The Effects of Benomyl and Glyphosate Treated Plant Litter on Nitrogen Mineralization in Mollisols

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Abstract

Cocoa production is bedevilled with low inherent soil fertility, pests and diseases, which cause substantial reduction in the potential yield of the crop. Despite these problems, farmers are often reluctant to use pesticides, due to lack of knowledge on the effect of the chemicals on non targeted organisms. The experiment is, therefore, aimed at finding out if Roundup (herbicide) and Benlate (fungicide), with Glyphosate and Benomyl, as their respective active ingredients recommended for use on cocoa plantations in Ghana, have any effect on nitrogen mineralization by affecting decomposition of plant litter. It is also to find out if the combined effects of the two pesticides are additive or interactive. Tomato served as a test plant, treated with three levels of each pesticide. A factorial experiment arranged in a completely randomized design with combinations of five incubation periods and 10 treatments was established. The 10 treatments were soil alone and nine factorial combinations of the fungicide and the herbicide at three levels each. The application rates of Roundup were 0, 1.2 and 12 kg Glyphosate ha^{-1}, and those of Benlate were 0, 1.13 and 11.3 kg benomyl ha^{-1}. The treated plant materials were dried, milled and incorporated into soils of *Rowland series* and incubated at 25 °C for 0, 7, 14, 28 and 42 days, after which NH_4^--N and NO_4^--N were determined. The results showed that increasing glyphosate concentration increased available NH_4^--N concentration at the day of application. NH_4^--N concentration at the day of application was significantly ($P < 0.05$) higher than those of days 7, 14, 28 and 42. Increasing glyphosate concentration increased nitrification. There was no net immobilization of soil mineral nitrogen at higher concentration of glyphosate treatment and their combinations. Benomyl treatment at normal concentration (1.13 kg benomyl h^{-1}) depressed nitrification for a longer period (up to day 28) than that at higher concentration (up to day 14). Net mineral nitrogen at day 42 was higher in the glyphosate and the benomyl treatments than the control. It is concluded that pesticide application affected litter decomposition as indicated by changes in mineral nitrogen. There were positive interactive effects between Glyphosate and Benomyl in relation to nitrogen mineralization.

Introduction

Crop production in the tropical world is bedevilled with low inherent soil fertility, pests and diseases. As a result, there is reduction in the genetic yield potential of the crops (White-Stevens, 1977). Despite the problems of pests and diseases, farmers are always reluctant in the use of pesticides. These situations are due to lack of knowledge on the effect of the chemicals on non targeted organisms.

The fears of the use of pesticides could be overcome by a complete understanding of their mode of action. In some farms, pesticides are used in the control of weeds, insects, fungi and nematodes. This means more than one chemical are used sometimes on the same piece of land within a growing season and, therefore, the need to understand the mode of action of these pesticides when applied in their combinations or sequences on non-targeted organisms. Unfortunately, work to investigate the effects of combinations of herbicides, or of combination of herbicides and other pesticides are scarce, and, therefore, the need to increased research in these areas (Greaves & Malkomes, 1986). These aspects of the studies become particularly important, as farming activities are partly influenced by some of these non-targeted living things and their inter dependencies.

In this experiment, the effects of chemicals are studied in relation to plant litter decomposition which contributes to nutrient cycling in plantations. Tomato is used as a test crop and nitrogen mineralization served as the index of litter decomposition (Dickinson & Pugh, 1974). Hence, the experiment is modelled to simulate what happens in a real farming situation under a controlled environment. A typical example is what happens in cocoa plantations where fungicide is sprayed nine times, insecticide four times and herbicide four times, all in a year. The aim of the studies, therefore, is to find out if the combined effects of pesticides are additive or interactive. The degradable product being assessed is mineral nitrogen. Hence, the Null Hypothesis to be tested states that 'Pesticides applied singly or in combinations to plants have no effects on ammonification and nitrification'.

Materials and methods

Pesticides

In the study Roundup served as a representative of herbicides whilst Benlate served as that of fungicide. Glyphosate ($C_6H_{17}N_2O_5P$) and Benomyl ($C_{14}H_{18}N_{14}O_3$) are the active ingredients of the candidate herbicide and fungicide, respectively. Glyphosate and Benomyl have relative molecular mass of 228.2 and 290.3, respectively. Benlate is the registered trade mark of E.I. Du Pont de Nemours, a company of USA, and Roundup is the registered trade mark of Monsanto Company of Leicester, UK.

Soils

The soil used in the experiment was *Rowland series* classified as Mollisol (USDA, 1990), sampled from the University of Reading Experimental Farm at Sonning woodlands (51^{0} 28' N, 0^{0} 55' W). The soil is brown earth, coarse loamy drift over non-calcareous river terrace gravel parent material. The soil was 62.7% coarse sand, 17.0% fine sand, 3.4% silt and 16.9% clay. The *p*H was 6.7 (1:2.5 soil:water ratio) and percent carbon was 1.4. Soil was sampled to a depth of 20 cm and large pieces of organic debris and stones removed. The soil was passed through 8 mm sieve, and the sub sample less than 8 mm used in filling the pots, which were used in growing the tomato plants. The soil used in the laboratory analyses was passed through 2 mm sieve, put in polythene bags, loosely tied and stored at 5 ^{o}C. Sub-sample of the stored soil was later air-dried and used for the determination of physico-chemical properties of the soil stated earlier.

Plant

Tomato plant, *Lycopersicon esculentum,* of Amateur variety, that grows to an average height of 15 cm, was used in the experiment. The seeds were sown directly in pots filled with 3 kg soil described earlier at three seeds per pot. The seedlings were thinned to one plant per pot during the second week, giving due consideration to uniformity of seedling sizes.

Experimental design.

The experimental design used was complete randomised design with 50 factorial combinations of five incubation periods and 10 treatments. There were 10 treatments, which are soil alone, and nine factorial combinations of fungicide levels (0, 1, 2) and herbicide levels (0, 1, 2). Each pesticide treatment was given three replications. With the pesticides, level 0 was where no pesticide was added, level 1 was the normal application rate and level 2 stands for 10 times the normal application rate. The following abbreviations, H_0, H_1, H_2, F_0, F_1 and F_2, are used to represent no herbicide, herbicide applied at the normal application rate, herbicide applied at 10 times the normal application rate, no fungicide applied, normal recommended application and 10 times recommended application of the fungicide, respectively.

In all the treatments, with the exception of soil alone, there were combinations of herbicide and fungicide from levels 0 to 2. H_0F_0 is no pesticide applied to plant material referred to as the control. The application rates of herbicides for the trials were 0, 1.2 and 12 kg Glyphosate h^{-1} while that of fungicide were 0, 1.13, 11.3 kg Benomyl h^{-1}. Spraying was done using hand held sprayer of 500 ml volume. The tomato plants were sprayed with Roundup when they were 8 weeks old. This marked the end of vegetative growth of the plants and the initiation of reproductive growth or flowering. Two weeks after Roundup (herbicides), the plants were sprayed with the various rates of the fungicide, using the same sprayer used for the Roundup application.

Preparation of plant material for analyses

The treated plants were allowed to dry under the greenhouse condition for the first 3 days at a minimum temperature of 11 °C and a maximum temperature of 36 °C, after which the plant materials were kept in an oven at 140 °C for 48 h to dry to a constant weight. Grinding (< 2.00 mm) was done using a Moulinex blender type 505 code 322 with frequency of 50/60 Hz at 150 W power rating from 220–240 voltage source.

Incubation study

Soil, sieved through the 2-mm mesh stored in the cold room at 5 °C was pre incubated for 7 days at a temperature of 25 °C. Three replicates of 100 g sieved (2.00 mm) samples of soil with 360 mg of the ground pesticide treated plant material were weighed into 250 ml conical flask. These were incubated for 0, 7, 14, 28 and 42 days at a temperature of 25 °C.

Determination NH_4^+-N and NO_3^--N.

10 g of the incubated samples were suspended in 100 ml of 1.0 *M* KCl and shaken for a period of 30 min using a horizontal shaker. The suspensions were filtered through Whatman number 1 filter paper. Aliquots of the filtrates were analyzed for NH_4^+-N, and NO_3^--N using Bremner & Keeney (1965) method. The calculations of the concentrations were based on the oven dry weight of the soil already determined.

Statistical analyses

The parameters analysed were concentrations of NH_4^+-N and NO_3^--N, as well as net mineral nitrogen of the soil applied with the herbicide and/or fungicide for the various incubation periods. The results were subjected to statistical analyses using the analysis of variance (ANOVA). These were to determine the effects of both the single and additive interactions of the herbicide and fungicide for the various incubation periods.

Results and discussion

Ammonium nitrogen (NH_4^+-N)

Table 1 shows the mean concentrations of NH_4^+-N (mg/100 g soil) of the soils incorporated with plant litter treated with the various pesticides for the various incubation periods. From the Table 1, it was observed that there were higher concentrations of NH_4^+- N in the soil at day 0, which is the day of applications in all the treatments than the subsequent sampling dates, with the exception of treatment H_0F_1. NH_4^+-N concentrations in soil incorporated with plant material with or without pesticides. The concentration of NH_4^+-N in the soils incorporated with the various pesticide treated plant materials at day 0, were highly significant ($P < 0.001$). In all the treatments, with the exception of the soil alone, the lowest levels of NH_4^+-N concentration was obtained in day 42 although ammonium concentrations of days 7, 14, 28 and 42 were

TABLE 1

Mean concentrations of NH_4^+- N (mg / 100 g soil) of the treatments for the various incubation periods (days)

	Incubation period (days)				
Pesticide treatments	*0*	*7*	*14*	*28*	*42*
H_0F_0	0.192	0.025	0.048	0.054	0.011
H_0F_1	0.012	0.023	0.046	0.088	0.013
H_0F_2	0.539	0.194	0.088	0.045	0.004
H_1F_0	0.969	0.132	0.068	0.148	0.013
H_1F_1	0.979	0.050	0.058	0.117	0.011
H_1F_2	0.964	0.607	0.060	0.160	0.012
H_2F_0	6.292	1.945	1.194	0.117	0.034
H_2F_1	5.702	1.346	1.160	0.083	0.025
H_2F_2	4.718	3.564	3.285	1.125	0.023
Soil alone	0.101	0.021	0.056	0.101	0.056

TABLE 2

Mean NO_3^-- N (mg/100 g soil) of the treatments for the various incubation periods (days)

	Incubation period (days)				
Treatments	*0*	*7*	*14*	*28*	*42*
H_0F_0	1.857	0.093	0.176	0.548	2.662
H_0F_1	1.351	0.464	0.450	0.444	3.867
H_0F_2	2.005	0.557	1.119	2.690	5.531
H_1F_0	2.442	0.172	0.486	1.032	3.975
H_1F_1	2.370	0.233	0.442	0.536	3.793
H_1F_2	2.147	0.951	1.961	3.894	5.550
H_2F_0	2.398	4.809	5.871	8.439	10.364
H_2F_1	2.265	3.080	4.168	7.161	8.543
H_2F_2	2.441	2.654	3.351	8.376	12.513
Soil alone	1.981	2.098	2.243	2.458	2.525

not obvious. Ammonium concentrations of these days were found to be significantly ($P < 0.05$) lower than that of day 0 (Table 3).

Comparing the concentrations of NH_4^+-N at day 0 and day 42, it was noted (Table 4) that the soil alone, which had almost the lowest concentration of NH_4^+-N (the last but one to H0F1) among the pesticide treatments, had the highest NH_4^+-N concentration at the 42nd day. The concentration of NH_4^+-N decreased steadily from day 0 to day 42 in H_oF_2, H_2F_o, H_2F_1 and H_2F_{2r} while those in the other remaining treatments fluctuated. The stability of ammonification in the soil alone treatment may be attributed to the 7 days pre-incubation which possibly reduced the handling effects. Also to be considered is why the 10 times increase in the initial application rate did not give corresponding 10 times increase in ammonium nitrogen concentration. This may be explained by the fact that the herbicide in plant is influenced by other additional factors apart from the

TABLE 3

Net mineral nitrogen[*] concentration (mg / 100 g soil) of the treatments for the various incubation periods (days)

Treatments	*Incubation period (days)* *0*	*7*	*14*	*28*	*42*
H_0F_0	-0.033	-2.001	-2.075	-1.957	0.092
H_0F_1	-0.719	-1.632	-1.803	-2.027	1.299
H_0F_2	0.461	-1.368	-1.092	0.176	2.954
H_1F_0	2.082	-1.815	-1.745	-1.379	1.407
H_1F_1	1.267	-1.836	-1.799	-1.906	1.223
H_1F_2	1.029	-0.561	-0.278	1.495	2.981
H_2F_0	6.608	4.635	4.766	5.997	7.817
H_2F_1	5.885	2.307	3.029	4.685	5.987
H_2F_2	5.077	4.099	4.337	6.942	9.955
Soil alone	0.000	0.000	0.000	0.000	0.000

* Net mineral nitrogen = (ammonium + nitrate ion concentrations in treatments) - (ammonium + nitrate ion concentrations in soil alone) for the various incubation periods.

TABLE 4

Summary of the analysis of variance of the model for the various incubation periods

Incubation period (days)	*F-value NH_4^+-N*	*F- Value NO^-_3-N*	*F-Value net mineral nitrogen*
0	3.779***	0.455**	0.633
7	2.164***	14.935***	2.063
14	3.285**	2.026***	3.921
28	0.200*	25.657***	4.102
42	0.990***	16.704***	8.200

Where *, ** and *** represent significant levels at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

physico – chemical properties of the herbicide. Some of the additional factors are vapour pressure, solubility, affinity for adsorption and the plant characteristics which determine the ultimate concentration around the chloroplast.

In addition, environmental conditions such as light, temperature, humidity, etc., indirectly affect transpiration, carbon dioxide and water diffusion (Shaw *et al.*, 1960). Also the degradation of the pesticide is partly by microorganisms which are being affected by other environmental factors like moisture, oxygen, soil reaction, nutrients and temperature (Wood, 1989; Cole, 1982; Nonura & Hilton, 1977). With Glyphosate, investigations had proved microflora to be responsible for degradation (Sprankle *et al*., 1975; Quilty & Geoghegan, 1976) although the dominant process of the co-metabolism of the microflora do not use the herbicide for growth (Torstensson & Aamisepp, 1977). This observation of initial higher NH_4^+-N concentration in the soil as a result of the addition of Glyphosate is in line with the observation of Marsh (1985). From the proposal of metabolic pathways of Glyphosate by plants (Rueppel *et al.*, 1975), it was found that Glyphosate in plants break down to glyoxylate and amino ethyl phosphonic acid which further break down into glyoxylic and citric acid to produce amino acids, carbohydrates and other natural substances. This means the breakdown of the pesticides contributed to the mineral nitrogen as both pesticides have nitrogen in their formulation. Another argument that could be raised is whether the herbicide hinders nitrification, which is the oxidation of NH_4^+-N into NO_3^--N, resulting in accumulation of, NH_4^+-N at the initial sampling date.

Table 2 shows that there was considerably higher nitrate nitrogen in some of the pesticide treatments than the non-pesticide treatments. Three possible sources are likely to contribute to the NH_4^+-N concentration. First is the NH_4^+-N nitrogen source from the decomposition of plant litter, and the second is that from the degradation of the pesticides, and lastly from the soil. That is from the time of spraying the live plants to the time it was incorporated into the soil and sampled. Satchell (1974) reported that decomposition of plant material starts from abscission of a leaf, which is followed by a more or less prolonged senescence when much of the mineral content is withdrawn to the stem and phyllophane fungi are already decomposing the available carbohydrate. Pesticide treated plant material gave significantly higher values, from 0.969 to 6.292 mg/100 g soil than what was obtained from the plant litter alone which is 0.192. If ammonification was hindered by the presence of Benomyl, then the degree of inhibition of ammonification should have been higher in the 10-folds than in the normal application rate but this was not found to be the case. This observation was in line with that of Pugh & Mainwright (1975).

Nitrate nitrogen NO_3^-*-N*

Table 2 shows the mean concentrations of NO_3^--N (mg/100 g soil) of the soils mixed with litter applied pesticides for the various incubation periods (days). It was observed that the NO_3^--N concentration was significantly ($P < 0.001$) higher in the 10-folds herbicide treatments and its combinations than the normal and the non-herbicide treatments at the 42nd day.

Although no significant difference ($P > 0.05$) was observed among the NO_3^--N concentrations at day 0 for the soils mixed with litter applied pesticides, NO_3^--N concentration in the soil alone increased steadily from 1.981 to 2.525 mg/100 g soil. Ten times herbicide applications also increased from 2.398 to 10.364 mg/100 g soil for H_2F_0, 2.265 to 8.543 for H_2F_1, and 2.441 to 12.513 mg/100 g soil for H_2F_2. From Table 3 higher concentrations of the fungicides in combination with herbicide highly increased the nitrate nitrogen. NO_3^--N concentrations increased from 0–42 days, for plant material treated with 10-folds the normal herbicide application rates and that of soil alone.

NO_3^--N concentration sampled in day 42 was significantly ($P < 0.05$) higher than those of day 28 in all the pesticide and non-pesticide treated soils. There was no significant ($P > 0.05$) differences in the NO_3^--N from 0 to 14 days whilst these concentrations were also significantly lower than those of day 28. Higher application rates of the herbicides were found to increase the NO_3^--N concentration in the soil whilst fungicide levels had influence in the nitrate ions concentrations. Although NO_3^--N concentrations of treatments H_2F_2 and H_2F_1 were found not to be significantly different, H_2F_0 was, however, significantly higher ($P < 0.05$) than H_2F_1 for 28 and 42 days. Nitrate nitrogen in treatment group (H_0F_2, H_1F_2 and soil alone) was significantly higher than that of group ($H_0 F_0$, H_1F_0, H_0F_1 and H_1F_1), but there was no significant difference within groups. With the pesticide treatments, the nitrate ion concentrations were always higher in the F_2 combinations than those of F_0, and those of F_1 combinations were always lower than those of F_0 combinations. This trend differs with single fungicide application. In this situation the nitrate ions concentrations were higher in $F_2 > F_1 > F_0$.

As mentioned earlier from other findings, the microorganisms which decompose Glyphosate did not use the metabolite for growth and, therefore, nitrate nitrogen from the plant material and Benomyl are possibly used for growth. As a result of increased in their energy, they are able to oxidise more of the ammonium in the soil incorporated with the pesticide-treated plant material than non-pesticide-treated plant material. This might possibly explained why ammonium nitrogen is higher in the soil alone and the non-pesticide-treated plant material incorporated in soil (day 42). Why $F_2 > F_1 > F_0$, could be explained by the fact that Benomyl, plant material and soil are the sources of the NO_3^--N in fungicide treatments whilst the soil and plant material are the only sources of the NO_3^--N in the non-pesticide treatments. This means the metabolite of the Benomyl contributed to the nitrate nitrogen.

In the non-fungicide and fungicide treatments at the normal application rates, the immobilisation continued from the initial incubation period to 28 days whilst that of 10 times the normal application rates ended on the 14th day. From the net mineral nitrogen results it could be realised that there was no net immobilisation probably because the microflora, which decomposed most of the Glyphosate, did not use the metabolite, which is the nitrate, as a source of energy, and, therefore, the products were left almost intact under laboratory condition where agents like leaching and fixation were not in operation.

Net mineral nitrogen

In this context net mineral nitrogen = (ammonium + nitrate ions concentrations) - (ammonium + nitrate ions concentrations in soil alone). In this calculation the negative values stand for immobilisation and the positive values are mineralisation. Soil alone treatment has 0 values as it serves as the 'bench mark' of the calculation. From the results in Table 3, it was observed that mineral nitrogen is immobilised when plant material without pesticide application was incorporated into the soil for 28 days. Mineralization recorded in day 42 was low and, therefore, found not to be significant. In all the normal pesticide application rates there was immobilisation from day 7 to day 28. Mineralisation occurred from day 0 to day 42 in 10-fold application rates, although the concentrations reduced in days 7 and 14. Net mineral nitrogen concentrations, observed in all the pesticide treatments in day 42, were higher than those of the control. This means that there were possibly additional substrates, which is the pesticide in the form of organic nitrogen compound in the plant.

From net mineral nitrogen in Table 3, it was observed that the greater the nitrogen input through the pesticide application, the greater the net mineral nitrogen value. But the greater the nitrogen input, the greater the nitrogen that is lost. There were always greater difference between higher pesticide input and the net mineral nitrogen. This means that the greater the input of nitrogen, the greater the difference between the input concentration and net mineral nitrogen concentration. This implies that, the greater the input nitrogen the lower the conversion efficiency of the input nitrogen to net mineral nitrogen. With the nitrogen input range of 15.403–35.208 mg nitrogen, between 19.3–35.4% of the input was mineralised, and with the nitrogen input range of 1.319–2.209 mg nitrogen, between 63.7–98% of the input was mineralised. Also, the conversion efficiency was always higher in single pesticide application than combined applications which may be due to interaction. The lower conversion efficiency of the nitrogen from higher input may be due to the maintenance of the C: N: P: S ratio in the soil and, therefore, the greater the lost of nitrogen through denitrification, volatilization in the form of ammonia or immobilization with the soil.

Conclusion and recommendation

The results from the experiment on the effects of pesticide on plant litter decomposition in soil showed that increase in the herbicide concentration increased NH_4^--N and the amount of nitrification. It was also realised that there was no net immobilization of soil mineral nitrogen at higher concentration of herbicide treatments and its combinations. Fungicide treatments at normal concentration retarded nitrification for a longer period (up to day 28) than that of higher concentration (up to day 14). The net mineral nitrogen at the end of the incubation period at day 42 was higher in all pesticide-applied treatments than the control. From the results, it may be concluded that pesticide affected litter decomposition as indicated by changes in mineral nitrogen. Also, it was found that there was an interactive effect between herbicide and fungicide in relation to nitrogen mineralisation.

The study may help to cast out fears that repeated use of pesticide might result in a build up of toxic residue that might affect the dynamic nature of soil and, therefore, lowering its fertility. The results had shown that even higher levels of 10 times the recommended application rate, which can hardly reach the soil, had no deleterious effects on ammonium and nitrate mineralisation. This means that mineralisation continues under such circumstances, releasing the various forms of mineral nitrogen. Prior to this experiment, one might think that, since microorganisms are involved in ammonification and nitrification, the availability of mineral nitrogen would be reduced as the microorganisms are exposed to this chemical but the results are contrary to this expectation.

Although the work was not done to measure the biomass of microorganisms responsible for the organic matter turnover, it could be assumed that the organisms were not adversely affected, as the process was not hindered. The next point to be considered is how the results may differ from soil to soil, pesticide to pesticide, and plant to plant with difference in the C:N ratio. An example is to consider how maize and cowpea are to differ in decomposition when pesticide is introduced. How clayey soil will behave differently from a sandy soil. These are necessary to be considered as they affect the level of nitrogen in the soil.

The next factor is how the greenhouse work is going to give results closer to what could be observed in the field, and how the greenhouse work could be extrapolated to the field. Also, how are rainfall, sunlight and temperature going to affect the levels of ammonium and nitrate nitrogen produced and the subsequent influence of leaching and erosion. During rainfall, there will be the possibility of nitrate leaching to the groundwater; with the expanding clays there will be fixation of ammonium ions. Finally, what is the extent to which plants are going to make good use of this source of mineral nitrogen. This could be based on the type of plant and what form of mineral nitrogen it prefers taking up and at what stage of the plant development. This could be worked out well so that the period of high nitrogen requirement of the plant coincides with the high availability of nitrogen through regulation of pesticide application, based on the period each is mineralised possibly to supplement the fertiliser use in the field.

Acknowledgement

The work was jointly funded by ODA and Ghana COCOBOD to whom the authors extend their appreciation. They are also grateful to Dr P. J. Harris and Dr D. L. Rowell of Soil Science Department, University of Reading, UK for their constructive criticisms and suggestions. This paper is published with the kind permission of the Executive Director, Cocoa Research Institute of Ghana.

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