Minufiya J. Agric. Res. Vol. 32 No.1: 323 - 333 (2007)

BIODEGRADATION OF PESTICIDES AND THEIR EFFECT ON ROOT NODULATION AND RHIZOSPHERE MICROFLORA OF CLOVER PLANTS

S.A. Abou El-Naga
Dep. of Soil Sci., Fac. of Agric. Minufiya University

(Received: July 17 , 2006)

***ABSTRACT** : A laboratory experiment was designed to study, the ability of the microflora of clover plants rhizosphere (bacteria, actinomycetes and fungi) to utilize three pesticides as sources of carbon and nitrogen, namely, Deenate (insecticide), Nemacur (nematicide) and Benlate (fungicide). Rhizosphere microflora were able to grow on the basal medium at the different concentrations of the insecticide Deenate as a sole source of carbon and nitrogen at variable degrees, however, they were unable to grow with the pesticides "Nemacur" and "Benlate" at their different concentrations used.*

In a fot experiment, the effect of such pesticides on rhizosphere microflora of the clover plants was examined. Results showed increases in the number of clover plant root nodules and total nitrogen content of the plants at the different concentrations tested of the insecticide "Deenate" as compared with control. The nematicide "Nemacur" and the fungicide "Benlate" had inhibitory effects on nodule numbers and total nitrogen content of the clover plants.

Results also showed an increase in the total number of microflora with "Deenate" as compared with control, while, the other pesticides, "Nemacur" and "Benlate" were inhibiting.

***Key words:** Nematicides, Insecticides, Root zone, Leguminous plants, Diazotrophy, Rhizobium sp.*

INTRODUCTION

Pesticides cause many pollution problems in the environment and affect the development of soil microorganisms. Degradation of pesticides by soil microorganisms is an important factor in reducing their deleterious effect. Many investigators have studied the biodegradation of pesticides by soil microorganisms (Abdel-Nasser *et al.,* 1981; Forrest *et al.,* 1981; Mahmoud *et al.,* 1988; Abo El-Naga, 1989 and Kremer *et al.,* 1996).

Some pesticides, had been found to be stimulatory to certain microorganisms especially rhizobia after their degradation by soil microbial

population (Alexander, 1985; Taha *et al.,* 1972). Salem *et al.* (1971) showed a stimulatory effect of insecticides on nodulation and symbiotic nitrogen fixation in some legume plants. Gamal El-Din *et al.* (1985); Aggarwal *et al.* (1986) and Abou El-Naga (1989) studied the deleterious effect of some pesticides on soil microorganisms, nodulation and symbiotic nitrogen fixation in some legumes. They found that higher doses of pesticides were injurious, to some extent to rhizobia. The utilization of these pesticides by the rhizosphere microflora as sources of carbon and/or nitrogen had been considered as an indication to the potentiality of microflora in degrading and utilizing pesticides.

This work was carried out to determine the potency of clover rhizosphere microflora in degrading some pesticides in a trial to evaluate their role in minimizing environmental pollution and to elucidate the influence of these pesticides on development of soil microorganisms and nodulation capacity of clover plants.

MATERIALS AND METHODS

Experiment I

Three pesticides were used, namely, insecticide "Deenate" (Methomyl diflubenzuron), nematicide "Nemacur" (fenamiphos; ethyl 3-methyl 4-"methlthio" phenyl) (N-isopropyl phosphoramidate) and fungicide "Benlate" (benomyl; methyl 1-"butylcarbamoyl") 2-benzimidazole-carbamate) see Table (1). Bunt and Rovira liquid medium was used as the basal culture medium for bacteria (Bunt and Rovira, 1955). Czapek's medium was used for actinomycetes (pH 6.6) and fungi (pH 6.0). (Page *et al.* 1982). The three pesticides used were supplied to the basal media without sucrose or nitrate as a carbon or nitrogen source, respectively, as required. The pesticides were supplemented at concentrations 9.0, 12.0 and 15.0 mg/L medium for Deenate and Nemacur and at 1000, 3000 and 6000 ppm for Benlate . The minimum doses of the applied pesticides were used herein according to the recommendations of the Egyption. The required amount of each pesticide was dissolved in a 2ml ethyl alcohol and added to the liquid medium after sterilization under aseptic conditions. Incubation period used was 10 days at 25-30 °C. Microbial growth rate was measured by turbidity of the liquid media, to determine the utilization of the pesticides by microflora. Microbial growth rate was described as: No growth (-), Feeble growth (+), Moderate growth (++) and Vigorous growth (+++).

Table (1): Pesticides used, their names, structures and formulas.

Trade name	Common name	Chemical formula	Chemical structure	Class
Consist of: Deenate Lannate	Methomyl	$C_5H_{10}N_2O_2S$	S-methyl N-(methyl carbamoyloxy) thioacetimide	Insectieide
Deenate Dimilin	Diflubenzuron	$C_{14}H_9N_2O_2F_2Cl_1$	1- (4 chlorophenyly) -3- (2,6 diflurobenzoyl) urea	Insect growth regulator
Nemacur	Fenamiphos	$C_{13}H_{22}NO_3PS$	Ethy 4-methtlthio-m-tolyl usopropyl phosphoramisate; -3- methyl -4- (methylthis) phenyl isopropylphosphoramidate.	Nematucude
Benlate	Benomyl	$C_{14}H_{18}N_4O_3$	Methyl 1- (butylearbamoyl) benzimidazol -2- ylearbamate.	Fungicide

Experiment II

This experiment was carried out in 25 cm-diameter earthenware pots which were packed with 3kg of clay loam soil (having 2.7% organic matter, 7.4 pH and 1252 ppm total nitrogen). The pesticides were applied to the soil at different concentrations which were calculated according to the pot area. Each pot was planted with 12 seeds of Egyptian clover (*Trifolium alexandrinum* L.) inoculated with a 3 day-old culture of *Rhizobium trifolii* .5 days after planting the seedlings were thinned to 8. Deenate and Nemacur were added at rates of 3.5, 4.5 & 5.5 kg/feddan (4200 m^2) and Benlate at 1000, 3000 & 6000 ppm. Each treatment was replicated 4 times. The cultivated pots were watered moderately every 2 days. The nodules on every plant root system were counted 50 days after planting. Total nitrogen content (by kjeldahl tecjrigue) and dry matter weight of the plants were determined according to Paga *et al.* (1982).

Experiment III

The rhizosphere microflora were determined by plate count technique using the mentioned media 25 days after planting. Each treatment was replicated 4 times. Fata obtained were statistical analized.

RESULTS AND DISCUSSION

Insecticide

Data presented in Table (2) "Laboratory Experiment" showed that the rhizosphere microflora were able to grow on the basal culture medium with the different concentrations of the insecticide "Deenate" as a source of carbon and nitrogen at variable degrees. The lower concentration of the insecticide (9.0 mg/L) was the most easily utilized by rhizosphere microflora followed by the higher doses (12.0 & 15.0 mg/L). Actinomycetes showed a vigorous ability to utilize this insecticide. These results are in agreement with Hankin and Hill (1978) and Gabr *et al.,* (1998), who reported that the decomposition of unusual synthetic compounds as pesticides by bacteria in the soil and rhizosphere could be explained by the capacity of these bacteria to produce the necessary enzyme systems to react with such substances. Matsumura and Benezet (1978) suggested that pesticides might be degraded incidentally by non-induced constitutive enzymes of the microbial cell. Many genera of fungi had been reported to use pesticides as a substrate, either cometabolizing the molecules or using them as nutrients (Kennedy and Talder, 1977). Data presented in Table (3) "pot experiment" showed an increases in plant root nodules and total nitrogen content at the different concentrations of the insecticide "Swwnate" used, as compared with control. These results confirm the finding of Abou El-Fadl and Fahmy (1958) and Abou El-Naga (1989), who reported that some pesticides were found to be stimulant to certain soil microbes especially rhizobia after their biodegradation. Results also showed an increase in the number of the rhizosphere microflora as compared with control. At "Deenate" concentration 9.0 mg/L, the total numbers of bacteria, actinomycetes and fungi were $15.5x10^8$, $5.5x10^7$ and $10.0x10^4$ g/soil while they were $10.5x10^8$, $2.6x10^7$ and $4.0x10^4$ g/soil in the control, respectively. This might be due to the ability of the insecticide "Deenate" to be readily degraded by rhizosphere microflora (Domsch, 1972).

Table (2): Utilization of the insecticide "Deenate" by rhizosphere microflora of clover plants (Laboratory Experiment).

Rhizosphere microflora	Deenate concentration, mg/L								
	9.0			12.0			15.0		
	C	N	C&N	C	N	C&N	C	N	C&N
Bacteria	-	+	+	-	+	+	-	+	+
Actinomycetes	++	+++	+	+	++	+	+	+	-
Fungi	++	++	+	+	+	-	+	+	-

- = No growth ++ = Moderate growth

+ = Feeble growth +++ = Vigorous growth

Table (3): Effect of the insecticide "Deenate" on nitrogen fixation and rhizosphere microflora of clover plants (Pot Experiment).

Treatment	Insecticide conc. mg/L	Total No. nodules/ plant	Dry weight g/plant	Nitrogen %	Total nitrogen mg/plant
Control	-	9.5	1.0	2.9	29.0
Deenate	9.0	14.1	1.2	3.6	43.2
	12.0	11.0	1.1	3.2	36.3
	15.0	11.0	1.1	3.1	34.1
L.S.D. at 5%		2.5	0.5	3.1	10.5

Treatment	Insecticide conc. mg/L	Numbers of rhizosphere microflora		
		Bacteria ($x10^8$/g soil)	Actinomycetes ($x10^7$/g soil)	Fungi ($x10^4$/g soil)
Control	-	10.5	2.6	4.0
Deenate	9.0	15.5	5.5	10.0
	12.0	12.6	4.2	6.2
	15.0	11.3	3.3	6.1
L.S.D. at 5%		3.1	1.8	3.5

Namaticide and fungicide

Data presented in Tables (4 and 5) "Laboratory Experiment" showed that the rhizosphere microflora were unable to grow on the basal medium with both the nematicide "Nemacur" and the fungicide "Benlate" as sole sources of carbon and/or nitrogen at their different concentrations. The chemical structure of the pesticides molecules plays a role in their degradation (Mathur *et al.,* 1980). According to this assumption, in this study pesticides are classified into, readily attached molecules. i.e. the insecticide "Deenate" and more resistant molecules, i.e. "Nemacur" and "Benlate". Venkateswarlu and Sethunatham (1978) found that the source for degradation differed in different pesticides depending on whether aerobic or anaerobic conditions prevail.

Table (4): Utilization of the nematicide "Nemacur" by rhizosphere microflora of clover plants (Laboratory Experiment).

Rhizosphere microflora	Nemacur concentration, mg/L								
	9.0			12.0			15.0		
	C	N	C&N	C	N	C&N	C	N	C&N
Bacteria	-	-	-	-	-	-	-	-	-
Actinomycetes	+	-	-	+	-	-	+	-	-
Fungi	-	-	-	-	-	-	-	-	-

- = No growth **+ = Feeble growth**

Table (5): Effect of the nematicide "Nemacur" on nitrogen fixation and rhizosphere microflora of clover plants (Pot Experiment).

Treatment	Nematicide conc. mg/L	Total No. nodules/ plant	Dry weight g/plant	Nitrogen %	Total nitrogen mg/plant
Control	-	9.5	1.0	2.9	29.0
Nemecur	9.0	5.3	0.8	2.1	17.0
	12.0	3.5	0.6	1.7	10.0
	15.0	2.1	0.6	1.3	8.0
L.S.D. at 5%		3.9	0.5		8.3

Treatment	Nematicide conc. mg/L	Numbers of rhizosphere microflora		
		Bacteria ($x10^8$/g soil)	Actinomycetes ($x10^7$/g soil)	Fungi ($x10^4$/g soil)
Control	-	10.5	2.6	4.1
Nemecur	9.0	7.4	1.6	3.7
	12.0	6.0	1.4	2.1
	15.0	4.4	1.0	1.6
L.S.D. at 5%		3.5	0.5	1.5

Data presented in Tables (6 and 7) "pot experiment" indicated that both pesticides, "Nemacur "and Benlate" had inhibitory effects at all concentrations used on root nodule numbers, dry weight of plant and its total nitrogen content. These results are in agreement with many investigators who had studied the deleterious effect of some pesticides on nodulation and symbiotic nitrogen fixation by rhizobia in some legumes (Aggarwal *et al.,* 1986; Siddiqui *et al.,* 1998 and Singh *et al.,* 1999). They came to the conclusion that application of higher doses of pesticides was injurious, to some extent, to rhizobia. Tables (5 and 7) "Field experiment", also showed the influence of "Nemacur" and "Benlate" pesticides on rhizosphere microflora of clover plants. Results revealed decreases in the number of rhizosphere microflora at their different concentrations, as compared with control. Some of the sensitive rhizosphere microflora showed decreases or no growth in the presence of such pesticides. Kojic et al. (1987) showed that the applied pesticide inhibited the intensity of soil respiration, which is one of the most reliable parameters for measuring the biological activities. Decreasing numbers of microflora in the presence of "Nemacur" and "Benlate" may be due to that both pesticides are more resistant to the attack by microflora than "Deenate" (Mathur et al., 1980) and/or to their toxicity to the assigned organisms (Cooper et al., 1978). Khalil et al. (1993); Gabr et al. (1998) and Abd El-Maksoud et al. (2003) found that fungicides completely inhibited the linear growth and sporulation of microorganisms. The inhibitory or stimulatory effect observed by previous investigators could not be generalized on using different pesticides and for all different groups of microflora. It depends on the chemical formula, concentration, and mode of action, on one hand and on sensitivity of rhizobia and rhizosphere microflora to these materials, on the other. Aggarwal et al. (1986) reported that the sensitivity to pesticides depends upon the species of rhizobia and also the type of pesticides.

Table (6): Utilization of the fungicide "Benlate" by rhizosphere microflora of clover plants (Laboratory Experiment).

Rhizosphere microflora	Benlate concentration, mg/L								
	9.0			12.0			15.0		
	C	N	C&N	C	N	C&N	C	N	C&N
Bacteria	+	-	-	+	-	-	+	-	-
Actinomycetes	-	-	-	-	-	-	-	-	-
Fungi	-	-	-	-	-	-	-	-	-

- = No growth **+ = Feeble growth**

Table (7): Effect of the fungicide "Benlate" on nitrogen fixation and rhizosphere microflora of clover plants (Pot Experiment).

Treatment	Fungicide conc., ppm	Total No. nodules/ plant	Dry weight g/plant	Nitrogen %	Total nitrogen mg/plant
Control	-	9.5	1.0	2.9	29.0
Benlate	1000	7.1	0.9	2.5	25.0
	3000	5.7	0.8	2.0	22.0
	6000	5.0	0.8	1.8	20.1
L.S.D. at 5%		3.1	0.2		5.5

Treatment	Fungicide conc., ppm	Numbers of rhizosphere microflora		
		Bacteria ($x10^8$/g soil)	Actinomycetes ($x10^7$/g soil)	Fungi ($x10^4$/g soil)
Control	-	10.5	2.6	4.1
Benlate	1000	8.3	2.0	3.9
	3000	6.1	1.7	3.0
	6000	5.2	1.2	2.3
L.S.D. at 5%		3.5	0.3	1.3

From the above-mentioned results, it can be concluded that application of the insecticide "Deenate", which was utilized by soil microbial population, led to positive effects on symbiosis and rhizosphere microflora of clover plants. While, the nematicide "Nemacur" and the fungicide "Benlate", soil microorganisms in general .

The present study also indicated that some of rhizosphere microflora of clover plants play an important role in the detoxification of pesticides via their degradation. On the other hand, pesticides should be applied to soil with care to avoid any probable harmful effect on inoculated rhizobia and soil rhizosphere microflora.

REFERENCES

Abdel-Nasser, M.; A.A. Makawi and A.A. Moneim (1981). Occurrence of certain Microorganisms in rhizosphere soils of maize, common bean and cotton as affected by the application of Temik or orthocide pesticides. Egypt J. Microbiol. 14 (1/2): 37-42.

Abd El-Maksoud, H.K.; I. Hosney and Fatma, Abd El-Zaher (2003). Ecological studies on Rhizobia indigenous to soils in Egypt. 2: Pestieides tolerance. Egypt. J. Microbiol. 38, No. 2, pp. 145-153.

Abou El-Fadl M.A. and M. Fahmy (1958). Effect of sodium 2, 4-D and MCPA on root nodulation of legumes and soil microorganisms. Agric., Res. 36: 339-344.

Abou El-Naga S.A. (1989). Influense of some nematicides on microbial processes in faba bean-planted soil. Proceedings of third World Conf. on Inviron. and Health Hazard of Pesticides Cairo, Egypt. VII. P. 13-222.

Aggarwal, T.C.; Neeru Narula and K.G. Gupta (1986). Effect of some carbamate pesticides on nodulation, plant yield and nitrogen fixation by *Pisum sativem* and *Vigna sinensis* in the presnec of their respective rhizobia. Plant and Soil 94: 125-132.

Alexander, M. (1985). Resistance and biological reactions of pesticides in soils. Soil Sci. Amer. Proc. 29: 1-7.

Bunt, J.S. and A.D. Rovira (1955). Microbiological studies of some subantarctic soils. J. Soil Sci. 6: 119-128.

Cooper, S.L.; G.I. Wingfield; R. Lawey and M.P. Greuves (1978). Miniaturized methods for testing the toxicity of pesticides to Microorganisms. Weed Res. 18 (2): 105.

Domsch, K.H. (1972). Interactions of soil microbes and pesticides. Symp. Biol. Hung. 11: 337-347.

Forrest, Margaret; K.A. Lord; N. Walker and H.C. Woodville (1981). The influence of soil treatments on the bacterial degradation of diazinon and other organophosphors insecticides, Environmental Pollution, Series A., (8): 412-419.

Gabr, M.R.; N.A. Hussein; O.I. Saleh; M.A. Kalil (1998). Some factors affecting rhizosphere fungal population of lupine plant. Anuals of Agric. Sci. Moshtohor. Fac. of Agric. Zagazig Univ. Egypt. 35, (2): 143-156.

Gamal El-Din, I.F.; N.A. Neweigy; M.I. Zeidan; Ehsan, A. Hanafy and M.A. Abou-Neama (1985). The influence of some fungicides on symbiotic nitrogen fixers (rhizobia) and nitrogenase activity of certain leguminous crops in soil infested with some pathogenic fungi. Anuals Agric., Sci., Fac., Agric., Ain Shams Univ. Cairo, Egypt. 30 (2): 793-804.

Hankin, L. and D.E. Hill (1978). Proportion of bacteria in agricultural soils able to produce degradative enzymes. Soil Science, 126: 40-43.

Kennedy, J.M. and R.E. Taldert (1977). Comparative persistence of dinitro aniline type herbicides on the soil surface. Weed Sci. 25 (5): 373.

Khalil, M.A.; M.M.F. El-Morshedy; M.H. Hashem and Aida M. El-Zawari (1993). Efficiency of certain pesticides on soil borne plant pathogenic fungi and root-knot nematode. Assiut J. of Agric. Sci. Vol. 24: 1.

Kojic, M.; D. Pejcinovic; E. Hodza and S. Gligorjevik (1987). Study of herbicide effect on the intensity of soil respiration in wheat. FRAGM HERBOL JUGOS 14 (1/2) 27 Bio. Abstr. 84 (9): 1155.

Kremer, R.J.; D.N. Sasseville and H.A. Mills (1996). Promotion of phytotoxic bacteria in rhizosphere of leatherleaf fern by benlate. J. Plant Nutrition. 19 (6), 939-953.

Mahmoud S.A.Z.; A.M. Abdel Hafez and M. El-Sawy (1988). Soil Microbiology in (Arbic), Cairo, Egypt.

Mathur, S.P.; A. Belanger; H.A. Hamilton and S.U. Khan (1980). Influence on microflora and persistence of field applied disulfoton, permethrin and prometryne in an organic soil, pedodiologia, 20: 237-242.

Matsumura, F. and H.J. Benezet (1978). Microbial degradation of insecticides, pp. 627-628 in Pesticide Microbiology (Edited by I.R. Hill and S.J.L. Wright), Academic press, London, New York, San Francisco.

Page, A.L. R.H. Miller and D.R. Keeney (1982). Methods of soil Analysis. Part 2: Chemical and Microbiological Properties. Amer. Soc. Agron. Madison. Wisconsin, USA.

Salem, S.H.; J. Szegi and Gulyas Ferenc (1971). Influence of some insecticides on the symbiosis of rhizobia and legume plants. Agriokemia es Talajtan 20: 581-589.

Siddiqui, I.A.; S. Ehteshamul-Hque and A. Ghaffar (1998). Effect of fungicides on the efficacy of *Rhizobium meliloti* and *Brady rhizobium* sp. In the control of root infecting fungi on chickpea. Pakistan J. Botany. 30 (1), 69.

Singh, G. and D. Wright (1999). Effect of herbicides on nodulation, symbiotic nitrogen fixation, growth and yield of pea (*Pisum sativum*). Agric. Sci. J. 133 (1): 21.

Taha, S.M.; S.A.Z. Mahmoud and S.H. Salem (1972). Effect of pesticides on rhizobia inoculation, nodulation and symbiotic N-fixation of some leguminous plants. Symp. Biol. Hung. 11: 423-429.

Venkateswarlu, K. and N. Sethunatham (1978). Degradation of carbofuran in rice soils as influenced by repeated applications and exposure to aerobic conditions following anaerobiosis. Journal of Agricultural and Food Chemistry, 26: 1148-1151.

التحلل الحيوى لبعض مبيدات الآفات وتأثيرها على ظروف التكافل واعداد الميكروبات المحيطة بجذور نبات البرسيم

صلاح على أبو النجا

قسم علوم الأراضى . كلية الزراعة . جامعة المنوفية . شبين الكوم

المخلص العربى

تم إجراء تجربة معملية لتحديد مقدرة الميكروفلورا المحيطة بجذور نبات البرسيم (البكتريا ، والأكيتنوميسيتس والفطر) للإسفادة من بعض مبيدات الآفات كمصادر للكربون أو النيتروجين أو كليهما معاً، للوقوف على مدى قابلية هذه المركبات للتحلل الميكروبى فى الأرض

وقد استخدم مبيد حشرى "دينات" ، ومبيد نيماتودى "تيماكور" ، وثالث فطرى "بنلات" . وأظهرت النتائج أن ميكروبات الريزوسفير يمكنها أن تنمو على البيئة المحتوية على تركيزات مختلفة من المبيد الحشرى "دينات" كمصدر للكربون أو النيتروجين كليهما معا بدرجات متفاوتة ومختلفة من النمو . بينما لا يمكن أن تنمو على البيئة المحتوية على تركيزات مختلفة من كلا من المبيدين النيماتودى والفطرى ، حيث لم تستطيع الاستفادة منهما كمصدر لأى من العنصرين . واتضح أن التركيز الأقل من المبيد الحشرى أكثر سهولة لإستفادة الميكروبات عن التركيز الأعلى .

وفى تجربة أصص تم دراسة تأثير هذه المبيدات الثلاث على مقدرة نباتات البرسيم الملقحة بذوره بالريزوبيا على تكوين العقد الجذرية وتثبيت النيتروجين الجوى تكافلياً . وأظهرت النتائج أن المبيد الحشرى "دينات" أدى إلى زيادة فى عدد العقد الجذرية والمحتوى النيتروجينى للنبات بتركيزاته المختلفة مقارنة بالكنترول. بينما ظهر تأثير مثبط على عدد هذين القياسيين الحيويين لدى المعاملة بكلا من المبيدين النيماتودى "تيماكور" والفطرى "بنلات".

وفى تجربة الأصص كذلك تم دراسة تأثير هذه المبيدات الثلاثة على أعداد الميكروفلورا المحيطة بجذور نبات البرسيم (البكتريا ، والأكتينوميسبتس والفطر) وقد وجد أن المبيد الحشرى "دينات" أيضا أدى إلى زيادة أعداد هذه الميكروبات ، بينما المبيدين النيماتودى والفطرى أظهرا تأثيراً مثبطاً على أعداد هذه الميكروبات مقارنة بالكنترول .