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# Impact of Azotobacter in Different Doses of Dichlorvos

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***Abstract:*** ***Azotobacter is free living, non–symbiotic, heterotrophic bacteria capable of fixing an average of 20kg N / hector year. Azotobacter species are Gram negative, free–living, aerobic soil dwelling, oval or spherical bacteria that form thick–walled cysts (means of asexual reproduction under favorable condition). Out of six species of Azotobacter, some of them are motile by means of peritrichous flagella while others are not. They are typically polymorphic and their size ranges from 2–10µm long and 1–2µm wide. A. chroococcum is the first aerobic free– living nitrogen fixer. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. Dichlorvos is an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored products from insects. In present study of dichlorvos in Azotobacter through different doses are effected of seed germination and observed the toxicity and harmful effect of dichlorvos solution***
***Keywords: Azotobacter, Dichlorvos, Biofertilizer, Mesophil.***

## I. INTRODUCTION

Nitrogen is the component of protein and nucleic acids and chlorophyll. Thus, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in crop. The atmosphere comprises of 78% nitrogen as an inert, in unavailable form. These nitrogen– fixers, life on this planet may be difficult.

Nitrogen (N) deficiency is frequently a major limiting factor for crops production.18 Nitrogen is an essential plant nutrient, widely applied as N–fertilizer to improve yield of agriculturally important crops. An interesting alternative to avoid or reduce the use of N–fertilizers could be the exploitation of Plant Growth–Promoting Bacteria (PGPB) capable of enhancing growth and yield of many plant species, several of agronomic and ecological significance. *Azatobacter* species are non–symbiotic heterotrophic bacteria capable of fixing an average 20kg N/hec./per year. Bacterization helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism. Seed inoculation *Azotobacter* and nutrient uptake seed inoculated with *Azotobacter* helps in uptake of N, P along with micronutrients like Fe and Zn, in wheat, these strains can potentially be used to improve wheat nutrition.

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate, commonly abbreviated as an DDVP[1]) is an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored products from insects. The compound has been commercially available since 1961 and has become controversial because of its prevalence in urban waterways and the fact that its toxicity extends well beyond insects.[2] The insecticide has been banned in EU since 1998.[3]

*Azotobacter* genus was discovered in 1901 by Dutch microbiologist and botanist Beijerinck et al. *Azotobacter* is a genus of free-living diazotrophic bacteria which have the highest metabolic rate compared to any other microorganisms. *Azotobacters* have generated a good deal of interest in the scientific community because of their unique mode of metabolism, by which they can fix nitrogen aerobically. *Azotobacter* Agar (Mannitol) is used for isolation and cultivation of mannitol positive *Azotobacter* species from soil. It is also useful for maintenance of *Azotobacter* species by adding extra 1% Mannitol to the medium as specified by the American Type Culture Collection. Out of six species of *Azotobacter*, some of them are motile by means of peritrichous flagella while others are not. They are typically polymorphic and their size ranges from 2–10µm long and 1–2µm wide. The *A. chroococcum* is the first aerobic free– living nitrogen fixer.

These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of *Azotobacter* cells thereby contributing towards the nitrogen availability of the crop plants. *Azotobacter* species is sensitive to acidic pH, high salts, and temperature. *Azotobacter* has beneficial effects on crop growth and yield through, biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phyto-pathogenic inhibitors. The present studies aim to evaluate the impact of different doses of dichlorvos on *Azotobacter* species.

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## II. MATERIAL AND METHOD

*1) Required Media: Azotobacter* agar (Ashby's agar media)

Plating and streaking method are use for isolation and incubated at $37^{O}$C temperature. Growth was observed and recorded after 3 days of incubation. A white colony was observed in different plats.

*2) Dichlorvos Dilution*

*a)* 1mg/20ml. distilled water
*b)* 3mg/20ml. distilled water and
*c)* 5mg/20ml.distilled water.

*3) Seed Germination Test and Use of Dichlorvos*

*a)* First set of 20 seed was dipped in *Azotobacter* broth media and add first dose of dichlorvos solution.
*b)* Second set of 20 seed was dipped in *Azotobacter* broth media and add second dose of dichlorvos solution.
*c)* Third set of 20 seed was dipped in *Azotobacter* broth media and add third dose of dichlorvos solution.

*4) Gram Staining of Azotobacter: Azotobacter* species are Gram-negative bacteria found in Neutral and alkaline soil.

*a) Azotobacter Reacts with IMVIC Test:* Indol test, Methyl red test, Voges-proskauer test and Citrate utilizing test.

## III. RESULTS AND DISCUSSION

*Azotobacter chroococcum* is isolated from the rhizospheric soil of rice plant. It is gram negative, motile, rod shaped bacteria. It shows positive result of IMVIC test and negative test of gram staining. In the experiment of different doses of dichlorvos solution of same incubation period and temperature, in first set of seed root germination was observe in 3 inch, in second set of germinated seed in second dose of 2.25 inch and third set of germination of third dose of 0.50 inch root. Now we conclude that the effect of dichlorvos is toxic shows on *Azotobacter* species.

Table – 1. (Biochemical characteristics)

| Characters | Results |
|---|---|
| Gram's reaction | - |
| Indol production | - |
| Methylered | + |
| Voges-proskauer | + |
| Citrate utilizing | + |

Table - 2. (Growth in different doses of dichlorvos)

| Doses | Growth of Azotobacter |
|---|---|
| 1mg/20ml dl | 3 inch |
| 3mg/20ml dl | 2.25 inch |
| 5mg/20ml dl | 0.50 inch |

Photo Plate

Culture plate Pure culture plate Gram staining



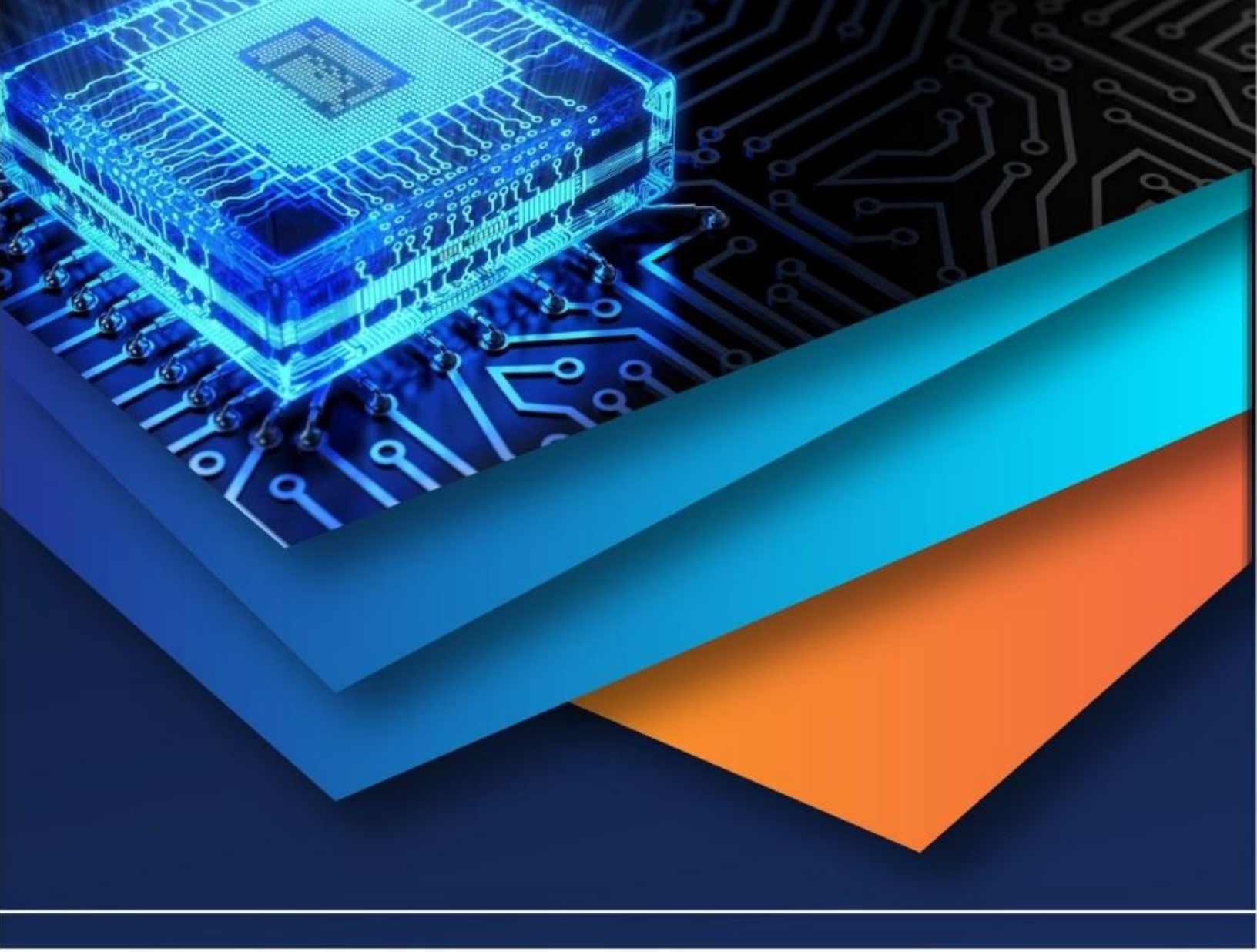
Dichlorvos, an organophosphate, is a predominant pesticide used in domestic insect control in developing countries. Acute and prolonged exposure may lead to death, genotoxic, neurological, reproductive, carcinogenic, immunological, hepatic, renal, respiratory, metabolic, dermal and other systemic effects (Henshaw and Iwara, 2018). Its toxicity is due to the ability of the compound to inhibit acetyl cholinesterase at cholinergic junction of the nervous system. Celik et. al. (2009) also concludes that the sub acute dichlorvos induced the level of damage marker enzymes and leucocytosis. Gillett et. al. (1972), Lu et.al. (1997); Pancetti et. al. (2007); Das (2013) and Shrivastava et. al. (2015) also studied effect of different dosages of dichlorvos.

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