



**EFFECT OF INSECTICIDE OXAMYL (VYDATE) ON *Escherchia coli* in
LABORATORY**

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ABSTRACT

This study included the determination of the effect of the insecticide (Oxamyl) on *Escherchia coli* isolated from the rizosphere of *Vicia faba* in the fields of Al-Nasiriya district at the range of 1.0,2.0,5.0 ppm concentration where the 5.0 ppm concentration represented to the initial concentration in the fields .

The results showed that the numbers of *E.coli* reached to 11.3×10^8 in control, but the numbers decreased to 1.8×10^7 , 5.5×10^7 , 5.6×10^7 with Oxamyl in 5.0 , 1.0 , 2.0 ppm .

The result showed that *E.coli* are not able to convert this insecticide to other compounds in the laboratory.

Keywords: Bacteria, Oxamyl, Insecticides, Rhizosphere, Soil ,Transformation

INTRODUCTION

Millions of tons of pesticides are applied annually, however less than 5% of these products are estimated to reach the target organisms , with the remainder being deposited on the soil and non target organisms , as well as moving into the atmosphere and 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 aresult 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.

The insecticide Oxamyl was low persistence in the environment and its highly toxic to human and animals, Oxamyl (Methyl 2-(dimethylamino)-N-[(methylcarbamoyl)oxy]-2-

oxoethanimidothioate **Figure.1**, Moreover, the toxicity of Oxamyl is increased by their breakdown to other products [2], and this insecticide is effected on biological processes during an important enzymes so as ATPase [3]. The determined of 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 insecticide Oxamyl in soil bacteria *E. coli* because this bacteria play an important role in environmental balance.

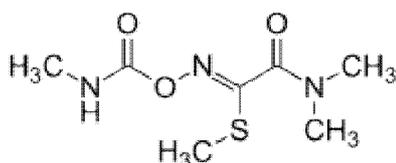


Figure.1 Structure of Oxamyl

MATERIAL AND METHODS

Organisms and culture condition

E. coli was isolated from the rhizosphere of *Vicia faba* in AI-Nasiriya fields,

south of Iraq by using serial dilutions on Mackonky agar [4]. Stock cultures were maintained on the Nutrient agar subcultured periodically and stored at 4°C. Mineral salts medium containing (g l⁻¹): K₂HPO₄, 1.71; KH₂PO₄, 1.32; NaNO₃, 0.42; MgSO₄·7H₂O, 0.42; CaCl₂, 0.02 was used for the incubation experiments. All media were autoclaved at 120°C for 20 min.

Chemicals

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

Study of effect Oxamyl on *E. coli* in mineral salts medium

2 ml from mineral salts medium was added to sterilized test tubes and transfer *E. coli* from growth on nutrient agar by one loopful. The test tubes were incubated at 37°C in an incubator to 48h, and in the similar conditions the test tubes incubated with 8-12h. After these period, the bacterial inoculated were transfer to 250 ml conical flasks containing 100ml mineral salt medium and incubated in 35°C to 12-18h even the growth reached to logarithmic phase, and these experiments were duplicate, 50 ml from bacterial cultured were transferred with sterilized method to 250 ml conical flasks and added insecticide

Oxamyl with 1.0,2.0,5.0 ppm respectively , fourth conical flask were used in control (without added Oxamyl), and repeated incubation with similar method. After incubation , serial dilution was made by using test tubes containing 9 mL distilled water with 1% peptone, and these experiments were duplicate . 1 mL from the last dilution(10^6),and were inoculated with pour plate count and calculated bacterial numbers after 24h from incubation in 35° and compared with other dishes (without treatment) , and these experiments were duplicate .

Study of metabolites of Oxamyl by

E.coli

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 Oxamyl with 5.0 ppm after decreased the temperature of mineral salts medium to 45° .

In these method Oxamyl was used as asole source of carbon , sulphure and energy , all flasks were inoculated with one loopful from culture of *E.coli* with age 48h . The control flasks were not inoculated (remain only Oxamyl) , Oxamyl sterilized by using Milipore filter paper 0.45μ [5]. All flasks were incubated in 28° 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 Oxamyl was carried by added 2mL from extraction solution (prepare by mixing 1:2 Hexane and Chloroform) to all vials and strongly mixing [6] . The upper layer (solvent layer) was transfer to 5mL glasses vial and stored under (-18°) even analysis and to determine insecticide levels . The degraded these insecticide were characterized by Infrared spectroscopy (PYE Unicam SP300 – England) IR .

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 *E.coli* in 1mL of mineral salts medium with Oxamyl reached to 11.3×10^8 CFU gm / soil in control ,but the numbers were decreased to 1.8×10^7 , 5.5×10^7 , 5.6×10^7 CFU gm / soil with 5.0,1.0,2.0ppm respectively **Figure 2**. The statistical methods showed no significance were

recorded between bacteria and concentrations of Oxamyl. The decreased of bacterial numbers in different concentrations refer to that this bacteria was an able to utilize Oxamyl to asource of carbon,sulfur and energy. [7] refer that carbamate pesticide(Methomyl) was killed *E.coli* ,but [8] found that *E.coli* can utilized Coumaphos after transfer the plasmied contain the biodegradation gene ,

and in the same time *E.coli* was not ability to degraded Propanil to other compounds [9].

Figure 3 shows that *E.coli* was not ability to transformed Oxamyl to other compounds in mineral salts medium by using (IR) , and this figure don't show any converted to the structure of Oxamyl when compared with standard Oxamyl **Figure 4** .

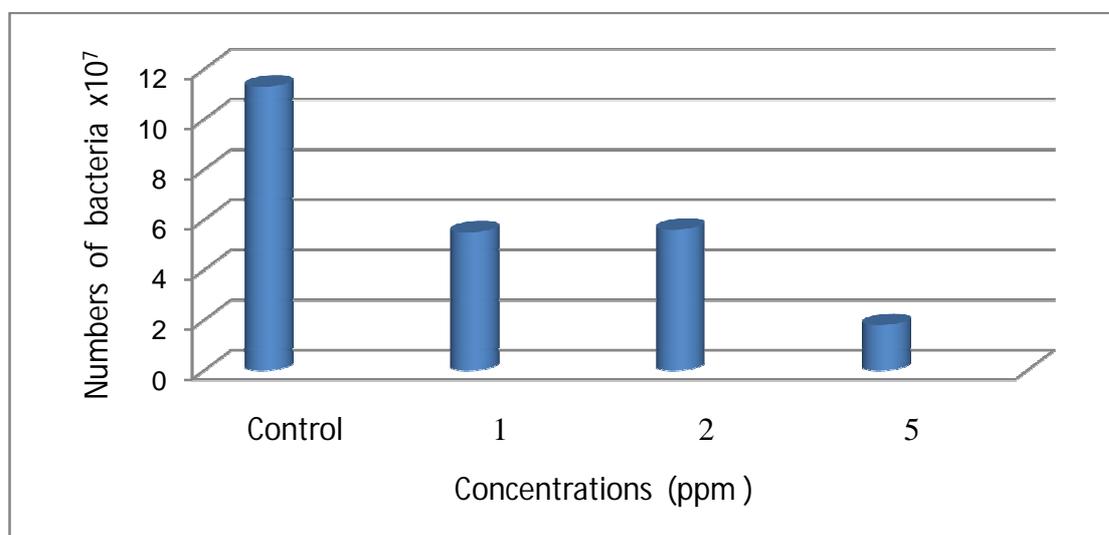


Figure 2: Effect of Oxamyl in numbers of *E.coli* / mI after incubation (12 – 18 h)

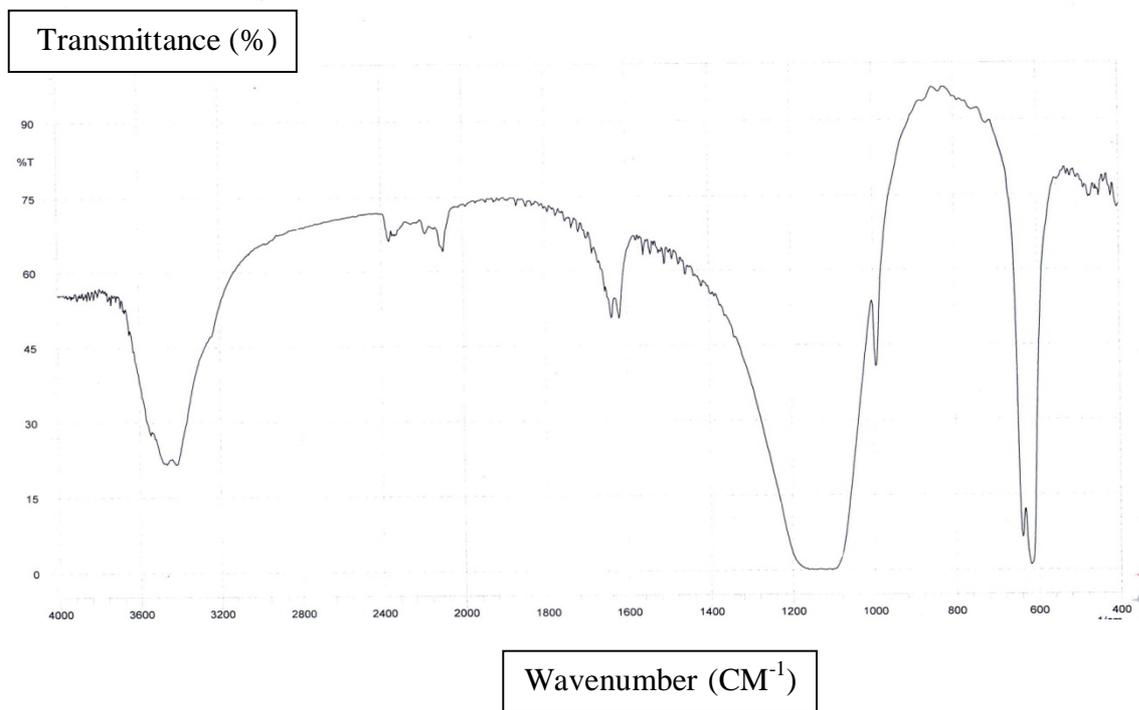


Figure 3: Infrared spectrum showed: Transformation of Oxamyl by *E.coli* in mineral salts medium

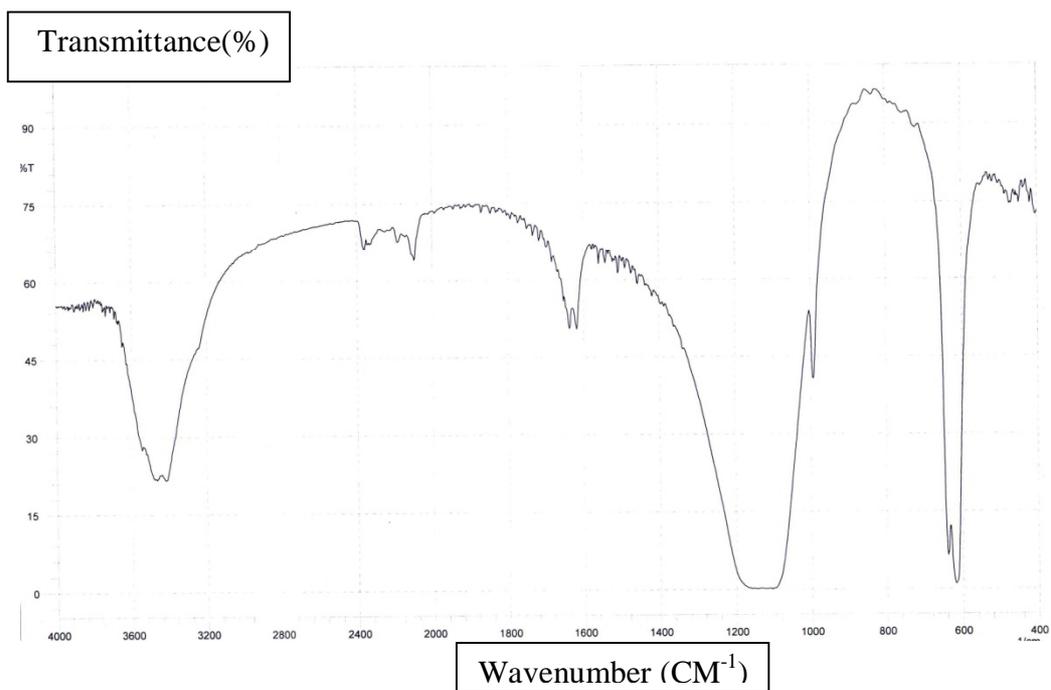


Figure 4: Infrared spectrum showed Standard Oxamyl

DISCUSSION

[10] found that parathion transformed to other compounds because activated to hydrolysis enzymes in non pure bacterial cultures, and in the same time [10] found these enzymes transformed eight pesticides. And in the same time [11] 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 .

[12] found that the pure enzyme extracted from cultures of *Pseudomonas* can hydrolysis pesticide contained phosphorous by cleavage aryl P -O bound . [13] 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 [14] found that *Bacillus subtilis* isolated from water polluted with Parathion was reduction this insecticide to amino Parathion. .

And [14] obtained that *B. subtilis* was reduced activation of Sumathion (Fenitrothion) in culture medium and

breakdown 93% from this insecticide (20 ppm) after fourth days .

But [15] determined that *B. subtilis* was breakdown Sumathion in a soil , and in the same time [16] isolated high numbers of microorganisms from a soil and that the 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 .

But [17] obtained that some species to genus *Pseudomonas* can hydrolysis Parathion in soil . And in the same time [9] found that *P. aeruginosa* , *Klebsiella* sp. can transformed herbicide (Propanil) to DCA (3,4 - dichloroaniline) in laboratory , but [18] 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 , photo degradation . In the same time [19] obtained that Oxamyl was remained in a very low quantities during four days after spray , and [20] explained that Oxamyl don't accumulated but speed degraded in 7day by aerobic and anaerobic bacteria.

CONCLUSION

The data obtained in the present study investigation advance our knowledge of Oxamyl inhibited in *E.coli* isolated from Iraqi soils and may make other researches about the toxicity of this insecticide in others non target organisms.

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