



COMPARATIVE STUDY TO DETERMINED THE EFFECT OF

DIAZINON AND VAPONA ON *Pseudomonas aeruginosa*

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ABTRACT

These study included the determination the effect of insectide Diazinon on *P.aeruginosa* isolated from the rizosphere of *Vicia faba* in the fields of Al-Nasiriya city at the range of 0.2 , 0.4, 0.6 ppm concentrations , Where the 0.6 ppm concentration represented to the initial concentration in the fields , and with Vapona (DDVP) in 0.1 , 0.3 , 0.5 ppm concentrations , where the 0.5 ppm concentration represented to the initial concentration in the fields . The results showed that the numbers of *P.aeruginosa* reached to 5.8×10^7 in control , but the numbers decreased to 3.3×10^7 with Diazinon in 0.2 ppm and the numbers increased to 3.7×10^8 , 1.1×10^8 in 0.4 , 0.6 ppm respectively . The numbers of *P.aeruginosa* reached to 5.8×10^7 with Vapona in control , but the numbers decreased to 6.4×10^6 in 0.3 ppm , and the same time the numbers increased to 1.2×10^8 in 0.1 , 0.5ppm respectively. The results showed that *P.aeruginosa* are able to convert these insecticides to other compounds in the laboratory

Keywords: Bacteria, Diazinon, Insecticides, Rizosphere, Soil, Transformation, Vapona

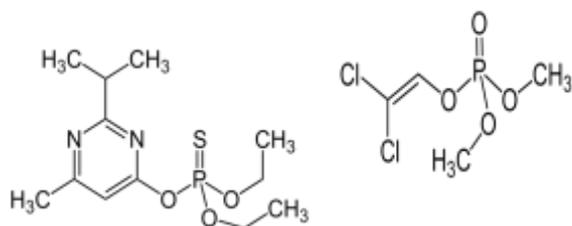
INTRODUCTION

Millions of tons of pesticides are remainder being deposited on the soil applied annually, however less than and nontarget organisms , as well as 5% of these products are estimated to moving into the atmosphere and reach the target organisms , with the water [1]. The metabolic fate of

pesticides is dependent on abiotic environmental conditions (temperature , moisture , soil pH , etc.), microbial community or plant species (or both) , and biological and chemical reactions . Abiotic degradation is due to chemical and physical transformations of the pesticide by processes such as photolysis , hydrolysis , oxidation , reduction , and rearrangements . Further , pesticides may be biologically unavailable because of compartmentalization , which occurs as a result of pesticide adsorption to soil and soil colloids without altering the chemical structure of the original molecule . However , enzymatic transformation , which is mainly the result of biotic processes mediated by plants and microorganisms , is by far the major route of detoxification Diazinon(O,O-diethyl- O - (2 - isopropyl - 4 - methyl - 6 - pyrimidinyl phosphorothionate) **Figure 1** ,and this insecticide is a commonly used thionophosphorus organophosphate (OP) pesticide to control a variety of insects in agriculture and household environment [2]. Despite its low persistence in the environment , it is a non-specific insecticide and highly toxic to animals and humans .

Moreover , the toxicity of OPS is increased by their break- down products , which may be bioactivated within an organism or through exposure to the sun light . The pathway of degradation is assumed to be substitution of sulfur by oxygen in P = S bond , cleavage of the pyrimidine ester bond , and the oxidation of isopropyl group [3],[4]. Diazinon undergoes fast hydrolysis at acidic and basic conditions [5],[6] ,in environment , is unstable under UV irradiation [7],and can be transformed to the more toxic diazoxon due to the enzymatic reaction in birds , fish , insects and mammals [6]. Vapona (DDVP) (2,2- dichlorovinyl dimethyl phosphate) is organophosphate **Figure 1** ,and this insecticide is widely used as an insecticide to control household pests , and protecting stored product from insect and it is effective against flies , aphids , spider , mites , thrips, and whiteflies in greenhouse , outdoor fruit , and vegetable crops. The determined efficiency of biological characteristic to any pesticide and indications of side effects were input in safe side during used. The present study investigated the environmental effects of insecticides Diazinon and Vapona

in soil bacteria *P.aeruginosa* because these bacteria play an important role in environmental balance.



Diazinon Vapona

Figure 1: Molecular structure of Diazinon and Vapona.

MATERIAL AND METHODS

Organisms and culture conditions

P.aeruginosa was isolated from the rhizosphere of *Vicia faba* in Al-Nasiriya fields, south of Iraq by using serial dilutions on cetrinide agar [8]. Stock cultures were maintained on the nutrient agar subcultured periodically and stored at 4°C. Mineral salts medium containing (g l^{-1}): K_2HPO_4 , 1.71; KH_2PO_4 , 1.32; NaNO_3 , 0.42; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.42; CaCl_2 , 0.02 was used for the incubation experiments. All media were autoclaved at 120°C for 20 min.

All chemicals used in the present study produced by (BDH) company. Insecticide Diazinon and Vapona with purity (98%) were from Pestanal (Germany).

Study of effect Diazinon and Vapona on *P.aeruginosa* in mineral salts medium

2 mL from mineral salts medium was added to sterilized test tubes and transfer *P.aeruginosa* from growth on nutrient agar by one loopful. The test tubes were incubated at 37°C in an incubator for 48 h, and in similar conditions the test tubes incubated with 8-12 h. After this period, the bacterial inoculated were transferred to 250 mL conical flasks and incubated in 35°C for 12-18 h even the growth reached to logarithmic phase, and these experiments were duplicate. 50 mL from bacterial culture were transferred with a sterilized method to 250 mL conical flasks and added insecticide Diazinon with 0.2, 0.4, 0.6 ppm respectively, fourth conical flask was used as control (without added Diazinon), and repeated incubation with similar method. After incubation, serial dilution was made by using test tubes containing 9 mL distilled water with 1% pepton, and these experiments were duplicate. 1 mL from all dilutions were inoculated with pour plate count and calculated bacterial numbers after 24 h from incubation in 35°C and compared with other dishes (without treatment), and these experiments were duplicate. The similar method was carried with insecticide Vapona in 0.1, 0.3,

0.5 ppm .

Study of metabolites of Diazinon and Vapona by *P.aeruginosa*

200 mL from mineral salts medium was divided and added 50 mL to fourth conical flasks. Mineral salts medium was sterilized by autoclaved and added Diazinon with 0.6 ppm after decreased the temperature of mineral salts medium to 45°C. In these method Diazinon was used as the sole source of carbon , phosphorus and energy, all flasks were inoculated with one loopful from culture of *P.aeruginosa* with age 48h. The control flasks were not inoculated (remain only Diazinon), Diazinon sterilized by using Milipore filter paper 0.45µ [9]. All flasks were incubated in 28 c° to 48h . After finishing the incubation period (24h), the containing in all flasks were filtered alone by Milipore filter paper 0.45µ , and the filtered was collected in 250 mL conical flask . 1mL from all treatments were transferred to 5mL sterilized vials . The determine of the residue of Diazinon was carried by added 2mL from extraction solution (prepaire by mixing 1:2 Hexane and Chloroform) to all vials and strongly mixing [10] . The upper layer (solvent layer) was transfer to 5mL glasses

vial and stored under (-18c°) even analysis and to determine insecticide levels . The similar method was carried with insecticide Vapona with 0.5ppm. The degraded these insecticides were characterized by Infrared spectroscopy (PYE Unicam

Statistical analysis

The present study conducted an Anova (analysis of variance) with two factors which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether or not, a significance difference.

RESULTS

The results showed that the numbers of *P.aeruginosa* in 1mL of mineral salts medium with Diazinon were decreased to 3.3×10^7 CFU gm / soil in 0.2 ppm when compared with control (5.8×10^7 CFU gm / soil) **Fig .2** , but the numbers increased to 3.7×10^8 , 1.1×10^8 CFU gm / soil in 0.4 , 0.6 ppm respectively. The statistical methods showed no significance were recorded between bacteria and concentrations of Diazinon . The increased of bacterial numbers in 0.4 , 0.6 ppm concentration refer to that Diazinon was activated these bacteria and used this insecticide as the sole source of carbon , phosphorous and

energy. [11] refer that 29 strain from *P.aeruginosa* was ability to consumed 76 – 82 compounds from 146 experiments compounds, and these bacteria depend on oxidation system and these bacteria contain a necessary amidase enzyme to consumed organic substances. **Figure 3** showed that the numbers of *P.aeruginosa* in the 1mL from mineral salts medium with Vapona reached to 5.8×10^7 CFU in control, but the numbers of *P.aeruginosa* were decreased to 6.4×10^6 CFU in 0.3 ppm concentration, although the numbers of these bacteria were increased to 1.2×10^8 CFU in 0.1, 0.5 ppm concentrations. The statistical methods showed no significance were recorded between these bacteria and concentrations of Vapona, and in the same time no significance was recorded between these bacteria and with two insecticides.

Figure 4 (A) show transformed of Diazinon by *P.aeruginosa* in mineral salts medium by using Infrared

spectroscopy (IR). This figure showed that converted of Diazinon when compared with standard of Diazinon, **Fig.4(B)**. In the same time **Fig.5 (A)** show transformed of Vapona by *P.aeruginosa* by using Infrared spectroscopy (IR), and this figure showed that converted of Vapona when compared with standard of Vapona, **Fig.5(B)**. From these results obtained that these bacteria has ability to transformed these insecticides and consumed as a sole source of carbon, phosphorus and energy. The similar observation reported around the ability of *P.aeruginosa* to consumed some phosphorus insecticides to a sole source of carbon, phosphorous and energy in laboratory, so as Disyston [12], Malathion [13], Parathion [14], Diazinon [15], Azordin, Aspon, Dasanit, Orthene, Trithion, Dimethoate, Dylox, Methyl parathion, Vapona [16].

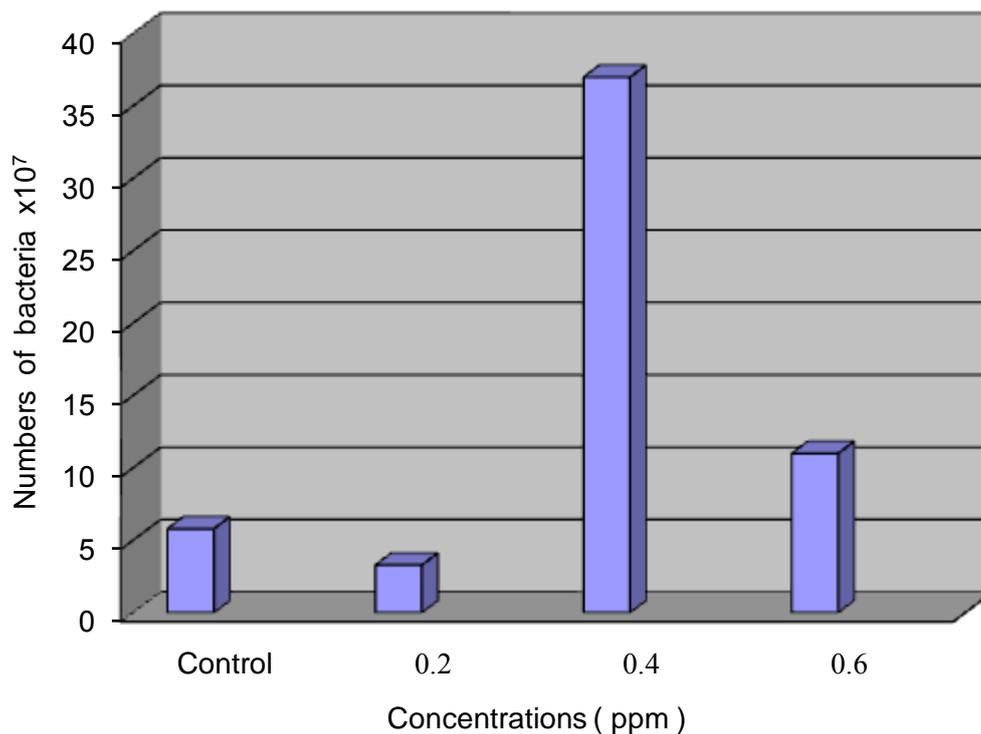


Figure 2: Effect of Diazinon in numbers of *P.aeruginosa* / mL after (12 – 18 h) from treatment

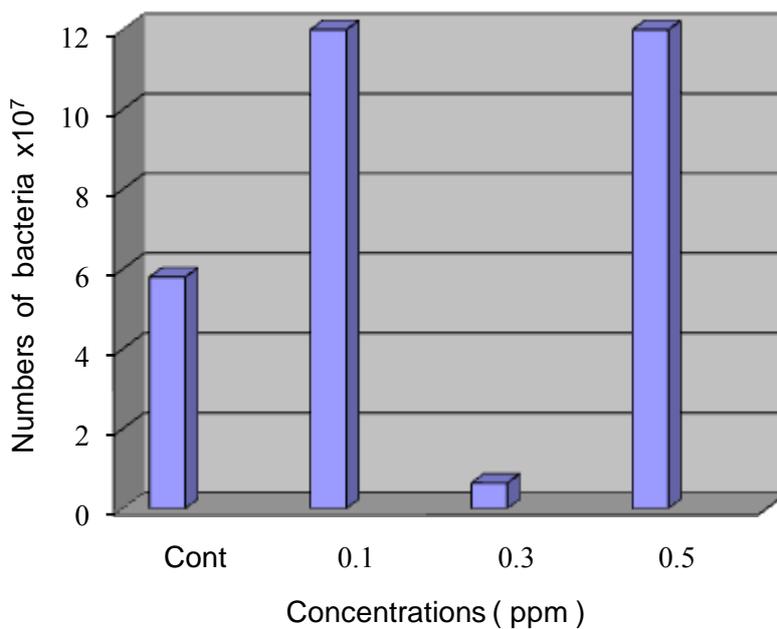
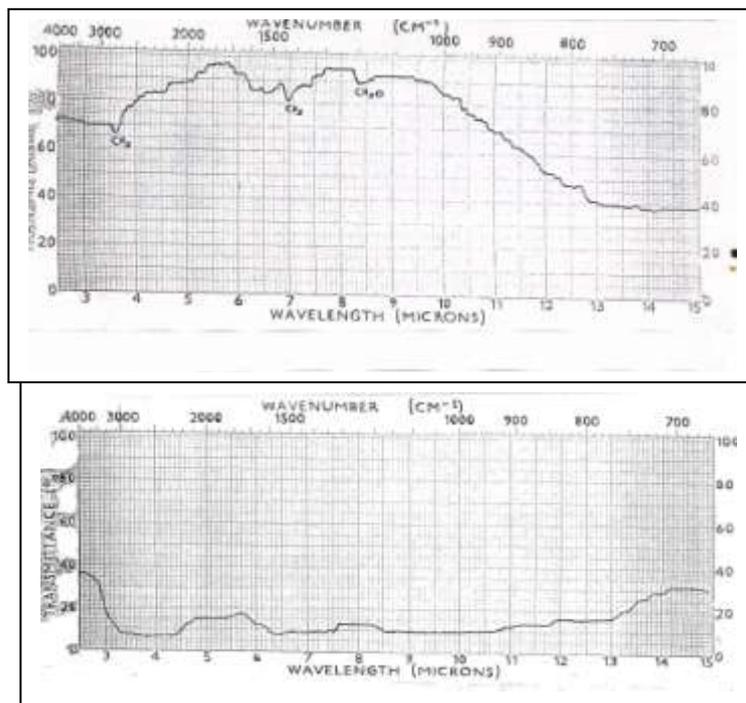
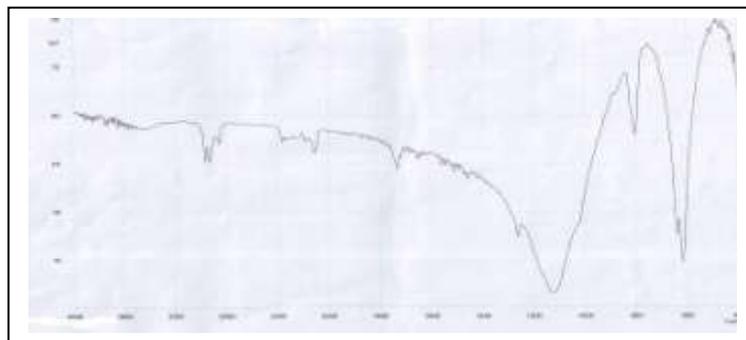
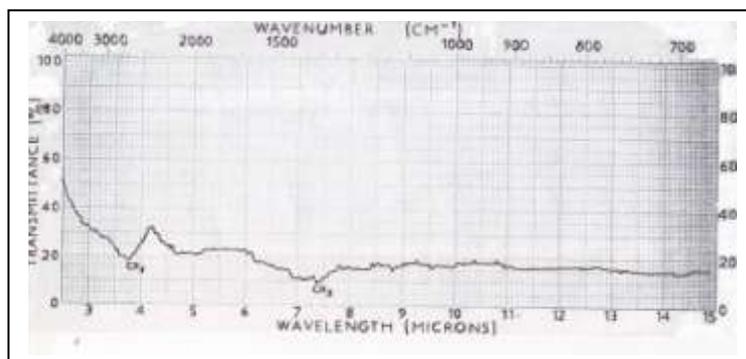


Figure 3: Effect of Vapona in numbers of *P.aeruginosa* / mL after (12 – 18 h) from treatment



B

Fig.4 Infrared spectrum showed : A : Transformation of Diazinon by *P.aeuginosa* in mineralal salts medium , B : Standard of Diazinon .



B

Fig.5 Infrared spectrum showed : A : Transformation of Vapona by *P.aeuginosa* in mineralal salts medium , B : Standard of Vapona .

DISCUSSION

[17] found that parathion transformed to other compounds because activated to hydrolysis enzymes in non pure bacterial cultures, and in the same time [17] found these enzymes transformed eight pesticides. [18] reported that the pure enzyme extracted from *Pseudomonas* on iso propyl - N - (3 - chlorophenyl) carbamate medium can hydrolysed many groups that similar to phenyl carbamates groups and although two acylanilide herbicide. [16] found that the pure enzyme extracted from cultures of *Pseudomonas* can hydrolysis pesticide contained phosphorous by cleavage aryl P - O bound. [19] explain that the first stage from metabolism of aromatic compounds were converted or breakdown groups attached on benzene ring and reduction to aliphatic chain with produced compounds reducing only carbon or two carbon atoms, and in the same time [20] found that *Bacillus subtilis* isolated from water polluted with Parathion was reduction this insecticide to amino Parathion [20] refer that *B.subtilis* reduced active of Sumathion (Fenitrothion) in culture medium and breakdown 93%

from this insecticide (20 ppm) after fourth days. [21] refer that *B.subtilis* breakdown Sumathion in soil, and in the same time [22] isolated high numbers of microorganisms from soil and to ability to biodegradation of DDT so as three species of *Bacillus* and one species of *Micrococcus* and all these microorganisms can transformed DDT to DDD and breakdown Andrin in soil. [23] found that some species cameback to *Pseudomonas* can hydrolysis Parathion in soil. [24] found that *P.aeruginosa*, *Klebsiella* sp. can transformed herbicide (Propanil) to DCA (3,4 - dichloroaniline) in laboratory, and in the same time [25] refer that the persistence of Diazinon and Parathion were long period under laboratory conditions, but the biodegradation of these insecticides were fast under field conditions, and these results explained that the pesticide in field was exposure to evaporation, leaching, photodegradation and the microbial degradation of Diazinon was studied by [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [36].

CONCLUSION

The data obtained in the present study investigation advance our knowledge of Diazinon and Vapona resistance in *P.aeruginosa* isolated from Iraqi soils and may make this promising candidates for further investigations regarding their ability to remove these insecticides from contaminated environments.

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