WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 1275-1280. Research Article ISSN 2277– 7105

RESIDUE ANALYSIS OF ORGANOPHOSPHORUS PESTICIDE MALATHION AFTER THE GROWTH OF *ANABAENA SPIROIDES* AND *NOSTOC PUNCTIFORMAE* IN CULTURE MEDIA UNDER LABORATORY CONDITION

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Article Received on
13 March 2018,
Revised on 03 April 2018,
Accepted on 24 April 2018
DOI: 10.20959/wjpr20189-12209

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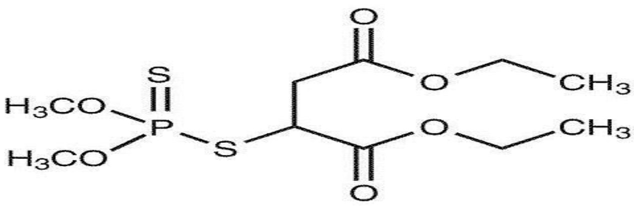
ABSTRACT
Residue analysis of malathion was investigated in laboratory condition. Different concentration of malathion was applied on agriculturally important cyanobacterial species Anabaena spiroides and Nostoc punctiformae under laboratory condition. Residue analysis was carried out using High Performance Liquid Chromatography (HPLC) and methanol was the solvent used as mobile phase to achieve the accurate result separation.

KEYWORDS: Residue analysis, Pesticide, malathion, *Anabaena spiroides*, *Nostoc punctiformae*, HPLC.

INTRODUCTION
Agrochemicals like pesticides becomes a part and parcels of modern day agricultural system. Pesticides are widely used against a range of pests infesting agricultural crops. Globally, about 3109kg of pesticides is applied annually with a purchase price of nearly $40 billions each year (Pan-UK, 2003). The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment (Carriger *et al.*, 2006; Pimentel, 1995). Pesticide which are used in the agricultural field is not intended to harm the beneficial microbes and blue green algae but the organisms become the non-target victim. The widespread use of pesticides in agriculture creates a necessity to study the effect of the pesticide on soil microorganisms. Blue-green algae or cyanobacteria are abundantly found in rice fields and these are of great economical and ecological significance due to fixation of atmospheric nitrogen which enrich the rice fields of tropics. Adverse impacts of pesticides on soil microbial diversity and activities have been described by many researchers

(Ingram *et al*., 2005; Littlefield-Wyer *et al*., 2008; Niewiadomska, 2004; Wang *et al*., 2006). The pesticides effect the various physiological process of the cyanobacteria and interact with their metabolic activities. Some bacteria and cyanobacteria are capable of using applied pesticide as a source of energy and nutrients to multiply, whereas the pesticide may be toxic to other organisms (*Johnsen et al*., 2001). Likewise sometimes, application of pesticides reduces microbial diversity but increases functional diversity of microbial communities (Wang *et al*., 2006)Malathion is a toxic organophosphate pesticide. The structural formula of malathion is $C_{10}H_{19}O_6PS_2$.



Chemical Name: O, O-diethyl O-quinoxalin-2-yl phosphorothioate

It is a wide-spectrum and non systemic organophosphate insecticide. It was introduced in 1950. Commercial grade malathion is generally used to control of sucking and chewing insects on fruits and vegetables, mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice. This pesticide can be found in formulations with many other pesticides. In the rice field it is used to control rice bug, grasshopper, rice sting bug, rice leaf miner. It is formulated as an emulsifiable concentrate (EC), wettable powder(WP), dustable powder(DP) etc. Trade names for products containing malathion include Dielathion, Emmaton, Exathios, Fyfanon and Hilthion, Karbofos and Cythion (Cynamid Agro), Dhanuka Malathion Luthion (Lupin).

Depending upon the type of application and pesticide formulation, malathion residue may be detectable in water and soil surface. In the last decade cyanobacteria were studied with numerous aspects especially with respect to their utility in bioremediation, human nutrition and as biofertilizer (Venkataraman, 1975). Bioremediation which includes the gainful utilization of microorganism for the metabolism or biodegradation of target pesticide into safer and innocuous products is amongst the potent technologies that are being used globally for the restoration of contaminated sites. Although organophosphates are biodegradable in nature, their residues are found in environment. Considering their toxicity, research on biodegradation of organophosphates is being carried out all over the world. Concerning the analysis of malathion residue on cyanobacterial species of rice filed of Jorhat district is rare.

So an attempt was made to study the residue analysis of malathion after application of some cyanobacterial species like *Anabaena spiroides* and *Nostoc punctiformae* isolated from the rice field of this region. This study is very new in this area Jorhat and it will helpful to update the scientific knowledge in the field of pesticide.

MATERIAL AND METHOD

A survey of different rice fields of Jorhat district, Assam was carried out and algal samples were collected. All soil samples were collected in sterile plastic bags and carried to the laboratory for further studies.Samples were cultured in laboratory to isolate it. Two species *Anabaena spiroides* and *Nostoc punctiformae* were selected out to continue the pesticidal study. These two were further cultured in 250ml conical flask containing 100ppm, 200ppm, 300ppm, 400ppm and 500ppm concentration or dose of pesticide malathion. The culture medium taken was BG-11 medium. Organic solvents (residue analysis grade) for dissolving and extraction of pesticide residue was methanol(HPLC grade). The reference analytical standard of malathion having purity ranging from 95.10 to 99.99 per cent were purchased from SIGMA ALDRICH, Germany. The stock standard solution of malathion was prepared by accurately weighting 0.0330gm of standard malathion and dissolved it in methanol and made the volume up to 100ml. The purity of the standard solution was 95%.The working standard solution of 100ppm, 200ppm, 300ppm, 400ppm and 500ppm was prepared by dilution with methanol. The stock standard solution and working standard solution were stored under refrigeration (4˚C).

HPLC-DAD Apparatus

A high performance liquid chromatography (HPLC) method where a Diode Array Detector (DAD) which have been widely used in pesticide residue detection. In the present investigation a high performance liquid chromatography (HPLC) manufactured by Waters, U.S.A. with binary Pump system, Water 515. It is well equipped with photodiode array detector. This detector was connected to the computer for computation of data. All these procedure was applied for analysis of residue of malathion containing s *Anabaena spiroides* and *Nostoc punctiformae* with the doses of 100ppm, 200ppm, 300ppm, 400ppm and 500ppm. For residue analysis of malathion HPLC column(Sunfire C18, 5 µm, 4.6 x 250 mm) was used mobile phase was methanol with with isocratic flow. The wave length selected for malathion was 210nm.

Sample extraction

The samples containing *Anabaena spiroides* and *Nostoc punctiformae* were homogenized and then measured out 10ml solution. In to it 2.5 gm of extra pure sodium chloride and 20 gm anhydrous sodium sulphate and were added. After this all were dissolved in 100ml of solvent (methanol) and blended it on high speed varying blender for 3-8 minutes. All the samples were filtered through 0.4µm filter paper. In these samples, added 25ml of above mentioned solvent and blended it for 10 minutes. All the samples were filtered again with Cellulose Acetate syringe filter (MICRO-POR Minigen syringe filter 25mm/0.2µm). All the samples were diluted twenty times (1:20) with methanol and then analyzed by HPLC-DAD aparatus.

RESULT AND DISCUSSION

The pesticide residue was measured by calculating the sample peak area and standard peak area of malathion. A total 10 culture samples containing *Anabaena spiroides* and *Nostoc punctiformae* with different concentrations(100ppm, 200ppm,300ppm,400ppm and 500ppm) of malathion were analysed by HPLC. Concentration of malathion in the analysed samples are outlined in table no.1.

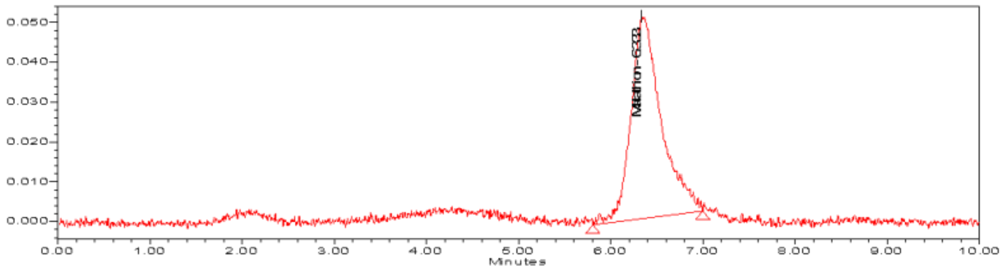


Figure no.1: Chromatogram of Analytical Standard of malathion.

Table no. 1: Concentration of malathion in the culture media after the growth of *Anabaena spiroides* and *Nostoc punctiformae*.

Cyanobacterial sample	Initial Pesticide Concentration (ppm)	Solvent used	Retention time (minutes)	Sample peak Area (m^3)	Pesticide residue detected (ppm)	% of loss of pesticide residue
Anabaen spiroides	100	Methanol	6.344	ND	ND	ND
	200	Methanol	6.344	ND	ND	ND
	300	Methanol	6.377	853970	147.23	50.92
	400	Methanol	6.342	1656829	285.65	28.58
	500	Methanol	6.392	2356086	406.21	18.75
Nostoc punctiformae	100	Methanol	6.344	ND	ND	ND
	200	Methanol	6.344	ND	ND	ND
	300	Methanol	6.366	973654	164.47	45.17
	400	Methanol	6.396	1798628	310.10	22.47
	500	Methanol	6.396	2490938	429.46	14.10

*ND-Not Detected

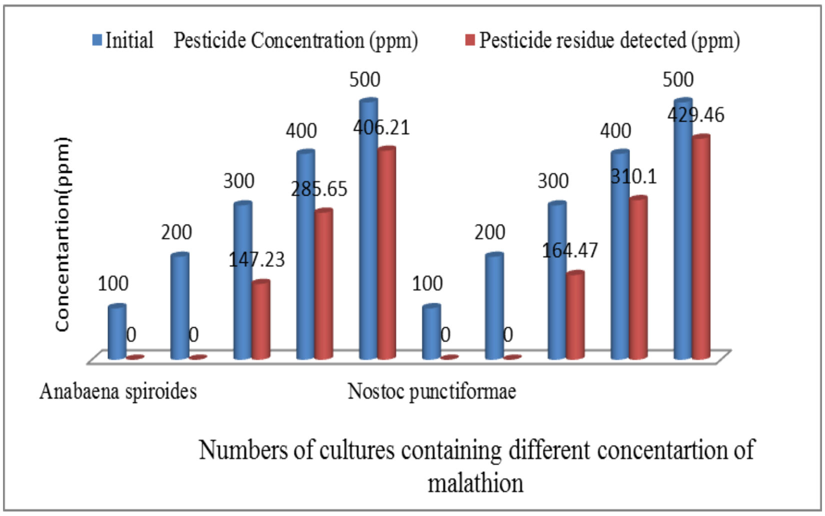


Figure no.2: Plots of concentration (ppm) of malathion residue in culture after blue green algal growth after 28 day of culture.

It was found from the result that after the growth of *Anabaena spiroides* and *Nostoc punctiformae* in 100ppm and 200ppm concentration of malathion no residue of malathion was detected after 28th day of culture. In *A.spiroides* the percentage of decline of malathion in 300ppm, 400ppm and 500ppm were 50.92%,28.58% and 18.75% respectively. After the growth of *N. punctiformae* its value were 45.17%,22.47% and 14.10% in 300ppm, 400ppm and 500ppm respectively. The decline of pesticide in the culture was due to biodegradation of ma; lathion after 28th day of culture as there was no other external factor for the degradation of pesticide. This fact is supported by Khalil and Mostafa, (1987) who showed that the blue green algae *Anabaena oryzae* and *Phormidium fragile* were able to break down malathion into malaxon and five additional metabolites. From the present study ita was clear that *Anabaena spiroides* degraded malathion more than that of *Nostoc punctiformae* after 28th day of culture(Figure no.2).

CONCLUSION

The present study showed that the blue green algae *A. spiroides* and *N.punctiformae* can totally degrade malathion in 100ppm and 200ppm concentration in the culture media. But in higher concentration like 300ppm, 400ppm and 500ppm the degradation process become slow and it was not completely degraded after 28th day of culture in the laboratory condition. In conclusion it can be emphasized that both these two blue green algae can play an important roles in the decontamination of organophosphorus pesticides like malathion and will be useful in the bioremediation of pollution caused by these pesticides. Malathion is often present in rice field soils and microbial degradation by these algae might be of importance for bioremediation.

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