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Isolation, Identification and Characterization of THIRAM Degrading Organisms from Soil of North Gujarat, India

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Abstract: ***Pesticides are often applied directly to soil. They may also reach the soil through foliage via drift spray, run-off, or wash of vectors (Racke et al.,1990). Thiram is widely used as an agricultural fungicide, used as seed protectant, animal repellant and contaminates the environment as a degradation product of widely used pesticides. These pesticides degrade to various toxic products. Thiram is also used in several medicated soaps and antiseptic sprays as ingredients. Thiram generates Thyroid toxin and carcinogenic in nature (Dalvi et al.,1986). Thiram is very highly toxic to both cold water and warm water fish (EPA, 1984). Thiram is moderately toxic to birds. In mammals subcutaneously toxicity is very high. Several microorganisms have capacity to degrade the chemicals under the certain condition. Some of pesticides seen resistant to microbial degradation, so they remain as such in environment were applied. This results in pest control failure (Racke et al.,1990).***

Keywords: soil of Himmatnagar,(A), Soil of Talod (B), Soil of Modasa (C), Soil of Vijapur (D), Soil of Visnagar (E), Soil of Tarabh (F), Soil of Nedara (G), Soil of Chanasma (H), Soil of Adiya (I).

I. INTRODUCTION

Farmers of North Gujarat widely used pesticides for their crops. Increasing awareness has resulted in regulatory measures that aim to remedy past mistakes and protect the environment from future contamination and exploitation. Pesticides are one of the source of water and soil pollution, so pesticide use reduces biodiversity and contributes to pollinator decline (Hackenberg, 2007)(Heafker, 2000), (Zeissloff, 2001). Pesticide destroys habitats especially for birds (Palmer, 2007). Pest has capacity to develop resistance towards pesticide, so there is need for new pesticide or higher dose of pesticide.

II. MATERIALS AND METHOD

A. Study Area and Data Collection

The study area was a three districts of North Gujarat i.e. Sabarkantha, Mahesana and Patan It was selected on the basis of the feasibility of available past data of Crops, yield and Pesticides. The Data collection was carried out by personal survey of farmers. Soil samples were taken vegetable farms of North Gujarat. This is Single season analysis and soil samples are taken after the application of pesticide. Aseptic technique and proper care is taken to prevent contamination and error.

B. Soil analysis, Crops policies and Yield

Soil analysis of the single season analysis was carried out in our Laboratory. It includes Physical and chemical parameters. e.g. organic Carbon , Inorganic Nitrogen, Sulfur, Chloride, Calcium carbonate, Total Hardness (Mg+ & Ca+).

C. Isolation, Identification and Characterization of Bacteria

Mineral salt medium (MSM) is used to isolate the bacteria. 1gm of soil is mixed with 10 ml of D/W to make suspension sample. This is serially diluted. Pesticide degrading bacteria were isolated using MSM agar plates with different concentration of Thiram.

D. *Degradation study of Thiram*

Extraction of pesticide from sample Inoculum is prepared in luria broth by inoculum of bacteria and incubated on rotary shaker at 120 rpm, Agriculture wiki in Gujarati was created using 28ºC temp, for 4-7 Days. 10 ml of inoculum and 90 ml of MSM which contain 50,100,150,200 PPM thiram pesticide. This is incubated at 28ºC at 120 rpm and after 48Hrs 10 ml broth is taken as sample this is homogenized. To this 3-4 drop of concentrated HCL is added and mixed well. Equal volume (10ml) Hexane ethyl acetate

mixture (20:80)is added and subject to vortex for 5min, then centrifuge at 2000rpm for 25 minutes. The organic phase is removed and kept at 4º C and immediately subjected to remove moisture from it. Sample is analyzed through HPTLC and UV-Visible Spectroscopy.

E. UV-visible Spectroscopic Analysis

The sample is diluted two times and scanned using range of 430nm wavelength (Nurretin and A.usame, 1999). Elico-164UV-visible Spectrophotometer.

F. High Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis of extracted pesticide was carried out as method described by (wei Fan *et al*.,2007). Before loading the sample HPTLC plates were activated for 1 Hr. 10µg of samples were loaded along with control. Plates were placed in HPTLC jar containing in standardize solvent system(hexane: Acetone 5:5).

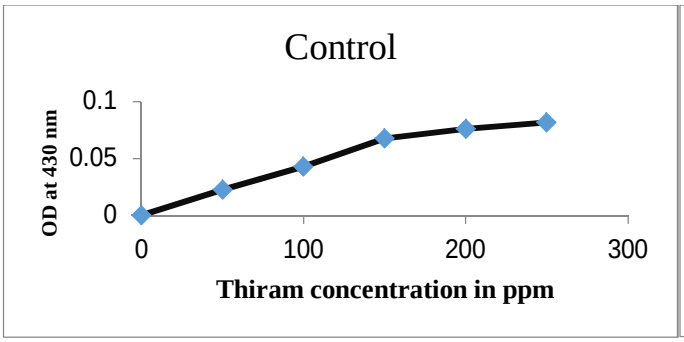

Figure 1 : Control.

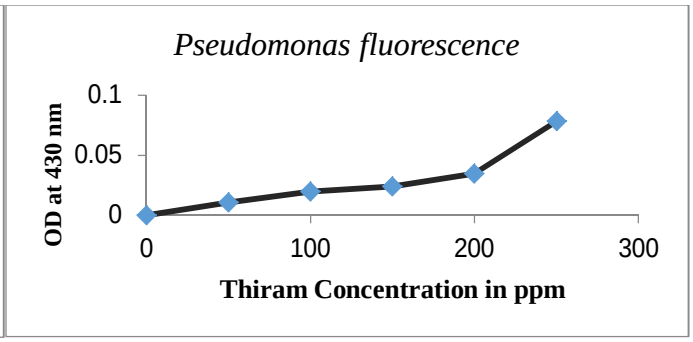

Figure 2: Thiram degradation by P. fluorescence.

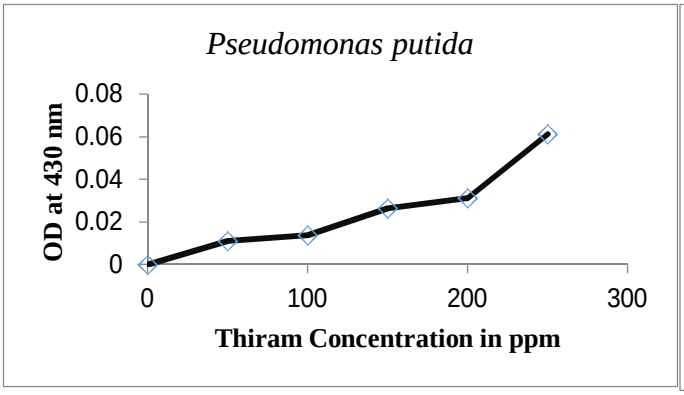

Figure 3: Thiram degradation by P.putida

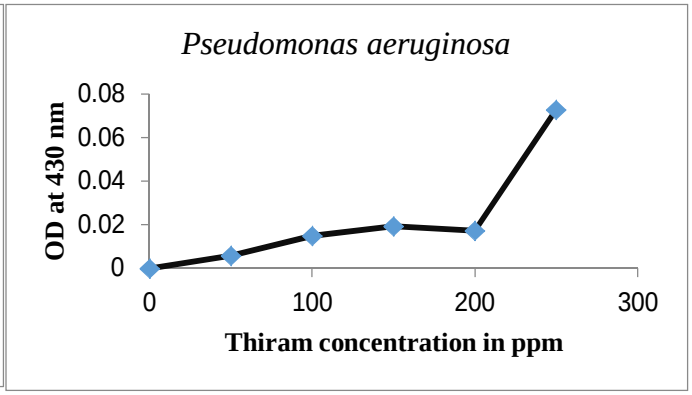

Figure 4: Thiram degradation by P.aeruginosa

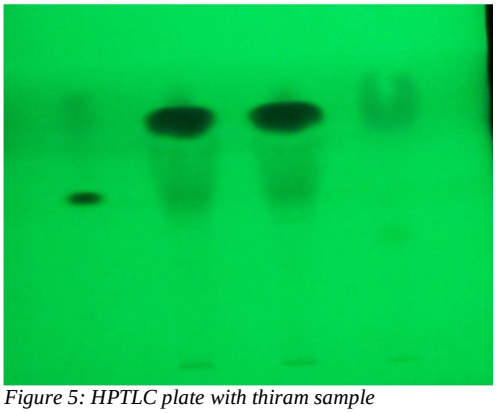

Figure 5: HPTLC plate with thiram sample

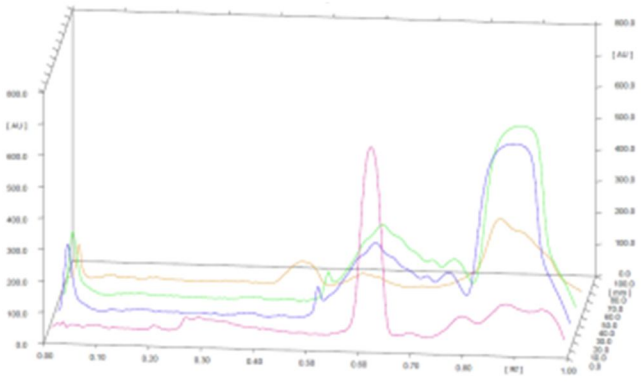

Figure 6: standard, 100ppm,150ppm,200ppm

III. RESULTS AND DISCUSSION

In present study on microbiological survey of soil contaminated with Thiram pesticide attempts were made to screen and isolate the potential microbial strain, able to degrade Thiram pesticide. As the major focus of the study was the microbiological survey of the soil contaminated with Thiram pesticide, treatment contain soil sample collection, isolation of microorganisms that are already present in contaminated soil, isolation, identification and potentiality to degrade thiram. As those microorganisms were already present in the soil contaminated with the thiram.

Physical soil characterization of two different district was studied and in Sabarkantha, course particles was more found in compare to Patan but slit particles were much found in Mehsana compare to Sabarkantha. The water holding capacity of Mehsana district soil was higher than the soil of Sabarkantha district because the amount of slit particle is higher in Mehsana district. Due to this reason humidity is more observed in Mehsana. (Table 1)

No more difference observed of pH and color between three districts. Concentration of carbon, nitrogen, chloride, total hardness, bicarbonate and carbonate results are varies in all three districts. Carbon is very high in Patan district and low in Sabarkantha. Nitrogen level is high in Mehsana and lowest in Sabarkantha. Sulfur level is high in Mehsana and lowest in Patan and Sabarkantha. Total hardness is highest in Patan and lowest in Mehsana. Bicarbonate is highest in Mehsana and lowest in Sabarkantha. Soil carbon and nitrogen ratio also is very important for biomass development. PH and nutrients are very important for the growth of microorganism.

By colonial characterization and performing gram staining different type of microorganism was identified. Among them one type of Bacteria was gram positive and other four were gram negative.

The identification is carried out by gram staining, Biochemical tests and Biolog. For more confirmation was done by 16s-rRNA sequencing. By confirmation found that the organisms are *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescence*.

Growth of all five isolates on different pesticides, different salt concentration, pH, and temperature to check the potential degrader and find the suitable organism which have capacity to degrade pesticide potentially. Among this five isolates three isolates are having potential growth on various pesticides, pH and temperature.

This three isolates used to check degradation of thiram by UV-Visible spectroscopy with compare to control. Pseudomonas aeruginosa have capacity to degrade the pesticide up to 200 ppm. It degrades thiram pesticide 80-85%. Pseudomonas putida degrades thiram 55-65% and beyond 200ppm it is not able to degrade thiram. Pseudomonas fluorescence degrades thiram 60-65 % and it does not degrade above the 200 ppm concentration.

Degradation of thiram with the help of Pseudomonas aeruginosa is very good and it is further checked by the help of HPTLC method (Wei Fan et al., 2007). HPTLC study gives the clear idea about the degradation and various other products formation. With comparison to standard thiram the pick of 100,150,200ppm shows degradation and peak area and Rf value of peak is low. Peak area and peak height is decreased in the samples containing various concentration of the thiram, and new peak formation is taken place in the graph. This results in the formation of new compound with the help of this microorganism. These degraded products are having different Rf values and different peaks.

IV. CONCLUSION

Use of pesticides is very high in agriculture field so this study can help in minimization of the soil, water pollution this kind of bacteria has ability to degrade the pesticide residues and prevents the pollution. The data thus indicates that the decay of thiram depends upon the nature of medium and the environmental conditions. Therefore large-scale variations are observed in the data on the decay profile. For any meaningful interpretation of results it is important to generate a database on the decay pattern of pesticide under controlled and field conditions simultaneously as the laboratory results cannot be necessarily extrapolated to field conditions. The results obtained in the present studies will help in understanding the persistence of this pesticide vis-à-vis the long-term effectiveness and toxicity of pesticide under different environmental conditions and also degradation capacity of bacterial community.

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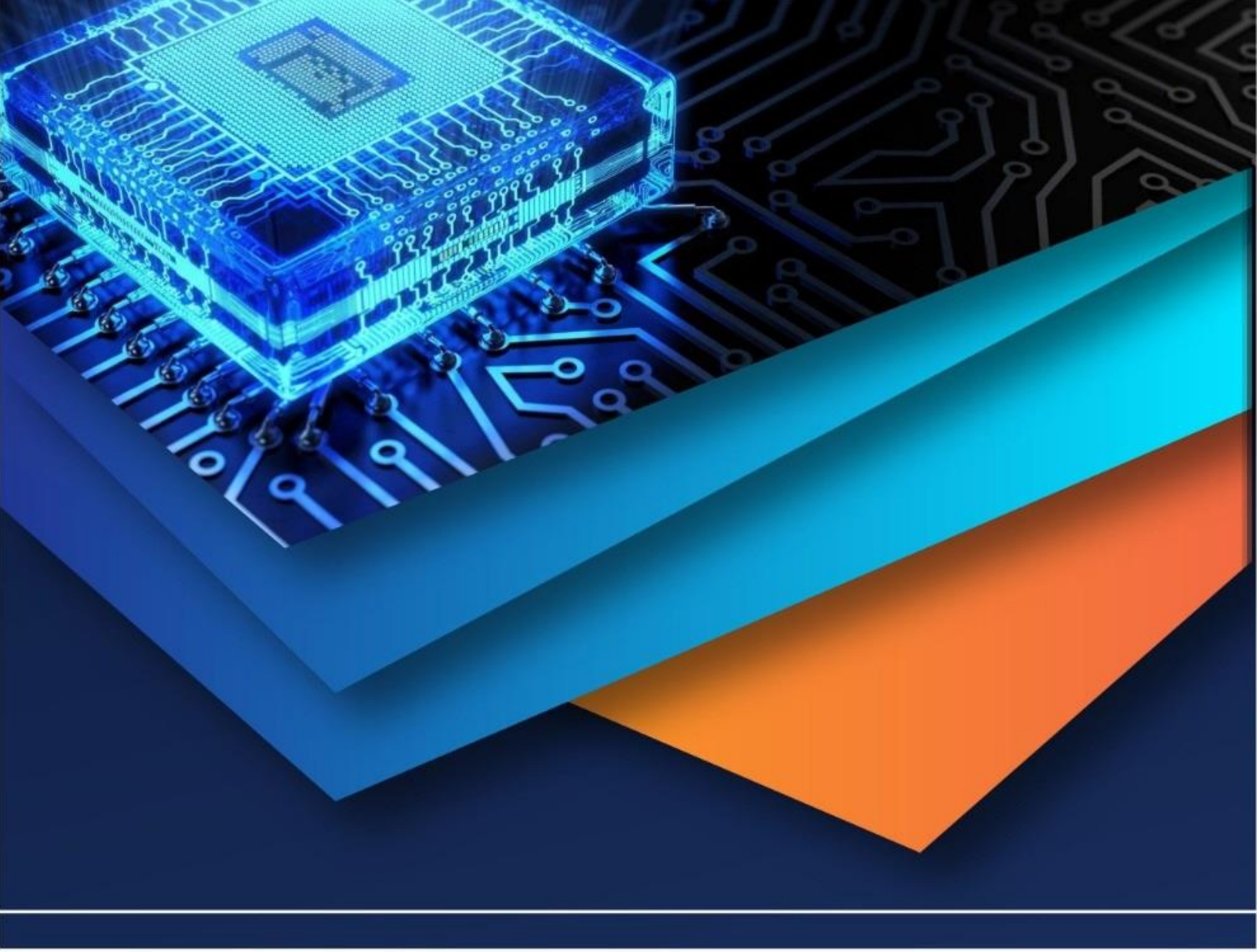

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