ORIGINAL RESEARCH

MICROBIAL DEGRADATION OF METHYL PARATHION PESTICIDE IN SOIL: A MICROCOSM STUDY

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ABSTRACT:

Methyl parathion is an Organophosphate insecticide; it is used to control boll weevils and a variety of sucking and chewing insect pests of agricultural crops. Apart from insects it is highly toxic to non- target organisms including human beings. These chemicals create diverse environmental problems via biomagnifications. Since most of these chemicals are mobile in soil they have the potential for groundwater contamination as well, thus isolation and selection of potential microorganisms which can degrade these chemicals thereby reducing their toxicity in a cost effective way is considered as a powerful means for environmental clean -up. In the present study a lab scale experiment that closely simulates the environmental conditions was set up, to study the degradation of methyl parathion in sterile and non sterile soil by indigenous soil bacterium *Achromobacter* sp. C1. The soils were spiked with Methyl Parathion (5 ppm final concentration). Cells of the test strain were inoculated in the beakers spiked with the concerned pesticide. To analyze the degradation % of methyl parathion, soil samples were withdrawn at various time intervals and analyzed with the help of Gas Chromatography coupled with mass spectrophotometer (GC-MS). Isolated soil bacteria had a significant effect on the degradation of methyl parathion (MP) and it was found that, the rate of degradation increases with the incubation time. It has been found that after 30 days of incubation, the degradation of MP in non sterile soil was (93.75%) and in sterile soil was (83.33%).

KEYWORDS: Pesticides, Gas Chromatography (GC-MS), Methyl Parathion (MP), Degradation

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INTRODUCTION:

The group of organic compounds mainly used as agriculture chemicals are called as pesticides[1]. These compounds are highly toxic and are widely used in plant protection throughout the world. When pesticides are dispersed in the environment, they become pollutants. The regular and excessive use of pesticides contaminate the atmosphere, as, when they reach the soil and persist for long periods, they cause harm to the soil microorganisms and also effect the plant growth. However their use decreased currently, but due to their recalcitrant nature, they remain active for longer periods in water bodies and soil , since it is hydrophilic in nature it is weakly absorbed by the soil particles and thus increases the threat of groundwater contamination due to leaching[2]. As a result these chemicals create diverse environmental problems via biomagnifications. Methyl Parathion (MP) (O-dimethyl O-4-nitro-phenyl phosphorothioate) is one of the most widely used organophosphate insecticides and acaricide used extensively in agriculture to control insects, fungi and herbs[3-5]. It is synthesized from diethyl dithiophosphoric acid. After being consumed by the insects, the parathion becomes oxidized by oxidases to give paraoxon, replacing the double bonded structure of sulphur with oxygen. It kills insects by contact, stomach and respiratory action [6-7]**.**Microbial degradation of pesticides in soil reduces their persistence and as a result reduces the risk of environmental contamination[8]. It has been suggested that biodegradation becomes an attractive option for destruction of pesticides since it utilizes a natural process and offers the potential for being cost effective as well as safe technology[9-11]. Bioremediation relies on biological processes to minimize an unwanted environmental impact of the pollutants. The microorganisms, in particular have the abilities to degrade, detoxify and even accumulate the harmful organic and as well as inorganic compounds. It has emerged during recent past as most ideal alternative, environment friendly and ecologically sounds technology for removing pollutants from the environment, and thus restoring contaminated sites and preventing further pollution. The purpose of this study was to develop an efficient bioremediation strategy for soils contaminated with methyl parathion by evaluating bio-degradative activity of the indigenous microorganisms in a soil microcosm, an experimental system that closely resembles the environmental conditions.

Many researchers reported in the past, that the microorganisms play a vital role in enhancing the degradation capability of pesticides[12-14]. Wan *et al.*[15] reported the degradation of para-nitrophenol (PNP) which is the first hydrolysis product of MP by *Achromobacter xylosoxidans* Ns from wet land sediments. PNP was degraded by the bacteria by forming 4- nitrocatechol and 1, 2, 4- benzenetriol as the key intermediates. Krishna *et al.* [16] reported degradation of mixed pesticides (lindane, carbofuran and methyl parathion) by mixed pesticides enriched cultures. After seven weeks of incubation enriched cultures were able to degrade 72 % lindane, 95 % carbofuran and 100 % of methyl parathion in facultative co-metabolic conditions. Wang *et al* .[17] reported biodegradation of methyl parathion (MP) and paranitrophenol (PNP) by a *Agrobacterium sp.* strain Yw12. The strain completely degraded MP and PNP and utilized MP as a sole source of carbon and energy for its growth. Kulshrestha *et al.*[18] studied the degradation of chlropyrifos – an organophosphorous pesticide by *a* fungus *Acremonium* sp. strain (GFRC-1) that was isolated from a laboratory –enriched red agricultural soil.Similarly Pradeep *et.al.*[19] reported a soil bacteria capable of degrading an organophosphorous pesticide chlropyrifos and endosulfan. Hindumathy *et al.*[20] reported the effect of pesticide- chlorpyrifos on soil microbial flora and studied the pesticide's degradation by strains isolated from the contaminated soil Gang *et al.*[21] reported the influence of kaolinite and goethite on microbial degradation of MP and observed that biodegradation was improved by kaolinite and depressed by geothite. As it was found that, the test strain C1 was able to degrade MP in aqueous condition, so an effort was made to take the study from flask conditions to soil conditions and so in this direction, the indigenous microorganism *Achromobacter* sp C1 which was isolated from the soil was evaluated for its efficacy in degrading the MP insecticide in soil conditions and thus, the microcosm study was conducted.

MATERIALS AND METHOD:

Soil samples were collected from the fields of Central Institute of Cotton Research

Institute, Nagpur (MS), which had the history of pesticide application of at least 10 years. Samples were taken randomly from a depth of 5-10 cm to minimize air contamination. Requisite soil was taken by means of sterilized spatulas and collected in sterile polythene bags. Metacid EC 50 a commercial formulation of MP was obtained from local distributors and all the other chemicals, media components used were of analytical grade that were obtained from Sigma-Aldrich (USA). The bacteria used in the study were isolated earlier from pesticide contaminated soil by enrichment technique (C1) and was identified up to genus level in the laboratory.

Experimental set-up: Microcosm experiment was performed with autoclaved and un-autoclaved soils:

1. **Autoclaved Soil**: To one set of beakers that contained 20 grams soil (un inoculated), 4 ml minimal medium (MM) was added and to another set with 20 grams soil (inoculated) 4 ml medium containing the cells of the isolated strain C1 was added, such that there were 1 x10 ^{5}cfu./ g (dry wt) of soil. Both the sets of autoclaved microcosm contained 5ppm concentration of MP. The beakers were covered with perforated aluminium foil and incubated at 35 ^{O}C for 30 days under sterile conditions. Moisture level was maintained periodically to compensate for the loss of water due to evaporation[22].

2. **Un-autoclaved Soil:** To one set of beakers that contained 20 gram soil each (un-inoculated), 4 ml minimal medium was added and to another set with 20 gram soil each (inoculated),4 ml medium containing the cells of the strain was added such that there were 1x 10 ^{5}cfu/g (dry wt) of soil. Both the sets of unautoclaved microcosms contained 5ppm concentration of MP. The beakers were covered with perforated aluminium foil and incubated at 35 ^{O}C for 30 days under sterile conditions. Moisture level was maintained periodically to compensate for the loss of water due to evaporation. Soil samples(1g) were withdrawn at various time intervals (0,5,10,15,20,25,and 30 days) , extracted and then analyzed through GC-MS for the degradation studies.

Extraction of MP: 1g soil sample was suspended in 10 ml of 5%NaOH and vortexed thoroughly followed by centrifugation at 1,500 rpm for 10 min. The supernatant was extracted with double the volume of ethyl acetate after acidification to pH 2.0 with HCL. The aqueous phase was again extracted with ethyl acetate; both the extracts were pooled together and passed through anhydrous sodium sulphate to remove the traces of water. The filtrate was air dried at room temperature and finally dissolved in 1ml acetone and then taken for the analysis by GC-MS[22].

RESULT AND DISCUSSION:

A total 10 strains were isolated from the pesticide contaminated soil, out of these only six strains viz., C1, C2 C3, C6, C8 and C10 were found to be potent strains and were further screened for their ability to tolerate maximum concentration of MP. Out of which, only one strain viz. C1 was found to be most potential strain as it was able to tolerate higher concentration of MP as compared to other isolated strains, and so finally it was selected for future analysis. On the basis of detailed biochemical tests, the test strain C1 was identified upto genus level and was found to be *Achromobacter sp* C1.

Since biodegradation is a biological process carried by microorganisms under suitable physiological conditions, therefore, different parameters such as effect of inoculum concentration, temperature, pH, effect of carbon, nitrogen sources and incubation period were optimized for rapid biodegradation of MP. It was found that the activity of the inoculated bacteria is influenced by multiple factors and was seen that 35 ^{0}C, 6.5 pH and 27 days of incubation are the best suitable conditions for rapid degradation of MP by the test strain. Better MP degradation was recorded when the medium was supplemented with glucose as carbon source and when NH_4Cl was used as a nitrogen source (data not shown).

It is evident from the data shown in the **Table-1** that the rate of MP degradation varied significantly with incubation period. It was

observed that degradation was gradually increases with incubation period and reached its maxima value after 30 days of incubation in sterile and non-sterile soil. It was observed that the degradation of MP was more in non-sterile soil as compared to sterile soil. In non sterile soil after 30 days of incubation, the isolate, *Achromobacter sp.C1* was able to degrade up to 93.75, it might be due to the presence of other micro flora which helped in the degradation of MP. In sterile soil, after 30 days of incubation the degradation of MP by *Achromobacter sp.C1* was 83.33%. The test strain alone was solely responsible for degradation of MP up to 83.33% after 30 days of incubation.

In this study, biodegradation of MP in soil microcosms was used as a model system to evaluate the efficiency of the strain C1 in degrading the concerned pesticide (MP).The aim of the present work was eventually to develop a strategy for bioremediation of MP from contaminated sites, for this two types of microcosms were used (sterile and non-sterile) so that, the efficiency of the indigenous strain C1 could be evaluated i.e. when it was used alone (sterile soil) and also when it was used in combination with the other microflora (non sterile soil). It was found that the strain C1 alone was able to degrade 83.33% of MP after 30 days of incubation. It was observed that the amount of MP detected after 5th day in sterile microcosm was 0.062ppm, which got reduced to 0.013ppm after 30th day of incubation as shown in Fig 1(a) and (b). Similarly, several other investigators have also reported 25 - 30 days as an optimum incubation period for rapid degradation of MP by *Pseudomonas diminuta*[23-25].

Labana *et al.*[22] carried out a microcosm study on the bioremediation of PNP contaminated soil using *Arthrobacter protophormiae* RKJ100 and effective biodegradation over the range 1.4-210 ppm, at 20-40 ^{o}C, 7.5 pH when applied @ 2x10^{8} CFU/gm soil. Pirie *et al.*[26] undertook a microcosm study on PNP biodegradation in soil slurry by *Alcaligenes faecalis* and stated that temperature, inoculums size, yeast extract concentration, pH and soil-water ratio were the most effective factors responsible for PNP biodegradation. Naqvi *et al.*[27] reported similar observation in case of biodegradation of carbaryl in soil. Greeshma *et al.*[28] reported the toxicity and bioremediation of pesticides in agricultural soil. Verma *et al.*[29] reported the pesticide relevance and their microbial degradation in soil. The present study is a model study that could be used for decontamination of sites contaminated with MP.

Table 1: Degradation % of MP in sterile and non-sterile microcosms

Incubation (Days)	% of Degradation in sterile sample	% of Degradation in non sterile sample
0-5	20.51	47.91
0-10	25.64	52.08
0-15	37.17	66.66
0-20	64.10	72.91
0-25	65.38	77.08
0-30	83.33	93.75
SD	25.185	16.896
SEM	11.263	7.556

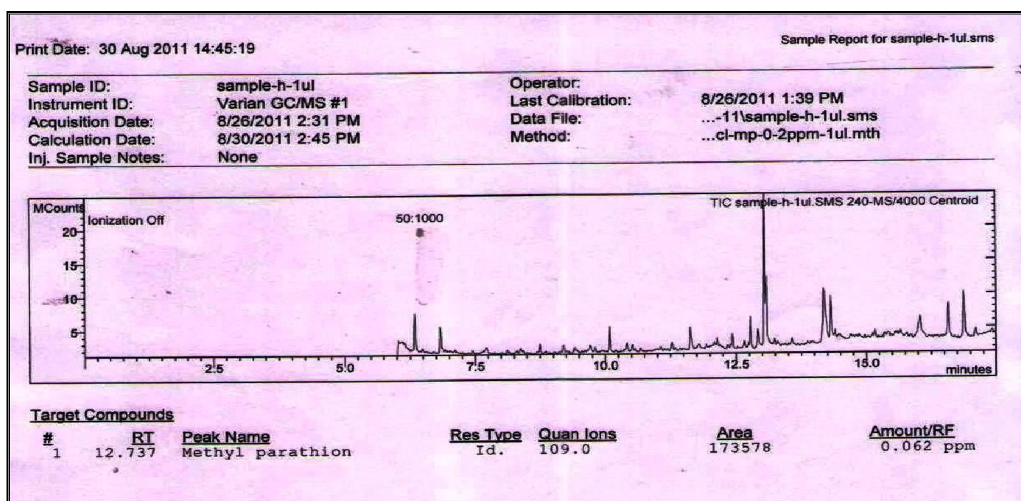

Fig.1 (a): The Chromatogram shows the detection of MP(0.062ppm) at 5th day in sterile soil sample

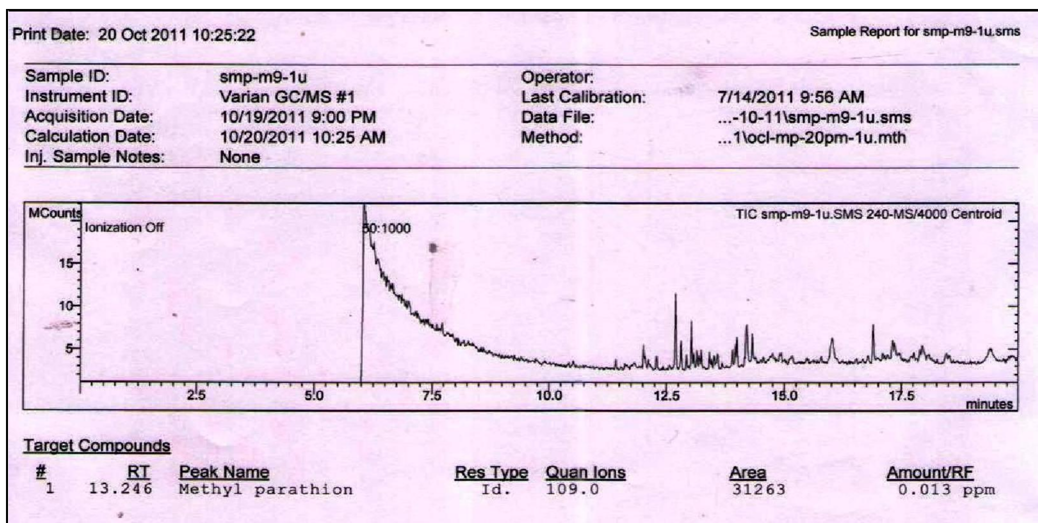

Fig.1 (b): The chromatogram shows the detection of MP(0.013) at 30th day in sterile soil sample

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