9

Uganda Journal of Agricultural Sciences, 2005, 11: 9 - 13 ISSN 1026-0919

Effects of carbamate and pyrethroid insecticides on cowpea bradyrhizobial population assessed in sand culture and on nitrogen fixation in soil

Josephine N. Ng'ang'a and G.P. Msumali
Department of Soil Science, Sokoine University of Agriculture
P.O. Box 3008, Morogoro, Tanzania

Abstract

The effects of two insecticides, *Lannate* (a carbamate) and *karate* (a pyrethoid) on nitrogen fixation by cowpeas (*Vigna unguiculata*) were evaluated after establishing the toxic effects of these insecticides on the microsymbiont bradyrihobia *in vitro*. In this (*in vitro)* experiment conducted in modified Leonard jars (LJ), seedlings from pre-germinated, surface sterilised cowpea seeds were inoculated with 1 ml each of five – fold (5^{-1} – 5^{-6}) dilute soil suspensions. Aqueous solutions of *lannate* or *karate*, each at a final concentration of 0.5 µg ml^{-1}were incorporated into the N-free solution in the LJ nutrient reservoirs. After 28 days, the plants were examined for nodulation on the basis of which, the most probable Number technique was used to estimate the cowpea bradyrhizobial count in the original soil from which the inoculant soil suspensions were prepared. Then, the effects of these insecticides each at 0.5 and 1.5 ppm were assessed on the cowpea-*Bradyrhizobium* symbiotic relationship in potted soil. Cowpeas were grown and harvested after 35 days. Nodule numbers and dry weight as well as shoot dry weight and nitrogen content were determined. Results of the Leonard jar investigations revealed that bradyrhizobial counts were reduced by the application of these insecticides, the effect being more pronounced with *lannate* than with *karate*. However, nodulation and nitrogen fixation by cowpeas grown in soil, were not significantly affected by any of these insecticides at any of the concentrations tested. It was concluded from this study that while these two insecticides appear to have no profound effects on the cowpea-*Bradyrhizobium* symbiotic association in the experimental soil, the very severe depressive effect of *lannate* on the microsymbiont population *in vitro*, may warrant further investigations into the effects of these insecticides on the *Bradyrhizobium* – cowpea symbiosis in other soils.

Key words: *bradyrhizobium, carbamates, cowpeas, insecticides, nitrogen fixation, pyrethroids*

Introduction

Biological nitrogen fixation (BNF) is one of the most important microbiological processes in nature because it has enabled man to utilize at very low costs, the (otherwise unavailable) atmospheric nitrogen (N_2) for plant growth. While there are numerous plant-microbe associations in which BNF is carried out, the one which involviug legumes and the nodule-forming bacteria has the greatest contribution in this regard. Cowpea (*Vigna unguiculata)* is one of the most important grain legumes in the tropics and has the unique advantage of tolerating many of the edaphic stresses e.g. drought and acid-soil infertility that are prevalent in the tropics. Through BNF, cowpea can derive at least 50% of its nitrogen requirements from atmospheric N_2 (Eaglesham *et al*., 1982, Ofori *et al*., 1987). The other advantage is that cowpea nodulates spontaneously with the native bradyrhizobia that abound in many tropical soils and hence, this crop does not require artificial inoculation. To many of the reource-poor farmers in the marginal areas of East Africa where beans do not perform well, cowpeas can be regarded as a dependable source of dietary protein. Unfortunately, cowpeas (and other grain legumes) are susceptible to numerous pests and diseases. Such pests can lower the crop yields substantially e.g, up to 85% in the case of cowpeas (IITA, 1990). Consequently, spraying with insecticides has become an essential and integral practice for successful production of cowpeas and even of other crops (Kaushik 1991; Medvecky and Zalom, 1992). Pesticides, however, could (and often do) have detrimental influences on non-target organisms.

Both soil micro- and macro-organisms, and indeed, numerous studies have been undertaken to determine the effects of pesticides on various microbial processes. Most of those studies have examined the effect of herbicides or fungicides on the microbial processes. Where insecticides were involved in such studies, greater emphasis has been placed on the chlorinated hydrocarbons and the organophosphates than on the other classes of insecticides. Two types of insecticides, *lannate* (a carbamate) and *karate* (a pyrethroid) are widely used in East Africa and, the effects of these insecticides on soil microbial processes notably the legume-*Bradyrhizobium* symbiosis are not well documented. The present study was therefore undertaken with the following objectives: (i) to determine the effects of the lannate and a karate insecticides on the ability of bradyrhizobia to proliferte/ nodulate with the host (cowpea) *in vitro,* (ii) to assess the effects of the insecticides on BNF in the cowpea – *Bradyrhizobium* association in soil.

Materials and methods

Soil and its characteristics

The soil used to prepare the dilute suspensions for the inoculation of cowpea seedlings (see methodology below), was a sandy clay loam with the following properties: pH (H_2O), 6.8 (Mclean 1982): total N, 0.12% (Bremner and Mulvaney, 1982); organic C 0.14% (Nelson and Sommers, 1982); extractable P (Bray I), 32.0 ppm (Juo, 1978) and exchangeable bases, 4.34, 1.78, 0.63 and 0.06 (cmol of Ca, Mg, K and Na respectively kg^{-1} soil) (Phoades, 1982).

The insecticides

The insecticides, whose effects were investigated in this study are *lannate* (a carbamate) and *karate* (a pyrethroid). *Lannate* (methomyl) contains 90% 1 (methylthio) ethylideneamino methyl carbamate. It is a systemic insecticide and acts on the target organisms by inhibiting cholinesterase activity. The other insecticide, *karate,* (lambda cyhalothrin) contains 17.5% alpha-cyano-3-phenoxybenzyl-*cis*-3 (z-2-chloro-3,3, 3-trifluoroprop-1-en-1-ny)-2,2-dimethyl cyclopropane carboxylate. It is a contract synthetic pyrethoid with improved properties to withstand photodegardation.

The legume host

Cowpea(*Vignaunguiculata*), variety SVS-3, widely adapted to Morogoro conditions was used in this study. (Price *et al.*, 1982).

The growth vessels

The vessels in which the cowpea plants were raised were bottle-bottle assemblies. These were modifications of the Leonard bottle-jar (LJ) assemblies described by Vincent (1970). In the modified LJ, two, 500-ml beer bottle were used. One of the bottles had its base chopped-off and the other had its neck chopped off. The former bottle was inserted into the latter, to constitute the modified LJ. Quartz sand was used as the plant supportive medium in the upper part of the LJ while a N-free mineral nutrient solution (Weaver and Frederick, 1982) was contained in the lower part of the LJ. The two parts of the LJ were connected with an absorptive cotton wick. All the fully-assembled LJ units were covered with protective jacket (stiff paper and aluminium foil) and sterilized by autoclaving before use.

Effects of *lannate* or *karate* on cowpea bradyrhizobia *in vitro*

100 ppm stock solutions each of *lannate* or *karate* were prepared and different amounts of these insecticide solutions were added into the N-free nutrient solution to achieve final concentration of 0.5 ppm of *lannate* or *karate.* Cowpea seeds of the variety specified above were surface-sterilized as described (Weaver and Frederick, 1982). Two of the surface-sterilized seeds were sown in each LJ and after germination, thinning was done to leave one healthy seedling per LJ. The seedlings were then inoculated with 1 ml each of soil suspensions diluted in a five-fold fashion, up to 6 dilution steps i.e, 5^{-1} - 5^{-6} (Woomer, 1993), in four replicate Leonard jars. A set of the LJ without the insecticides was used as the control. The LJ were then transferred to the glass-house where the plants were left to grow and examined at 28 days for the presence or absence of nodules after 28 days. Based on the nodulation pattern of the test seedlings, the Most Probable Number (MPN) concept was used to estimate the cowpea bradyrhizobial population in the original soil, that is, the soil from which suspensions for inoculating the test seedlings were prepared. The MPN estimates were generated from the Most Probable Number Enumeration System (MPNES – Bennett *et al.,* 1991 – uncatalogued manual) and, for each MPN estimate, an associated confidence interval (CI: $P<0.05$) was assigned from existing MPN tables in MPNES.

Table 1. The Most Probable Number (MPN) count of cowpea *Bradyrhizobium* in soil, as affected by *lannate* and *karate* insecticides *in vitro.*

Insecticides		MPN Count	Confidence Interval (CI) (P<0.05)	% reduction of MPN count due to insecticides
Type	Concentration (ppm)			
Control	0.0	24,000	6,937-57,657	-
Karate	0.5	10,500	3,035-25,225	56.2
Lannate	0.5	2,812	95-8,108	88.3

Effects of *lannate* and *karate* on the cowpea – *Bradyrhizobium* symbiosis *in vivo*

Here, we examined the effects of the lannate and karate insecticides on the cowpea *Bradyrhizobium* symbiotic relationship in soil. Before use, the soil was sieved (2 mm) to remove large stones and plant debris. 4 kg of the soil in plastic pots, were thoroughly mixed with 0.24 g of finely-ground triple superphosphate (TSP). This amount of TSP per pot was equivalent to 24.6 kg P ha^{-1}, a rate recommended for cowpeas in Morogoro region (Price *et al.*, 1982). The soil was then watered and left for two days, to allow for the fertilizer(P) to equilibrate before planting. Then, four seeds of cowpeas cv SVS-3 (Price *et al.*, 1982) were sown in each pot. Water containing the respective insecticide *lannate* or *karate* was applied to the soil in the respective pots. The amounts of the insecticides added were those which gave final concentrations of 0.5 and 1.5 µg g^{-1} soil of each of the insecticides. Control treatments were included in which no insecticides were applied. This experiment was set up in a completely randomized design with three replications. Seven days after emergence, the seedlings were thinned to two healthy plants per pot. During the remainder of the experimental period, the soil was kept moist using insecticide – free distilled water. Harvesting was done 35 days after planting, the time taken for cowpea plants to accumulate maximum amount of nitrogen (Summerfield *et al.*, 1985). The following parameters were determined: (i) nodulation data (numbers and dry weight per plant) (ii) nitrogen fixation indicators (i.e. shoot dry weight at 60°C) and, nitrogen accumulated in the shoots.

Results

Effects of *lannate* and *karate* on cowpea-*Bradyrhizobium* counts *in vitro*

Both *lannate* and *karate* appeared to be detrimental to bradyrhizobial viability when the insecticides were applied in the nutrient solution. *Lannate* reduced the bradyrhizobial numbers by 88.3% while *karate* reduced the same by 56.2% (Table 1). This observation suggests that *lannate* was more toxic to the microspymbiont than *karate*.

Effects of *lannate* and *karate* on the cowpea-*Bradyrhizobium* symbiosis *in vivo*

Both *lannate* and *karate* caused slight but non-significant reductions in cowpea nodulation. However, nodule dry weight was slightly increased by *lannate* while it was reduced by *karate* (Table 2). The effects of these insecticides on the nitrogen fixation indicators i.e. shoot dry weight and nitrogen content are also shown in Table 2. While *lannate* caused a slight reduction in shoot weight, *karate* tended to increase shoot weight when this insecticide was applied at the higher concentrations. In addition karate reduced plant shoot weight at lower concentrations (Table 2). The insectside effect on shoot N content showed reverse partten to that of shoot dry weight in that *lannate* tended to increase shoot N content while *karate* reduced it (Table 2). In either case, the effects were statistically not-significant.

Table 2: Effects of *lannate* (L) and *karate* (K) on the cowpea-*Bradyrhizobium* symbiosis assesed *in vivo* (n = 3)

Insecticide concentration (ppm)	Nodulation (Numbers/plant)		Dry weight (mg/plant)		N-fixation Shoot dry weight (g/plant)		Shoot N content (%)	
	L	K	L	K	L	K	L	K
0	86a	73b	28c	43d	3.54e	3.13f	3.72g	3.67h
0.5	66a	61b	30c	23d	3.41e	2.71f	2.80g	3.63h
1.5	76a	62b	38c	36d	3.12e	3.73f	3.08g	3.08h
Standard error	8	6	6	10	0.23	0.24	0.18	0.13

NB: Within a column, means followed by the same superscriptletter do not differ significantly (P<0.05), the LSD test.

Discussion

Effects of *lannate* and *karate* on soil cowpea bradyrhizobial numbers determined *in vitro*.

Both *lannate* and *karate* had detrimental effects in bradyrhizobial incorporated into the N-free nutrient solution. *Lannate* caused a greater reduction (83.3 %) in the bradyrhizobial count than *karate* a (56.2%) reduction in the numbers of the microsymbiont, relative to the numbers in the insecticide-free (control) treatment (Table 1). very widely *lannate* was more toxic to the cowpea bradyrhizobia than *karate*. Little information is available on the effects of pyrethroid insecticides on rhizobia or the BNF process. Never the less the toxic effects of *lannate* are consistent with those of earlier workers who reported that other members of the carbamate class of insecticides showed toxic effects on different species of *Rhizobium*. Carbaryl, for example, was shown to have inhibitory effects on *Bradyrhizobium japonicum* (Kapusta and Ron-wenhorst, 1973). Aldicarb was also reported to have inhibted the growth of *B. japonicum, Rhizobium leguminosarum, R. meliloti* and *R. trifolii* (Lin *et al.*, 1972). The nature of the toxic effects of the insecticides in this

The nature of the toxic effects of the insecticides investigated in this study remain to be established. It should however be noted that in the present study, the numbers of cowpea bradyrhizobia which were found to have been adversely affected by *lannate* and *karate*, were MPN estimates of counts present in the soil, the dilute suspensions of which were used to inoculate cowpea test seedlings. The MPN procedure is therefore an indirect method of counting legume root-nodule bacteria present in a given soil because such numbers cannot be determined directly by plate counts on agar media (Woomer, 1993). The insecticides *lannate* and *karate* which were supposedly toxic to the cowpea bradyrhizobia as indicated in this study, were not previously applied to the soil. The MPN concept is based on the ability of the test host seedlings to form nodules when such seedlings are exposed to dilute soil suspensions supposed to contain an unknown population of the relevant root-nodule bacteria. Reduced or lower incidences of nodulation of the test seedlings are interpreted as low MPN counts. When such low MPN estimates are due to the effects of the insecticides added to the N-free nutrient medium, it could then be argued that the insecticides may have prevented (in some way), proper nodulation of the test seedlings in the LJ's, thus, giving rise to low MPN estimates. Since the process of nodule formation is a joint influence of the host legume and the microsymbiont, it could then be argued that reduced, or an altered pattern of nodulation of a test seedling could either be due to the failure of the microsymbiont (e.g, toxic effects of insecticides and hence reduced microsymbiont numbers) or, the insecticides may have influenced the host root system in such a way as to make it less receptive to bradyrhizobial infection. The latter hpothesis is not so easy to test. The former hypothesis i.e, the direct influence of the insecticides on the microsymbiont could have been examined by incorporating successive doses of the test insecticides to rhizobial culture (broth) media and determine the degree of toxicity and in series establish the minimum inhibitory concentration of *lannate* and *karate* on the cowpea bradyrhizobia. We are aware of the limited usefulness of the information on the response of rhizobia to antimicrobial agents when such information is exclusively based on agar or broth (liquid) culture studies.

Effects of *lannate* and *karate* on the cowpea – *Bradyrhizobium* syminosis *in vivo* (i.e. in potted soil)

Results of this study revealed to a large extent, only inconsistent effects of *lannate* and *karate* on cowpea nodulation as well as on the nitrogen fixation indicators when the insecticides were directly applied into the soil. While both *lannate* and *karate* caused a slight reduction in nodule numbers, the nodule dry matter tended to increase in the *lannate*-treated soil but was slightly lowered by *karate* application into the soil (Table 2). The observed effects of the insecticides on cowpea plant nodulation were however, statistically non-significant (Table 2). It could therefore be argued that except for the minor variations, *lannate* and *karate* had no major effects of the nodulation of cowpea plants grown in soil to which the insecticides in question, were directly applied. Regarding the effects of ~~these insecticides~~ on the nitrogen fixation indictors in cowpea, Table 2 shows that while *lannate* caused a slight but consistent decrease in shoot weight, *karate* caused a slight decrease and then an increase in shoot weight as the insecticide concentration increased from 0.5 to 1.5 ppm. Shoot nitrogen content exhibited a reverse trend to that of shoot weight in response to the applied insecticides. Together, the findings revealed that *lannate* and *karate* had no major influences on the cowpea-*Bradyrhizobium* symbiosis when the insecticides were directly applied into the soil. Little information is available regarding the effects of pyrethroids applied to soil, on the bradyrhizobia – legume association. However, results of this study (on the effects of *lannate*) are in agreement with those of Schnelle and Hensley (1990) who found no significant differences in nodule number and dry weight between carbaryl-treated and the untreated bean plants. The results of this study, regarding the effects of *lannate* on the nitrogen fixation indicators are also consistent with the report by Rodell *et al.* (1977) which indicated that propoxur and carbofuran did not affect nitrogen fixation (assessed by acetylene reduction) in *Glycine max*. However, our results (for *lannate*) are not in agreement with those of Lin *et al.* (1972) which indicated that four types of carbamates (approcarb, carbofuran, dursban and aldicarb) significantly reduced the growth and nitrogen accumulation in alfalfa (*Medicago sativa*) and sweet clover. It is therefore possible that different legume-rhizobial associations respond differently to different insecticides even if those insecticides are grouped in the same class, in terms of their chemical composition.

It should also be noted that while the insecticides studied here, notably *lannate*, caused a marked reduction in the population of the cowpea bradyrhizobia when evaluated *in vitro* (Table 1), this inhibitory effect was not evident when the same insecticides were directly applied into the soil in that, the host legume symbiotic attributes were largely unaffected by those insecticides. The depressive effects of the insecticides, particularly that of *lannate in vitro*, was of much interest. One would have expected this reduction in bradyrhizobial numbers *in vitro*, to result in reduced nodulation and symbiotic performance by the host plant grown in the soil treated with the same insecticides. Correlations have been reported between (brady) rhizobial numbers in a medium and the numbers of subsequently – formed nodule (Mathews *et al.*, 1993). It would therefore appear that the depressive effects of the two insecticides *in vitro* either did not occur *in vivo* (i.e. in the soil) or, the bradyrhizobial numbers may have been reduced but up to a level that may have left a sufficient population to cause successful nodulation, plant growth and nitrogen accumulation. It is important to note, however, that the phenomenon whereby the depressive effects of pesticides *in vitro* was not evident *in vivo*, was also reported by other workers (Diatloff, 1970; Lin *et al.*, 1972) who observed that the numbers of *Rhizobium leguminosarum*, R. *trifollii*, *R. meliloti* and nitrifying bacteria were reduced by pesticides in artificial growth media but were not reduced in similarly – treated soil under field conditions.

Insecticides (or other pesticides) in the soil may, and actually, do interact with soil particles and microbes in ways which may reduce or increase their toxicity or even alter the spectrum of their toxicity (Nicholls, 1988, Alexander, 1991). The insecticides evaluated in this study may have undergone some of the above-mentioned interactions. Future studies could therefore, examine the adsorptive characteristics and other transformations these pesticides undergo in soil and possibly, determine their persistence in soil. Because of the numerous interactions insecticides could undergo in soil, the effects of such insecticides applied to soil on biological processes including the legume – *(Brady)rhizobium* associations, may be difficult to critically evaluate. However, the very depressive effects of *lannate* on the cowpea bradyrhizobia observed *in vitro* in the present study, induces curiosity to examine the influence of this insecticide on a wide range of legume – (brady) rhizobial associations or on other vital microbial processes in a wide range of soils.

Acknowledgements

We are grateful to the Norwegian Agency for Development Co-operation (NORAD) for financial support in the form a scholarship to the senior author. This paper is part of the senior author's M.Sc. Dissertation in Soil Science and Land Management submitted to Sokoine University of Agriculture.

References

Alexander, M. 1991. *Introduction to soil microbiology* (2nd edition). Krieger Publishing Company, Malabar, Florida, 467 pp

Bremner, J.M. and Mulvaney, C.S. 1982. Nitrogen-Total. In: *Methods of soil analysis*. Part 2. *Chemcial and Microbialogical properties Agronomy Series N. 9*, 2nd edition (Edited by Page, A.T.; Miller, R.H. and Keeney, D.R.). American Society of Agronomy-Madison, Wisconsin, U.S.A. 595-622.

Diatloff, A. 1970. The effects of some pesticides on root-nodule bacteria and subsequent nodulation. *Australian Journal of Experimental Agriculture and Animal Husbandry* **10**:562-597.

Eaglesham, A.R.J., Ayanaba, A., Rao, V.R. and Eskew, D.L. 1982. Mineral nitrogen effects on cowpea and soybean crops in a Nigerian soil II. Amouots of N fixed and accrual to the soil. *Plant and Soil* 68: 183 – 192.

IITA (International Institute of Tropical Agriculture) Annual Report, 1990

Juo, A.R.S. (Editor) 1978. Selected methods of soil and plant analysis. Manual series No. 1, Revised edition. I.I.T.A., Nigeria, 3-52.

Kapusta, G. and Ron-wenhorst, D.L. 1973. Interaction of selected pesticides on *Rhizobium japonicum* in pure culture and under field condition. *Agronomy Journal* **65**:112-155.

Kaushik, C.P. 1991. Persistence and metabolism of HCH and DDT in soil under sub-tropical conditions. *Soil Biology and Biochemistry* **23**: 131-134.

Lin, S.C., Funke, B.K. and Schulz, J.T. 1972. Effect of some organophosphorus and carbamate insecticides on nitrification and legume growth. *Plant and Soil* **37**: 489-496.

Methews, A., Bauer, W.O. Brodlwell, D., Carrol, B.J., Gault, R.R. and Gresshoff, P.M. 1993. Rhizosphere colonization by *Bradyrhizobium japonicum* is related to extent of nodulation of *Glycine max* cv Bragg and its supenodulating mutant. *Soil Biology and Biochemistry* **25**: 613-619.

McLean, E.O. 1982. Soil pH and lime requirements. In: *Methods of soil analysis, Part 2.* (As in Bremner and Mulvaney above). 199-223.

Medvecky, B.A. and Zalom, F.G. 1992. Convetional and alternative insecticides, including a granular formulation for *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in Kenya. *Tropical Pest Management* **38**:186-190.

Nelson, D.W. and Sommers, L.E. 1982. Total carbon, organic carbon and organic matter. In: *Methods of Soil Analysis.* Part 2 (As in Bremner and Mulvaney-above) 539-577.

Nicholls, P.H. 1988. Factors influencing entry of pesticides into soil water. *Pesticide Science* **22**:123-137.

Ofori, F., Pate, J.S. and Stern, J.S. 1987. Evaluation of N fixation and N economy of a maize/cowpea intercrop system using ^{15}N dilution methods. *Plant and Soil* **102**:149-160.

Price, M., Machange, F.M. and Asenga, J.A. 1982. *Growing cowpea in the Morogoro Region, Tanzania.* Ministry of Agriculture, Miscellaneous publications.

Rhodes, J.D. 1982. Cation exchange capacity. In: *Methods of soil analysis. Part* 2. (As in Bremner and Mulvaney-above) 149-157).

Rodell, S., Funke, B.R. and Schulz, J.T. 1977. Effect of insecticides on acetylene reduction by *Azotobacter vinelandii* and soybean nodules. *Plant and Soil* **47**: 375-381.

Schnel, M.A. and hensely, D.L. 1990. Effect of herbicides upon nitrogen fixation and nodulation by dry bean. *Pesticide Science* **28**: 325-329.

Summerfied, R.J., Pate, J.S., Roberts, E.H. and When, H.C. 1985. The physiology of cowpeas. In: *Cowpeas: Research, production and utilization.* (Edited by S.R. Singh and K.O. Rachie). John Wiley and sons. pp 65-102.

Vincent, J.M. 1979. *A manual for the practical study of root-nodule bacteria.* IBP Handbook No. 15. Blackwell Scientific Publications, 164 pp.

Weaver, R.K. and Frederick, L.R. 1982. *Rhizobium. In: Methods of soil analysis. Part 2.* (As in Bremner and Mulvaney-above) pp. 781-802.

Woomer, P. 1993. Most Probable Number counts of rhizobia in soils – In: *Tropical soil biology and fertility: A Handbook of methods.* CAB International, pp. 172-178.