Biodegradation of the Neonicotinoid Pesticide, Spiromesifen Using the Natural Bacterial Isolate, *Serratia* sp.

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Abstract

Background: Application of pesticides in modern agricultural practices is unavoidable which helps to increase the productivity through the reduction of pest attack. Simultaneously, enduring persistence of pesticides is the major issue in terms of environmental and health hazards. Hence, there is an urge to discover an ideal strategy to conquer this trouble.

Objectives: The present study has been designed to isolate a bacterial strain effectively degrading spiromesifen and to test its efficiency of degradation.

Materials and Methods: The bacterial strain was isolated from soil samples collected from agricultural fields after serial dilution. Based on biochemical tests, it was identified. In 2000, 3000, 4000 and 5000 ppm concentrations of spiromesifen in minimal broth, parameters like pH, CO_2 released, ammonia produced and biomass were determined for every 48 hours for ten days.

Results: The selected bacterial strain was identified as *Serratia* sp. At the time of biodegradation of spiromesifen by *Serratia* sp. significant level of variations were noted in the tested parameters.

Conclusion: From the above findings it can be concluded that the isolated bacterial strain could be used in the treatment of pesticide contaminants in agricultural fields.

Keywords: *Neonicotinoid, Spiromesifen, Serratia, bacteria, pesticide, biodegradation.*

Introduction

Pesticides are most commonly employed in agricultural practices for several decades in order to control or inhibit plant diseases and insect pests[1]. The merit of application of pesticides is increase of crop/food productivity and reduction of vector-borne diseases. Concurrently, vast utilization of these chemicals show the way for the existence of microbial imbalance, environmental and health hazards. Because of these issues, there is a great need for the development of low-cost technologies with more efficiency in safest abolition of pesticides[2,3].

Biodegradation is an ideal method through which a pesticide can be converted into a compassionate component with environmentally compatible nature. The degradation or breakdown of pesticides can proceed in the soil and water or inside the body of plants and animals. However, a large amount of

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degradation is taking place only in soil by the action of microorganisms like fungi and bacteria that utilize pesticide as food source. Methyl bromide (a soil fumigant), dalapon (a herbicide) and choloneb (a fungicide) are some of the examples for pesticides which are degraded by microorganisms[4-6].

Microbial degradation is very effective, minimally hazardous, economical, versatile and environment-friendly. Microorganisms have theability to transform and/or degrade xenobiotics. Scientists have been exploring the microbial diversity, particularly of contaminated areas in search of organisms that can degrade a wide range of pollutants[7]. The biochemical and genetic basis of microbial degradation has been substantially noticed in recent times. Enzymes have an enormous power of transformation and detoxification of polluting agents because they have been identified to be able to transform pollutants at a measurable rate and are potentially appropriate to re-establish the polluted environments. Several genes/enzymes, which provide microorganisms with the ability to degrade pesticides, have been identified and characterized[8,9]. Therefore, microorganisms supply a prospective prosperity in biodegradation of pesticides. In the present study, an attempt has been made to isolate and identify a bacterial strain, which degrades the neonicotinoid pesticide, spiromesifen and also to test its efficiency of degradation.

Materials and Methods

Spiromesifen [2 –oxo -3- (2, 4, 6- trimethylphenyl) -1 –oxaspiro [4.4] non -3 –en -4 -yl] 3, 3-dimethylbutanoate, was selected for the present study based on its broad range of application in the agricultural fields and present market trends. Spiromesifen applied soil samples were collected from agricultural fields in Paravai, Madurai, Tamil Nadu, India in sterile containers and immediately brought to the laboratory for analysis. The bacteria capable of degrading spiromesifen were isolated from the collected soil with varying concentrations of spiromesifen in the medium. The bacterial strain exhibiting highest tolerance to spiromesifen was isolated, identified and preserved for further studies. The identification and characterization of the selected bacterial strain were carried out using morphological, cultural and biochemical tests according to Bergey's manual of determinative bacteriology[10].

Isolated culture was maintained on nutrient agar slants and stored at 4°C. The maximum concentration of spiromesifen for bacterial growth was determined by the inoculation of the selected bacterial strain on minimal medium containing 2000, 3000, 4000 and 5000 ppm concentrations of commercial grade spiromesifen. The plates were incubated at 37°C for 24-48 hrs. The samples taken were then subjected for the estimation of pH, CO_2 released, ammonia produced and biomass[11,12]. The above mentioned parameters were measured every 48 hours for 10 days. All the values calculated represent the means of three observations.

The pH of the sample was checked in every two days using a pH meter. Free CO_2 was determined by titrating the samples against a strong alkali (NaOH) to pH 8. Sodium hydroxide was prepared in CO_2 free distilled water (boiled) from which 50 ml was diluted in 1000 ml CO_2 free distilled water and titrated against 100 ml of the sample. Phenolphthalein was used as the indicator and the end point is the appearance of pink colour. The free CO_2 was determined by the following formula[12].

$$\text{Free } CO_2 \text{ (mg/ml)} = \frac{\text{Titre Value} \times \text{Normality of NaOH} \times 1000 \times 44}{\text{Vol. of sample}}$$

In order to estimate ammonia, 1 ml of sample was taken in a test tube and a few drops of Nessler's reagent were added and the OD was read at 570 nm.

For estimation of biomass, 10 ml of sample was centrifuged and the pellet was collected. The pellet was dried using hot air oven at 80ºC for three hours. The dry weight of bacterial culture was determined. Two way ANOVA was performed for the parameters, pH, CO_2 released, ammonia produced and biomass using MS-Excel (Version: 12.0.6611.1000). Variability was considered significant only when the test statistic value was greater than the tabulated value at P is less than (or) equal to 0.05.

Findings

Bacterial strain isolated from the spiromesifen applied soil was identified as *Serratia* sp. based on morphological, cultural and biochemical studies. It is a Gram-negative, rod-shaped, motile bacterium and exhibited positive results in catalase, hydrogen sulphide gas production, Voges-proskauer and citrate tests. It showed negative results in oxidase, lactose fermentation, glucose fermentation, sucrose fermentation, indole and methyl red tests.

The efficiency of *Serratia* sp. on the biodegradation of spiromesifen was studied by analyzing the changes in pH, CO_2 released, ammonia produced and biomass in culture medium during the degradation. Figure 1 illustrates the changes in the pH recorded on the 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} days of treatment by *Serratia* sp. The pH of the medium increased from the 4^{th} day to 6^{th} day and decreased on 10^{th}. Thus, the increasing pH represents the degradation of spiromesifen by *Serratia* sp.

The biodegradation of spiromesifen resulted in the production of CO_2which was found to increase linearly with the concentration of spiromesifen during the degradation of spiromesifen by *Serratia* sp. The amount of CO_2 released during the 8 days treatment of spiromesifen by *Serratia* sp is shown in Fig.2. The amount of CO_2 was the maximum for 5000 ppm of spiromesifen on 10^{th} day.

Release of ammonia as a result of biodegradation of spiromesifen by *Serratia* sp. is shown in Fig.3. *Serratia* sp. effectively produced a lot of ammonia on 8^{th} day in 2000 ppm concentration and maximum was observed in 2000 ppm. Figure 4 depicts the changes in biomass of *Serratia* sp. during the study period. There was a moderate increase in the biomass which represents the increase in the bacterial growth due to utilization of pesticide by the bacteria as energy source. *Serratia* sp. exhibited the highest biomass in 4000 ppm initially but at the end of 10th day, 2000 ppm had the highest biomass and the lowest biomass was observed in 5000 ppm.

Two way analysis of variance for the factors like pH, CO_2 released, ammonia produced and biomass are given in Table 1. Variations due to spiromesifen concentration and treatment period were statistically significant at 5% level for the parameters biomass, CO_2 released, and ammonia production. In the case of pH, variations due to concentration were not statistically significant while they were significant for treatment period.

Table 1. Results of two way analysis of variance (ANOVA) for the various factors during the biodegradation of spiromesifen using *Serratia* sp.

Factor	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	Calculated F Value	Table F Value	Level of Significance
pH	Spiromesifen Concentration	0.850	3	0.283	3.317	3.490	Not Significant
	Treatment Period	5.675	4	1.419	16.610	3.269	Significant [P < 0.05]
CO2	Spiromesifen Concentration	4015.8	3	1338.6	6.368	3.490	Significant [P < 0.05]
	Treatment Period	24982.3	4	6245.6	29.71	3.259	Significant [P < 0.05]
Ammonia	Spiromesifen Concentration	0.168	3	0.056	5.245	3.490	Significant [P < 0.05]
	Treatment Period	0.150	4	0.038	3.515	3.260	Significant
Biomass	Spiromesifen Concentration	0.36	3	0.120	3.646	3.490	Significant [P < 0.05]
	Treatment Period	1.373	4	0.343	10.43	3.260	Significant [P < 0.05]

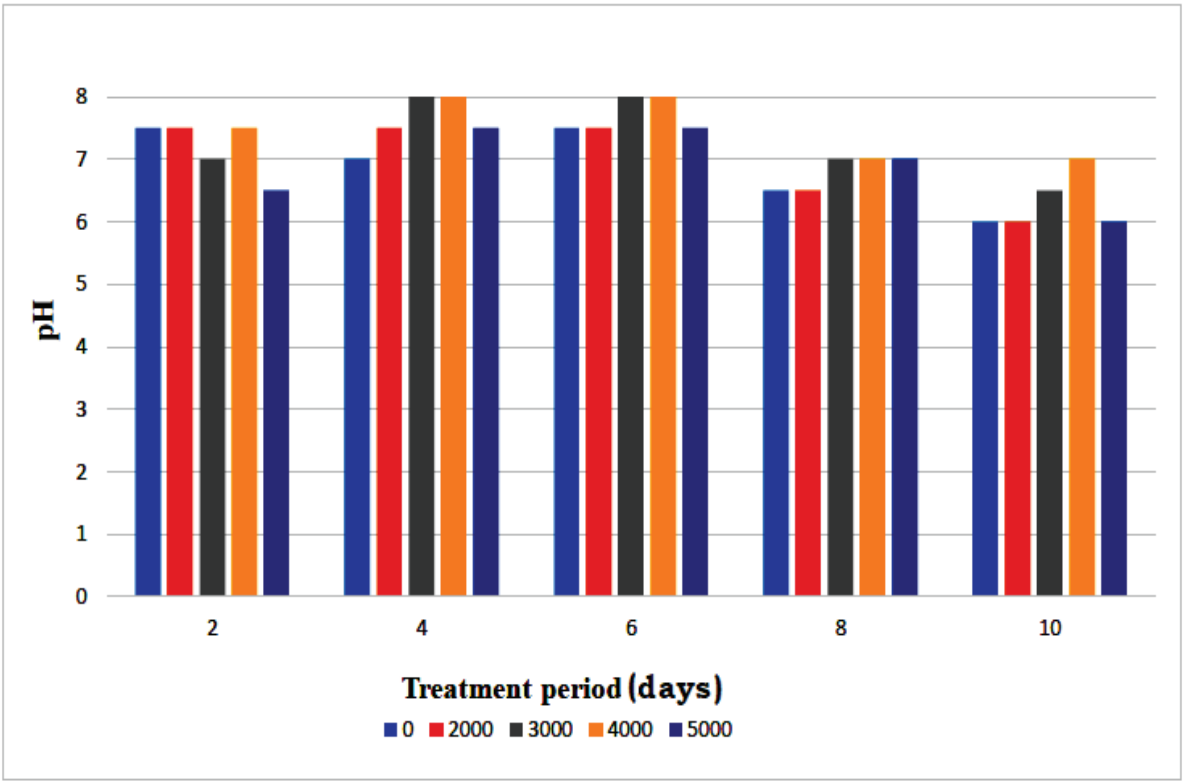

Fig. 1. Changes in pH of the medium during degradation of spiromesifen *by* *Serratia* sp.

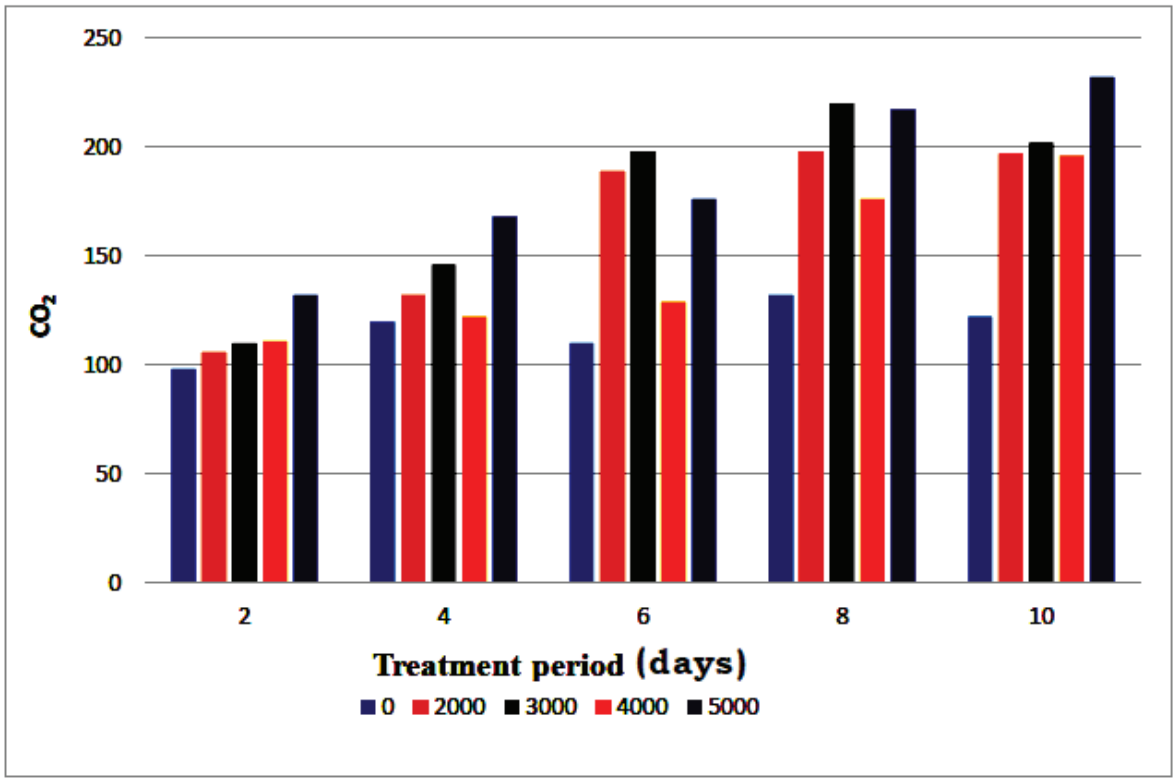

Fig. 2. Changes in CO_2 of the medium during degradation of spiromesifen *by* *Serratia* sp.

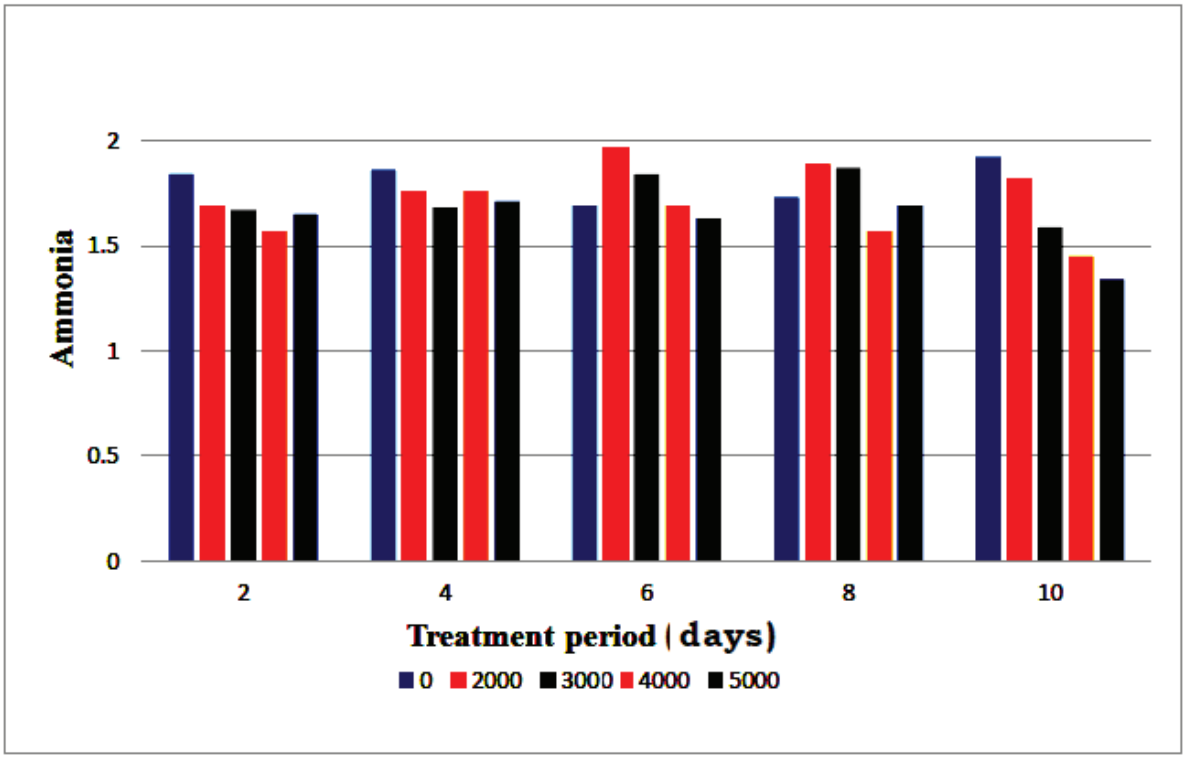

Fig. 3. Changes in ammonia concentration of the medium during degradation of spiromesifen *by* *Serratia* sp.

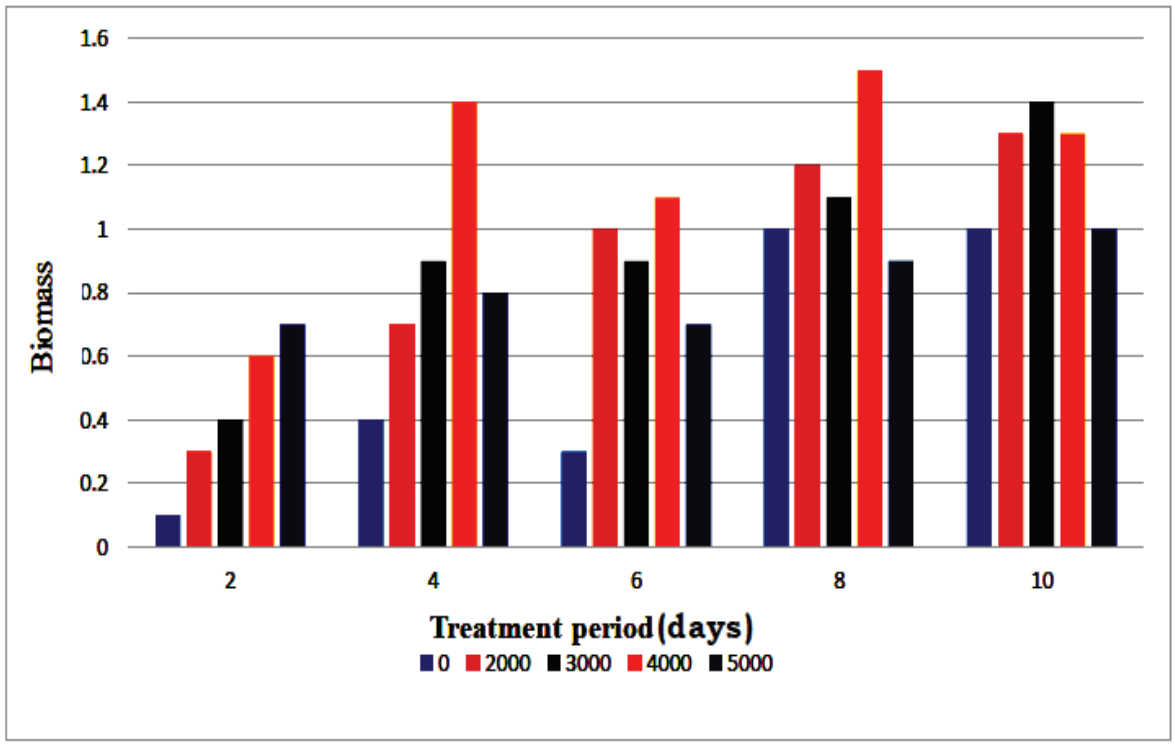

Fig. 4. Changes in biomass of the medium during degradation of spiromesifen *by* *Serratia* sp.

Discussion

Microorganisms provide a potential wealth in biodegradation and their ability to reduce the levels of xenobiotics is directly linked to their long-term adaptation to environments where these compounds are applied[13]. Moreover, genetic engineering may be used to enhance the performance of such microorganisms that have the preferred properties essential for biodegradation[14]. About 30% of agricultural produce is lost due to pests. Hence, the use of pesticides has become indispensable in agriculture. The indiscriminate use of pesticides has inflicted serious harm and problems to humans as well as to other nontarget organisms. The agricultural pesticides that are exhaustively applied to the land surface travel long distances and can move downward until reaching the water table at detectable concentrations, reaching aquatic environments at significantly longer distances. Therefore, the fate of pesticides is often uncertain; they can contaminate other areas that are distant from where they were originally used. Thus, remediation of the pesticide-contaminated areas has become a very complex task[15-17].

Neonicotinoid insecticides are developed and synthesized based on nicotine structure research with better insecticidal capabilities[9]. Various researchers have studied metabolites of degradation of neonicotinoids. Metabolism of spiromesifen by microorganisms less studied. Neonicotinoids are a kind of neuroactive insecticides. Their insecticidal principle involves the action of nicotine acetyl bile on the postsynaptic membrane of insect nicotinic acetylcholine receptors (nAChRs) while the surrounding nerves stimulate excitation resulting in paralysis and death[18-20]. The aim of the present work is to study the possibility of using the selected bacterial strain in removing the neonicotinoid insecticide.

In the present study the pH is alkaline during spiromesifen degradation. The increase in pH has led to the degradation of spiromesifen. Thus, *Serratia* sp. was able to breakdown spiromesifen effectively. Increase in biomass during the degradation period indicates the increase in bacterial growth due to utilization of pesticide by the bacteria as energy source. A moderate increase in biomass was observed during the degradation period. This indicates that the pesticide is utilized by the bacteria as an energy source. *Serratia* sp. showed an increase in biomass during the degradation study. Thus the release of CO_2 during the degradation indicates the utilization of pesticide by bacteria. The increase in CO_2 thus indicates that the pesticide is being degraded and the bacterial growth is increasing. *Serratia* sp. exhibited a gradual increase in CO_2 levels thus indicating the degradation of Spiromesifen. The ammonia produced during degradation indicates that the spiromesifen is being utilized by the bacteria. The spiromesifen break down into nitrogen compounds thus indicates the degradation. In this study, the level of ammonia increased gradually and hence it is confirmed that the spiromesifen has been degraded well by *Serratia* sp. Thus in the present study, the pH, CO_2 released, ammonia produced and biomass were monitored periodically and the results have shown a significant degradation of spiromesifen. Thus the bacteria have served good in the biodegradation of spiromesifen.

Conclusion

This study discovered the efficiency of *Serratia* sp. on the biodegradation of spiromesifen that can be beneficial for bioremediation programmes for restoring soil quality. This study will help the researchers to uncover the critical areas of using the natural isolate, *Serratia* sp. for biodegradation of pesticides. Thus a new theory on using *Serratia* sp. for treating pesticide polluted soil may be arrived at.

Acknowledgement:

The authors thank the authorities of The American

College, Madurai, Tamil Nadu, India, for the facilities and encouragement.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical permission: No ethical issues were involved.

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