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

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Abstract

The effects of two insecticides, Lannate (a carbamate) and karate (a pyrethroid) on nitrogen fixation by cowpeas (Vigna unguiculata) were evaluated after establishing the toxic effects of these insecticides on the microsymbiont bradyrhizobia 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 (5^1-5^5) 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 (N₂) for plant growth. While there are numerous plant-microbe associations in which BNF is carried out, the one which involves 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, cowpeas can derive at least 50% of their nitrogen requirements from atmospheric N₂ (Eaglesham et al., 1982; Ofori et al., 1987). The other advantage is that cowpeas nodulate spontaneously with the native bradyrhizobia that abound in many tropical soils and hence, this crop does not require artificial inoculation. To many of the resource-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 (Kausik 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 proliferate/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 (H₂O), 6.8 (Mclean 1982); total N, 0.12% (Bremner and Mulvaney, 1982); organic C 0.14% (Nelson and Sommers, 1982); extractable P (Bray 1), 32.0 ppm (Juo, 1978) and exchangeable bases, 4.34, 1.78, 0.63 and 0.06 (mol 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-phenoxycinnamyl-cis-3 (2-chloro-3,3,3-trifluoroprop-1-en-1-ny)-2,2-dimethyl cyclopropane carboxylate. It is a contract synthetic pyrethroid with improved properties to withstand photodegradation.

The legume host

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

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

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>MPN Count</th>
<th>Confidence Interval (CI)</th>
<th>% reduction of MPN count due to insecticides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Concentration (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>24,000</td>
<td>6,937-57,657</td>
</tr>
<tr>
<td>Karate</td>
<td>0.5</td>
<td>10,506</td>
<td>3,035-25,225</td>
</tr>
<tr>
<td>Lannate</td>
<td>0.5</td>
<td>2,812</td>
<td>95-8,108</td>
</tr>
</tbody>
</table>
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 vivo

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 83.3% while karate reduced the same by 56.2% (Table 1). This observation suggests that lannate was more toxic to the microsymbiont than karate.

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

Both lannate and karate caused slight but non-significant reductions in cowpea nodulation. However, nodules 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 insecticide effect on shoot N content showed reverse pattern 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>
<thead>
<tr>
<th>Insecticide concentration (ppm)</th>
<th>Nodulation (Numbers/plant)</th>
<th>Dry weight (mg/plant)</th>
<th>N-fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>K</td>
<td>L</td>
</tr>
<tr>
<td>0</td>
<td>86a</td>
<td>73b</td>
<td>28c</td>
</tr>
<tr>
<td>0.5</td>
<td>66a</td>
<td>61b</td>
<td>30c</td>
</tr>
<tr>
<td>1.5</td>
<td>76a</td>
<td>62b</td>
<td>38c</td>
</tr>
</tbody>
</table>

Table 2: Effects of lannate (L) and karate (K) on the cowpea-Bradyrhizobium symbiosis assessed in vivo

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's (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 Ronenhorst, 1973). Aldicarb was also reported to have inhibited 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 hypothesis 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 symbiosis 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 module numbers, the module 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 indicators 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 module 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 vivo, 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. trifolii, R. meliloti and nitifying 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 insecticides undergo in soil and possibly, determine their persistence in soil. Because of the numerous interactions insecticides 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 lin dane on the cowpea bradyrhizobia observed in vitro in the present study, induce 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.

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References


