Research Journal of Agriculture and Forestry Sciences _____ ISSN 2320-6063
Vol. **1(5),** 13-17, June **(2013)** *Res. J. Agriculture and Forestry Sci.*

Optimization of soil parameters for Benzyl Benzoate degradation by *Pseudomonas desmolyticum* NCIM 2112

Rokade Kedar B.[1*] and Mali Gajanan V.[2]
[1]JJT University, Vidyanagari, Jhunjhunu, Rajasthan, 333001, INDIA
[2]Bharati Vidyapeeth's, MBSK Kanya Mahavidyalaya, Kadegaon, Dist. Sangli-415304, MS, INDIA

Available online at: www.isca.in
Received 5th April 2013, revised 16th April 2013, accepted 21st May 2013

Abstract

Additives are the chemical compounds generally used in the insecticides formulations. Benzyl benzoate is one of such additive generally used in the formulation of neonicotinoid insecticides. Similar to insecticide, additives are also responsible for the potential toxicity in soil environment. The present research work describes Optimization of soil parameters for benzyl benzoate degradation by Pseudomonas desmolyticum NCIM 2112. Soil degradation study shows the complete degradation of benzyl benzoate in soil with increase in number of soil microflora and CO_2 evolution rate. Due to biodegradation of benzyl benzoate by Pseudomonas desmolyticum NCIM 2112 increase in soil fertility was observed.

Keywords: Additive, benzyl benzoate, soil, biodegradation, pseudomonas.

Introduction

Insecticides are widely used in most areas of the crop production to minimize infestations caused by insects to crops[1]. Use of insecticides constitutes an important aspect of modern agriculture, as they are routinely used by farmers for pest management[2]. Additives are generally used in insecticide formulation and are responsible for toxicity to the insects[3]. Benzyl benzoate is one of such additive mainly used to eliminate lice and mites [4]. Benzyl benzoate is generally used in many products and formulations therefore it is of major environmental concern[5].

To avoid the loss in agriculture, it is imperative to use insecticides with its additives in the agriculture field to such an extent that will not arise any other ecological factors. But the overexposure of insecticide in the agriculture field results into bioaccumulation and biomagnification of these compounds in the environment[6]. Addition of insecticide affects the microbial components of an ecological niche and therefore effect is observed on biotransformation reaction occurring in soil[7]. In case of soil, smaller particles i.e. clay is responsible for adsorption of insecticide additives[8]. Immobilization of insecticidal residues takes place by the clay particles and if the overexposure of insecticide residues takes place it shows adverse effect on the growth of microorganisms[9]. Due to adsorption of insecticide additives to clay particles decrease in number of soil microorganisms has been reported and concluded that it results into slower decomposition of organic matter in soil[10]. Humic acid is one of the most active ingredients of soil organic matter. Presence of insecticide residues reduces the mobility of humic acid in agriculture soil[11,12].

Persistence of the particular insecticide additive in the soil depends upon the microbial systems present in that soil. In such cases, where innate microbial population of the soil is unable to degrade insecticides, the external addition of insecticide degrading microflora is recommended[13]. In such case, biodegradation of organophosphorus insecticide chlorpyriphos by means of *Pseudomonas desmolyticum* NCIM 2112 has been reported and concluded that by means of external addition of such insecticide degrading bacteria it is possible to reduce the residual toxicity of insecticide in soil[14].

Contaminated land due to repeated use of insecticide with its additives is a potential threat to human health and its continual use leads to adverse effect on environment. This investigation deals with Optimization of soil parameters for benzyl benzoate degradation by *Pseudomonas desmolyticum* NCIM 2112.

Methodology

Insecticide additive: Benzyl benzoate of 99.99% purity was purchased from Loba Chemicals, Mumbai, India.

Growth of Bacteria: Growth of *P.desmolyticum* NCIM 2112 was monitored in mineral based medium[14], having composition as, 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 28^0C under aerobic conditions on rotary shaker at 120 rpm.

Toxicity of benzyl benzoate on different soil parameters: 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 was determined[15]. As soil energy transfer processes takes place in presence of moisture its maintenance during the experiment was done by adding five milliliters of distilled water in the soil.

Benzyl benzoate at 10µg.g^{-1} of concentration was added in soil and mixed well. Microbial inoculum of *P.desmolyticum* NCIM 2112 at 3.5 x 10^5 cfu /ml cell density was used throughout the experiment to study the biodegradation of benzyl benzoate in soil. A control set of fertile soil containing neither benzyl benzoate nor *P.desmolyticum* NCIM 2112 was run parallel to the experiment.

Toxicity on organic carbon and organic matter content of soil: The toxicity of benzyl benzoate on organic carbon and organic matter content of soil was assessed by preparing two sets as - one containing the benzyl benzoate but without *P.desmolyticum* NCIM 2112 while another set contains benzyl benzoate and *P.desmolyticum* NCIM 2112. The soils samples were analyzed after every 5 days interval up to 15 days[16].

Toxicity on microbial flora of soil: The toxicity of benzyl benzoate on soil microorganisms was studied by treating the soil with benzyl benzoate and compared with the soil having *P.desmolyticum* NCIM 2112 inoculum. Effect of degradation on soil microbial flora was studied at every 5 days interval up to 15 days, for both soil samples.

Toxicity on humic acid and trace element content of soil: Initial humic acid content and trace elements of soil was determined as per the methods described previously[17]. Toxicity of benzyl benzoate on different trace elements mainly Cu^{++}, Fe^{++}, Mn^{++}, Zn^{++} at 10µg.g^{-1} of concentration alone and in presence of *P.desmolyticum* NCIM 2112 was studied. Detection of trace elements was done after 5, 10 and 15 days of incubation with *P.desmolyticum* NCIM 2112 by using atomic absorption spectrometer (Perkin Elmer A analyst 300).

Soil respiration study: Amount of CO_2 evolved from soil in presence of benzyl benzoate as well as in presence of both benzyl benzoate and *P.desmolyticum* NCIM 2112 inoculum was determined as per the method described previously[18]. In this experiment, 5 ml of benzyl benzoate solution having 10 ppm concentration was mixed with 100 gms of sieved soil. This mixture was taken in 250ml flask. To correlate the degradation of benzyl benzoate with soil respiration, 10 ml cell suspension of *Pseudomonas desmolyticum* NCIM 2112 with 3.5 x 10^5 cfu /ml cell density was added at the bottom of flask. A control set containing only 100 gms of sieved soil was also run parallel to it. To trap the CO_2 evolved due to microbial activities during incubation, *30ml* of 1M NaOH solution was taken in small glass beaker and it was placed inside the flask. The flask were then sealed and incubated at room temperature (27±1^{0}C). During incubation, the NaOH was renewed at every 2, 5 and 10 days of interval. The trapped CO2 was titrated with standard 0.1N HCl and pH was measured by pH meter (PICO, Lab India). The amount of CO_2 was expressed as µg of CO_2-C evolved g^{-1} of soil.

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

Effect of benzyl benzoate on various soil parameters: Effect on Organic carbon and Organic matter content of soil: Effects of benzyl benzoate on soil parameters before and after degradation are summarized in table-1. It indicates that soil containing benzyl benzoate shows constant percentage of organic carbon as well as organic matter. On the contrary in presence of *P.desmolyticum* NCIM 2112 the organic carbon and organic matter content decreases along with the biodegradation of benzyl benzoate.

Effect on microbial flora of soil: The effect of benzyl benzoate on soil microorganisms indicates that 10µg.g^{-1} of benzyl benzoate concentration is toxic for the growth of soil microorganism whereas in the presence of *P.desmolyticum* NCIM 2112, the number of soil microflora was found to be increased, indicating degradation of benzyl benzoate in soil.

Table-1
Effect on Organic carbon and Organic matter content of soil

Day	Sample	Clay (%)	pH	Temperature (^{0}C)	Standard plate count (SPC) cfu/ml	Organic carbon (%)	Organic matter (%)
-	Agricultural Fertile soil (Control)	37.4±0.115	7±0.333	30±0.333	180±0.577	2.27±0.008	1.32 ±0.011
5	WB	37.4±0.115	7±0.333	30±0.333	180±0.577	2.27±0.008	1.32±0.011
-	WBI	37.4±0.115	7±0.333	30±0.333	190±1	2.15±0.008	1.25±0.011
10	WB	37.4±0.115	7±0.333	30±0.333	167±0.666	2.27±0.008	1.32±0.011
-	WBI	37.4±0.115	7±0.333	30±0.333	240±0.333	1.70±0.008	0.99±0.005
15	WB	37.4±0.115	7±0.333	28±0.333	67±0.333	2.15±0.008	1.32±0.020
-	WBI	37.4±0.115	7±0.333	30±0.333	300±0.333	1.24±0.008	0.72±0.020

-Values are mean of ±SEM of three experiments, WB- With benzyl benzoate, WBI- With benzyl benzoate and *P.desmolyticum* NCIM 2112 inocula.

As shown in table-2, presence of *P.desmolyticum* NCIM 2112 in soil results into biodegradation of benzyl benzoate which reduces the soil toxicity level and thus simultaneous effect was observed on increase in trace element content of soil. Thus, correlation is observed in between organic matter degradation and increase in trace element content of soil. The ecotoxicity of insecticide additive with reference to organic matter, organic carbon and phytotoxicity has been reported recently and found that these additives are responsible for loss in soil fertility[19].

Soil respiration study: The effect of benzyl benzoate on organic matter content of soil in absence as well as in presence of the *P.desmolyticum* NCIM 2112 was determined in terms of CO_2-C evolved in $\mu g.g^{-1}$ of soil after 2, 5 and 10 days of incubation. The amount of CO_2 evolved was increased in presence of *P.desmolyticum* NCIM 2112 indicating the degradation of organic matter along with benzyl benzoate. Presence of benzyl benzoate in soil shows inhibitory effect on soil environment on the contrary in presence of *P.desmolyticum* NCIM 2112, stimulatory effects on soil microbial respiration was observed as shown in (figure-1).

Degradation of the insecticide along with its additive by the microorganisms not only depends upon the enzyme systems but also on the different environmental conditions such as, temperature, pH, water potentials and available nutrients[20]. Some of the insecticides are readily degraded by the microbes however; some are recalcitrant in nature[21]. It was previously reported that plants can fulfilled the phosphorus requirement because of the soil microorganisms having capability of insecticide degradation[22-24]. Presence of insecticides along with its additives affects this mineralization process which ultimately results in to inhibition of nitrification or N_2 fixation in soil[25]. It has been previously reported that it is possible to degrade such hazardous insecticide additives by microbes which have genetic diversity and metabolic versatility[26]. Biodegradation of organophosphorus insecticide additive tallowamine ethoxylate has been reported recently and it was concluded that such additives shows soil toxicity as well as phytotoxicity[27]. Presence of xenobiotic compound such as insecticides, dyes in the environment are always responsible for ecotoxicity, degradation of such compound was previously reported and concluded that by means of bacterial degradation, toxicity of such compounds can be lowered[28]. Use of fungi in the biodegradation of recalcitrant compounds from the environment has also been reported and concluded that fungi plays important role in the biodegradation of xenobiotic compounds[29, 30].

Table-2
Effect on Humic acid and trace element content of soil

Day	Sample	Clay (%)	Humic acid (465nm)	Leachability (ppm)			
				Cu^{++}	Fe^{++}	Mn^{++}	Zn^{++}
-	Agricultural soil (Control)	38.4±0.115	0.15±0.005	0.56±0.003	3.1±0.066	1.64±0.003	1.21±0.003
5	WB	38.4±0.115	0.15±0.005	0.45±0.003	2.1±0.066	1.54±0.003	1.15±0.003
-	WBI	39.4±0.115	0.15±0.005	0.52±0.003	4.1±0.066	1.84±0.003	1.31±0.003
10	WB	38.4±0.115	0.15±0.005	0.35±0.003	1.97±0.066	1.24±0.003	1.05±0.003
-	WBI	38.4±0.115	0.19±0.005	0.54±0.003	3.27±0.003	1.95±0.003	1.63±0.003
15	WB	38.4±0.115	0.14±0.005	0.10±0.003	0.97±0.066	1.00±0.003	1.00±0.003
-	WBI	38.4±0.115	0.20±0.003	0.56±0.003	3.96±0.003	2.16±0.003	1.92±0.003

-Values are mean of ±SEM of three experiments, WB- With benzyl benzoate, WBI- With benzyl benzoate and microbial inocula, Cu- Copper, Fe- Ferrous, Mn-Manganese, Zn-Zinc.

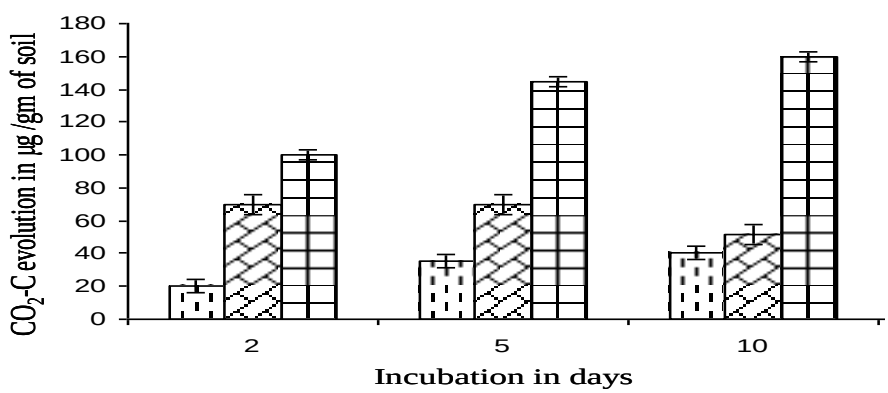

Figure-1
Soil respiration study in terms of CO_2-C evolution in µg / gm of soil
- Control, with benzyl benzoate, with benzyl benzoate and microbial inocula. Results Obtained are mean values ± SD, n = 5.

Conclusion

Soil parameters influence the degradation of insecticide additives in soil. By means of external addition of microorganisms like *P.desmolyticum* NCIM 2112 biodegradation of benzyl benzoate in soil is possible. Significant correlation is observed in between presence of benzyl benzoate with organic matter content of soil. By means of biodegradation, toxicity of benzyl benzoate in soil can be lowered. Similarly, the soil respiration study shows that the organic matter decomposition rate is enhanced with more CO_2 evolution due to degradation of benzyl benzoate by *P. desmolyticum* NCIM 2112. To avoid the problem concerned with bioaccumulation and biomagnfication of insecticide residues through soil by plant uptake we recommend the use of *P. desmolyticum* NCIM 2112 for the remediation of insecticide contaminated land.

Acknowledgement

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

References

1. Damalas C.A., Understanding benefits and risks of pesticide use, *Sci Res and Essay.,* **4(10),** 945-949 **(2009)**

2. Gouma S., Biodegradation of mixtures of pesticides by bacteria and white rot fungi, Ph.d. Thesis, School of Health Cranfield University, 416 **(2009)**

3. Ravier I., Haouisee E., Clement M., Seux R. and Briand O., Field experiments for the evaluation of pesticide spray-drift on arable crops, *Pest Manag Sci.,* **61(8)**, 728-36 **(2005)**

4. Olsen O.T., Jacobsen L. and Svendsen U.G., Effectiveness of benzyl benzoate in elimination of house dust, *Aerobiol.,* doi : 10.1007/BF02248117 **(1996)**

5. Tovey E. and Marks G., Methods and effectiveness of environmental control, *J Aller Clini Immunol.,* **103**, 179-191 **(1999)**

6. Singh J., and Singh D.K., Dehydrogenase and phosphomonoesterase activities in groundnut (*Arachis hypogaea* L.) field after diazinon, imidacloprid and lindane treatments, *Chemosphere,* **60**, 32–42 **(2005)**

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

8. Frederick T., Soils and Soil Fertility, 6th Edition, John Wiley & Sons, ISBN 978-81-265-1787-9 **(2008)**

9. Bashan Y., Inoculants of plant growth promoting bacteria for use in agriculture, *Biotech adv.,* **16(4)**, 729-770 **(1998)**

10. Omar S.A., Availability of phosphorus and sulfur of insecticide origin by fungi, *Biodegrad.,* **9**, 327-336 **(1998)**

11. Senesi N., Binding mechanisms of pesticides to soil humic substances, *Sci Total Environ.,* **123/124,** 63–76 **(1992)**

12. Wang Q. and Lemley A.T., Kinetic effect of humic acid on alachlor degradation by anodic Fenton treatment, *J. Environ Qual.,* **33,** 2343–2352 **(2004)**

13. Singh D.K., Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments, *Indian J of Microbiol.,* **48**, 35-40 **(2008)**

14. Rokade K.B., and Mali G.V., Biodegradation of chlorpyrifos by *Pseudomonas desmolyticum* NCIM 2112**,** *Int J Pharm Bio Sci.,* **4(2)**, 609–616 **(2013)**

15. 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)**

16. Walkely A., and Black I,A., An examination method for determining soil organic matter and a proposed modification of the chromic acid titration method, *Soil Sci.,* **37**, 29-38 **(1934)**

17. Faithfull N.T., Methods in agricultural chemical analysis: a practical handbook, 1st edn. CABI Publishing, UK, 68-70 **(2002)**

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

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

20. Karpouzas D.G. and Walker A., Factors influencing the ability of *Pseudomonas putida* epI to degrade ethoprophos in soil, *Soil Bio and Biochem.,* **32**, 1753-1762 **(2002)**

21. Richins D., Kaneva I., Mulchandani A. and Chen W., Biodegradation of organophosphorus pesticides by surface expressed organophophorous hydrolase, *Nature Biotechnol*, **15,** 984-987 **(1997)**

22. Subramanian G., Sekhar S. and Sampoornam S., Biodegradation and utilization of organophosphorus pesticides by cyanobacteria, *Int Biodeter and Biodegrad.,* **33**, 129-143 **(1994)**

23. Omar S.A., Effect of some pesticides on soil-borne fungi and some soil microbial biochemical processes with special reference to some processes involved in the nitrogen cycle. Ph. D. Botany Dept, University of Assiut, Egypt **(1991)**

24. Abd-Alla M.H., Use of organic phosphorus by *Rhizobium leguminosarum* biovar *viceae* phosphatase, *Biol Fertil Soils.,***18,** 216–218 **(1994)**

25. Sardar D., and Kole R.K., Metabolism of chlorpyrifos in relation to its effect on the availability of some plant nutrients in soil, *Chemosphere*, **46**, 506-510 **(2005)**

26. Siddavattam D., Khajamohiddin S., Manavathi B., Pakala S.B., and Merrick M., Transposon – like organization of the plasmid-borne organophosphate degradation (opd) gene cluster found in *Flavobacterium* sp., *Appl and Environ Microbiol.*, **69**, 2533-2539 **(2003)**

27. Rokade K.B., and Mali G.V., Biodegradation of tallowamine ethoxylate by *Pseudomonas desmolyticum* NCIM 2112, *I. Res. J. Biological Sci.*, **1(4),** 1-8 **(2012)**

28. Praveen Kumar G.N. and Bhat Sumangala K., Decolorization of Azo dye Red 3BN by Bacteria, *I. Res. J. Biological Sci.*, **1(5)**, 46-52 **(2012)**

29. Praveen Kumar G.N. and Bhat Sumangala K., Fungal Degradation of Azo dye- Red 3BN and Optimization of Physico-Chemical Parameters, *I. Res. J. Biological Sci.*,**1(2)**, 17-24, **(2012)**

30. Namdhari B.S., Rohilla S.K., Salar R.K., Gahlawat S.K., Bansal P., and Saran A.K., Decolorization of Reactive Blue MR, using *Aspergillus* species Isolated from Textile Waste Water, *I. Res. J. Biological Sci.*, **1(1),** 24-29, **(2012)**