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EVALUATION OF PHYOTO AND AQUATIC TOXICITY OF INSECTICIDE ADDITIVE TALLOWAMINEETHOXYLATE AND ITS METABOLITES PRODUCED BY *PSEUDOMONAS DESMOLYTICUM* NCIM 2112

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ABSTRACT

Insecticide additives are added into the insecticide formulations to increase the efficiency of insecticides. However, they are responsible for considerable ecotoxicity. Talloamineethoxylate, a nonionic surfactant is used as an insecticide additive in organophosphorus insecticide formulation. The present study was carried out with the objective of checking the potential of *Pseudomonas desmolyticum NCIM 2112* in the biodegradation of toxic insecticide additive tallowamineethoxylate into non toxic metabolites as well as the evaluation of phytotoxicity and aquatic toxicity of the tallowamineethoxylate and its metabolites produced by *Pseudomonas desmolyticum NCIM 2112*. The results revealed the phytotoxicity as well as aquatic toxicity of tallowamineethoxylate but reduced or no toxicity of metabolites indicating the potential application of *Pseudomonas desmolyticum NCIM 2112* in biodegradation of ecotoxic tallowamineethoxylate into their nontoxic metabolites.

KEY WORDS: Insecticides, Insecticide additives, Tallowamineethoxylate, Metabolites, *Pseudomonas desmolyticum*, Phytotoxicity, Aquatic toxicity

INTRODUCTION

Insecticides are the substances intended to prevent deterioration by insects and can be used as the plant growth regulators. They are mainly classified as Organophosphates, Neonicotinoids, Organochlorines, Carbamates and Pyrethroids. Organophosphate insecticides are the most widely used and available insecticides today. Most of them are registered for use and run the risk of toxicity tests. Insecticide additives are the part of insecticide and generally added along with the active ingredient of insecticide during formulation. They are added to compensate the time required before the action of main insecticide begins. Insecticides along with its additives remain eco healthy when their use is in a limited concentration. But if they are used in excessive amount in agriculture field, they show hazards not only to the plants but also to all living biota present on earth. The insecticide has desired value only when it controls the insects without damaging the crops. Insecticide additives show a marked effect on the growth of soil microorganisms. They not only reduce their number but also are responsible for germination inhibition of seeds (Racke *et al.*, 1997).

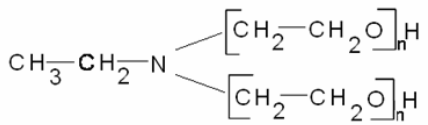

Fig. 1. Structure of tallowamineethoxylate

Tallowamineethoxylate is commonly used insecticide additive in organophosphorus insecticide formulation. Structurally, it consists of a

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amide group having at least one long alkyl chain and 2 to 50 polyoxyethylene groups covalently linked to a nitrogen atom. It enhances the efficacy of the crop protection chemicals (Roberts *et al.,* 1999). It provides good wetting of the leaf surface and facilitates the foliar penetration of the biocide under a wide range of climatic conditions and thereby enhance the activity of insecticide. Stewart *et al.* (2007) suggested the use of tallowamineethoxylate in many agrochemical formulations. It can be used in the range of 1- 99% during insecticide formulation and in preparing sprayable and bioactive agrochemical systems for controlling the pests.

Tallowamineethoxylate is a surfactant, generally used in the formulation of organophosphorus insecticides. Surfactants are a class of xenobiotics which due to their chemical nature, accumulate at interfaces including the solid/ liquid interface of stones and sediment particles in rivers. Tallowamineethoxylate is responsible for considerable ecotoxicity. It is more toxic than the active ingredient of insecticide. It is toxic to aquatic organisms particularly to the amphibians. The LC_{50} value reported is about 1.1mg/L for the single amphibian species (Howe *et al.,* 2004). It persists for longer time in soil and its high concentration hampers the growth of other soil microorganisms (Rokade and Mali, 2012).

Keeping in mind all the environmental issues associated with insecticide additive tallowamineethoxylate, the study was carried out to find out the potential of *Pseudomonas desmolyticum* NCIM 2112 in the biodegradation of insecticide additive tallowamineethoxylate into non toxic metabolites. The present work deals with the evaluation of phytotoxicity and aquatic toxicity of the tallowamineethoxylate and its metabolites produced by *Pseudomonas desmolyticum* NCIM 2112.

MATERIALS AND METHODS

Insecticide additive

The organophosphorus insecticide additive tallowamineethoxylate having 99% purity was obtained from Rhodia Chemicals Pvt. Ltd, Raigadh.

Bacterial culture

Pseudomonas desmolyticum NCIM 2112 was obtained from National Chemical Laboratory, Pune, India. It was maintained on the nutrient agar slant containing (g L^{-1}) NaCl 5.0, bacteriological peptone 10.0, yeast extract (2.0), beef extract 1.0 and agar 15.0

Biodegradation of tallowamineethoxylate

The biodegradation of tallowamineethoxylate was carried out by inoculating *Pseudomonas desmolyticum* NCIM 2112 in 100 mL mineral based medium with tallowamineethoxylate at 10 mg L^{-1} of concentration. The broth was incubated at 30 ^{o}C on orbital shaking incubator at 120 rpm. After 7 days of incubation the degradation was confirmed by using HPLC/MS method. The metabolites of biodegradation were identified as ethylenimine and acetamide on the basis of results of mass spectrometry.

Metabolites

The metabolites were extracted from the invitro biodegradation experiment using organic solvent extraction method with ethyl acetate as a solvent. The ethyl acetate fraction was collected in 100 mL beaker and allowed to evaporate in water bath at 30 ^{o}C. The obtained residues of degraded metabolites were diluted with equal amount of distilled water and used to study the phytotoxicity and aquatic toxicity.

Plant seeds

The seeds of *Sorghum bicolor, and Oryza sativa* were obtained from Maharashtra Hybrid Seeds Company Limited, Jalna.

Phytotoxicity study

The toxicity of the tallowamineethoxylate and its metabolites on germinating seeds was assessed by treating the seeds of *Sorghum bicolor* and *Oryza sativa*. The study was carried out at room temperature (27± 2^{0}C). The parameters used to determine the toxicity were germination inhibition percentage with respect to the height of roots and shoot of germinating seeds. Distilled water was used as a control throughout the study.

Aquatic toxicity

The use of insecticide with its additives in agriculture land results their leaching into water reservoirs. As these sources are linked with river, the insecticide additives can easily cause water contamination. Therefore, it is important to assess the toxicity of tallowamineethoxylate on fishes. The aquatic toxicity study was assessed by studying the effect of tallowamineethoxylate and its metabolites

on the fish *Labeo rohita* by the method as described by Sakr and Lail (2005).

Maintenance of fish

Fishes (*Labeo rohita*) were collected from fish breeding pond having no known insecticidal exposure history. They were 14 cm in length and 50 g in weight. They were kept in glass tanks filled with dechlorinated tap water at 27±1 °C, pH 7.0 and dissolved oxygen 6.4±0.1mgL^{-1}.The dissolved oxygen concentration was measured as per the method described by Welsh and Smith (1953). The fish density was two fishes per liter. They were allowed to acclimatize under such conditions for about 4 days. During this period fish were fed with rice bran and oilcake. After acclimatization, fishes of about same size (weight and length) irrespective of sexes were selected for the experiment. Feeding was stopped one day before the commencement of the experiment.

Effect on oxygen consumption by the fish *Labeo rohita*

The toxicity was assessed in terms of rate of oxygen consumption by fish *Labeo rohita.* The amount of dissolved oxygen consumption is related with the metabolic rate and mortality of fishes. Decrease in oxygen consumption rate by fish indicates disturbance in metabolism that results in mortality.

Determination of LC_{50} value

To study the LC_{50}value of tallowamineethoxylate and its metabolites, they were dissolved in the reagent grade acetone. Five different concentrations from 0.1 to 0.3 ppm were prepared and used. Control set of water having no exposure of any chemical was run in parallel. The concentration of additive and metabolite where mortality of *Labeo rohita* is half was considered as LC_{50} value.

Histopathological changes in liver of fish *Labeo rohita*

After the LC_{50} value determination, the fishes they were dissected. The liver was removed and small pieces were fixed in 10% formalin and Carnoy's fluid. The fixed samples were dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Sections of 6 microns thickness were cut, mounted and stained with different stains according to the target of investigation. For histopathological investigations, 10% formalin - fixed sections were stained with haematoxylin and eosin.

Statistical analysis

All the experiments were carried out in triplicate. Analysis of the variants was carried out on all data at $P< 0.05$ using Graph Pad software. (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA) and LC_{50} was determined by Trimmed Spearman-Karber (TSK) Program Version 1.5.

RESULTS AND DISCUSSION

Phytotoxicity of tallowamineethoxylate and its metabolites

The phytotoxicity of talloamineethoxylate and its metabolites in terms of germination inhibition percentage of *Sorghum bicolor* and *Oryza sativa* is shown in Table 1. As indicated in the table, the seeds of *Sorghum bicolor* shows 90% germination inhibition in presence of tallowamineethoxylate at 10 ppm of concentration while there was germination equal to that of control in metabolite treated set. In all other parameters like shoot length and root length tested, metabolites were found to have almost negligible effect on both the plant seeds. In control and in presence of metabolites, better root and shoot length was observed. Similarly, in case of *Oryzas ativa*, 80% germination

Table 1. Phytotoxicity study of tallowamineethoxylate on *Sorghum bicolor* and *Oryza sativa*

Compound	*Sorghum bicolor*			*Oryza sativa*		
	Germination Inhibition (%)	Shoot length (cm)	Root length (cm)	Germination Inhibition (%)	Shoot length (cm)	Root length (cm)
Distilled water	0.0	7.6±0.333	16.6±0.333	0.0	7.2±0.057	10.5± 0.166
Tallowamineethoxylate (10ppm)	90	1.1±0.288	3.2±0.333	80	2.0± 0.057	1.2±0.152
Metabolites	0.0	7.2±0.333	18.5±0.577	0.0	15.5±0.288	12.2±0.057

-Values are mean of ±SEM of three experiments.

inhibition was observed in presence of 10ppm of tallowamineethoxylate while there was no germination inhibition in control set and in presence of the metabolites. Further, there was better root and shoot length in presence of metabolites (Fig. 2 and 3).

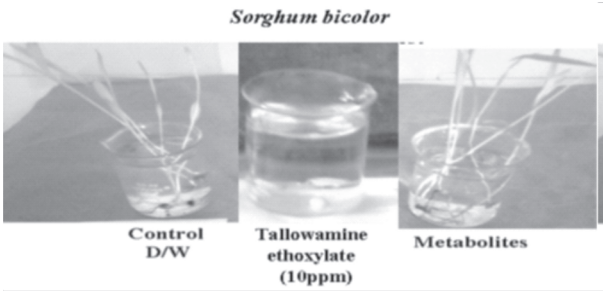

Fig. 2. Phytotoxicity of tallowamineethoxylate and its degraded metabolites on *Sorghum bicolor*

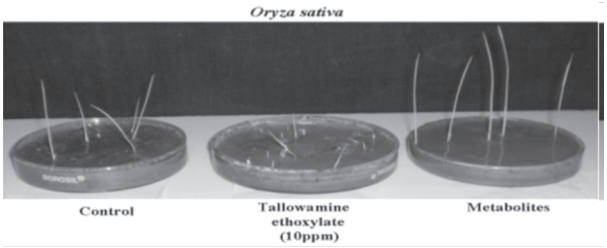

Fig. 3. Phytotoxicity of tallowamineethoxylate and its degraded metabolites on *Oryza sativa*

Aquatic toxicity study of tallowamineethoxylate and its metabolites

Effect on oxygen consumption rate (mL/g/hr) by the fish *Labeo rohita*

Table 2 indicates rate of oxygen consumption at the lethal concentration of tallowamineethoxylate during 1, 2, 3 and 4 hrs of exposure. It is found to be decreasing with increased period of exposure indicating the toxicity of the compound to fish *Labeo rohita*. However, comparatively, more oxygen consumption rate in presence of metabolites and that of control was observed.

Determination of LC_{50} value of tallowamineethoxylate

As given in the **Table 3**,4 hrs LC_{50} value of tallowamineethoxylate for the fish *Labeo rohita* was 0.08 ppm. The symptoms of toxicity were drastic change in fish behavior with agitated swimming, rolling movement, swimming on the back and change in colour of the skin from normal dark pigmentation to very light pigmentation with a scald type of lesions. The fish became very weak, settled at the bottom and died. There was no behavioral change in the control and in presence of degraded metabolites.

Histopathological study of fish *Labeo rohita*

Histopathological changes in the liver of fish *Labeo rohita*

Figure 4A and 4B shows histology of normal fish liver and changes in fish liver after exposure to tallowamineethoxylate at 10 ppm concentration respectively. The main histopathological changes recorded were necrosis and vacuoles with increased number of blood sinusoids. However, in presence of degraded metabolites, any histopathological changes were not observed.

Histopathologicalchanges in hepatocytes of fish due to the exposure to xenobiotic compounds is a powerful tool to reveal sublethal effects of chemicals and to elucidate underlying modes of action. Liver and hepatocytes are considered as efficient biomarkers of contaminant exposure because the liver organ is central to numerous vital functions in basic metabolism.

Many reports on the ecotoxicity of various insecticides including organophosphates with respect to different plants and aquatic life are available. Similarly the degradation of different insecticides into nontoxic metabolites by different bacteria has been documented by many researchers. However, very little data is available on the

Table 2. Study of oxygen consumption rate (mL/g/hr) by the fish *Labeo rohita*

Toxicity introduced	Exposure periods	Control	Experiment (with tallowamineethoxylate)	Experiment (with degraded metabolites)
Acute toxicity	1 hr	0.737±0.33	0.428±0.33	0.732±0.33
	2 hr	0.626±0.33	0.325±0.33	0.621±0.33
	3 hr	0.515±0.33	0.210±0.33	0.512±0.33
	4 hr	0.315±0.33	0.165±0.33	0.315±0.33

-Values are mean of ±SEM of three experiments

Table 3. LC_{50} determination for *Labeo rohita*

Toxicity introduced	Concentration (ppm)	Number of exposed fishes	Mortality
Acute toxicity	0.00	10	0.00
	0.01	10	0
	0.02	10	0
	0.03	10	0
	0.04	10	0
	0.05	10	1
	0.06	10	2
	0.07	10	4
	0.08	10	5
	0.09	10	6
	0.1	10	8

Spearman Karber Trim : 20.00%
LC_{50} : 0.08
95% Lower confidence : 0.07
95 % Upper confidence : 0.09

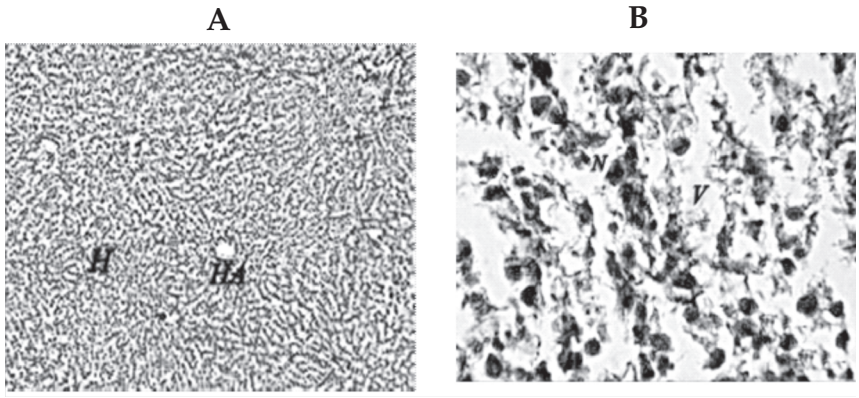

Fig. 4. Histopathology of fish liver exposed to tallowamineethoxylate
A) Section in the liver of a control fish. H: hepatocytes, HA-Hepatic artery
B) Shows necrosis (N) and vacuoles (V) in liver with increased blood sinusoids

biodegradation of insecticide additives as well as on the ecotoxicity of insecticide additives and their metabolites. Therefore, the results of the present investigation aware about the ecotoxicity of tallowamineethoxylate and indicate the potential application of *Pseudomonas desmolyticum* NCIM 2112 in biodegradation of eco toxic tallowamine-ethoxylate into their nontoxic metabolites.

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