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Biodegradation of Organo phosphorous Pesticide Dichlorvos by bacteria isolated from field sample

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Abstract- The extensive use of pesticides is considered to be one of the major causes of soil and water pollution in agricultural sector. As it enters the food chain, humans on the other hand are directly or indirectly victimized. In order to counteract the situation, such contaminants need to be removed from the environment. The safest approach to clean the environmental pollution in an eco-friendly way is by employing microorganisms, a process termed as biodegradation. Dichlorvos is an organophosphorus pesticide widely used as insecticide to control pest in agriculture.In the present study an attempt was made to screen for potential Dichlorvos degrading bacteria. The soils collected from various field samples are subjected to enrichment culture technique using various concentrations of pesticides in minimal salt medium. Of all the organisms screened only four isolates labelled as SA, SB, SC and SD showed degradative activity. Organophosphate enzyme assay was performed to confirm the degradation by using UV-Visible spectrophotometer. However further studies are to be done to check whether the cultures are showing complete degradation, partial degradation or bioconversion.

Keywords: *Biodegradation, Dichlorvos, Organophosphate assay, Organophosphorus pesticide.*

I. INTRODUCTION

Pesticides constitute the key control strategy for crop pests and disease management and have been making significant contribution towards improving the crop yields per hectare. At present, a total of 145 technical pesticides have been registered in the country, of which 93 technical grade pesticides are being manufactured indigenously [1] [2]. Presently, the chemical pesticides consumption is found to be highest in Andhra Pradesh (33%), followed by Punjab (14%) and Karnataka (11%) [3]. Pesticides are classified into five chemical groups which includes organochlorine, organhosphate, carbamate, synthetic pyrethroids, avermectin and formamidine [4][5][6].

Currently, among the various groups of pesticides that are being used, organo phosphorus group forms a major and the most widely used group. These OPs are highly toxic heterogeneous compounds.They share a phosphoric acid derivative and are widely used for plant protection and pest control. OPs are acutely toxic and act by inhibiting acetylcholine esterase, an important enzyme in the nervous system [7]. On exposure to OPs, the enzyme is unable to function causing accumulation of acetylcholine, which interferes with the transmission of nerve impulses at the nerve endings. Although organophosphates are biodegradable in nature, their residues are found in environment and causes high toxicity in humans and animals.

Many of these Organophosphorus pesticides are biodegradable with a few exceptions. Some of these compounds leaves toxic residues in the environment due to its extensive usage. One such pesticide is Dichlorvos. The chemical name of Dichlorvos is 2,2-dichlorovinyl dimethyl phosphate. It is an organophosphate that is mostly used to control agricultural and household pest and in protecting stored product from insect. These compounds are powerful inhibitors of acetylcholinesterase, a vital enzyme involved in neurotransmission, in the form of acetylcholine substitutes [8]. It also is known to cause neck muscle weakness, diarrhoea etc [9] [10]

The extensive use of pesticides leads to accumulation of a huge amount of residues in the environment, thereby causing a substantial environmental health hazard [11]. Biodegradation is a naturally occurring process which uses microorganisms to degrade pollutants present in environment. This is a common method for the removal of organophosphate pesticides because of its low cost and low collateral damage of indigenous plants and animal organisms Many microorganisms that are able to degrade Dichlorvos pesticide have been isolated from soil samples. Previous research has shown that Malathion, Chlorpyrifos, Dichlorvos degrading bacteria used as single strain or in consortium to increase the rate of degradation of pesticide [12] [13]. The main objective of present study focuses on Screening of Dichlorvos degrading micro-organisms from pesticide contaminated soil sample and to study the efficacy

of degradation potential of Dichlorvos degrading microorganisms by enzyme assays.

II. RELATED WORK

Agarry *etal* in 2013 studied on isolation of Dichlorovos degrading bacteria from agricultural field samples. The results of study showed that use of consortium of bacteria which included mainly *Serratia sps,Proteus vulgaris, Acinetobacter and Vibrio sps* could effectively degrade the pesticide when supplemented with NPK fertiliser[14]. Similar studies by Sony Yadav *etal* in 2015 demonstrated the efficacy of Dichlorvos degradation by six bacterial cultures belonging to *Micrococcus, Bordetella, Staphylococcus, Klebsiella,Pseudomonas and Enterobacter sp* respectively. Also the results showed that there is an increase in Dichlorvos degradation activity when medium is supplemented with Sucrose[15].

III. MATERIALS AND METHODS

Collection of Soil sample: - The soil sample used for the isolation of pesticide degrading bacteria was collected from a bottle guard field from Dammaiguda, Hyderabad where dichlorvos pesticide is used extensively. Samples were collected at a depth of 5cm to 10cm in the field. The soil sample was air dried at room temperature to the remove excess moisture and it was passed through a 2mm sieve to remove the unwanted debris and stored in a polyethylene bags in refrigerator for further experimental study [16].

Preparation of stock pesticide: Commercial grade Nuvan Dichlorvos (76%) pesticide was procured from local pesticide shop from ECIL. Stock solution was prepared and stored in refrigerator for further use.

Enrichment culture Technique:

Enrichment culture technique was used to isolate Dichlorvos degrading bacteria. 10g of soil sample is supplemented with Mineral Salt Medium(MSM) containing different concentrations of pesticide *i.e.* 10ppm, 20ppm, 40ppm, 80ppm and 100ppm respectively and incubated at 37^{o}C for 40 days. Enrichments were done for every 7 days.

Isolation and characterization of bacteria:

After enrichment the bacteria grown with Dichlorvos pesticide as solesource of carbon were isolated on Nutrient Agar medium. The isolated cultures were purified and stored until analysis. The isolated bacteria were subjected to different Morphological, Cultural and biochemical tests for tentative identification as per Bergeys manual.

Dichlorvos degradation studies:

MSM broth supplemented with various concentrations of pesticide were inoculated with test isolates and also consortium of cultures and incubated at 37ºC for 24hrs and 48 hrs respectively. Dichlorvos is the sole source of carbon in MSM. Controls were setup for every concentration tested. After incubation the degradation capacity was monitored by observing the growth at 600nm in UV Visible spectrophotometer (Model NoBK UV 1000). All experiments were done in triplicates.

Organophosphorus phosphatase enzyme Assay (OPP assay):

The OPP assay was done as described by Wang et al., 2008. The cells grown in MSM broth with pesticides were harvested and pelleted by centrifugation at 10,000 rpm for 20 min. The supernatant was separated and is used for extracellular organophosphorous phosphatase activity. 100 µL of crude enzyme was added to 900 µL of Tris HCl containing 10 mg/mL p-nitrophenol phosphate. This step is followed by incubating the mixture for 15 min at 37°C. After that reaction was terminated by addition of 1 mL of 10% TCA and 1 mL of 10% sodium carbonate and the liberated yellow coloured end product p-nitrophenol was measured in a spectrophotometer at 410 nm. One unit (U) of OPP activity is defined as the amount of enzyme liberating 1mol of p-nitrophenol per minute at 37°C [17].

III. RESULT AND DISCUSSION

Four bacterial isolates were obtained from soil samples enriched with Dichlorvos and were coded as SA, SB, SC and SD respectively. All the four bacterial isolates were unable to grow at 40ppm, 80ppm and 100ppm pesticide concentration. The growth was monitored at OD 600nm in UV Visible spectrophotometer (Table 1)

Table 1. Growth of different isolates at OD 600 nm

S.No.	Strain Code	Bacterial growth on MSM containing Dichlorvos as the sole carbon and energy source					
		Control	10ppm	20ppm	40ppm	80ppm	100ppm
1	SA	0.456	0.779	0.799	0.0	0.0	0.0
2	SB	0.593	0.768	0.786	0.0	0.0	0.0
3	SC	0.524	0.0	0.0	0.0	0.0	0.0
4	SD	0.558	0.0	0.0	0.0	0.0	0.0

The morphological and physiological characteristic of the isolate were noted which showed that all the four isolates are different cultures (Table 2)

Table 2. Morphological characteristics of Dichlorvos degrading bacterial strains

S. No.	Strain code	Cell shape	Gram reaction	Colony Morphology
1	SA	Spore forming Rod	Gram positive	Cream coloured, wrinkled, entire, flat and dry colony
2	SB	Rod	Gram negative	Elevated, mucoid, irregular, white, slimy
3	SC	Rod	Gram negative	Round, flat, entire
4	SD	Rod with central spores	Gram positive	White, wrinkled, entire

All the four isolates were subjected to Biodegradation studies and OPP enzyme activity was recorded.

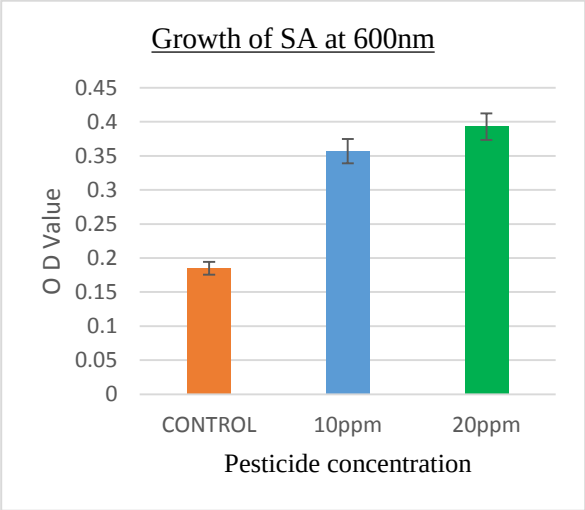

Fig.1a: Growth of SA at 600nm

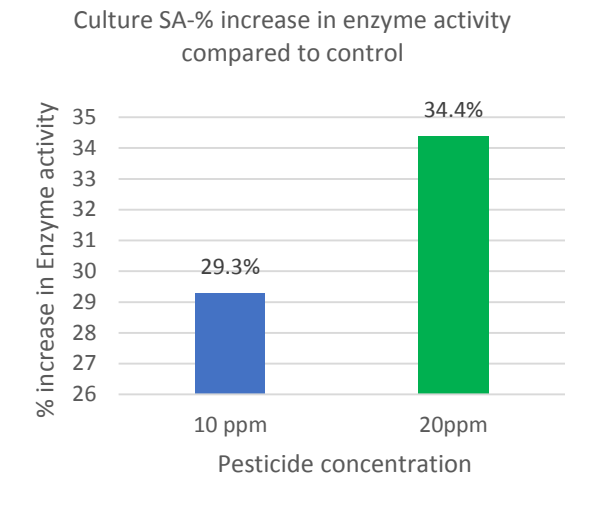

Fig.1b: Culture SA- % increase in enzyme activity

Culture SA showed maximum growth at 20ppm pesticide concentration as compared to control (Fig 1a). The % increase in OPP activity was found to be more at 20ppm concentration (Fig1b). This result indicates that enzyme activity is more at higher concentration of pesticide.

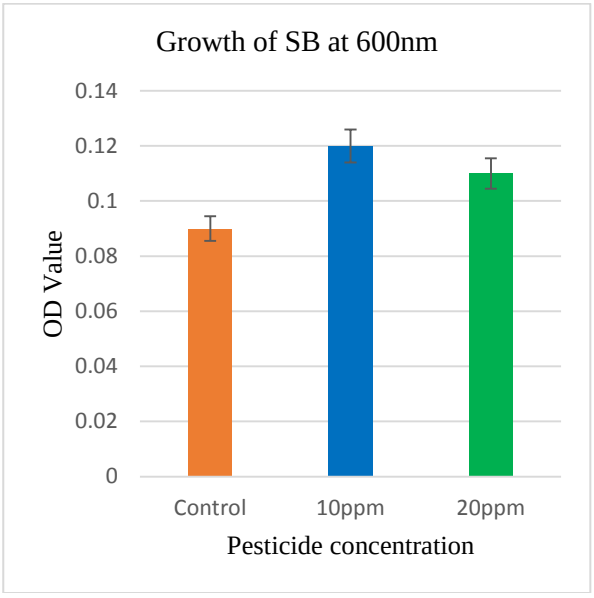

Fig.2a: Growth of SB at 600nm

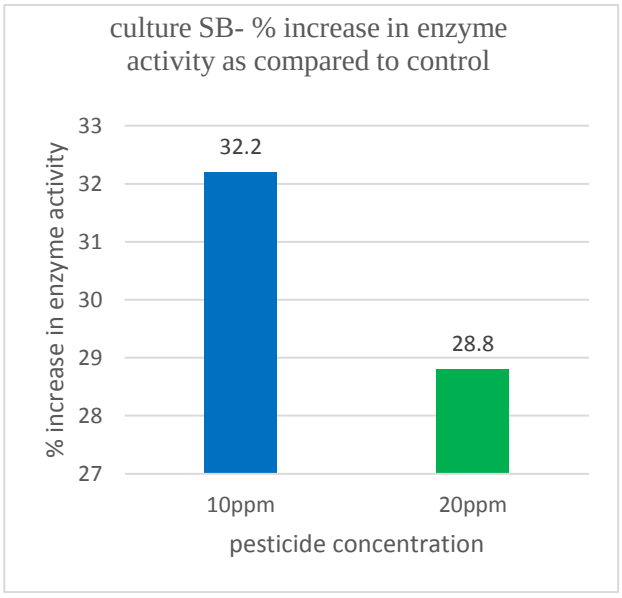

Fig.2b: Culture SB- % increase in enzyme activity

Culture SB showed maximum growth at 10ppm pesticide concentration as compared to control (Fig.2a). The % increase in OPP activity was found to be more that 10ppm concentration (Fig 2b). This result is in contrast with the result obtained for Culture SA where growth and enzyme activity were more at 20ppm concentration.

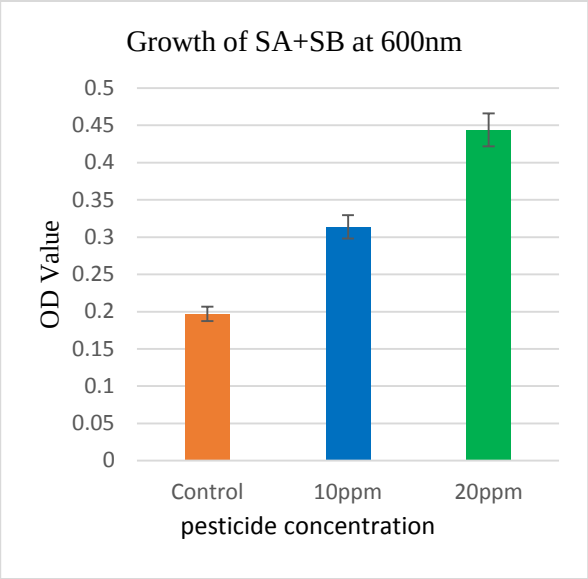

Fig.3a: Growth of SA+SB at 600nm

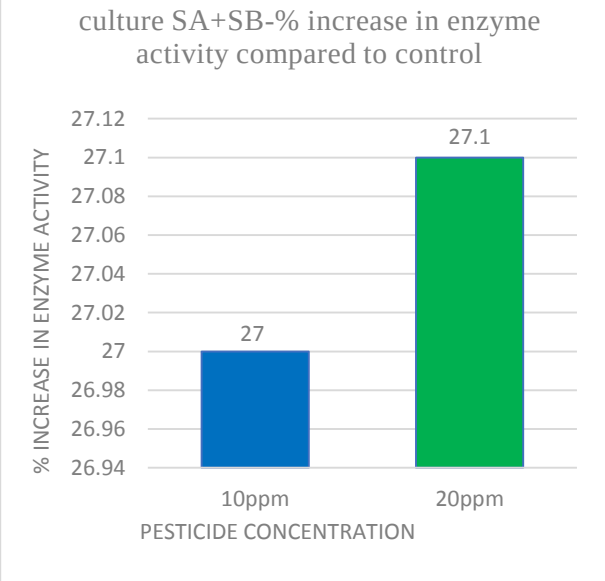

Fig.3b: Culture SA+SB- % increase in enzyme activity

As four different isolates were isolated after enrichment culture technique an attempt was made to check the ability of cultures SA and SB in degrading the dichlorvos pesticide. The results demonstrated that there is an increase in growth and enzyme activity when pesticide is used as sole source of carbon. This result indicates the synergistic action of the two isolates SA and SB(Fig 3a and Fig 3b). However, a detailed study has to be done to know the exact interaction between the cultures. From the figures 3a and 3bit was clear that maximum activity was observed at 20ppm concentration. However, no growth was noticed above 20ppm concentration.

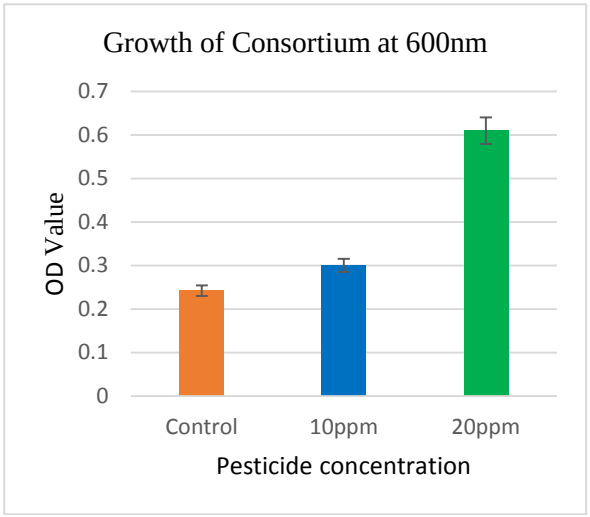

Fig.4a: Growth of consortium at 600nm

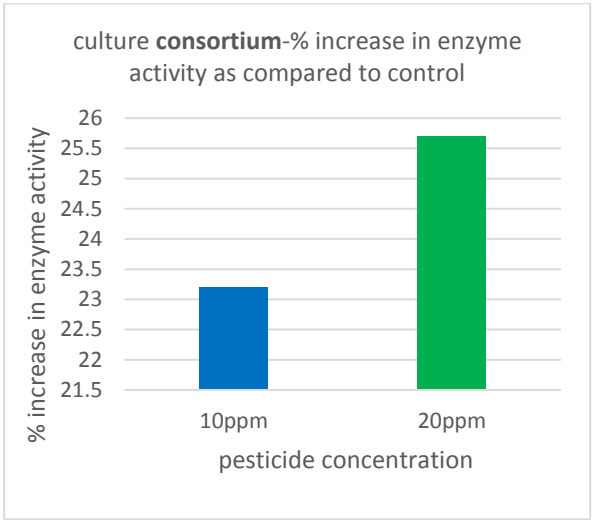

Fig.4b: Culture consortium- % increase in enzyme activity

From the figure 4a and 4b it was noted that the consortium of all four cultures were able to grow synergistically as the growth and enzyme activity increased when compared to the previous three tests. Even this consortium of four isolates could not grow beyond 20ppm concentration. This indicates that beyond 20ppm concentration it is toxic to all four isolates. Further analysis have to be done invitro and in field conditions to know the exact mode of action of these isolates and their interaction with other organisms.

IV. CONCLUSION AND FUTURE SCOPE

The present study shows that use of consortium of cultures could effectively degrade Dichlorvos pesticide. Removal of xenobiotic compounds which are a major threat to environmental pollution are of major concern as they enter the food chain and are the major causes of various diseases. The study shows that the bacterial isolates are able to use Dichlorvos as sole source of carbon. Therefore these four cultures can be used effectively for Bioremediation of contaminated sites. Future studies aim in studying the biochemistry of pesticide degradation and its significance in fieldconditions.

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