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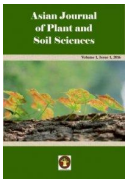

EFFECT OF PESTICIDE (CHLORYPYRIPHOS) ON THE GROWTH OF NITRIFYING BACTERIA (*Nitrosomonas* AND *Nitrobacter* species) ISOLATED FROM THE SOIL

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research investigated the effect of pesticides application on the growth of two soil nitrifying bacteria species, *Nitrosomonas* and *Nitrobacter* respectively. The bacteria were isolated from soil samples obtained from Bingham University, Karu local government area of Nassarawa State, Nigeria. The soil bacteria were cultured using the Manniol Yeast Extract Agar (MYEA). The bacteria isolates where identified and characterized based on their morphology and chemical characteristics. The growth count of the bacteria after four (4) days isolation showed increase in the bacteria growth. *Nitrosomonas* obtained from MYEA ranged from 23 to 102 ($x10^5$) cfu/g, and the growth of *Nitrobacter* count obtained from MYEA ranged from 24 to 97 ($x10^5$) cfu/g. there was an increase in bacteria growth at both control level and decrease in bacteria growth at concentration level of 20% to 100% within 120 hours of observation for *Nitrosomonas* species. The result shows that *F– cal 0.830* was greater than *F-tab 0.05,* and *Nitrobacter* species. F– cal 1.360 was greater than f-tab 0.05. Since the F- cal value is greater than F- tab value for both the *Nitrosomonas* and *Nitrobacter* species. The Null hypothesis is rejected and alternate is accepted. This shows that the nitrifying bacteria could not survive or grow under high pesticides concentration. It is therefore very important to examine pesticides in order to determine it toxicity to soil bacteria before application.

Keywords: Soil; bacteria; pesticides; growth and isolates.

1. INTRODUCTION

Nitrifying bacteria are gram-negative, chemoautotrophic, aerobic bacteria that oxidize ammonia to nitrate in the soil in a process known as nitrification [1]. The ammonia-oxidizing bacteria convert and the nitrite oxidizing bacteria convert ammonia to nitrate in a two step process that is dependent on the action of two separate species of nitrifying bacteria, the first stage of ammonia oxidation is carried out mainly by the genera, *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosocytis* and *Nitrosogloea* while in the second stage, nitrite formed is converted to nitrate by the genera, *Nitrobacter, Nitrocystis, Nitrococcus, Nitrospina and Nitrospira* [2]. Nitrifying bacteria play a very important role in soil fertility. Nitrogen, which is a common soil nutrient element required in large quantity by plant, is largely made available to plants in the form of nitrate ion by the activities of nitrifying bacteria through the process of nitrification [3].

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Modern agriculture and industry are dependent on a variety of synthetic chemical compounds, including pesticides, e.g. zoocides, fungicides, herbicides and others. Their extensive exploitation has resulted in the contamination of natural environment, e.g. natural aquifers which are both major and intermediate receivers of the applied pesticides. While pesticides elicit an acute or chronic toxic effect upon microorganisms, the latter are capable of accumulating, detoxifying and metabolizing these compounds and, additionally, use them as a source of carbon [4,5]. The detrimental effect of pesticides on the species composition of microorganisms triggers changes in higher trophic levels. This modification involves both qualitative and quantitative changes. It seems indispensable, therefore, to identify the impact of the mentioned xenobiotics on soil and aquatic microorganisms, and thus on the process of primary production, nutrient circulation and decomposition of matter, in which bacteria serve an important function. In addition, due to the significant role of bacteria in the degradation process of toxic compounds in the natural environment, gaining knowledge on the decomposition of these compounds in pure and mixed cultures is also of key significance, as well as their effects on natural populations of microorganisms.

Pesticides do not necessarily distinguish between pests and other living things. The use of pesticides decreases the general biodiversity in the soil. Pesticides can kill beneficial soil bacteria, earthworms, snails, frogs, birds, and other valuable species. Soil microorganisms play a key role in maintenance of soil structure, transformation and mineralization of organic matter, making nutrients available for plants.

The identification of nitrifying bacterial strains with biological capacities and metabolic capacities to degrade or utilize pesticides as carbon sources is considered one of the promising approaches to enhancing soil fertility in an ecosystem contaminated with these pollutants. Thus, it is important to examine the response of these organisms to these pesticides so that less toxic and more readily biodegradable pesticides may be developed especially if the current ones in use are toxic, persistence and thus do not meet regulatory requirements in terms of their pollution effects in our environment.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Bingham University, Soil samples were collected from agricultural soil free from pollution within the Bingham University. Bingham University is approximately 25km from the Federal Capital Territory (FCT), Abuja. Bingham University is located at longitude 7.6^{o} E and latitude 8.9^{o} N in Karu, Nasarawa State, Nigeria [6]. The soil samples were collected at a depth of 0-20cm, using sterile spatula and transferred into sterile polyethene bags and transported to the laboratory for use as described by Ferrari et al. [7].

2.2 Determination of Soil Temperature

A rod was pushed into the ground till 3 cm mark is reached. The thermometer was inserted into the hole made with the rod and the temperature was recorded [8].

2.2.1 Preparation of media

The Mannitol Yeast Agar was prepared by adding 15.0g agar to 100ml of water and sterilized at 121^{0}c for 15minutes at 15psi.The media was allowed to cool to about 45^{0}c before they were dispensed into the sterile petri dishes. All the agar plates were aseptically inoculated with 0.1ml of the appropriate dilution of the soil suspension using spread plate technique. The inoculums were spread over the entire surface of the solid Mannitol yeast agar with a sterile glass rod. All the inoculated Petri dishes were incubated aerobically at room temperature for 4 days [9]. Pure isolates from the corresponding agar slants were characterized and identified using morphological (cell and colonial morphology, shape, motility, and gram reaction), biochemical attribute [10].

2.3 Morphological Identification

2.3.1 Colonial morphology

A small portion of the isolate was picked and streaked on the corresponding Mannitol yeast solid medium and incubated aerobically for 2 days at room temperature. The shape, size and color were observed using hand lens. Isolates were tested for motility using the hanging drop method. In this method, wet suspensions of the isolate were made on a cover slip. A thin ring of plasticine were made and placed round the sample suspension on the cover slip. Microscope slides were gently and firmly pressed to the plasticine ring on the cover slip, taking care not to break coverslip and the slide. The slides were quickly inverted through 1800 so that the cover slip is now on top and the wet suspension hanging on it. This arrangement was mounted on a microscope and observed with the x40 objective lens [10].

2.4 Biochemical Characterization

2.4.1 Catalase test

Colonies of isolates where introduced into test tube containing 3ml of 3% of hydrogen peroxide solution and it was observed for gas bubbles [10].

2.4.2 Oxidase test

Oxidase reagent of two to four drops were placed in a sterile petri dish containing Whatman no 2 filter paper. Colonies of the isolates where smeared on the filter paper and was kept for 10 seconds. There was deep purple coloration observed which was an indication that the oxidase converted the reagent to deep purple coloration [10].

2.4.3 Sugar fermentation test

The mannitol and glucose were the sugar used to determine the ability of the isolate to ferment, at the end an acid / gas was produced. 0.5g of each sugar where dissolved in 50 ml of peptone water and was sterilized by membrane filtration [10].

2.4.4 Indole test

Certain species of bacteria are able to split the aromatic amino acid, tryptophan, into indole, pyruvic acid and ammonia. The indole can easily be detected using the Kovac's reagent. The isolates were inoculated into bijou bottles containing 4 ml of sterile peptone water and incubated at 37^0c for 48 hours. Indole was then tested by adding 0.5 ml of Kovac's reagent. The mixture was thoroughly shaken and examined for the development of red colour on the surface layer within 10 minutes [10]

2.4.5 Methyl red test

Glucose phosphate broth was prepared by dissolving 3g of peptone and 3g of dihydrogen phosphate in one liter of distilled water. The mixture was distributed in 5 ml volume into test tubes and sterilized at 121^0c for 10 minutes at 15 psi. Then 5 g of glucose was dissolved in 500 ml of distilled water and sterilized by filtration. 3ml of this glucose solution was aseptically dispensed into the same test tubes containing the peptone and dihydrogen phosphate. After cooling, test organism was inoculated into the broth and incubated for five days at 37^0c. After incubation, 5 drops of 0.04% solution of alcoholic methyl red was added into each test tube and mixed thoroughly. The contents were observed immediately for the development of bright red color [10].

2.4.6 Effects of chlorypyriphos on the growth of isolates

Changes in population of the two nitrifying bacteria isolated from soil samples were monitored following their exposure to different concentrations of Chlorypyriphos for 120 hours. Five different concentrations of Chlorypyriphos (20%, 40%, 60%, 80%, and 100%) were, respectively, used. The control will be set up without Chlorypyriphos. The growths were counted using a colony counter after every 24 hours during incubation for 120 hours [11].

3. RESULTS

Table 1 shows the result of the test on the two bacteria isolates. Two bacteria were isolated from the soil samples. The first bacteria showed tiny colonies which were colorless, round and raised, long rod. The bacteria are gram negative, non-motile, catalase positive, oxidase negative, indole negative, methyl red negative, and non-fermentative to glucose and mannitol sugar. Based on the result of the identification test carried out on the isolate, it was identified as *Nitrosomonas* spp. The second bacteria *Nitrobacter* spp showed tiny colonies which were whitish, round and raised, short rod. The bacteria are gram negative, non-motile catalase positive, oxidase negative, indole negative, methyl red negative and non-fermentative to glucose and mannitol sugar. Based on the result of the identification test carried out on the isolate, it was identified as *Nitrobacter* spp.

Table 1. Morphological and biochemical characteristics of the isolates

Tests	Isolate A	Isolate B
Color of colony	Colorless	Whitish
Shape of colony	Round and Raised	Round and flat
Size of colony	Tiny	Tiny
Cell shape	Long rod	Short rod
Gram Staining	_	_
Motility	_	_
Catalase	+	+
Oxidase	_	_
Indole	_	_
Methyl red	_	_
Glucose	_	_
Mannitol	_	_
Organism	*Nitrosomonas* spp	*Nitrobacter* spp

Key: *+ = Positive, - = Negative*

3.1 Effects of Concentrations of Chlorypyriphos on the Growth of *Nitrosomonas* spp.

Fig 1 shows the growth of *Nitrosomonas* spp. increased in the control experiment without chlorpyriphos. At 20%, *Nitrosomonas* spp decreases in growth from 96-61 within the period of 24hours – (120 hours), at 40% the growth of the organism decreases from 98-53 within the period of 24 hours – (120 hours), at 60% the growth of the organism decreases from 82-50 within the period of 24 hours – (120 hours), at 80% the growth of the organism decreases from 88-39 within the period of 24 hours – (120 hours), and at 100% the growth of the organism decreases from 82-25 within the period of 24 hours – (120 hours).

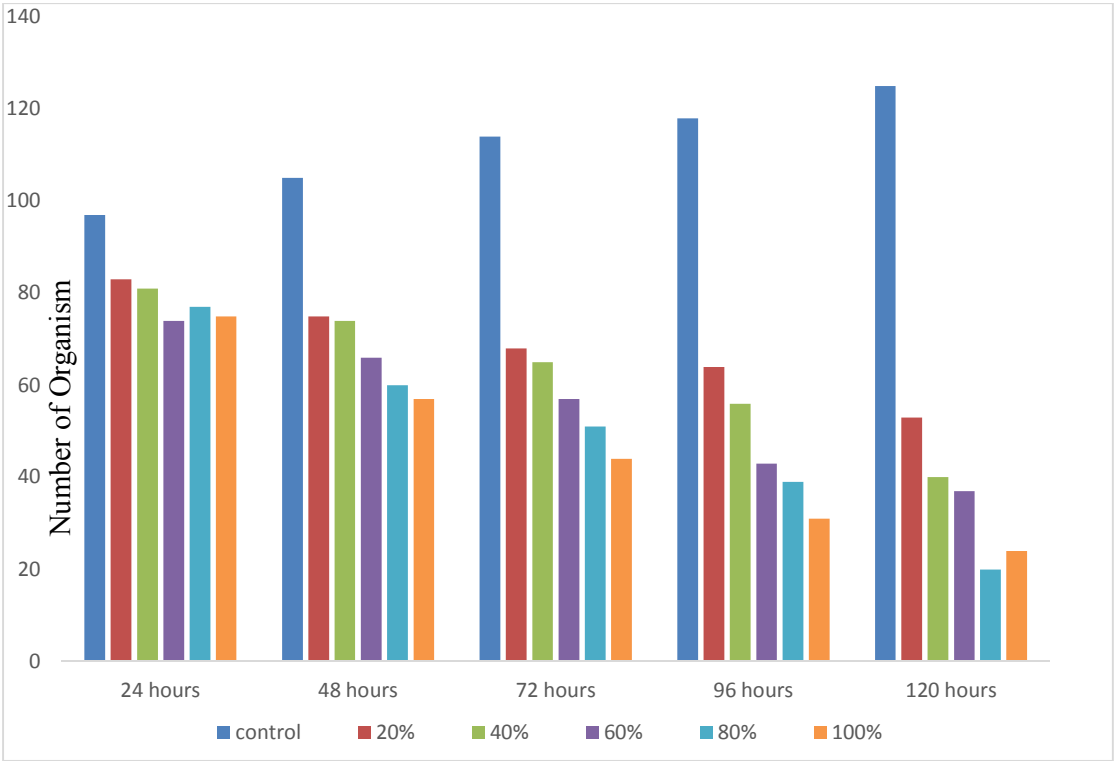

Fig. 1. The effect of different concentrations of chlorypyriphos on the growth of *Nitrobacter* spp.

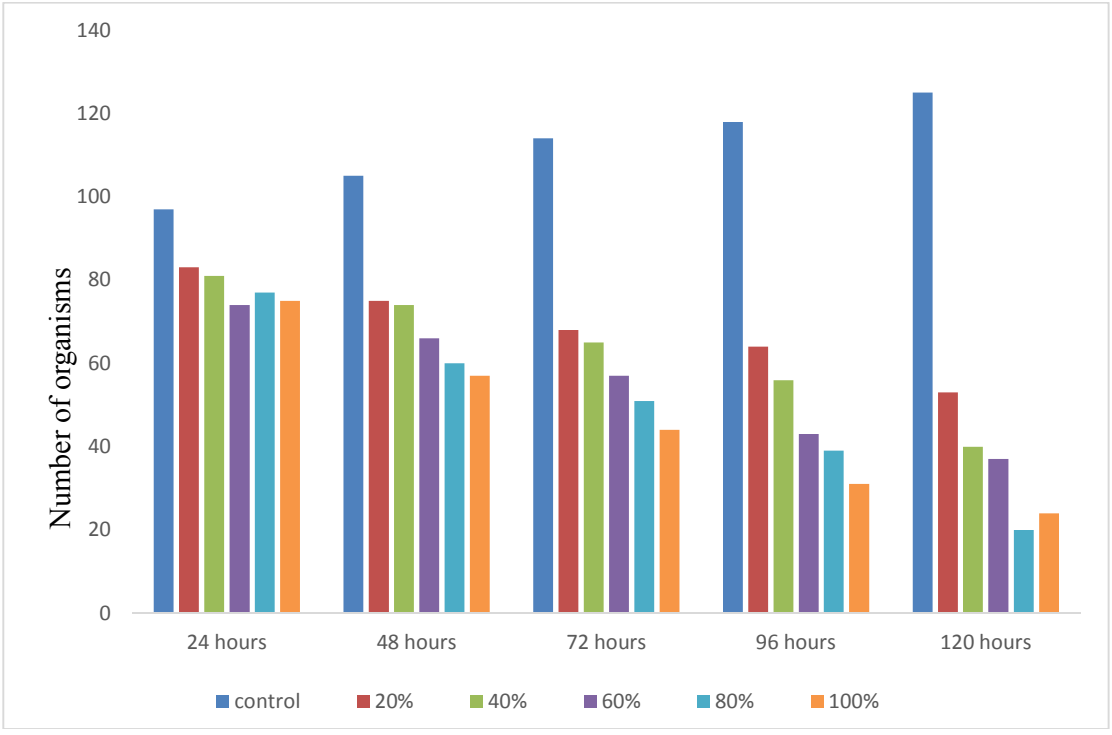

Fig. 2. The effect of different concentrations of chlorypyriphos on the growth of *Nitrobacter* spp

3.2 Effects of Concentrations of Chlorypyriphos on the Growth of *Nitrobacter* spp

In Fig 2, The growth of *Nitrobacter* spp. increased in the control experiment without chlorpyriphos. At 20%, *Nitrobacter* spp. decreases in growth from 83-53 within the period of 24 hours -120 hours, at 40% the growth of the organism decreases from 81-40 within the period of 24 hours-120 hours, at 60% the growth of the organism decreases from 74-37 within the period of 24hours-120 hours, at 80% the growth of the organism decreases from 77-30 within the period of 24 hours-120hours, and at 100% the growth of the organism decreases from 75-24 within the period of 24 hours-120 hours as shown in Fig. 2.

4. DISCUSSION

Two nitrifying bacteria: *Nitrosomonas* spp. and *Nitrobacter* spp. were isolated from the soil samples collected from different locations of agricultural soil in Bingham University, Nasarawa State, Nigeria. Although both bacteria may have similar cell wall morphology as Gram negative rods, the difference in response of these bacteria to the chlorypyriphos may be due to their genetic differences. It is also known that toxicity of some chemicals is dependent on the properties of the chemicals and the organisms [12]. Higher toxicity could be expected from compounds easily soluble in water. On the other hand, hydrophobicity could indicate toxicity because some compounds tend to bio-accumulate in the organisms [13].

The results of the study of the effect of different concentrations of chlorypyriphos on the growth of *Nitrosomonas* spp., indicate that the growth of the organism increased successively throughout the 120 hours exposure time at 0% (control experiment), the highest increase in growth of the organism was observed at the control experiment followed by 20%, 40%,60%, 80% and 100% respectively (Fig. 2). The result shows that F^{-} cal was $0.830 < 0.05$. Since the F-cal value is greater than F- tab value, the null hypothesis (there is no significance effect of chlorypyriphos on the growth of *Nitrosomonas* spp.) was rejected, and the alternate was accepted. The results of the study of the effect of different concentrations of chlorpyriphos on the growth of *Nitrobacter* spp indicate that the growth of the organism increased successively throughout the 120 hours' exposure time at 0% (control experiment), the highest increase in growth of the organism was observed at the control experiment followed by 20%, 40%, 60%, 80% and 100% respectively (Fig. 2). The Analysis of Variance (ANOVA) was carried out to find out if there was significance effect of chlorypyriphos on the growth of *Nitrobacter* spp. The result shows that F^{-} cal was $1.360 < 0.05$. Since the F-cal value is greater than F- tab value, the null hypothesis (there is no significant effect of chlorypyriphos on the growth of *Nitrobacter* spp.) was rejected, and the alternate was accepted.

The adverse effects of chlorpyriphos concentrations on the nitrifying bacteria may also be attributable to the fact that the organisms were subjected to a sudden shock effect of exposure to high chlorpyriphos concentrations as opposed to a gradual build up, since these organisms were isolated from soils that have not been previously exposed to pesticide contamination. This is why the organisms could not grow at concentration above 20%.

A decrease in the number of survivors of *Nitrosomonas* spp. and *Nitrobacter* spp. With increase in the exposure time and concentrations of chlorpyriphos was a general pattern observed in this study. As in *Nitrosomonas* spp. and *Nitrobacter* spp., the effect was more obvious at higher concentrations (20%, 40%, 60%, 80% and 100%) of the pesticide. Controls showed an increase in the population of viable cells (apparent absence of mortality) with increase in exposure time. This showed the bactericidal properties of the pesticide obviously decrease in the number of survivors of the two bacteria with increasing contact time (exposure period) and concentrations of the two pollutants. Similar observations have been made by Okpokwasili and Odokuma (1997) who observed a decrease in percentage log survival with increase in contact time and concentrations when *Nitrobacter* spp. was exposed to three spill dispersants and five domestic detergents. The adverse effects of chlorpyriphos on *Nitrosomonas* spp. and *Nitrobacter* spp. may be attributed to the soluble surfactant which may be present in these two pollutants which may dissolve out lipid component of cell membrane which results in the leakage of cell contents. It has also been reported that increasing concentrations of some chemicals reduced total viable count of marine nitrifying bacteria [14].

5. CONCLUSION

The identification of nitrifying bacteria strains with biological potentials and metabolic capacities to degrade or utilize pesticides and metabolic sources is considered one of the promising approaches to enhancing soil fertility in an ecosystem contaminated with these pollutants. Thus, it is important to examine the response of these organisms

to these pesticides so that less toxic and more readily biodegradable pesticides may be developed especially if the current ones in use are toxic, persistence and thus do not meet regulatory requirements in terms of their pollution effects in our environment.

6. RECOMMENDATIONS

Future investigation on soil chemicals is likely to improve understanding of the interaction between soil and soil microorganisms, and soil contaminated with certain levels of chemicals should not be used for agricultural purposes/practices.

Farmers and other food institute should be encouraged not to depend solely on chemical fertilizers for crops and food production. Also more research should be encouraged on the dangers of pesticide to nitrifying bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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