EFFECTS OF CARBOFURAN-3% PESTICIDE APPLICATIONS TO TWO SOILTYPES MICRO-FLORA ORGANISMS IN NEW BUSSA, NIGER STATE, NORTH CENTRAL, NIGERIA

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ABSTRACT

Soil contamination from pesticide is now a global issue that needs immediate attention. Cases of pesticide spillage and drift have been reported by many researchers to contaminate the soil thus causing effect and shift in the diversity of microbial community. The aim of the study was to determine the effects of Carbofuran 3% pesticide on soil microorganism of two soil types, clay and sandy soil. Serial dilution technique was employed up to 10^3 using nutrient agar, potato dextrose agar and sabourand agar plates. The isolation of both bacterial and fungal organism was made before and after treating the soil types with the pesticide. The experiment was carried out at the Government Day Secondary School Farm and Government Technical College Farm in New Bussa, Niger State for sand and clay soil respectively. The experimental design used was Complete Randomized Design fitted into factorial experiment, using carbofuran and two types of soil. Soil sample were taken according to the treatment designed? analyzed for bacteria and fungi isolation, identification and determination of population present in the clay and sand soil after exposure to the pesticide. Data collected were subjected to analysis of Variance (ANOVA), means were later separated using DMRTat 5% probability level.) The result showed that, the fungal species isolated were Aspergillus , Syncophatastrum , Penicillum and Mucor . The population of both bacteria and fungi were higher in clay (98.27 x 10^6 and 83.37 x 10^4 respectively at 3 weeks after spraying (WAS)) than sand (79.13 x 10^6 and 65.03 x 10^4 respectively) soil. The populations of both bacteria and fungi were not significantly different in the treated and untreated soil. The population of fungi in the sampled soil was

lower than that of the bacteria before and after exposure. It can be concluded from this research work that, the population of bacteria and fungi decrease with increased exposure to carbofuran. Thus, care must be exercised, so that the microbial population will not be affected by the addition of this pesticide.

Keywords: *Soil, Contamination, Carbofuran, Drift, Microflora, pesticide, Spillage*

INTRODUCTION

Soil can be described as a complex natural material derived from decomposed rocks and organic materials (Chandrosekaran *et al*., 2015). According to Walter *et al*., (2015), soil isthat portion of the surface of the land which is essential for plant growth. Plants are anchored in the soil by their roots spread in all directions and which by holding on to the soil keep the plant in position. Plants draw all their water and most of their food or nutrient from the soil. Soil is therefore the source of nutrient or food for plants, animals and man. The living organisms in the soil include both the animal (fauna) and the plant (flora) (Encyclopiedia Britannica, inc 2021; Beketov *et al*., 2008). These organisms engineer a myriad of biochemical changes as decay takes place. They also physically chum the soil and help stabilize soil structure. A vast number of organisms live in the soil, by far the greater portion of these belong to plant (flora). Animals are not to be underestimated especially in the early stage of organic decomposition, such as soil invertebrates. Christorpher (2017) and Beketov*et al*. (2008) explained that the activities of specific group of soil organisms are commonly identified by; their numbers in the soil, weight per unit volume or area of soil (biomass) and their metabolic activity. Although, the relative metabolic activities are not shown, they are generally related to biomass of the organism. Soil microorganisms, so great are their number that they dominate the biomass inspite of the minute size of each individual organism. The soil microorganism monopolizes the metabolic activity in soil. It is estimated that 60 to 80% of the total soil metabolism is due to microorganism (Buol, 2010). Soil microorganisms besides their role in soil forming processes, they make an important contribution to plant growth through their effect on the fertility level of the soil. They are essential for maintenance of soil structure, transformation and mineralization of organic matter, making nutrients available

for plants. Soil microorganisms are also able to metabolize and degrade a lot of pollutants and pesticides and thus are of great concern for use in biotechnology, among the soil microorganisms are fungi, bacteria and virus. Fungi are saprophytic or parasitic non- green plants. The saprophytic fungi are beneficial in Medicine, industries and in nature, while, the parasitic fungi are harmful via cause of disease, spoilage of food, deterioration of materials and causes death Christorpher (2017) Bacteria are microorganism that can easilybe seen with the aid of light microscope. They occur in clusters or colonies. There are three classes of bacteria; Aerobic bacteria: require oxygen for their respiration, Anaerobic bacteria: do not require oxygen for their respiration while facultative bacteria: can live under aerobic and anaerobic conditions. Bacteria is also classified on the bases of their shape; cocci, bacilli, vibria and spririllae. Bacteria have beneficial effects via; nature, medicine, and in industries.It also has some harmful effects which include spoilage of food, causing diseases, deterioration of materials and causes death Christorpher (2017) A lot of factors affect microbial activities in the soil such as agricultural activities, temperature and applications of Agricultural synthetic chemicals such as fertilizers and pesticides (Christorpher (2017) ; Ayansina, et al, 2003). Many of the Agricultural synthetic chemicals used as pesticides are persistent soil contaminant whose impact may endure for decades and adversely affect soil conservation. The use of pesticides in recent researches showed that pesticides decrease the general biodiversity in the soil. Non use of the chemicals results in higher soil quality with the additional effect that more organic matter in the soil allows for higher water retention (https: // www. Biologydiscussion.com). According to Walter *et al*., (2015), Agricultural practices have a significant positive and negative impact on soil, such as wrong application ofpesticide with active ingredient Carbofuran 3% G, which is detrimental to soil microbes. Therefore, it is necessary to determine the effect of Carbofuran 3% G on the microbial population and species composition of the two soil types. Micro-flora is a living microorganism that is so small that it can be seen with a microscope and that maintain a more or less constant presence in a particular area e.g. bacteria, virus, protozoa and fungi (Farcex, 2003, Medical dictionary for health professionals and Nursing Farlex, 2012). Collins English Dictionary unabridged (2003) define microflora community is the community of micro- organisms including algae, fungi and bacteria that live in or on another living organism or

a particular habitat. Pesticide according to the United States Environmental Protection Agency (2012), a pesticide is a chemical used to prevent, destroy or repel pests. Pests can be insects, mice or other animals, weeds, micro-organism such as virus, fungi and bacteria. A pesticide can be naturally derived plant derivatives, animals or mineral or synthetically produced substances. It can also be an organism like Bacillus thuringiensis which is used to control a number of insect pests, or even a genetically modified crop, example are Bollard ilexternal link cotton. Pesticides are also a kind of agrochemicals. Many of the chemicals used (synthetic chemicals) in pesticides are persistent soil contaminants whose impact may endure for decades and adversely affect soil conservation. The use of synthetic pesticides in recent research shows that synthetic pesticides decreases the general bio diversity in the soil (Environment Canada, 2001; Damalas et al., 2011; external Links, 2015). All pesticides act alike in blocking some metabolic activities of the organisms they come in contact with (Hayes et al., 1990). They differ however in composition, potential mode of action, speed of effects, dosage requirement and stage of pest against which they may be used. They mayoften differ according to the type of organism they are principally intended to control or kill. More than 800 biocide compounds are now used as pesticides. These compounds include the organochlorine (chlorinated hydrocarbons), organophosphate pesticides and carbamate, pyrethrin and pyrethroids (Dalshad, 2012). He furthered confirmed that some pesticides are naturally occurring while other are said to be synthetic organic matter. Carbofuran belongs to carbamate insecticides and is in granules or encapsulate formulation. In the mid-1940s, Geigy chemical company attempted to develop an insect repellant. They tested a series of carbamate compounds, discovering that these compounds were poor repellants but that they were toxicto houseflies, aphids and other small insects. At this point Geigy decided to pursue developing carbamates insecticides rather than carbamates repellants. In1953, Union Carbide Corporation synthesized another class of carbamate compounds in which dimethyl carbamoylmoiety was replaced with a monomethyl moiety. These aryl-N-methylcarbamates were shown to have superior insecticidal activity compared to the dimethyl carbamates acids(Story and Cox, 2001). It was from these aryl-N-methyl carbamate insecticides that carbofuron was derived. Carbofuran is exclusively a field applied insecticide and nematicide used for various crops. Some of the more important ones include

corn and rice.

Table 1: Physical and chemical properties of Carbofuran

Chemical formula	**$C_{12}H_{15}NO_3$**
Molecular mass	**221.256 g/mol^{-1}**
Appearance	**white crystalline solid**
Density	**1.18g/Cm^3**
Melting point	**151 ^{O}c (304ºF, 424)**
Density	**1.180 (20ºC)**
Boiling point	**313.3ºC (595.9ºF, 586.5kg)**
Solubility	**Highly solubale in N-methy-2-pyrolidone,dimethl,formamade.**
Water soluble	**25ºC 320mg/L**
Koc	**9-36ml/g**

Ravichandra, N. G (2018)

MATERIALS AND METHODS

Serial dilution technique was employed up to 10^3 using nutrient agar, potato dextrose agarand sabourand agar plates by using standard laboratory procedure. This was carried out for both the sandy and clay soils. The land was neither cleared nor weeded before collection of the soil samples. The initial soil sample that was collected was three (3) for each sandy and clay soils. Further samples were taken three times, 3, 6 and 9 weeks after spray with 12 samples in each case for the two soil types. Thus, there were 39 samples for clay and sandy soils. The total samples taken were 78 on each of the soil samples. Initially, the analysis for physical and chemical properties of the soil was done. The serial dilution techniques was employed up to 10^3 using nutrient agar, potato dextrose agar and sabourand agar plates. The isolation of both bacteria and fungal organism were made before treating the soil types with Dichlorvos pesticide.

Data analysis

Data were subjected to Analysis of Variance (ANOVA) and means were separated usingDuncan's Multiple Range Test (DMRT)

Experimental Layout

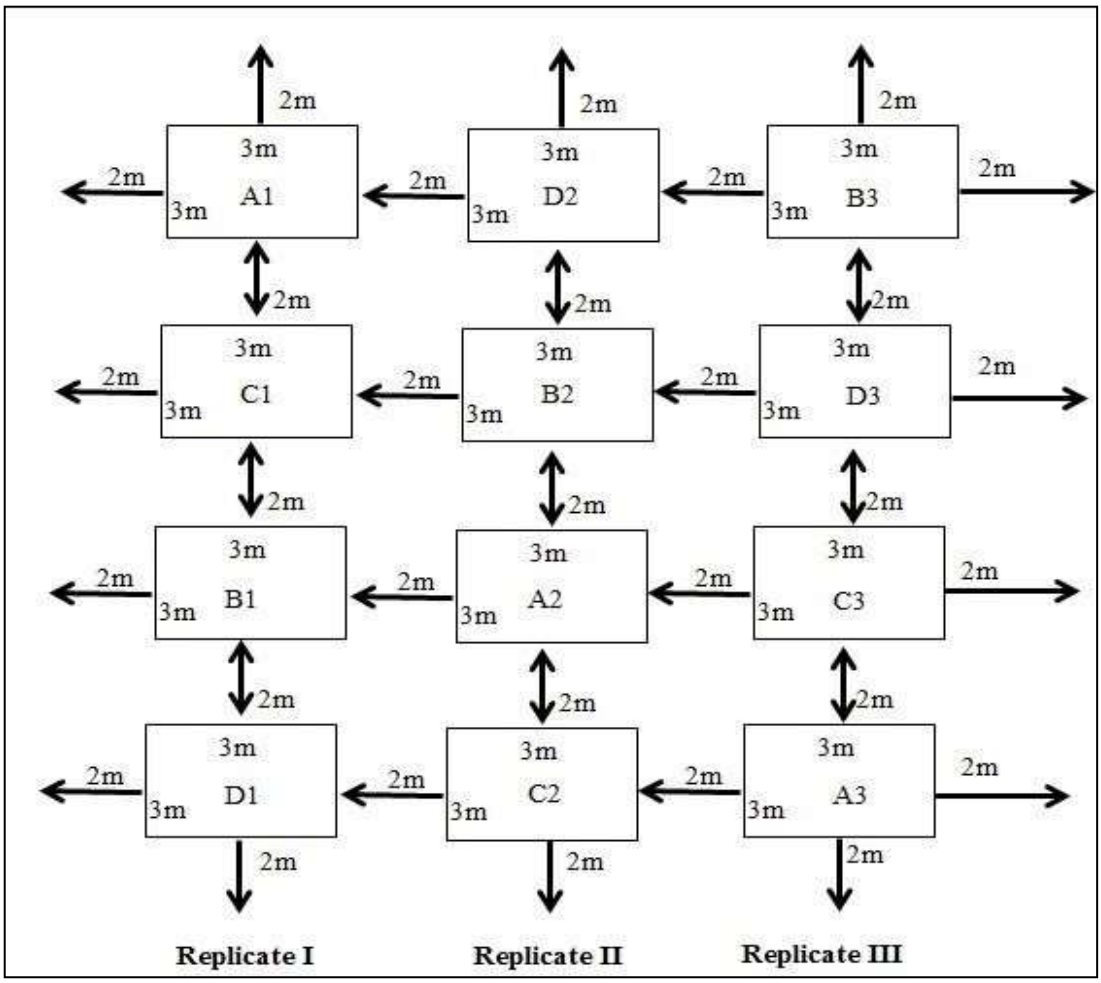

Fig 1: Plot Layout

Carbonfuran-3% = A1, B1, B2, C2, B3, C3Control = C1, D1, D2, A2, D3, A3

The two types of soil; sandy and clay were collected from plots laid out in Complete Randomized Design (CRD). This was carried out for both the sandy and clay soils. The land was neither cleared nor weeded before collection of the soil samples. The initial soil sample that was collected was three (3) each for sandy and clay soils. Further samples were taken three times, 3, 6 and 9 weeks after spray with 12 samples in each case for the two soil types. Thus there were thirty nine (39) samples for clay and sandy soils. The total samples taken were seventy eight (78) on each of the soil samples. Initially, the analysis for physical and chemical properties of the soil was done. The serial dilution technique was

employed up to 10^3 using nutrient agar, potato dextrose agar and sabourand agar plates. The isolation of both bacterial and fungal organism were made before treating the soil types with the three pesticide.

Treatment and application methods Dichlorvos= A1, B1, B2, ,C2, B3 and C3
Control = C1, D1, D2, A2,D3 and A3

Preliminary collection of the two types of soil

Samples of clay and sandy soil were taking after the crops have been harvested from the schools farm with sterile containers? The sandy soil samples were collected from the school farm of Government Day Secondary School, Dogo-Ngeri District, New Bussa and the clay samples were collected from the farm of Government Technical College, Dogo-Ngeri District, New Bussa. Both schools are opposite to each other with the major road (Trunk A) from Mokwa to New Bussa passing in between the two schools.

Materials

Materials used includes; autoclave, colony counter, incubator, inoculators, hand gloves, hot air oven, digital pH meter, Bunsen burner, retort stand, spatula, weighing balance, wire loop, respirator, goggles, face shield and tyvek clothing.

Glass wares

Glass material used are; beaker, conical flask, petri dishes, biyour bottle, Durham tubes, thermometer, test tubes, universal bottles.

Preparation of the media

The media for culturing was aseptically (free from harmful bacteria) as when necessary according to the manufacturers instruction and autoclaved at 121^{o}C for fifteen minutes at 151 bs pss. The remaining media in flask was stored at 40^{o}C.

Preparation of bacteriological culture media

Nutrient Agar (N. A) (oxides)Composition

Agar N_3	**15.0g**
Sodium chloride	**5.0g**
Yeast Extract	**2.0g**
Distilled water	**1 Litre**

Peptone	**5.0g**
Lablemco powder	**1.0g**

The distilled water added to 28g of Agar powder to make pH of 7.4
N.A can be used for culturing both bacteria and fungi

Reagents

Reagent used for the research includes; gram stains, methyl red indicator, phenol red,hydrogen peroxide, lactophenol cotton blue, omeara reagent, X-naphtholand Naphthol.

Preparation of sabouraud dextrose agar oxide

Composition

Agar	**15.0g**
Glucose (Dextrose)	**40.0g**
Mycological peptone	**10.0g**
Distilled water	**1 Litre**

The 1litre distilled water is added to make pH 5.2 (use for culturing and growing offilamentous bacteria)

Preparation of potato dextrose agar (pda)

Composition

Potato extracts	**4.0g**
Dextrose D-glucose	**20.0g**
Agar	**15.0g**
Distilled water	**1 Litre**

The 1 Litre distilled water was added, acidified later by addition of 1 ml of lactic acid 10% SR0021 to each 100 ml of sterilized medium at 50^{o}C to acidify the medium to pH 3.5. This isused for culturing fungi (Japanese pharmacopoeia, 2006).

Composition of media for biochemical test

Peptone

Methyl red test reagent (MR)

Dipotassium hydrogen phosphate	**5.0g**
Glucose 10% solution sterilized separately	**50ml**
Distilled water	**1 Litre**

The 1 Litre distilled water was added to make pH 7.6 (used to detect the continued existenceof microbes) (IFI claims patent service, 2016)

Voges proskauer test reagent (vp)

Composition

Potassium hydroxide	**40.0 g**
Cretonne	**0.3 g**
Distilled water	**100ml**

Glucose phosphate peptone water as in methyl red test reagent

V.P reagent was prepared in the same way as methyl red test plus Omearas reagents were used to detect acetoin in a bacteria broth culture (About microbeonline.com, 2016).

Indole test reagent

Composition

DMACA	**10.0g**
Hydrochloric acid 37%	**100.0ml**
De-ionized water	**900.0ml**

It was used for identification of entero bacteria (About microbeonline.com, 2016).

Nitrate reduction test reagent

Composition

Potassium nitrate	**0.2g**
Distilled water	**1 Litre**
Peptone	

The 1 Litre of distilled water was added (It was used for differentiation of members of enterobacteriaceae on the basis of their ability to produce nitrate reductase) (About microbeonline.com, 2016).

Sterilization

Properly washed Petri dishes, byour bottles, test tubes, conical flasks, beakers, universal bottles, pipettes, spatulas, wire lops, masculating needs, 50 ml capacity bottles, Durham tubeswere sterilized in hot air oven at 160^{o}C for one hour and stored at 4^{o}C.

Precision

Special attention was paid to the analysis of all the chemical components, it was also ensuredthat the distilled water used were neutral.

Land preparation

Land demarcation, experimental design and treatment application.

Plating technique

The serial dilution techniques were employed; sandy soil was dissolved in 9 ml of water and dilution of up to 10^{-3} was made. This process was adopted and used separately for clay and sandy soil. Nutrient agar, potato dextrose agar (PDA) and Sabouraud dextrose agar oxide plates were prepared in this order: three for untreated sandy soil and three for untreated clay soil and three plates for the treated sandy soil and three plates for the treated clay soil separately with the pesticides. This was done for all the solutions. The soil samples; sandyand clay soils were dilution serially up to 10^{-3} was taken from the untreated sample and treated sample of each soil type and was run into the agar plate while still molten. The plates were swung clockwise and anticlockwise for even distribution and the plates were allowed to solidify, inverted and incubated for 18 to 24 hours at 37^{o}C, replicate plates were prepared from each serial dilution up to 10^{-3} for each soil types, for both untreated and treatedand incubated at 28^{o}C. Seventy eight samples were analyzed for the soil types separately for untreated and treated samples.

Microbial counts staining and microscopic work

After incubation, the number of colonies on the Petri dishes was counted using colonycounter. The average total (mean) and differential standard plate count

(data) was taken. The colonies were placed in to the group based on pigmentation colony morphologies and gram reactions. The gram stained colonies was examine microscopically with the aid of microscope, representative colonies was separately subculture on nutrient agar slope for confirmatory characterization of the organism.

Identification of moulds

Potato dextrose agar (PDA) was acidified by addition of 10% of lactic acid after the agar has been sterilized just prior to being poured into the plates. The culture plate of acidified potato dextrose agar was incubated at 37^{o}C for 24 hours. After incubation, the resulting colonies were counted and reported as mould count per ML. The colonies were further examined with the aid of microscope using Lactophenol cotton blue.

Biochemical tests for identification catalase test

A small amount of the culture was picked from the agar slope using a clean sterile platinum wire loop, this was inserted in drops of H_2O_2 (hydrogen peroxide) on a clean microscopic slide and production of gas bubble indicated a positive reaction.

Indole test

Water medium was inoculated for 48 hours at 37^{o}C. The tube was further allowed to stay for more 48 hours in the incubator for the accumulation of indole after this period. 0.5 ml of Koran reagent was added separately to each tube and swung gently, the appearance of red colour in the alcohol layer indicate a positive reaction.

Methyl red test

The sterile glucose phosphate peptone water medium was slightly inoculated from an agar slope, cultured and incubated at 37^{o}C for 48 hours. Then, five (5) drop of methyl red reagent was added into each tube, the content was mixed and read immediately, the positive result was bright red and negative is yellow.

Voges proskauer test

Sterile medium used was inoculated and incubated at 37^{o}c for some hours, 0.5

ml of Omearasreagent was added in to each tube after incubation, the tubes was shaken at interval to ensure maximum aeration by shaker, after 2-5 minutes, they were read, a positive reaction is indicated by development of a pink colour which become crimson in 30 minutes.

Nitrate reduction test

The prepared and sterilized peptone KNO_3 medium was inoculated and incubated for 96hours at 37°C, equal volume of solution A (0.8g of sulphuric acid dissolved in 1 Litre of 5N Acetic acid) and B (5.0g of Naphthylamine dissolved in 1 Litre of 5N acetic acid) was the testreagents. Then 0.1 ml of the test reagent was added to the test culture. A red colour developing within a few minutes will indicate the presence of Nitrate hence, the ability of the organisms to reduce nitrate to nitrite.

Motility test

About 2-3 drops of peptone water with growth of the organism was placed on a clean slide with a loop. The cover slip was placed over the slide; the slide was left for some time andthen examined microscopically with the high power

RESULT AND DISCUSSION

Preliminary indication of bacteria isolates

Four microbial species was presumptively identified after 24 hrs. incubation (Table 2).*Pseudomonas* and *Rhizobim* spp was gram negative while *Bacillus* and *Actinomycetes* spp were gram positive. *Pseudomonas* spp were greenish blue colouration on the media, rod shaped, *Bacillus* spp were gray-white colouration spherical rod, *Actinomycetes* spp were hard and chalky creamy white colourarion and branching filamentous cocci and *Rhizobium spp* werebluntly rod shape, oval and spherical cells, non sporulating rods. The biochemical tests that were carried out on *Pseudomonas* species, *Bacillus* species, *Actinomycetes* and *Rhizobium* species were presented in table 2.

Table 2: Preliminary indication of bacteria isolates

Code	Morphology after 24 hrs. at 30°c incubation	Gram Reaction	Presumptive Identification
A	Greenish blue colouration on the media, rod shaped	Gram Negative	***Pseudomonas*** **Species**

B	Gray-white colouration spherical rod	Gram Positive	***Bacillus* Species**
C	Hard and chalky creamy white colouration and branching filamentous cocci	Gram Positive	***Actinomycetes* Species**
D	**Bluntly rod shape, oval andspherical cells, non sporulating rods**	**Gram Negative**	*Rhizobium*

A= *Pseudomonas* species, B= *Bacillus* Species, C = *Actinomycetes* Species, D = *Rhizobium*.

Biochemical test for identification of bacteria isolates from soil types

On glucose,*Pseudomonas* species, the glucose was oxidized in oxidation and fermentation and good growth. *Bacillus* species was Air bubbles which indicate gas (Motile),*Actinomycetes* species was aerial hypa and good growth while on *Rhizobium* species there was indication of gas. On lactose, *Pseudomonas* species (Motile) aerial growth was indicated,*Bacillus* species high production of the species was indicated (Motile). *Actinomycetes* species growth was indicated (+), while on rhizobium species there was indication of growth (Motile). Onsucrose, *Pseudomonas* species was indication of the utilization of sucrose (Motile).*Bacillus* species was inhibition of growth (Non-motile) thus very low production. *Actinomycetes* species enhancement of growth was indicated (Motile), while *Rhizobium*species was evidence of species, there was evidence of transportation of the disaccharide by the fast growing rhizobia (Motile). On catalase, *Pseudomonas* species was evidence of growth (Motile).*Bacillus* species; the reaction was (+), growth was indicated.*Actinomycetes* species was reacted (Motile) there was formation of bubbles. *Rhizobium* species reacted (Motile) there was indication of drastic sensitivity. On indole,*Pseudomonas* species reacted negatively (Non-motile), the result appears yellow.*Bacillus* species reaction was negative (Non-motile), the result appears yellow.*Actinomycetes* species reaction was positive (Motile). The result shows red, while *rhizobium* reaction was (Motile), the result shows in the appearance of pink colour in the surface alcohol layer of the broth. On methylred, all the bacteria isolate degraded (Motile) methyl red in the test process. On vogesprokauer, *Pseudomonas*species reaction was negative (Non-

motile), *Bacillus* speciesreaction was positive (Motile), *Actinomycetes* speciesreaction was positive (Motile), while *Rhizobium*species reaction was negative (Non-motile). Onnitratereduction,*Pseudomonas* species using nitrate as an electron (Non-motile) acceptor instead of Oxygen,*Bacillus* species reaction was positive (Motile), *Actinomycetes* species reaction was negative (Non-motile), while *Rhizobium* species reaction was positive (Motile). On motility, On Pseudomonas species; the reaction was positive (Motile), on Bacillus species the reaction was negative (Non-motile), on Actinomycetes species the reaction was positive (Motile), while on Rhizobium species the reaction was positive (Motile).

Table 3: Biochemical test for identification of bacteria isolates from soil types

Identification of organism	Glucose	Lactose	Sucrose	Catalase	Indole	Methyl red	Voges proskauer	Nitrate reduction	Motility
Pseudomonas Species	AG	+	+	+	-	+	-	-	+
Bacillus Species	G	+	-	+	-	+	+	+	-
Actinomycetes Species	AG	+	+	+	+	+	+	-	+
Rhizobium Species	G	+	+	+	+	+	-	+	+

AG = Air and Gas, G = Gas, + = Motile and - = Non-motile

Isolated fungal organism from soil types

The four fungi spp. isolated were stain with Lactophenol cotton blue. *Aspergillus* Species were circular colony with colourless thread like growth, *Syncophalastun* Species were crayfish fluffy colony with coarse hanging mycelia,*Penicilirium*spp were Circular colony with packed mycelia while *Mucor* Species were Cloudy white with round compact mycelia dustered threads (Table 4).

Table 4: Isolated fungal organism from soil types

Identification of organism	**Stain used**	**Morphological character**
***Aspergillus* Species**	Lactophenol Cotton Blue	**Circular colony with colourless thread like growth**
***Syncophalastun* Species**	Lactophenol Cotton Blue	**Crayfish fluffy colony with coarse hanging mycelia**
Penicilirium	Lactophenol Cotton Blue	**Circular colony with packed mycelia**
***Mucor* Species**	**Lactophenol Cotton Blue**	**Cloudy white with round compact mycelia dustered threads.**

Table 5: Bacteria and fungi population (cfug^{-1}) in clay and sand soils treated withCarbofuran

Pesticide	**Soil type**	**Sampling period (Weeks)**	**Bacteria**	**Fungi**
Carbofuran	Clay	3	79.07 x10^{6}a	**72.33 x10^{4}b**
Carbofuran	Clay	6	90.43 x10^{6}a	**84.10 x10^{4}a**
Carbofuran	Clay	9	39.03 x10^{6}bcd	**48.27 x10^{4}c**
Carbofuran	Sand	3	32.47 x10^{6}bcde	**22.53 x10^{4}d**
Carbofuran	Sand	6	31.37 x10^{6}cde	**20.10 x10^{4}de**
Carbofuran	Sand	9	19.47 x10^{6}ef	**13.97 x10^{4}f**
Control	Clay	3	88.20 x10^{6}a	**75.00 x10^{4}a**
Control	Clay	6	43.47 x10^{6}bc	**50.33 x10^{4}c**
Control	Clay	9	43.93 x10^{6}bc	**52.97**

				x10^{4}c
Control	Sand	3	29.87 x10^{6}ed	**22.23 x10^{4}d**
Control	Sand	6	21.90 x10^{6}ef	**16.47 x10^{4}ef**
Control	Sand	9	14.80 x10^{6}f	**13.43 x10^{4}f**
LDS (0.05)			**13.0 x10^{6}1**	**13.01**

Value followed by the same alphab*et al*ong the column were not significantly different

DISCUSSION

Sample taken of 6wks from Carbonfuran treated clay plot had the highest bacteria population [90.4 x 10^6 cfug^{-1}], fungi [84.10 x 10^4 cfug^{-1}] while the control plot bacteria population was [43.47 x 10^6 cfug^{-1}], fungi [50.33 x 10^4 cfug^{-1}] indicated that the population of the microorganism is more in the treated plot than in the control, thus Carbonfuran-3% enhanced the growth and increased in the population of the microorganism. 3wks treated plot of carbonfuran-3% bacteria population was [79.07 x 10^6 cfug^{-1}], fungi [72.33x 10^4 cfug^{-1}], control plot of clay 3 wks of bacteria population was (88.20 x 10^6 cfug^{-1}), fungi [75.00 x 10^4 cfug^{-1}] indicated that there are more population of the microorganism in the control plotwhich implied that there was inhibition of growth after application. 9wks treated plot, the population of bacteria was [39.03 x 10^6 cfug^{-1}], fungi [48.27 x 10^4 cfug^{-1}], while 9 wks at control plot bacteria population was [43.93 x 10^6 cfug^{-1}], fungi [52.97 x 10^4 cfug^{-1}] which implied that there are more population in the control plot than treated plot indicating that there was growth inhibition in the control plot. Sand treated plot with Carbofuran-3% 3 wks bacteria population was [32 x47 10^6 cfug^{-1}], fungi [22.53 x 10^4 cfug^{-1}], while at the 3 wks the control plot bacteria population was [29.87 x 10^6 cfug^{-1}], fungi [22.23 x 10^4 cfug^{-1}] there was no much significant different, 6 wks bacteria population at treated plot was [31.3 x 10^6 cfug^{-1}], fungi [20.10 x 10^4 cfug^{-1}], while the control plot bacteria population was [21.90 x 10^6 cfug^{-1}], fungi [16.47 x 10^4 cfug^{-1}] indicated that application of Carbofuran-3% enhanced growth ofmicroorganism. 9 wks of treated plot, bacteria population was [19.47 x 10^6 cfug^{-1}], fungi [13.97 x 10^4 cfug^{-1}], while the control plot

bacteria population was [14.80 x 10^6 $cfug^{-1}$], fungi
[13.43 x 10^4 $cfug^{-1}$] indicated that there is little significant difference in population. Carbofurana has acted as stimulator for the population growth of bacteria which was confirmed by the finding of Edward (1965) and Das and Mukherjee et al. (1998). They reported that carbofuran significantly stimulated the populations of bacteria as well as N^2 fixing bacteria in the agricultural soil while other tested insecticides reduced proportions of Micrococcus and Rhizopus in the soil. Later the population of the microorganism started to decrease from 6 week after application. Reduction in number of microorganisms in different soil types was investigated to inhibit growth of microorganisms (Harley et al., 1969; the Royal Society of Chemistry (1983); Digrak, 1998; Kazanici, 2001). Reduction of fungi and bacterial population was due to inability to survive and multiply well in presence of pesticide.It has been reported that one of the primary metabolites of (3,5,6-trichloro-2-pyridinol) possesses antibacterial properties (Yohh, 2011). Significant decline in bacterial populations observed in the present study could be attributed to generation of such antibacterial metabolites (Singh et al., 2003; Singh et al., 2006). Pesticides may be toxic to some importantbacterial groups; other microorganisms are able to use same pesticides as energy and nutrient sources (Johnsen, 2001; Mokiedje and Spiteller, 2002). This could be the reason for highest population of microbes recorded in the first 3 weeks. In addition, some metabolites ofpesticides may even be more adverse to microorganisms than the original compound (Topp, 2004). Different effects of pesticides on soil living components have been reported. Many of pesticides have shown adverse impacts on numbers and functions of diverse range of microorganisms. The reduction of bacterial and fungal biomass, may affect soil respiratory activities, soil enzymes and microbial diversity as well as rates of carbon and nitrogenturnover (Johnson, 2001; Mokiedje and Spiteller, 2002; Yair, 2008). However, many factors such as soil structure and texture, PH, organic matter content, temperature and moisture influence pesticides effects on soil microorganisms (Beulke and Malkomes, 2001; Kim,2002; Mokiedje and Spiteller, 2002). This could lead to higher population of microbes found in clay than sand soil as observed in this study. It had been reported that, Carbofuran have some inhibitory effects on soil micro-flora temporarily (Kalyanee and Hemen, 2011). Organophosphate insecticides affect soil diversity of microbes; had specifically toxic effect on

one type of microorganisms but stimulated the growth of another type (Worthing, 1987; Digrak and Kazanici, 2001).

SUMMARY, CONCLUSION AND RECOMMENDATIONSUMMARY

Repeated application of Carbofuran-3% to soil leads to less of efficacy of the pesticide; thus resulting to its effects on the population of the microorganism which as a result also affect thebiological importance of the microorganism in the soil which serves as a source of nutrientsto plant growth.

CONCLUSION

It has been recognized since 1981 that repeated use of Carbofuran 3% G in soil result in the loss of pesticide efficacy of carbofuran, which is attributed to the enhanced degradation of thepesticide in soils. The study shows that loss of pesticidal efficacy in enhanced soil has been attributed to carbofuran degrading microorganism as shown in the table. It is hoped thatfuture works on the ecology, physiology and genetics of the carbofuran degrading microorganism will yield insight into cellular and molecular mechanism of enhanced degradation of carbofuran by soil organism.

RECOMMENDATION

Frequent application of Carbofuran-3% to the soil should be minimized/reduced because ofits side effects on soil microorganism.

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