

ENVIRONMENTAL STUDY TO THE EFFECT OF SOME TYPES OF PESTICIDE ON *RHIZOBIUM SP.* BACTERIA

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ABSTRACT

This study aimed to know the effect of different concentration of alphacypermethrin insecticide on *Rhizobium sp.* bacteria that were isolated from alfalfa root plant. Which is the one of the important bacteria in soil? The results appeared increasing in average numbers of bacteria in concentration 0.0001 and 0.0005 ml/l, in percentage growth number 107 and 109 % compared to control, while decreased in average numbers of bacteria at concentration (0.001-0.05) mL/l in average growth numbers (229, 216, 161, 129) colonies/ml. But the results didn't show significant differentiation in level of probability 0.005 with negative correlation coefficient.

KEY WORDS : *Rhizobium*, Bacteria, Soil, Colony

INTRODUCTION

Insecticides are used excessively in agriculture and public health effective insects, and these pesticides move everywhere in different environments such as soil to settle there. In the soil these pesticides affect the original bacterial flora of the soil.

Bacteria of the nodule roots or *Rhizobium sp.* are genus of gram-negative soil bacteria (Gouda *et al.*, 2017), which are groups of coexistence bacteria with roots of some leguminous plants to fix atmospheric nitrogen in soil by using specific enzyme to fix nitrogen (Kiers *et al.*, 2003), some studies refer to these bacteria as soil biofertilizers (Shefali, 2011). Generally, these bacteria classify depending on the type of legumes that can be formed in its roots, which are usually one strain of *Rhizobium* infected many types of legumes while the specific gene of active enzyme can determine which legumes can be infected, on the another hand there are particular strain can hit species of legumes and formed nodules that are not able to fix nitrogen (Andrews and Andrews, 2017).

Alphacypermethrin which is pyrethroid synthetic insecticides with high activity and most potential toxic pollutants that can affect on wide range of insects. It is insoluble pesticide in water and can

quickly be adsorbed in water-soil systems (Maund *et al.*, 2002). It is an unsystematic pesticide that can work by contact with the target or through digestive or respiratory system of insect (EPA, 2013). It can control on chewing and sucking insects especially that belong to lepidoptera, Hemiptera and coleoptera that can be found on fruits, cereals, beets, potatoes, corns, soya beans, cottons and grapes. Also, it used in combat insects that effect on public health such as flies, cockroaches and mosquitoes and the insects that can be found parasitically on animals (WHO, 2019).

This study aimed to know the effect of alphacypermethrin 5 % insecticides concentrations on *Rhizobium sp.* bacteria, which is a useful bacteria in soil and plant, that can be isolated from alfalfa roots plants.

MATERIALS AND METHODS

Isolation of root nodes bacteria

As per Otieno *et al.* (2017) and Yashita (2013) ten of alfalfa plant have been taken and separated the roots from another parts then washed the roots carefully first with tap water then with distilled water and left to dry, after that the nodes isolated from roots in clean petri dish, 20 nodes have been taken randomly

and transported to a bottle containing ethanol 95% for 5-10 second, then washed by distilled water and dried to transport to sodium hypochlorite 1% for 6 minutes, the nodes washed six times in distilled water until remove all trace of sterilizers. Before crushing the nodes in petri dish, the nodes were dipped in ethanol 95% and inflamed to remove alcohol and ensure perfect sterilization to prepare for crushing on petri dish by sterile forceps tapered head. finally, parts of crushing nodes have been selected to culturing on Yeast extract mannitol broth that has 8 % of potassium nitrate for growth and diagnosis purpose.

Preparation of culture media

The method of Hossain *et al.* (2019) has been followed after diagnosis of bacteriaby using some kind of biochemical tests. After preparation serial dilution of bacteria, 0.1 ml of bacteria have been transported to group of petri dish containing Yeast extract mannitol agar that had been sterilized by autoclave and mixed well with different concentrations of alphacypermethrin insecticides that had been sterilized by filtration. The pour plate method has been used with control sample for comparing the gathering results (Kenasa *et al.*, 2014). The bacteria sample and the media were mixed well and petri dishes left to harden before flipped. Finally, petri dishes were kept at 28 °C for 2-5 days or until the colonies appeared.

Data analysis

A completed randomized design (CRD) has been used. Data analyzed statistically by using less significant differentiation (LSD) at 0.05 after subjecting them to the analysis of variance (Devore *et al.*, 2014).

RESULTS AND DISCUSSION

Figure 1 shows the effect of alpha cypermethrin pesticide (0.0001-0.05) ml/l on *Rhizobium sp.* bacteria. Where the results were variation on average number of *Rhizobium sp.* colonies bacteria growth on culture media, an the results showed increasing in average number of colonies in concentration 0.0001 and 0.0005 ml/l, then showed decreased in average number of colonies in concentration (0.001-0.05) ml/l, also, results did not show a significant differentiation between data, and uncertain negative correlation coefficient, which could mean that the first increasing (Figure 2) in

average number of colonies in concentration 0.0001 and 0.0005 ml/l, in percentage of growth 107 and 109 % respectively, could be attributed to bacterial ability on avoid low concentration of toxic components pesticide and used parts of these components in its activities, Torres-Gutiérrez *et al.* (2017) and Mehana *et al.*, (2002) pointed to ability some type of *Rhizobium sp.* bacteria to transform some types of toxic compounds to useful forms for soil life. Thus low concentration of alphacypermethrin insecticides was used as nutrition to bacteria. Also, Sarnaik *et al.* (2006) pointed to some bacterial soil activities including rhizobial bacteria that can cause break down or decay toxic pesticide components that lead to release some of its components for reproduction or bioactivity purpose. Which could be according to Fig. 2 depending on the structure and concentration of pesticide to response to these compounds. Other study referred to the importance of soil microorganisms to make nutrient metals bioavailability (Sethi *et al.*, 2013) because of these bacteria are flora in soil and nitrogen fixation, for that it can use nitrate compound in pesticide (Fox *et al.*, 2013) but according to the results (Figure 1) is insignificant form for activity or reproduction.

The results in Figure 3 showed decrease in average number of *Rhizobium* colonies bacteria that growth on culture media treated with concentration (0.001-0.005) ml/l of alpha cypermethrin with LC₅₀ (0.15) ml/l in insignificant form according to results. Also, in spite of the data pointed to find negative correlation coefficient but that is not mean increasing in pesticide concentrations can cause decrease in growth of *Rhizobium* bacteria or caused kelling to bacterial cells, which is according to study of Fox *et al.*, (2013). Some kind of pesticide can cause delay or

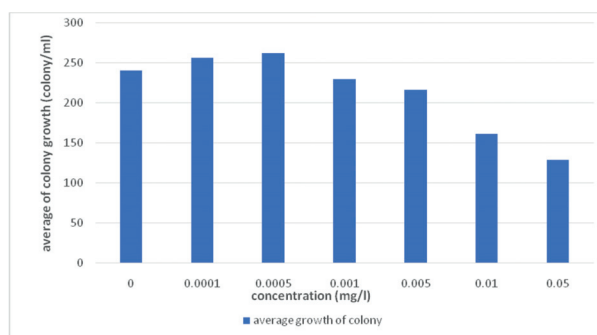


Fig. 1. Showed the average of *Rhizobium sp.* colonies (colony/ml) that growth on yeast extract mannitol agar that treated with different concentration of alpha cypermethrin insecticides.

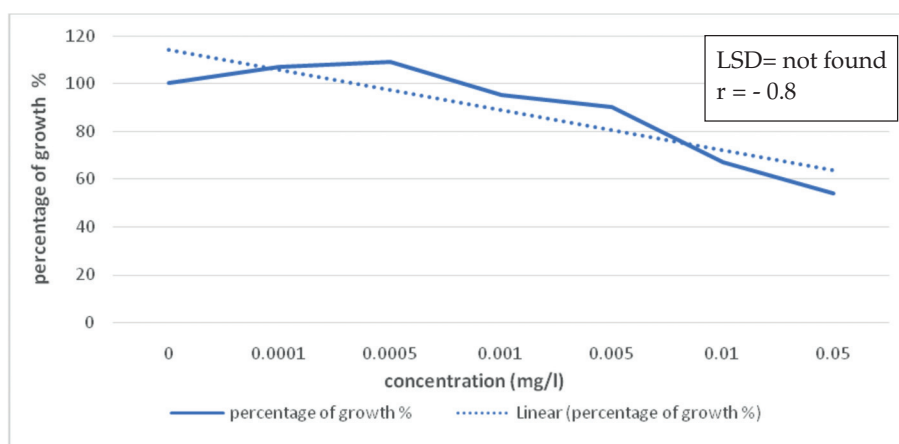


Fig. 2. Showed the percentage growth of *Rhizobium sp.* colonies (colony/mL) compare to control that growth on yeast extract mannitol agar that treated with different concentration of alpha cypermethrin insecticides.

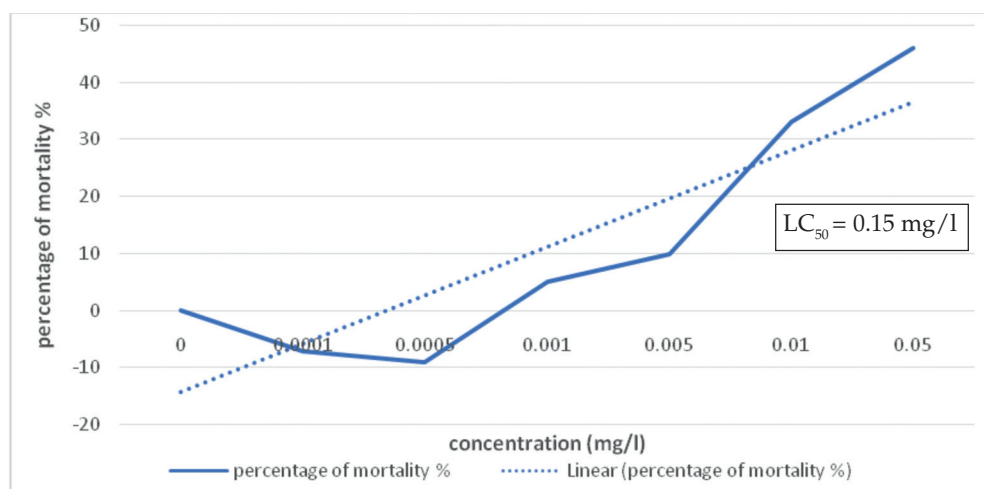


Fig. 3. Showed LC_{50} for *Rhizobium sp.* colonies (colony/ml) after exposure to different concentration of alphacypermethrin insecticides.

reduce in number of radical nodes but that not necessarily means it killed rhizobial nodes bacteria. Other study referred to type of plant that had the *Rhizobium* isolated from which can effects on response of bacteria to pesticides (Ravikumar, 2012; Patriarca *et al.*, 2002), or in some studies depending on type of soil (Martanil *et al.*, 2011). Also, Wilpiseski *et al.* (2019) showed a synergistic effects of soil bacteria on some kind of pesticide to release compounds useful to bacteria growth which can lead to significant increase in bacteria numbers even in high concentration of pesticide. But in the current study there is no synergistic effect of another genus of bacteria in culture media that has been used.

CONCLUSION

1. There is no significant differentiation between

concentration of pesticide and average numbers of *Rhizobium sp.* colonies bacteria

2. In high concentrations of pesticide appeared decrease in average number of *Rhizobium sp.* bacteria colonies
3. In low concentrations of pesticide showed increase in average numbers of *Rhizobium sp.* bacteria colonies

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