EFFECTS OF *CARBAMATE* AND *PYRETHROID* INSECTICIDES ON MICROBIAL NUMBERS AND ON *LEUCAENA* DECOMPOSITION IN SOIL

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ABSTRACT

The effects of lannate (a carbamate) and karate (a pyrethroid) on the decomposition of Leucaena green manure were evaluated after establishing the inhibitory effects of these insecticides in vitro, on the heterotrophic micro-organisms (bacteria, fungi and actinomycetes). In this study, the manure, loosely-packed in perforated plastic litter bags, was buried (10 cm) in soil contained in wooden troughs (450 x 35 x 20 cm) and the insecticides in aqueous solution, were applied on the soil surface at the final concentrations of 0.5 and 1.5 ppm for lannate or 0.5 and 1.0 ppm for karate. The decomposition process was monitored at 0,1,3,5, and 7 weeks of incubation using various parameters, namely, residual dry matter, residual organic carbon and residual total nitrogen of the incorporated manure. The total heterotrophic microbial count was also determined in the treated soil at each sampling time. Results of these investigations indicated that when evaluated in vitro, lannate and karate each at 0.5 ppm concentration, depressed the growth of the heterotrophs. However, this inhibitory effect was surprisingly not evident at much higher (10x) insecticide concentrations. It was further observed that the two insecticides, when applied to soil even up to 2-3 times of their respective field recommended rates, did not affect the populations of the decomposer micro-organisms, nor did they influence the rates and magnitude of the Leucaena decomposition, regardless of the parameters used in assessing the decomposition process. It was, therefore, concluded that this type of insecticides could be used even at much higher concentrations, without upsetting the ecological balance or activity of the soil heterotrophic micro flora.

Key words: Carbamates - Green manure decomposition – Insecticides - Pyrethroids

INTRODUCTION

The increasing pressure on available land for food and cash production has changed shifting cultivation to more settled and intensive agriculture in most parts of the densely populated East African highlands. Intensification of agricultural production often requires the use of external inputs which, in many cases, are not accessible to the majority of resource-poor farmers. Such farmers are often advised to resort to the exploitation of locally-available resources such as nitrogen fixing trees or shrubs as sources of green manure and/or compost. Green manure from leguminous plants improves soil fertility and thus, reduces or eliminates the need for costly artificial fertilizers (Szott *et al.*, 1991; Lehmann *et al.*, 1995). However, the benefits of green manure can only be realized after the manure is decomposed to release the organically-bound nitrogen. Decomposition is a microbial process. Hence, any factor that affects the microbial number will directly affect their activity and hence crop production (Wardle and Parkinson, 1990). Pesticide use is an integral part of intensive agriculture. Thus, although efforts are currently being directed towards exploiting locally-available resources for soil fertility improvement as stated above, the pest pressure (particularly insects) is often so intense that use of insecticides becomes inevitable particularly on vegetable

production. Pesticides however, may, and often do influence non-target organisms both soil flora and fauna. The effects of pesticides may therefore counteract those efforts geared towards soil fertility improvement using locally available resources when such pesticides affect the nitrogen and carbon transformations in soil.

Numerous studies have been undertaken to assess the effects of pesticides on various soil microbial processes including denitrification (Yeomans and Bremner, 1985); legume nodulation (Clark and Mahanty, 1991) microbial biomass (Wardle and Parkinson, 1992) and other transformations involving sulphur, phosphorus and carbohydrate metabolism (Megharaj *et al.,* 1999). Most of these studies have involved herbicides. Relatively little has been done on the effects of insecticides on soil microbial activities, particularly the decomposition process *per se*. Of the few studies undertaken, greater emphasis has been placed on the effects of the chlorinated hydrocarbons and the organophosphates than on the other classes of insecticides e.g. carbamates and pyrethroids. Two types of insecticides, a carbamate (*lannate*) and a pyrethroid (*karate*) are extensively used in East Africa and their effects on microbial activities are not well-documented. The present study was therefore undertaken with the following objectives:

- To determine the effects of *lannate* (methomyl) and *karate* (lambda cyhalothrin) on the ability of heterotrophic bacteria, actinomycetes and fungi, to proliferate in their respective culture media.
- To determine the effect of the above insecticides on the decomposition of *Leucaena leucocephala* green manure and,
- To determine the effects of the above insecticides on the populations of the decomposer microflora during the decomposition of the incorporated manure in soil.

MATERIALS AND METHODS

Soil and its characteristics

Top soil (0-15 cm) was sampled from the Sokoine University of Agriculture (SUA) horticultural unit. It was a sandy clay loam with the following chemical characteristics: pH (H_20) 6.8; total N 0.2%; organic C 1.14%; extractable P (Bray I) 32.0mg kg^{-1}; exchangeable bases (Ca, K, Mg and Na) 4.34, 0.63, 1.78 and 0.06 cmol kg^{-1}, respectively.

Green manure and its chemical composition

Leaves and twigs of *Leucaena leucocephala* were used as the green manure material. It had the following chemical composition: total N 4.52%, organic C 47.16% (hence C:N ratio, 10:1).

Insecticides

The insecticides used in this study were *lannate* and *karate*. *Lannate* (methomyl) contains 90% 1-(methylthio) ethylideneamino methylcarbamate. This systemic insecticide acts on the target organism by inhibiting cholinesterase activity. The second 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 synthetic pyrethroid with improved properties to withstand photodegradation. *Karate* is a contact insecticide.

Determination in vitro, of the inhibitory concentration of lannate and karate on the soil heterotrophic micro-organisms

Each of the two insecticides was incorporated into the respective isolation media (specified below) at concentrations

equivalent to the field recommended rates (FRR) or higher. The FRR for each insecticides is 0.5 [g g^{-1} (ppm). The higher concentrations tested were 2, 3, 4 and 10 times the FRR. The concentrations above the FRR were tested to simulate situations where accumulation of the insecticides could occur due to prolonged use and/or frequent use in the field within short-term intervals.

The media used for the isolation of micro-organisms were soil-extract agar, peptone-glucose agar and starch-casein agar for the isolation of bacteria, fungi and actinomycetes, respectively (Wollum, 1982).

In the control treatments, no insecticides were added to any of the microbiological media. The spread-plate method (Wollum, 1982) was used to inoculate the media with dilute soil suspensions and each of the experimental treatments was replicated three times. The bacterial and fungal colonies formed were counted after 5 days of incubation. Actinomycete colonies were counted on the 14^{th} day after plating. Results of this experiment are shown in Tables 1 and 2.

The decomposition experiment

Leucaena prunings were loosely-packed in plastic litter bags (30x30 cm) with 7 mm openings (Anderson and Ingram, 1993). The litter bags (with the manure) were then buried 10 cm deep in soil contained in rectangular wooden troughs (450x35x20 cm) each of which was partitioned into 10 compartments (45x35x20 cm). Based on the moisture and nitrogen contents (72.24 and 4.52%, respectively) of the *Leucaena* prunings and the amount of soil (26 kg) contained in each trough compartment, the quantity of manure per compartment (92.27 g) was determined. This amount of manure gives an equivalent rate of 90 kg N ha^{-1} assuming complete decomposition, and, is a rate generally applicable when green manure is used on a field scale, as a sole source of N (FAO, 1983; Onium *et al.*, 1990).

The respective insecticides (in aqueous solutions) were applied on the soil surface after burying the manure. *Lannate* was applied at the field recommended rate (FRR) of 0.5 [g g^{-1} (ppm) and at three times this rate. *Karate* was applied at rates comparable to those of *lannate*. As indicated in the results of the *in vitro* experiment (Tables 1 and 2), each of these insecticides caused, a noticeable depression in microbial populations at the 0.5 ppm concentration. Although this depression was not progressive at higher insecticide concentrations, the effects of these insecticides on the decomposition of *Leucaena* was still evaluated in soil. This is because the response of micro-organisms to antimicrobial substances when evaluated *in vitro* does not necessarily relate to the reaction of the same *in vitro* (Diatloff, 1970).

The control treatments were those in which no insecticides were applied. Sufficient litter bags were prepared so as to allow for three replications of each treatment and four sampling occasions, namely 1, 3, 5 and 7 weeks of incubation. The experimental soil was maintained moist throughout the study. Effectively, this experiment, set up in a complete randomized design, had a factorial treatment structure incorporating the two insecticide application rates and the four sampling times. The first sampling interval was justified as follows: In instances in which insecticides show inhibitory effects on soil microbes, this is reported to occur during the first seven days after the insecticide application (Lin *et al.*, 1972; Tu, 1978).

The rate of manure decomposition was inferred from determining the following parameters (in absolute amounts), on the retrievable, undecomposed manure material at each sampling time: remaining (residual)

dry matter, residual organic carbon, and residual total N.

Other measurements made in the soil adjacent to the litter bags were microbial numbers and the inorganic N content (NH_4 and NO_3) although the latter parameter is reported in a separate communication. At each sampling occasion, the undecomposed manure was retrieved according to the procedure described by Okalebo *et al.* (1993).

RESULTS

The effects of *lannate* and *karate* on the proliferation of heterotrophic microorganisms *in vitro.*

Effect of lannate

Results of the effect of *lannate* on microbial numbers are shown in Table 1. *Lannate* reduced bacterial numbers compared to those in the untreated soil thus, indicating an inhibitory effect. However, this reduction was not statistically significant and was actually not progressive with successive increases in the insecticide concentration. Regarding the fungal numbers, a significant (P=0.05) reduction was observed at the 0.5 ppm concentration relative to the untreated control (Table 1).

Surprisingly, *lannate* at higher concentrations significantly (P=0.05) increased the fungal numbers although the trend (as for bacteria), was not progressive. The actinomycete numbers were reduced significantly (P=0.05) by *lannate.* However, as was the case with bacterial and fungal numbers, the reduction in the actinomycete population was not progressive with the successive increases in the insecticide concentration.

Effect of karate

Table 2 shows the effect of *karate* on the populations of the different microbial groups. *Karate* significantly (P=0.05) reduced both bacterial and actinomycete numbers when this insecticide was applied at the 0.5 ppm concentration and higher. However, this inhibitory effect on both of the above microbial groups was not progressive with successive increases in *karate* concentration (Table 2). Fungal numbers did not appear to be affected by *karate* except at the 5.0 ppm concentration where the fungal numbers were substantially (though not statistically) reduced.

The effects of *lannate* and *karate* on the decomposition of *Leucaena* green manure

In this study, the rate of decomposition of *Leucaena* green manure was monitored using various parameters as stated above. The parameters were: residual dry matter (RDM), residual organic carbon (ROC) and residual total nitrogen (RTN) in the retrievable manure fractions. These different parameters were used in order to obtain a more informative picture about the decomposition process and to examine how external factors affect such a process. This multiple-parameter analysis was deemed necessary because there are instances in which trends of RDM, ROC and RTN are not consistent (Christensen, 1985; Wangari, 1995) although in other instances, such parameters are consistent in describing the pattern of green manure decomposition (Msumali *et al.,* 1996).

Using residual dry matter (RDM) as an indicator of decomposition

The effects of *lannate* and *karate* on the rate of manure decomposition using RDM as a parameter, are shown in Figure1. There was an initial rapid loss of the manure dm during the first week but, this loss slowed

down subsequently. By the 7th week, about 80% of the dm had been lost. Remarkably, the rate of dm loss was almost the same in pattern and magnitude, both in the soil treated with *lannate* and in untreated soil [Fig. 1(a)]. Regarding the effect of *karate* on the manure dm loss, Fig. 1(b) shows that the rate and pattern of the dm loss in the soil treated with *karate* is almost identical to that in the untreated soil and also, in the soil treated with *lannate* [c.f. Figures 1(a) and 1(b)], indicating that neither of these insecticides had any effect on the rate and pattern of the manure dm loss.

These observations are further supported by the very similar decomposition rate constants (k values) of the manure incorporated into the soil treated or not treated with the insecticides. These k values (Table 3) were obtained from the following exponential function, according to Wieder and Lang (1982).

$$\frac{W_t}{W_0} = W_0 e^{-}$$

Where:

W_t is the amount of mass remaining at time t from the initial mass (W_0) at the beginning of the incubation.

Using residual organic carbon (ROC) and residual total nitrogen (RTN) as indicators of decomposition

As was the case for the dm loss implied from RDM values, carbon and nitrogen mineralization rates were implied from ROC and RTN values respectively. When these (latter) values were plotted against incubation time, patterns of decomposition (not presented here) very similar or almost identical to dm loss, emerged, both for the treated (*karate* and *lannate*) and the untreated soil. Thus, it would appear that any of these parameters (RDM, ROC and RTN) could singularly be used to monitor *Leucaena* green manure decomposition in soil. These similar patterns of manure C and N mineralisation in treated (*lannate* and *karate*) or the untreated soil support the above findings (using dm loss) that the manure decomposition rate was not influenced by any of the two insecticides. This contention is confirmed by the similar C and N mineralization (release) rate constants (k values) in the treated and the untreated soil (Table 3). The C and N release rate constants were calculated using the same approach as that used above (Wieder and Lang, 1982) where RDM was the indicator of decomposition. The notations C_t and C_0 or N_t and N_0 of the manure carbon and nitrogen respectively, replaced W_t and W_0 in the above equation. The very similar k values for C and N release, obviated the need for graphic presentations of ROC or RTN against incubation time.

Changes in microbial numbers in soil in response to *lannate* and *karate* application

The changes in microbial numbers (combined bacterial, fungal and actinomycete counts) in soil treated with *lannate* are shown in Figure 2(a). There was a modest increase (0.6 $\log_{10}$ units g^{-1} soil) in microbial numbers during the first three weeks of incubation, followed by an overall decline in numbers towards the end of the incubation period [Fig. 2(a)]. As evident from this figure, the microbial numbers did not substantially differ during the entire period or at any sampling time in the soil treated or not treated with *lannate*. The numbers tended to spread out at the first and fifth week of incubation but these differences were only 0.3 $\log_{10}$ g^{-1} soil in each case.

The effect of *karate* on the numbers of the decomposer micro-organisms in soil, is shown in Figure 2(b). The population dynamics of the decomposer micro-organisms did not appear to be much affected by *karate* application. In both the treated and the control soil, a modest increase in microbial numbers occurred in the first five weeks of incubation followed by a slight decline towards the end of the

incubation period [Fig. 2(a)]. The higher concentration of *karate* tended to favour higher microbial numbers than those found in the soil which received a lower concentration of *karate* or that not treated with this insecticide. However, these differences were numerically small and also, statistically non-significant. This indicates that *karate*, like *lannate* had no inhibitory effect on the soil heterotrophic microflora *in vivo,* a response similar to that observed in the *in vitro* evaluation of the effect of the same insecticides as reported above (Tables 1 and 2).

DISCUSSION AND CONCLUSIONS

Effects of *lannate* and *karate* on the heterotephic microflora in the *in vitro* experiment

Effect of lannate

The results in Table 1 show that *lannate* at the 0.5 ppm concentration, caused a noticeable reduction in bacterial, fungal and actinomycete numbers relative to numbers in the control treatment. However, the magnitude of response to this concentration of the insecticide and higher, appeared to differ among the three microbial groups. While the reduction in bacterial population was found to be statistically non-significant at the 0.5 ppm *lannate* concentration, the reduction in fungal and actinomycete numbers was found to be statistically significant (P=0.05) (Table 1). If this insecticide is indeed toxic to the decomposer microorganisms as can be deduced from the reduction in numbers of all groups at the 0.5 ppm insecticide concentration, progressive reduction in numbers of all the three microbial groups would have been expected to occur as the level of the insecticide was progressively increased up to 10 times in the isolation media. This was not found to be the case for bacteria and actinomyceters. Surprisingly, higher levels of *lannate* were in fact, found to have significantly (P=0.05) increased fungal numbers above those in the 0.5 ppm concentration but not in the control treatment (Table 1).

This pattern of response by the fungi is clearly difficult to explain. It should however be noted from Table 1 that larger standard errors were associated with the bacterial counts than with the fungal and actinomycete counts in that order. Thus, relatively large differences between treatment means for bacterial counts were found to be statistically non-significant while relatively small differences in the actinomycete counts were found to be statistically significant. The degree of variability in microbial counts in the order in which it occurred for bacterial, fungal and actinomycete counts (Table 1), is difficult to explain since such a trend in variability did not repeat itself (Table 2) in the case where the effect of *karate* was evaluated (Table 2).

Undoubtedly, such unexplained variability causes problems in objectively evaluating the relative response of these groups of microorganisms, to different pesticides or other agro-chemicals with potential antimicrobial activity. It should however, be noted that studies done on the effect of carbofuran (another member of the carbamate class of insecticides) revealed that at 5 ppm, this insecticide caused significant reduction in bacterial and fungal populations (Tu, 1972). This implies that different soil microbial groups may be responding differently to specific types of insecticides even if they belong to the same class.

Effect of karate

The different microbial groups appeared to have responded differently to the effect of *karate* (as was the case with *lannate*). While bacterial and actinomycete numbers were significantly reduced (P=0.05) when this insecticide was added to the isolation media at a concentration of 0.5 ppm or higher, this inhibitory effect was not evident on the fungi except at the highest

karate concentration (5.0 ppm) at which the inhibitory effect appeared to manifest itself substantially though at a level not statistically significant (P=0.5) (Table 2).

If indeed *karate* is toxic to the heterotrophs, then the fungi appear to show greater tolerance to this insecticide than the bacteria and actinomycetes although, even for these latter groups, the toxicity effect was not evidently progressive with successive increases in the concentration of the insecticide (as was the case with *lannate*). As it was argued in the case of assessing the effects of *lannate*, evaluation of the differential response of the different microbial groups to various types of antimicrobial substances is often made difficult by the inherent variability in microbial counts using the soil dilution plating.

Although the standard errors associated with bacterial, fungal and actinomycetes counts did not differ much in the *karate* experiment (Table 2), comparison of the fungal counts in the *lannate* and *karate* experiments indicates substantial differences in the fungal numbers in the same untreated or control soil (c.f. Tables 1 and 2). If there are such noticeable differences in fungal populations in the untreated soil between the two instances, it becomes difficult to critically assess the effect of any subsequent treatments on the fungal populations. It should, however, be noted that inherent variability in microbial numbers between two samples of the same soil, is common in the soil dilution plating. In the authors' experience, such variability is not substantially reduced even with 10 replicate determinations or, when the pour plate procedure is used instead of the spread plate method. However, a $\log_{10}$ transformation of the counts tends to compress such variability, an approach suggested for this kind of data (Little and Hills, 1978) and one which was employed in this study.

It should, however, be noted that scanty information generally exists regarding the effects of pyrethroids on soil microflora. It would appear from the available (limited) literature that natural and synthetic pyrethrins tend to show low toxicity to soil microflora. Inglesfield (1989) reported that permethrin, cypermethrin, fenvelate and deltamethrin had short-term depressive effects on fungal and bacterial populations when each of these insecticides was applied at 0.5 and 5.0 ppm in soil. Results of the present study with *karate* would thus appear to be somewhat inconsistent (for fungi) with those of Inglesfield (1989). The discrepancy could be attributed to differences in the assay media i.e. soil in the case of Inglesfield versus laboratory agar media in the present study. The other cause of the discrepancy could be attributed to the differences in the types of pyrethroids studied between the present study (*karate*) and those studied by Inglesfield (1989) as specified. As argued above for the case of *lannate* (a carbamate), different soil microbial groups appear to be affected differently by the various members of the pyrethroids.

Effects of lannate and karate on the decomposition of Leucaena and on the populations of the associated microflora in soil

The pattern and rate of *Leucaena* green manure decomposition was generally unaffected by the application of the two insecticides regardless of the parameter used to monitor the decomposition process e.g. RMD (Figure 1) or ROC and RTN (Table 3). The lack of an effect of these insecticides on the manure decomposition is not surprising since both the *in vitro* (Table 1) and the *in vivo* (Fig. 2) experiments did not provide evidence that any of the two insecticides had consistently affected the decomposer microbial populations.

Scanty literature however exists, on the effect of the tested insecticides on the

decomposition process although the effect of other carbamate are documented. Weary and Merriam (1978) reported that carbofuran reduced by 42%, the decomposition rate of red maple leaf. In that study, however, the reduction in maple leaf decomposition rate was attributed more to a reduction in the number of decomposer soil fauna than the microflora. In the present study, some representative members of the soil fauna (earthworms, centipedes and millipedes) were found both in the treated and the untreated soil indicating that neither of these insecticides had any toxic effects on the above-mentioned soil faunal groups.

The contention that *Leucaena* manure decomposition pattern and rate were not influenced by these insecticides is further supported by the other parameters used to monitor the process (i.e. ROC and RTN), having shown very close agreement with RDM (Table 3). These findings (using ROC) are consistent with those of Murthy *et al.* (1991) who found that other types of carbamate insecticides namely carbofuran and carbaryl had no effect on the decomposition of ^{14}C- labelled rice straw (using residual radioactive C as an indicator of decomposition). It is interesting however, to note that in a separate study (Bartha *et al.,* 1967) in which CO_2 evolution was used as an indicator of the decomposition of native soil organic matter, carbaryl and isolan (other types of carbamates) decreased the CO_2 production in soil, implying a reduction in the mineralization of the native soil organic carbon. It would thus appear that other than the type of insecticide in question, the parameter and the nature of substrate (e.g. fresh organic amendment vs native organic matter), could give varying impressions of the trends of decomposition of organic substrates in soil. Results of this study would lead to the conclusion that *lannate* (a carbamate) or *karate* (a pyrethroid), when used at their respective recommended rates or higher, have no effect on the population and activities of the heterotrophic soil mcro-organisms.

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