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Nematicidal Effects of Carbofuran and GC-MS Analysis of its Residue in Pineapple Fruits

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Abstract

Concerns over the safety of food items from fields treated with nematicides had risen in recent times. In this study, two field experiments arranged in a Randomized Complete Block Design were conducted to assess the efficacy of poultry manure and carbofuran in suppressing nematode population and determine the residual presence of the nematicide in pineapple fruits. Three poultry manure rates (0, 20 and 25 metric tonnes per hectare) and carbofuran treatments (0, 3.0 kg a.i/ha and 3.4 kg a.i/ha) were applied to two naturally infested pineapple fields. Twenty core soil samples per plot were collected from plants rhizosphere at 3, 6, 9, 12, 15 and 18 months after planting. Fifty grams each of chopped pineapple samples from the carbofuran-treated and untreated plots was extracted with 20ml of ethyl acetate solution for fruit analysis to determine the residual presence of carbofuran using Gas Chromatography-Mass Spectrometry (GC-MS). The study indicated that poultry manure and carbofuran significantly ($P \leq 0.05$) suppressed nematode population in both locations and promoted crop yield. The GC-MS test showed that carbofuran and its metabolites were not detected in the pineapple fruits, suggesting that carbofuran is not likely to constitute dietary risks to consumers of fruits from treated plots.

Keywords: Pineapple; Carbofuran; Nematode population; Pesticide residue; Gas Chromatography-Mass Spectrometry

Introduction

Pineapple (*Ananas comosus* Meer) is one of the most delicious tropical fruits. It is the most economically important plant in the family Bromeliaceae being a rich source of bromelain, a complex proteolytic enzyme that has been found extremely useful as a natural anti-inflammatory. It is important in the pharmaceutical industry because of its ability to inhibit the growth of malignant cells, and also serve as antithrombotic and fibrinolytic agents (Bhattacharyya, 2008). The proteolytic enzyme-bromelain, has also demonstrated significant health benefits ranging from anti-inflammatory and digestive benefits to antioxidant protection, immune support, as well as treatment of cardiovascular diseases and lowering the risk of age-related macular degeneration (ARMD) (Tochi *et al*., 2008; Joy, 2010).

Plant-parasitic nematodes have been implicated as a major cause of economic loss to horticultural and field crops all over the world (Stirling and Pattison, 2008) and particularly in pineapple fields, damage by these nematodes is a major limitation to production and often result in significant crop losses (Rohrbach and Apt, 1986; Caswell *et al*., 1990; Stirling and Nikulin, 1993; Sipes and Schimitt, 1994).

Nematode control in pineapple fields has been based on the use of nematicides (Caswell *et al*., 1990) since the end of the Second World War. They provide effective and immediate solution to nematode problems in agricultural fields, especially when crop failure is imminent. Currently, the use of nematicides is confronted with much criticism and several useful chemicals are currently being phased out of the market and beyond the reach of farmers, thus creating the need for alternative measures for effective management of parasitic nematodes of pineapple.

Nematode population in the field has been suppressed and kept at levels below the economic threshold by nematicide application, planting resistant crop varieties, fallowing, intercropping, heat treatment, use of biological agents, soil amendments and all cultural practices that are inhibitory to nematode development and reproduction (Rohrbach and Apt, 1986). Farmland materials such as plant residues, poultry droppings and cow dung, which otherwise would have been regarded as wastes could also be incorporated into soils as organic amendment, thereby contributing greatly to the improvement of the soil fertility, enhancement of the activities of the soil bio-fauna and at the same time help in suppressing the population of plant-parasitic nematodes present in the soil (Babatola, 1988). However, management of plant-parasitic nematodes with organic manure was reported to be less effective and slow

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acting on pineapple than control normally achieved by nematicides (Daramola *et al*., 2013a).

Carbofuran, a broad-spectrum non-cumulative carbamate, is a systemic nematicide that is non-persistent and does not pass along food chain. Like other carbamates, it is metabolised rapidly into less toxic and finally into non-toxic metabolites (Barnthouse *et al*., 1990). The use of granular carbofuran was found to be associated with avian toxicity, and consequently, its use was prohibited. It is however still available as a restricted pesticide in many parts of the world (Chitwood, 2003).

This study attempts to compare the effects of poultry manure and carbofuran on soil population of nematodes and also determine the residual presence of carbofuran in harvested pineapple fruits.

Materials and Methods

Experimental Site

The field experiment was conducted at two locations. One site was at the Teaching and Research farm of the University of Agriculture, Abeokuta (Longitude 3º 21'E, Latitude 7º N and 66 m.a.s.l.) while the other location was at the National Horticultural Research Institute, Idi-isin, Ibadan (Longitude 3º 50' E, Latitude 7º 23'N and 239 m.a.s.l).

Experimental Design

Pineapple suckers (cv Smooth cayenne) were planted at a spacing of 60 cm between lines and 30 cm between rows using a double row spacing method of 1 m. The experimental plot size was 2.4 m × 2.1 m. There were 40 plants per plot and 15 plots at each location. The treatments consist of three rates of Carbofuran 3G (0, 3.0 kg a.i/ha and 3.4 kg a.i./ha) applied at three weeks after planting (WAP) and at every three month interval and three rates of weathered poultry manure (0, 20 tonnes/ha and 25 tonnes/ha) which were incorporated into the soil at 3 WAP and at 6 months interval. Each treatment was replicated three times and the experiment laid out in a Randomized Complete Block Design (RCBD). The data on nematode population was subjected to analysis of variance and the means partitioned by Duncan's multiple range tests at 5% level of probability.

Soil Sampling and Extraction of Nematodes from Soil

Soil samples for nematode analysis were collected from each experimental plot prior to planting, and at 3, 6, 9, 12, 15 and 18 months after planting. Twenty core samples of soil collected with a soil auger to a depth of 15 cm and within a 25 cm radius from the base of the pineapple plants were taken from each plot for bulking, thorough mixing and extraction of nematode. The nematode population in all the plots were monitored throughout the period of the experiment.

The Extraction Tray method of Whitehead and Hemning (1965) was used for the extraction of vermiform nematodes from the soil samples. Two hundred grams (200g) of each of the composite samples were placed in the upper sieve of a modified Baermann tray set-up which was made up of a double-ply facial tissue, sandwiched between a pair of plastic sieves and placed in a bowl of water with about 500 mL of water in it. The set-up was allowed to remain undisturbed for 24 h, after which the sieves were gently lifted off. The resulting nematode suspension in the bowl were poured into a 500 mL Nalgene wash bottle and left undisturbed for 5 h.

The suspension was concentrated to 25 mL by siphoning excess water (supernatant) using the settling-siphon method (Caveness, 1975). The siphoning process automatically stops at a factory-fixed siphon-breaking level and the remaining nematode suspension were poured into a Doncaster (1962) counting dish for identification and counting of the different nematode genera and species.

Assessment of Crop Performance

At 18 months after planting (MAP), the pineapple plants were induced to flower using the method of Bartholomew *et al.* (2003). The plant maturity indices; plant height, number of leaves, 'D' leaf length, and 'D' leaf width were determined per plot (Van de Poel *et al*., 2008). At harvest, fruits were picked and size-classed. The fresh fruit weights, number of fruits and percentage fruiting were also recorded for each plot.

Laboratory Analysis for Nematicide Residue in Pineapple Fruits

Gas Chromatography-Mass Spectrometry (GC-MS) was used for the analysis of carbofuran residue in the pineapple fruits. Two pineapple fruits were randomly picked from each treatment (3.4 kg a.i/ha, 3.0 kg a.i/ha of carbofuran and the control). A total of 12 pineapple fruits were selected for the analysis for residual presence of carbofuran from both Ibadan and Abeokuta locations.

The extraction and clean-up method of Iqbal *et al.* (2007) for the determination of pesticide residue in Brinjal fruits was modified for the extraction of pineapple juice from the fruits. Fifty grams each of the freshly chopped pineapple samples was homogenized and extracted with 20 mL of ethyl acetate solution. Thereafter, 20 g anhydrous sodium sulphate was added into it. The solvent was filtered through Buchner funnel with Whatman flute filter paper. Then these extracts were cleaned-up by activated charcoal (the charcoal was activated at 105^{o}C for 4 h) and were concentrated to about 5 mL per sample. The extracts were analyzed at the Lagos State Teaching Hospital (LASUTH).

Result

Effects of Carbofuran and Poultry Manure Treatments on Nematode Suppression in Pineapple Fields

The results show that PM and carbofuran treatments suppressed the population of plant-parasitic nematodes, which were found in the two experimental fields (Table 1). Soil treatment with 3.4 kg a.i/ha of carbofuran recorded the most effective nematode control when compared with poultry manure-ammended and the untreated plots in both fields. Population of the non-parasitic nematodes were more abundant in PM-treated plots while higher numbers of parasitic and non-parasitic nematodes were recorded from the untreated plots in both locations (Table 1).

In the Abeokuta field, the population of the plant-parasitic nematodes was significantly higher (p<0.05) in the untreated plots than in the carbofuran and poultry manure-treated plots. Carbofuran at 3.4 kga.i/ha gave the highest nematode suppression, which was significantly higher than the effects of carbofuran at 3.0 kga.i/ha, PM at 20 and 25 metric tonnes/ha. Similar trend was observed in the Ibadan field where high nematode population was recorded on untreated plots, and higher nematode suppression recorded with 3.4 kga.i/ha carbofuran-treated plots. There was however no significant difference in the effects of carbofuran at 3.4 kga.i/ha and 3.0 kga.i./ha on the nematode population in the soil.

Effects of Poultry Manure and Carbofuran on Fruit Yield of Pineapple

At harvest, PM treated plots recorded higher fruit yield in terms of fruiting percentage, number of fruits harvested and the total fruit weights per plot at 25 t/ha in Ibadan field (Table 2). This was significantly higher than those recorded on the carbofuran and the untreated plots. There was however no significant treatment effect on the total fruit weights of pineapple from plots with PM at 20 t/ha and those treated with carbofuran at 3.4 kga.i/ha. Higher yield and more uniform fruiting of pineapple were recorded on 3.4 kg a.i/ha carbofuran-treated plots at Abeokuta field (Table 3). This was significantly higher than those from the PM and untreated plots. Very small fruit sizes and poor fruiting percentage were recorded on the untreated plots.

Residual Presence of Carbofuran in Pineapple Fruits

The result of the fruit analysis using Gas Chromatography Mass Spectrometry (GC-MS) analytical method to determine the residual presence of Carbofuran in the pineapple fruits is shown in Fig. 1. The GC-MS Chromatograms of the analytical tests show that there was no trace of the carbamate (carbofuran) or any of its metabolites in the pineapple fruits analysed. The presence of volatile and non volatile compounds such as amino-acids, alcohols, esters, saturated and unsaturated fatty acids that were resident in the pineapple fruits was however evident from the chromatography test. The peak retention times on the GC-MS chromatographs were recorded for some of the fatty acid components of the fruits (Table 4).

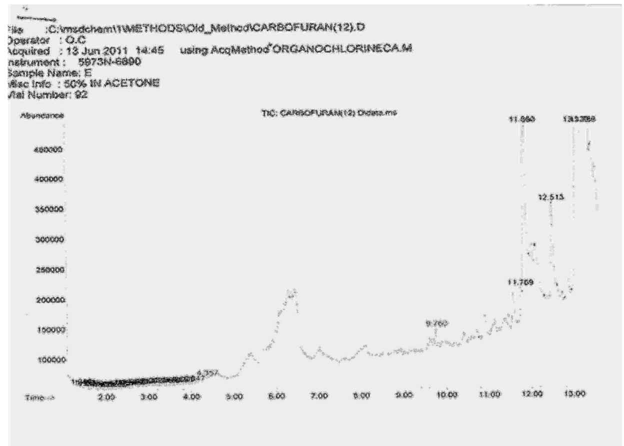

Fig. 1: GC-MS Chromatogram of a pineapple fruit with carbofuran treatment.

Discussion

In the quest to promote the use of environmentally safe but effective and sustainable strategies for the management of plant-parasitic nematodes of pineapple, the nematicidal properties of carbofuran and poultry manure were compared. The study showed that soil amendment with poultry manure and carbofuran treatments apart from having suppressing effects on nematode population, also promoted yield of pineapple. However, 3.4 kg a.i./ha of carbofuran in the Abeokuta field suppressed nematode population, promoted vegetative growth and yield of pineapple than the more gradual effect of the poultry manure. This is in agreement with the findings of Tanimola (2008) who reported the best plant growth, higher yield and nematode control on cowpea plots treated with carbofuran at 2 kg a.i/ha, as compared with those treated with poultry manure in nematode-infected cowpea. The positive effects of poultry litter on the vegetative growth of pineapple have also been variously reported (Bartholomew *et al*., 2003; Agu, 2008; Daramola *et al.,* 2013b).

Growing concerns in recent times about potential environmental hazards and health risks associated with the use of chemical nematicides has led to restrictions in the use and outright withdrawal of many effective chemicals that have successfully been used in controlling plant-parasitic nematodes. Likewise, the development of new classes of nematicides with novel activity, which are environmentally benign and specific to target pests which is perhaps an idealistic hope is still in its infantry especially in the developing nations. Farmers are therefore left with very few

Table 1: Suppressive Effects of Poultry manure and Carbofuran treatments on Nematode Population at 18 MAP

Treatment	Abeokuta			Ibadan		
	Plant-parasitic nematodes/250ml soil	Non- parasitic nematodes/250ml soil	Total nematode population/250ml soil	Plant-parasitic nematodes/250ml soil	Non-parasitic nematode/250ml soil	Total nematode population/250ml soil
T1=20 metric tonnes PM	154.67[b]	1611.70 [b]	1766.30 [b]	259.33 [b]	821.67 [ab]	1081.00 [a]
T2=25 metric tonnes PM	119.00 [c]	1709.70 [b]	1828.70 [b]	229.00 [c]	872.00 [a]	1101.00 [a]
T3=3.0 kga.i/ha C	50.33 [d]	1091.00 [c]	1141.30 [c]	104.67 [d]	761.67 [bc]	866.33 [b]
T4=3.4 kga.i/ha C	40.67 [e]	1092.30 [c]	1131.70 [c]	90.00 [d]	682.67 [d]	772.67 [c]
T5=control	868.67 [a]	2623.00 [a]	3491.30 [a]	386.67 [a]	721.67 [cd]	1108.33 [a]

PM=Poultry manure
C=Carbofuran
MAP= Months after Planting
*Means followed by the same letter in the same column do not differ significantly according to Duncan's Multiple Range Test ($P < 0.05$)

Table 2: Mean squares of yield parameters of pineapple as affected by Poultry manure and Carbofuran treatments at Harvest

IBADAN				
Source of Variation	df	Fruit Number/plot	% Fruiting/plot	Total Fruit weight(g/plot)
Block	2	3.07[NS]	10.75[NS]	39517.1[NS]
Treatments	4	64.43**	385.41**	39850560.7**
Error	11	2.18	9.13	47671.3

Table 3: Mean squares of pineapple yield parameters as affected by Poultry manure and Carbofuran treatments at Harvest

ABEOKUTA				
Source of Variation	df	Fruit Number/plot	% Fruiting/plot	Total Fruit weight(g/plot)
Block	2	0.17[NS]	49.45*	93799.5[NS]
Treatments	4	219.13**	1802.85**	66883083.8**
Error	11	0.70	7.70	136187.4

** Significant at $P<0.001$
NS = Not significant

Table 4: Some Chemical Components Identified from GC-MS Pesticide Analysis of Pineapple Fruits

Identified Compounds	Retention time (minutes)
cis- vaccenic acid	9.65
Heptadecane	9.76
Octadecanoic acid	10.399
Oleic acid	11.763
n- Hexadecanoic acid	11.860
n- Hexadecanoic acid	11.899
Nonadecanoic acids	11.957
Eiocosanoic acid acid, Docosanoic acid	12.603
Oleic acid	13.120
Octadecanoic acids	13.294

Acetone/water 1:1

alternatives to effectively control plant parasites that are ravaging their agricultural products.

The mode of action of carbofuran in nematode control and plant response to carbofuran treatment has not received enough attention. Moreover, the broad-spectrum activity of most nematicides has resulted in much of their basic biochemical effects being documented on insects or mammals instead of nematodes (Chitwood, 2002). Whereas fumigant nematicides cause a high degree of nematode mortality in the soil, carbamates, at concentrations presently used in the field, do not actually cause direct mortality (Wright, 1981; Hartwig and Sikora, 1991) but inhibit acetyl-cholinesterase at the nerve synapse, causing malfunction of the muscular and other organic systems in the nematode (Draber, 1970; Wright, 1981). Because of their cholinesterase inhibiting activity, carbamates may disrupt juvenile orientation and host recognition, and subsequently, the disruption of these systems can greatly affect nematode movement and behaviour and ultimately alter the infection process of the parasite, either by delaying or reducing penetration (Sikora and Hartwig, 1991). As with other carbamate compounds, carbofuran's cholinesterase-inhibitory effect is short-term and reversible. Symptoms of mild poisoning are short lasting and in case of occupational over-exposure, they occur at doses well below the fatal dose. Because of its rapid metabolism and excretion, it does not accumulate in the tissues.

Bunt (1987) described the nematostatic effects of the systemic nematicides as causing an inhibition of egg hatch and paralysis of the juveniles by poisoning their nervous system, which results in disorientation, inhibited movement, and protrusion of their stylets whose muscles are very sensitive to nerve poisons like cholinesterase inhibitors, thereby ensuring that plants are well protected from infection. However, the concentration of systemic nematicides decreases rapidly in soil and as a result, nerves and muscle tissues within nematodes begin to recover and show increasing orientation and movement. The nematodes still find it difficult to penetrate and feed on plant tissue, thus they die more of starvation and the side effects of the nematicide and not directly from the toxic effects of the nematicide.

Carbamate effects on nematodes have been described as reversible, therefore nematode activity is restored after degradation or dilution of the carbamates in the plant rhizosphere (Hartwig and Sikora, 1991; EXTOXNET, 2001). Since carbofuran is systemic in plants, and is quickly metabolized and degraded in the soil, chances remain that the reduction in nematode population recorded on the carbofuran-treated plots in the experiment was due to starvation resulting from the inability of the second stage juveniles to penetrate and initiate feeding sites on the pineapple roots and not necessarily due to the acute toxicity of the nematicide. Possibility also exists that the pineapple plant itself may be affected so that attractants are not produced as proposed by Di-Sanzo (1973).

The result of the residual pesticide analysis of carbofuran in pineapple fruits from this present study revealed that carbofuran residue was not detected in the pineapple fruits from the nematicide treated soil. Hexadecanoic acid, an aromatic component which had been linked to the flavour of pineapple fruits (Liu *et al*., 2011) was predominant in all the samples analysed.

Previous researches into the residual analysis of carbofuran in crop and food commodities (FAO, 2003; Trevisan *et al.,* 2004; PRIF, 2012) had shown that carbofuran is rarely detectable in food commodities and in the few cases where they were detected, they were always found in levels below the minimum residue limit (MRL) and so could not constitute a health risk when fruits are consumed.

The United State Environmental Protection Agency (U.S. EPA) initiated a ban on all granular formulations of carbofuran due to its avian toxicity. This is because bird kills have occurred when birds ingested carbofuran granules, which resemble grain seeds and when predatory or scavenging birds have ingested small birds or mammals, which had eaten carbofuran pellets. Coupled with this is the incidence mortality recorded on some predators that have ingested poisoned baits resulting from misuse and deliberate abuse of the pesticide by some farmers (EXTOXNET 1996; Otieno *et al*., 2010).

This present investigation seeks to re-appraise the appropriateness of outright removal of this useful nematicide from the reach of farmers especially when provision of equally effective alternatives is still in its infantry. Farmers could be advised to avoid misuse and deliberate abuse by applying recommended rates and conform to manufacturer's instructions in order to ensure safety and reduce health risk due to over-exposure during application. In addition, when re-packaged in forms that are not attractive to birds which could lead to avian toxicity, the merits of using carbofuran for effective management of plant-parasitic nematodes is enormous and immediate until equally effective alternatives are possible.

The GC-MS test for residual presence of carbofuran in the pineapple fruits confirmed that neither carbofuran residue nor any of its metabolites were detected in the fruits from the carbofuran-treated plots, indicating that it is not likely to pose a dietary risk when fruits are consumed.

Conclusion

Soil treatment with 3.4 kg a.i./ha carbofuran is recommended for effective nematode control in infested pineapple fields. Soil amendment with poultry manure is also recommended as a suitable alternative to chemical control of plant-parasitic nematodes especially when access to nematicides is not feasible for resource-poor farmers. The GC-MS test for residual presence of carbofuran in the pineapple fruits confirmed that neither carbofuran residue nor any of its metabolites were present in the fruits from the carbofuran-treated plots, implying that it is not likely to pose a dietary risk when fruits are consumed. Therefore, the residual presence of carbofuran in fruits and food items from carbofuran-treated soils, its environmental fate, plant and nematode response as well as human toxicity should be given a more holistic approach while re-appraising the need for outright removal of this useful pesticide from the global market.

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