International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **1(4),** 56-60, August **(2012)** *I. Res. J. Biological Sci.*

Biodegradation of tallowamine ethoxylate by *Pseudomonas desmolyticum* NCIM 2112

Rokade Kedar B.[1*] and Mali Gajanan V.[2]
[1]JJT University, Vidyanagari, Jhunjhunu, Rajasthan, INDIA
[2]Bharati Vidyapeeth's M.B.S.K. Kanya Mahavidyalaya, Kadegaon, Dist. Sangli, MS, INDIA

Available online at: www.isca.in
Received 17th July 2012, revised 27th July 2012, accepted 30th July 2012

Abstract

Nonionic surfactants such as alkylamine ethoxylates is generally added in insecticide formulation as an additive. Tallowamine ethoxylate is one of such additive used in the formulation of many insecticides. Large deposition of insecticide additives in agriculture farm results in the infertility of soil. The deteriorating health of soils and plants has therefore, drawn the attention of researchers as to how the soil fertility and associated activities of microorganisms can be protected. The present research work describes the biodegradation of tallowamine ethoxylate by Pseudomonas desmolyticum NCIM 2112. Soil degradation study shows the complete degradation of compound with decrease in organic matter content and increase in number of soil microflora and CO_2 evolution rate. The phytotoxicity indicates retarded growth and germination inhibition of treated seeds of Sorghum bicolor and Oryza sativa. Pseudomonas desmolyticum NCIM 2112 degrades tallowamine ethoxylate into compounds like ethylenimine and acetamide which are nontoxic in nature.

Keywords: Insecticide, tallowamine ethoxylate, pseudomonas, soil fertility, phytotoxicity.

Introduction

Insectides are used to control the pests in agriculture field and they are more commonly used for those agricultural crops which are commercially important[1,2]. Pests cause a considerable agronomic damage to the crops and depending upon the climatic changes on agriculture the pest concentration also changes[3]. Most frequently used insecticides to control the pests are from organophosphates and neonicotinoids class[4]. Arthopod pests such as Myzus *persicae, Tetranychus urticae, Pluetella xylostella, Culex spp., Tribolium castaneum* etc. are commonly controlled by the use of organophosphrous group of insecticide.[5]

The insecticides are usually formulated in such a way that they are effective in less duration of time. To compensate the time required for controlling the pest, the active ingredient of insecticide is generally mixed with additives[6]. Use of additives like benzothiazole, benzyl benzoate, tallowamine ethoxylate is common in controlling the pest[7]. These additives generally found in neonicotinoid class of insecticides[8, 9]. Similarly, the Organophosphrous class of insecticide is also formulated with additive like tallowamine ethoxylate and its use in glyphosate formulation is common[10]. As far as the structure of ethoxylated fatty amines is concerned, it consist of a amide group having at least one long alkyl chain and 2 to 50 polyoxyethylene groups covalently linked to a nitrogen atom. These chemicals are used in a wide range of agro-adjuvants, wetting agents and emulsifiers. The tallowamine ethoxylate is more toxic than the active ingredient of insecticide. It is more toxic for aquatic organisms particularly for the amphibians. The LC50 value reported is about 1.1 mg/L for the single amphibian species[11]. Addition of pesticides affects the microbial components of an ecological niche and therefore effect is observed on biotransformation reaction occurring in soil[12]. Certain bacteria posses the ability to survive under pesticide contaminated soil[13]. The tallowamine ethoxylate persist for longer time in soil and its high concentration hampers the growth of other soil microorganisms. Because of such toxic properties of this additive in soil and aquatic habitat, it is necessary to study its biodegradation to make ecotoxic free environmental practices. As the bioaccumulation of pesticide by plants is increased, use of organic food in diet became necessary[14,15]. The biological detoxification and biodegradation is the only way to minimize the toxicity of this compound from environment. This investigation deals with biodegradation of tallowamine ethoxylate by *Pseudomonas desmolyticum* NCIM 2112.

Material and Methods

Insecticide additive: The tallowamine ethoxylate (99.99%) was purchased from Rhodia chemicals, Raigad, India.

Growth of Bacteria: *P.desmolyticum* NCIM 2112 was grown in a mineral based medium containing 0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.0001% $FeSO_4$, 0.05% yeast extract, glucose 1.0 % and pH 7.00 at 28°C under aerobic conditions on rotary shaker at 120 rpm.

Growth on tallowamine ethoxylate: To study the degradation of tallowamine ethoxylate, *P.desmolyticum* NCIM 2112 was grown in the mineral based medium as mentioned above, but without glucose and with 10 mg L^{-1} concentration of tallowamine ethoxylate as the sole sources of carbon and

nitrogen. The flasks were incubated on rotary shaker at 120 rpm at 30^{o}C.

Extraction of the metabolites: After 7 days of incubation the broth (100 ml) was centrifuged at 10000 x g for 15 min. The supernatant obtained was used to extract metabolites with petroleum ether (1:1). The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in rotary evaporator. The obtained residue was analyzed by HPLC/MS method as reported [16,17]. The solvent system used for analysis consist of 10 g/l Magnesium chloride hexahydrate in water-methanol-dioxane (6:3:1, v/v/v) plus 9, 10-dimethoxyanthracene-2 sulphonate (DAS) as the pairing ion reagent in (34 mg/l) concentration at pH 2.5.

Effect of degradation on soil parameters and phytotoxicity: Soil sampling and preparation: 100g (dry weight equivalent) of soil sample having no known insecticide exposure was sieved through a mesh with 2mm diameter pores. The clay content and other properties of soil were studied[18]. The moisture content was adjusted to 60% for optimum mineralization of tallowamine ethoxylate. The tallowamine ethoxylate was added at a concentration of 10µg.g^{-1} and mixed well. Two sets were prepared - one containing the test compound but without any microbial inoculums while another set containing test compound and microbial inoculum having 3.5 x 10^5 cfu /ml cell density. The soils were checked after every 5 days interval up to 15 days.

Effect on organic carbon and organic matter content of soil: To study the effect of degradation on organic carbon and organic matter content of soil, the organic matter and organic carbon content of soil before and after degradation was determined [19].

Effect on microbial flora and moisture content of soil: To study the effect of degradation on microbial flora of soil, the standard plate count of soil untreated and treated with tallowamine ethoxylate, both with and without degrading organism was carried out. The effect on moisture content of soil was determined by adjusting the moisture to 60% at the beginning of the experiment (the maximum water holding capacity of soil) and then checking the change in moisture content at interval of 5 days interval up to 15 days, for both test and control soil.

Soil respiration study: The amount of CO_2 evolved with decrease in organic matter content of soil in presence of only tallowamine ethoxylate as well as in presence of both tallowamine ethoxylate and microbial inocula was determined [20]. A control set containing soil without addition of tallowamine ethoxylate and microbial inocula was run parallel.

Phytotoxicity: The improper use of insecticide causes bioaccumulation in crops which directly affect their growth. Phytotoxicity was studied by observing the effect on germination ability of seeds of *Sorghum bicolor* and *Oryza sativa*. It was studied at room temperature (27 ± 2^{o}C). Here an aqueous solution of tallowamine ethoxylate was mixed with 10g of air dried soil to get a final concentration of 10µg.g^{-1} on attaining equilibrium. Observations recorded after 15 days were percent inhibition of germination and height of root and shoot of the germinating embryo.

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)

Results and Discussion

Degradation of tallowamine ethoxylate: Culture grown on shaker at 120 rpm showed rapid decrease in concentration of tallowamine ethoxylate. This was confirmed by using HPLC/MS.

Effect of tallowamine ethoxylate degradation on soil parameters

Effects of tallowamine ethoxylate on soil parameters before and after degradation are summarized in table-1. It indicates that organic matter and organic carbon content of soil containing tallowamine ethoxylate remains constant, due to its toxicity whereas in presence of *P.desmolyticum* NCIM 2112 the organic carbon and organic matter goes on decreasing along with the biodegradation of tallowamine ethoxylate.

Soil respiration study: The effect of tallowamine ethoxylate on organic matter content of soil in absence and in presence of the microbial inoculum was determined in terms of the CO_2 evolution in µg per gram of soil after 2, 5 and 10 days of incubation. The amount of CO_2 evolved was enhanced in presence of microbial inoculum which indicates the rapid rate of organic matter degradation with tallowamine ethoxylate degradation, as compared to control samples containing only tallowamine ethoxylate. The 10 µg.g^{-1} concentration of tallowamine ethoxylate in soil was inhibitory whereas in presence of microbial inoculum soil microbial respiration was stimulated as shown in figure-2.

Phytotoxicity: As shown in table-2, germination was strongly inhibited in both the plant seeds treated with tallowamine ethoxylate whereas there was germination equal to that of control in metabolite treated set. In all tested parameters, metabolites were found to have almost negligible effect on both the plants as compared to that of tallowamine ethoxylate.

Biodegradation: The extracted tallowamine ethoxylate showed retention time of 2.625 minutes when analyzed by HPLC/MS as shown in figure-1a. Upon incubation for 7 days with *Pseudomonas desmolyticum* NCIM 2112 under aerobic condition, the newly formed compounds were detected at retention times of 1.128 and 2.825 minutes on the chromatogram figure -1b.

Proposed degradation pathway: Based on the results obtained from HPLC/MS the biodegradation pathway for tallowamine ethoxylate is proposed as shown in figure-3.

Surfactants are a class of xenobiotics which, due to their chemical nature, accumulate at interfaces including the solid/liquid interface, such as that which occurs on the surface of stones and sediment particles in rivers[21]. The bacteria *Pseudomonas* sp. DESl is capable of degrading surfactant by using enzyme like alcohol dehydrogenase and alkylsulfatase complements which are the part of its metabolism. *Pseudomonas* sp. DESl degrades surfactant dodecyl triethoxysulfate by cleaving each of the three ether bonds including the dodecyl ether bond[22]. Preliminary studies using [1-^{14}C] C_{12} $(EO)_3$ found evidence for the production of an aldehyde metabolite which was more transient in *Pseudomonas* sp. DES2 [23]. The total mineralization of the alcohol ethoxylates, alkylphenol ethoxylates by means of bacterial strain showed that it is converted into carbon dioxide, water and moderately stable intermediates. It has been previously reported that the various soil environmental conditions such as pH, moisture, temperature, nutritional factors are affected by the application of insecticides causing hazards to soil microbial activities[24]. Sonocatalytic degradation of water pollutants has been previously studied and reported the helpful strategy for the degradation of phenol and pesticide[25]. Enzyme like Glutathione S - transferase also plays an important role in detoxification of many insecticides including organophosphate pesticides and acaricides[26]. The ecotoxicity of insecticide additive with reference to organic matter, organic carbon, humic acid, trace elements of soil and phytotoxicity has been reported recently and found to be responsible for loss of soil fertility [27]. Degradation of pesticides by fungi has been previously studied and found that the fungal genera belonging to phyla ascomycota, deuteromycota and zygomycota posses ability to degrade the pesticides in certain amount for its growth and energy substrate[28].

Conclusion

P. desmolyticum NCIM 2112 degrade the tallowamine ethoxylate into ethylenimine and acetamide. The formed metabolites are non toxic in nature. Due to biodegradation of tallowamine ethoxylate its toxicity in soil as well as phytotoxicity is lowered. Similarly, the soil respiration study shows that the organic matter decomposition rate is enhanced with more CO_2 evolution due to degradation of tallowamine ethoxylate by *P. desmolyticum* NCIM 2112.

Acknowledgement

The authors are very grateful to the Principal, Bharati Vidyapeeth's MBSK Kanya Mahavidyalaya, Kadegaon, Dist. Sangli (M.S.) for extending the laboratory facilities to complete the investigation.

References

1. Uttam Kumar., Sharma P.K., and Shrivastava S., Spectrum of insect pest complex of soybean (*Glycine max* (L) Merrill) at Lambapeepal village in Kota region, India, *ISCA J Biological Sci.,* **1(1),** 80-82 **(2012)**

2. Iyer K., and Khan Z.A., Depression – A Review, *Res.J. rececnt sci.,* **1(4),** 79-87 **(2012)**

3. Suryavanshi P., Babu S., Baghel J.K., and Suryavanshi G., Impact of Climate Change on Agriculture and their Mitigation Strategies for Food Security in Agriculture: A Review, *ISCA J Biological Sci.,* **1(3),** 72-77*,* **(2012)**

4. Gerson U., Smiley R.L., Ochoa R., Mites (Acari) for Pest Control, Blackwell, Oxford, 539 **(2003)**

5. Blade R.J., Peek R.J. and Cockerill, United States Patent. Pesticides, Patent no. US 5,114,940 **(1992)**

6. Stewart J.F. and W.G. Brown., United States Patent. Agrochemical formulation aid composition and uses thereof. Patent no.US7,247,602 B2 **(2007)**

7. Pompeo M.P., Hodge R.L., United States patent. Biocidal azole emulsion concentrates having high active ingredient content, Patent no. US 2007/0270612A1 **(2007)**

8. Hansen E.J., Ada J., Williams., Hudsonville. United States Patent. Aqueous miticide containing benzyl benzoate, Patent no.6, 107,341 **(2000)**

9. Merlet S., Essonnes C., Abribat B., Marques M.D.S. and Penil V.L., United States Patent. Adjuvant compositions, Patent no.US 2011/0082037A1 **(2011)**

10. Owen S.A., Sorption and biodegradation of surfactants by biofilms on river sediment*s,* PhD thesis, University of Wales,UK **(1995)**

11. Howe C.M., Berrill M., Pauli B., Helbing C.C., Werry K. and Veldhoen N., Toxicity of glyphosate-based pesticides to four North American frog species, *Environ. Toxico.l Chem.* **23,** 1928–1938 **(2004)**

12. Bhardwaj V., and Garg N., Importance of Exploration of Microbial Biodiversity, *ISCA J Biological Sci.,* **1(3),** 78-83 **(2012)**

13. Majumdar T., Chakraborty R. and Raychaudhuri U., Study on Enzyme-Linked Immunoassay and Polymerase Chain Reaction for the Identification and Quantification of *Staphylococcus aureus* in Pure Culture and Food Samples, *ISCA J Biological Sci.,* **1(3),** 30-36 **(2012)**

14. Tena M. T., Rios A., Valcarcel M., and Sanchez-Alarcon M., Supercritical fluid extraction of organophosphorus pesticides from organge samples: Effect of solid additives on recovery, *Chromatogr.,* **46(9-10),** 524-528 **(1997)**

15. Yosini D., Market Segmentation for Organic Products in Bandung West Java, Indonesia, *Res.J.Recent Sci.,* **1(3),** 48-56 **(2012)**

16. Schreuder R.H. and Martijn A. Determination of the composition of ethoxylated alkylamines in pesticide formulation by liquid chromatography using ion pair extraction detection, *J. of Chromatogr.,* **368**,339-350 **(1986)**

17. Nakovich L., Analysis of biogenic amines by GC/FID and GC/MS, PhD thesis, Virginia Polytechnic Institute and State University, USA **(2003)**

18. Day P.R., Particle fractionation and particle-size analysis, Methods of soil analysis, Part 1: American Society of Agronomy, Inc., Madison, Wisconsin, *in* Black, C.A, (eds), 545-567 **(1965)**

19. Pompeo M.P. and Hodge R.L., United States patent. Biocidal azole emulsion concentrates having high active ingredient content, Patent no. US 2007/0270612A1 **(2007)**

20. Latif M.A., Razzaque M.A. and Rehman, Impact of some selected insecticide application on soil microbial respiration, *Pakistan J. Biol. Sci.,* **11(16)**, 2018-2022 **(2008)**

21. Stewart J.F. and W.G. Brown., United States Patent. Agrochemical formulation aid composition and uses thereof. Patent no.US7, 247, 602 B2 **(2007)**

22. Hales G., Dodgson K.S., White G.F., Jones N. and Watson G. K, Initial stages in the biodegradation of the surfactant sodium dodecyltriethoxy sulfate by *Pseudomonas* sp. strain DES1, *Appl. Environ. Microbiol.,* **l44**, 790-800 **(1982)**

23. Suryavanshi P., Babu S., Baghel J.K. and Suryavanshi G., Impact of Climate Change on Agriculture and their Mitigation Strategies for Food Security in Agriculture: A Review, *ISCA J Biological. Sci.,* **1(3),** 72-77 **(2012)**

24. Subhani A.H., Changyong X., Zhegmiao L., Min A.M., El-Ghamry., Impact of soil environment and agronomic practices on Microbial/Dehydrogenase enzyme activity in soil, *Pakistan J. Biol. Sci.,* **4**, 333-338 **(2001)**

25. Anju S.G., Jyothi K.P., Sindhu J., Suguna Y., and Yesodharan E.P., Ultrasound assisted semiconductor mediated catalytic degradation of organic pollutants in water: Comparative efficacy of ZnO, TiO_2 and ZnO-TiO_2, *Res.J.Recent Sci.,* **1 (ISC-2011),** 191-201 **(2012)**

26. Uttam Kumar., Sharma P.K., and Shrivastava S., Spectrum of insect pest complex of soybean (*Glycine max* (L) Merrill) at Lambapeepal village in Kota region, India, *ISCA J Biological Sci.,* **1(1),** 80-82 **(2012)**

27. Ghosh J.S. and Rokade K.B., Biodegradation of 2-mercaptobenzothiazolyl- (Z)-(2-aminothiazol-4-yl)-2-(tert butoxycarbonyl) isopropoxyiminoacetate by *Pseudomonas desmolyticum* NCIM 2112, *Appl. Microbiol. Biotechnol.,* **93,** 753–761 **(2012)**

28. Rohilla S.K.,and Salar R.K., Isolation and Characterization of Various Fungal Strains from Agricultural Soil Contaminated with Pesticides, *Res.J.Recent.Sci.,* **1(ISC-2011),** 297-303 **(2012)**

Table- 1
Results of treated and untreated soil

Day	Sample	Clay (%)	pH	Temp.(^{0}C)	Moisture	SPC cfu/ml	Organic matter (%)	Organic carbon (%)
-	**Agricultural soil sample**	37.4 ±0.115	7 ±0.333	30 ±0.333	60 ±0.333	180 ±0.577	0.65 ±0.003	1.25 ±0.011
5	**WT**	37.4 ±0.115	7 ±0.333	30 ±0.333	60 ±0.333	180 ±0.577	0.65 ±0.003	1.25 ±0.011
	WTI	37.4 ±0.115	7 ±0.333	30 ±0.333	60 ±0.333	180 ±1	0.65 ±0.003	1.25 ±0.011
10	**WT**	37.4 ±0.115	7 ±0.333	30 ±0.333	43 ±0.003	157 ±0.666	0.65 ±0.003	1.25 ±0.017
	WTI	37.4 ±0.115	7 ±0.333	30 ±0.333	55 ±0.333	180 ±0.333	0.48 ±0.003	0.90 ±0.005
15	**WT**	37.4 ±0.115	7 ±0.333	27 ±0.577	31 ±0.577	47 ±0.333	0.65 ±0.003	1.25 ±0.020
	WTI	37.4 ±0.115	7 ±0.333	30 ±0.333	55 ±0.881	180 ±0.881	0.34 ±0.003	0.60 ±0.014

-Values are mean of ±SEM of three experiments, SPC- Standard plate count, WT- With test compound, WTI- With test compound and microbial inocula

Table- 2
Phytotoxicity study on *Sorghum bicolor* and *Oryza sativa*

Compound/Metabolites	Plants studied					
	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
Tallowamine ethoxylate (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.

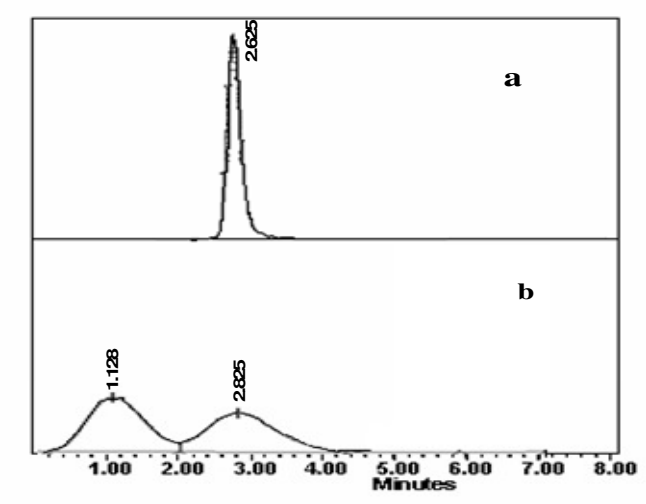

Figure-1
(a) HPLC peak of tallowamine ethoxylate,
b) HPLC peak of 7 days incubated cell free broth showing newly formed compounds

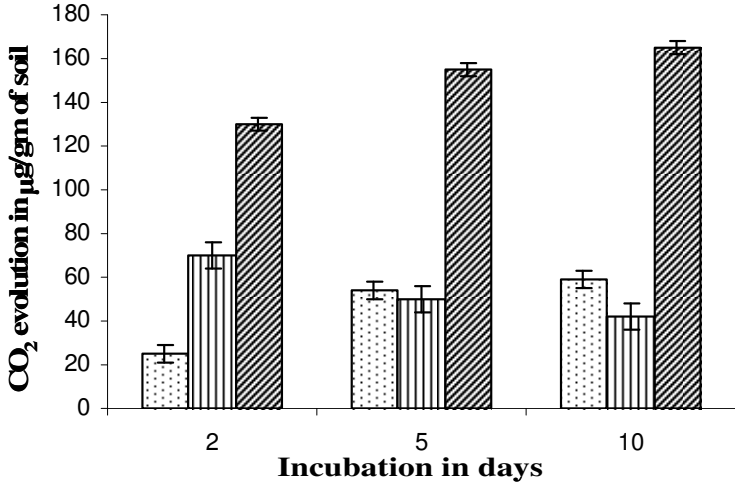

Figure-2
Soil respiration study in terms of CO_2 evolution per gram of soil
Control, with test compound, with test compound and microbial inocula. Results Obtained are mean values ± SD, n = 5.

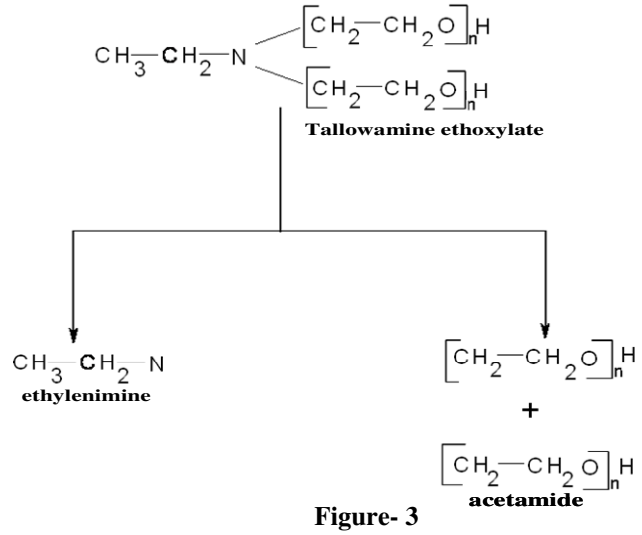

Figure- 3
Proposed pathway of tallowamine ethoxylate by *Pseudomonas desmolyticum* NCIM 2112