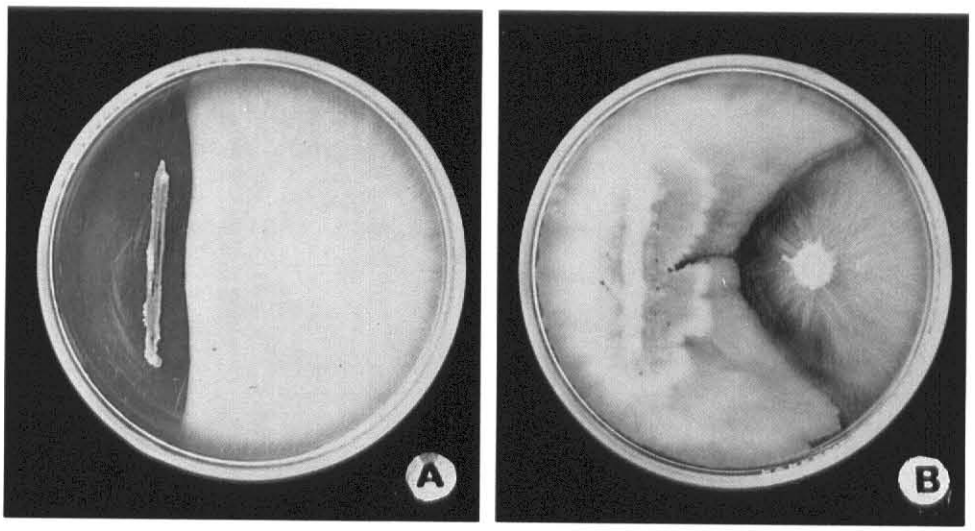

Fig. 2: Types of reactions between *F. moniliforme* and its antagonistic bacteria.

to the control plates. Atrazine at high concentrations, 1.0 NRR and ametryn at all tested concentration (0.125-1.0 NRR) had high inhibitory effect to this pathogen. Slight effect of herbicide to *F. moniliforme* was observed in paraquat. The inhibitory reaction of herbicide to *F. moniliforme* is shown in Fig 3. The ED50 (Effective Dose at 50%) of herbicides to *F. moniliforme* calculated from DR (Dosage Response) curve of probit value revealed that ED_{50} of ametryn, atrazine and paraquat were 33.5 ppm., 2985.4 ppm. and 3981.1 ppm., respectively. The correlations of probit values and all tested concentrations of these herbicides were shown in Fig.4.

Table 2. **The antagonistic reactions of soil bacteria from sugarcane field on *Fusarium moniliforme* in laboratory.**

Bacterium	No. of isolate	Type of reaction		
		A	B	O
Gram negative	25	8	2	15
Gram positive	15	7	-	8
Total	40	15	2	23

Type A: The test bacterium produced some substances inhibit to mycelial growth of *F. moniliforme*
Type B: The test bacterium grew better than the growth of mycelium of *F. moniliforme*
Type O: No reaction between the test bacterium and *F. moniliforme*

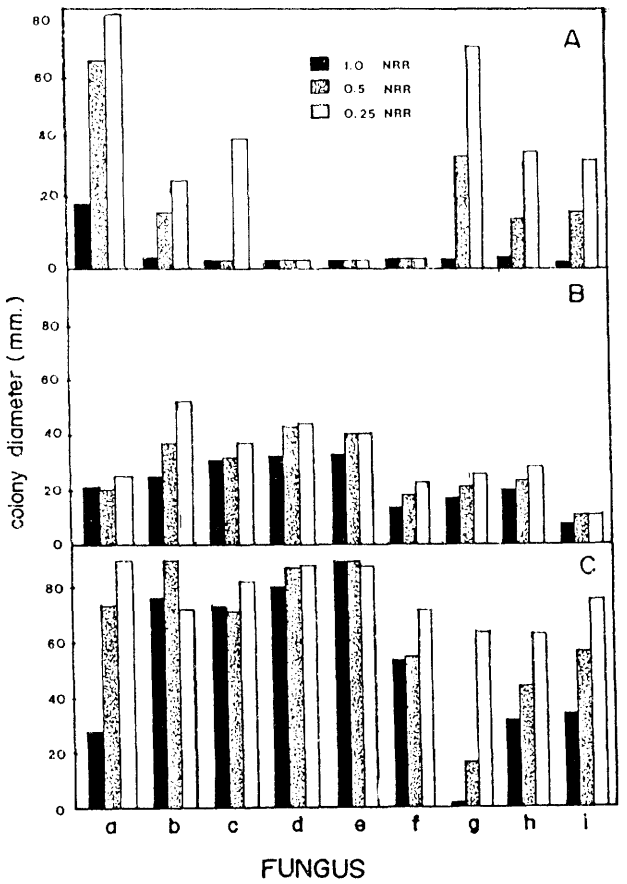

Fig. 3: **Inhibitory effect of atrazine (A), ametryn (B) and paraquat (C) on mycelial growth of *Fusarium moniliforme* (a) and its antagonists (b-i)**
b: *Aspergillus niger* ;c: *Aspergillus flavus* isolate 1
d: *Aspergillus tamarii* ;e: *Aspergillus flavus* isolate 2
f: *Aspergillus fumigatus* ;g: *Trichoderma* sp.
h: *Curvularia* sp. isolate 1 ;i: *Curvularia* sp. isolate 2
NRR = Normal Recommended Rate

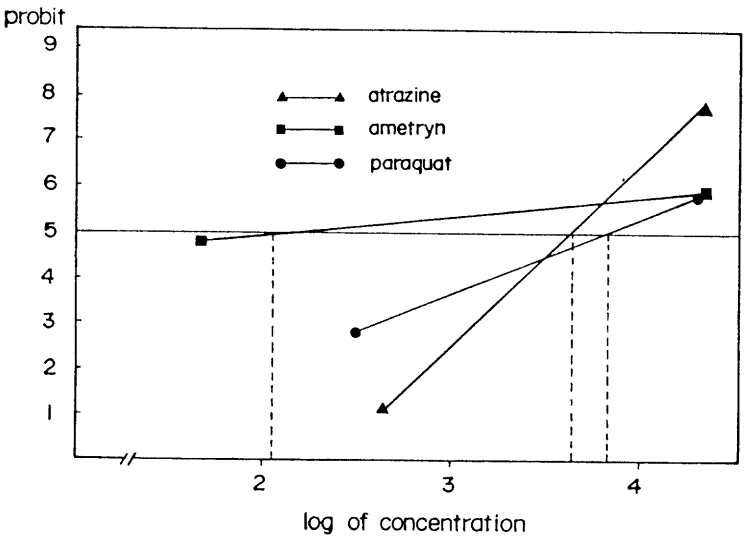

Fig. 4 : The correlation of probit value of atrazine, ametryn and paraquat at different concentrations.

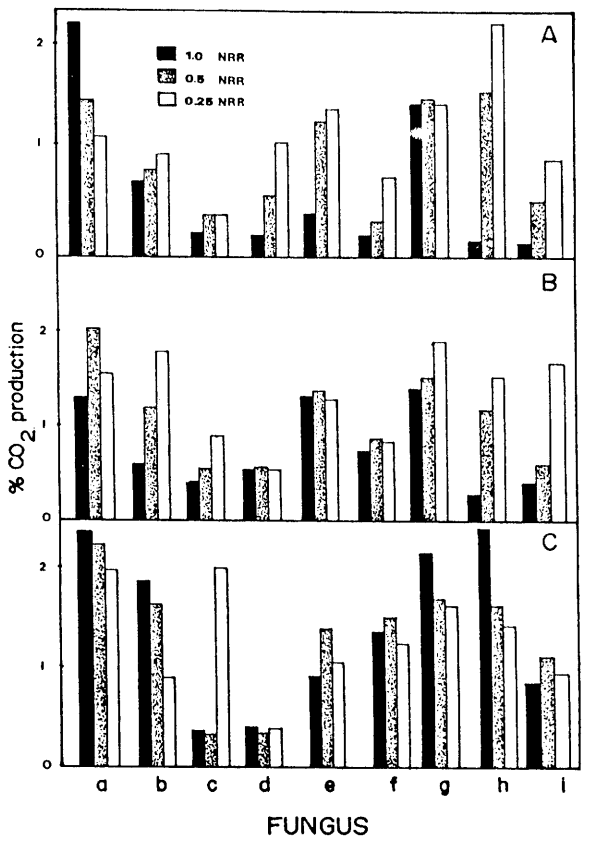

Fig. 5: Effects of three herbicides, atrazine (A), ametryn (B) and paraquat (C) on CO_2 production (%) in anaerobic condition of *Fusarium moniliforme* (a) and selected microorganisms (b-i)
b: *Aspergillus niger* ;c: *Aspergillus flavus* isolate 1
d: *Aspergillus tamarii* ;e: *Aspergillus flavus* isolate 2
f: *Aspergillus fumigatus* ;g: *Trichoderma* sp.
h: *Curvularia* sp. isolate 1 ;i: *Curvularia* sp. isolate 2
NRR = Normal Recommended Rate.

The relationship of CO_2 production during the growth period of *F. moniliforme* in treated soil was used as a parameter for calculating the growth rate of microorganisms. Under optimum condition, high growth rate of microorganisms occurred, which correlated with the production of high CO_2. At concentration 1.0 NRR of atrazine and paraquat in soil samples containing *F. moniliforme*, CO_2 production were 2.374 and 2.378 % in 1 ml of air sample taken from air tight container, respectively, while 2.533 % and 1.244 % CO_2 were observed in the control (not shown in a Fig. 5) and ametryn treated soil, respectively (Fig.5). This indicated that all herbicides affected the biological activity of *F. moniliforme* but the effect of atrazine and paraquat were slightly less than ametryn.

Side effect of herbicide on antagonistic microorganisms

Fungi

The mycelial growth of all selected antagonistic fungi was inhibited by herbicides. Atrazine, at high concentration (1.0 NRR), completely affected on mycelial growth of the test antagonists, where less effect was detected in paraquat and ametryn treatments, respectively *Trichoderma* sp. and 2 isolates of *Curvularia* spp. tolerated to atrazine, compared to most isolates of *Aspergillus* spp. as shown in Fig. 3.

For CO_2 production, all test herbicides affected on CO_2 production or biological activity of the antagonists. *A. tamarii* was sentitive to all herbicides at all concentrations. Low inhibitory effect of herbicides to antagonists was observed in *Trichoderma* spp. and 2 isolates of *Curvularia* spp. (Fig. 5 B-b-i)

Bacteria

Investigation on the inhibitory effect of herbicide on four bacteria isolates, revealed no inhibition (clear) zone on the surface of PDA amended with each of all concentrations of atrazine and ametryn. However, inhibition zone was detected when isolate #8 and #12 were spread on the surface of PDA containing with each of the tested samples (Table 3).

The biological activity (CO_2 production) of antagonistic bacteria isolate No. 33 and 36 were inhibited by all concentrations of atrazine and ametryn. Similar effect was also found when paraquat at 1.0 and

Table 3. Effect of different concentrations of three herbicides on inhibition (clear) zone development of selected antagonistic bacteria.

Herbicide/rate	Diameter of clear zone (mm)			
	Bacterial isolate No.			
	8 (G+)	12 (G-)	33 (G+)	36 (G-)
atrazine				
1.0 NRR	n	n	n	n
0.5 NRR	n	n	n	n
0.25 NRR	n	n	n	n
ametryn				
1.0 NRR	n	n	n	n
0.5 NRR	n	n	n	n
0.25 NRR	n	n	n	n
paraquat				
1.0 NRR	8.8	9.3	n	n
0.5 NRR	7.2	7.1	n	n
0.25 NRR	6.5	7.0	n	n

NRR = Normal Recommended Rate
G = Gram reaction (positive or negative)
n = no clear zone

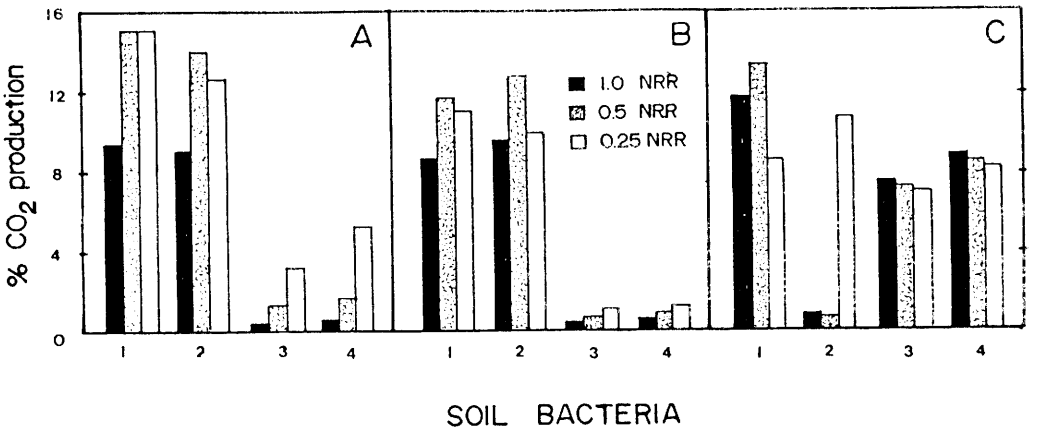

Fig. 6: **Effect of three herbicides, atrazine (A), ametryne (B) and paraquat (C) on CO_2 production of selected bacteria (1:isolate 8, 2:isolate 12, 3: isolate 33, 4: isolate 36)**
NRR = Normal Recommended Rate.

0.5 NRR were used against bacteria isolate No.12 (Fig.6).

LITERATURE CITED

Altman, J. and C.L. Campbell. 1977. Effect on herbicide on plant disease, Ann. Rev. Phytopathol. 15 : 361-385.

Bozarth, G.A and B.G. Tweedy. 1971. Effect of pesticide on growth and sclerotial production of *Sclerotium rolfsii*. Phytopathology 61 : 1140-1142.

Deshmukh, V.A. and J.G. Shrikhande. 1974 Effect of pre-and post emergence treatment of herbicides on soil microflora and two microbial process. Jour. Ind. Soc. Soil Sci. 22 : 36-42.

Haber, D.M.,C.I. Seely and R.D.Watson. 1966. Effect of the herbicide diuron on foot rot of winter wheat. Plant Dis. Reptr. 50 : 852-854.

Heitefuss, R. 1970. The effect of herbicide on field crop. Proc. Eur. Weed Res. Cont. Syny. Herbicide - soil. pp. 99-128.

Jone, D.G. and J.R. William. 1971. Effect of paraquat on growth and sporulation of *Septoria nodorum* and *Septoria tritici*. Tans. Br. Mycol. Soc. 57 : 351-357.

Kabana, R.R. and E.A. Curl. 1970. Nontarget effects of pesticides on soilborne pathogen and disease. Ann. Rev. Phytopathol. 18 : 311-321.

Kabana, R.R., E.A. Curl. and H.H. Funderburk. 1966. Effect of herbicide on growth of *Rhizoctonia solani*. Phytopathology 56 : 1332-1333.

Korpraditskul, V., S. Jiwajinda, R. Korpraditskul, S. Wicharn and C. Rattanagreetakul. 1988. Side effect of three herbicides on soil microorganism. Kasetsart J. (Nat. Sci) 22: 54-66.

Quilty, S.P. and M.J. Geoghegan. 1975. Effects of glyphosate on fungi. Proceedings of the Society of General Microbiology 2:87.

Romig, W.R. and M.Sasser. 1972. Herbicide predisposition of snapbeans to *Rhizoctonia solani*. Phytopathology 62 : 785-786.

Ross, J.P. 1965. Predisposition of snapbeans to Fusarium wilt by *Heterodera glycines*. Phytopathology 75 : 684-687.

Wilkinson, V. and R.L.Lucas. 1969. Gramoxone W:its effects on spores and mycelia of *Rhizoctonia stolonifer* Trans. Brit. Mycol. Soc. 53 : 297-299.