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Effect of Imidacloprid on the Soil and Rhizosphere Microflora of Tea Agro-Ecosystem

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Abstract: Observations were made to determine the effect of Imidacloprid (pesticide) on the microbial population (i.e. fungi, actinomycetes and bacteria) of tea soil and rhizosphere. The experiment was conducted over a period of 65 days and the samples were taken at different interval of days (5, 20, 35, 50 and 65 days) for both rhizosphere and non rhizosphere soil. The fungal, bacterial and actinomycetes population of both rhizosphere and non rhizosphere soil was able to recover with time from the initial inhibitory effect. In case of both fungal and actinomycetes population, the pesticide treatment affected the rhizosphere microbial population more compared to the non rhizosphere. The dehydrogenase activity of the treated soil also showed a decrease at the initial stage but was able to recover with time. The overall experimental results suggest that Imidacloprid significantly affects the total microbial population of the rhizosphere adversely (i.e. fungi, actinomycetes and bacteria), which can be compared with the dehydrogenase activity of the said soil. However, the inhibitory effect was observed only at the initial stage and the population of microorganisms recovered with time.

Key words: Actinomycetes · Bacteria · Dehydrogenase activity · Fungi · Imidacloprid · Rhizosphere · Tea soil

INTRODUCTION

Rhizosphere is the hot spot of microbial interactions as the exudates released by plant roots is the main food source for microorganisms and a driving force for their population density and activity [1]. Soil is the most important site for biological interactions [2]. Rhizosphere is the region of the soil that is subjected to the influence of plant roots [3]. It encompasses the millimeters of soil surrounding a plant root where complex biological and ecological processes occur [4]. Due to the presence of large number of microorganisms in the rhizosphere, there is considerable interaction between the soil in the rhizosphere and the microorganisms which fulfill important nutritive needs, both for the plant and for the associated microorganisms [5].

Pesticides are used in the modern agriculture in large quantities to control pests and increase crop yield [6]. Despite its necessity and beneficial impacts, these chemicals eventually contaminate the soil ecosystem and pose threat to balanced equilibrium among various groups of soil inhabited microorganisms [7]. The pesticides induce stress on the plant rhizosphere and influence the microflora. These effects change the activity of the soil microorganisms and have either beneficial or deleterious effect on the crop plants and their productivity. Soil enzymes are significant because of their major contribution to recycle essential plant nutrients and degrade organic matter in soil. Dehydrogenase is thought to be an indicator of overall microbial activity because it occurs intercellularly in all living microbial cells [8]. It is considered to be a significant parameter for assessing the

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side effects of herbicide treatments on the soil microbial biomass [9]. This paper reports on the impact of Imidacloprid, an insecticide, which is widely used in tea agroeceosystem to control various insect pests, on the soil and rhizosphere microflora, estimation of the rhizosphere effect and on the soil dehydrogenase activity in the tea agro-ecosystem.

MATERIALS AND METHODS

Soil and tea samplings were obtained from the agricultural field (i.e, tea agro ecosystem) of Rosekandy Tea Estate. The soil was sieved prior to treatment with Imidacloprid. 5 kg of soil per earthen pots was taken and Imidcaloprid was applied @ 10, 100 and 1000 ppm. Healthy seedlings of tea were planted in each pot. Soil and rhizosphere soil samples for analysis were collected at 5, 20, 35, 50 and 65 days interval after the application of the said pesticide. Three pots were taken for each treatment and control. To obtain the rhizosphere samples, the complete root system was dug out and put in the polythene bags where it was tapped gently to remove loosely attached soil. Approximately 5g of the root was transferred to a 500 ml conical flask and mixed for 10 minutes. Serial dilution was prepared from this suspension. Corresponding non rhizosphere soil samples were also collected at each sampling time from the depth in the soil corresponding to the root zone. Serial dilution was prepared from this sample for the non rhizosphere fungal, bacterial and actinomycetes population. The total number of microorganisms was determined using the dilution plate method [10].

The number of colony forming units (CFU) in a gram of sample was calculated by the formula given by Angle *et al.* [11] and the rhizospheric effect was quantified by calculating the ratio between the number of microorganisms in the rhizospheric soil (R) and the corresponding number of microorganisms in the root free soil (S) i.e the R/S ratio [5].

Nutrient agar medium was used for determining the bacterial population while Rose Bengal Agar and Starch Casein Agar were used for fungal and actinomycetes population respectively.

The dehydrogenase activity of soil microbes was assayed by the method as described by Casida *et al.* [12].

RESULTS

The physicochemical properties of the experimental soil were determined and presented in Table 1.

Table 1: The Physicochemical characteristics of experimental soil

S. No.	Characteristics	Observation
1	pH	5.3
2	Moisture Content	15.15%
3	Water Holding Capacity	49%
4	Bulk Density	0.28 g/cm^3
5	Soil Porosity (%)	0.10%
6	Volumetric Water Content (g/cm^3)	0.04g/cm^3

Effect of Imidacloprid on the Rhizosphere and non Rhizosphere Soil Microbial Population: Compared to the control which showed a higher count of fungal population, the treated soil showed an inhibitory effect (Fig. 1). The population did ultimately recover and increase from the first observation on the 5th day after the application of the insecticide to the last day of observation on the 65$^{th\ day}$. The treatment showed highly significant results on the 20th day (<0.005) and 35th day (<0.01). The total population of the treated soil was lower than that of the control soil in case of the non rhizosphere soil (Fig. 2). Significant inhibitory effect was seen on the 20th day of observation (<0.04). With time it recovered from the initial inhibitory effect, which increased and maintained a constant population. The fungal population was comparatively higher in the non rhizosphere soil compared to the rhizosphere soil which shows that the rhizosphere fungal population is more sensitive to Imidacloprid than those of the non rhizosphere soil.

In the treated soil, the actinomycetes population was observed to be comparatively higher during the first observation on the 5th day which decreased suddenly during the next observation on the 20th day (Fig. 3). It however recovers from the inhibitory effect and maintains a constant population. The population of the non rhizosphere soil showed an almost similar trend as the rhizosphere soil (Fig. 4). There was no significant effect on the total population due to the insecticide.

As observed in the effect of the insecticide on the fungal population, the actinomycetes population is also higher in the non rhizosphere soil compared to the rhizosphere. This also indicates that the rhizosphere actinomycetes are more sensitive to Imidacloprid.

The bacterial population of the rhizosphere soil was inhibited by the insecticide treatment in the initial stages, however, the population increased gradually with time (Fig. 5). A slight increase in population was observed during the later observations. Immediately after the application of the insecticide, the population of the non rhizosphere bacteria reduced significantly (Fig. 6). As observed in the rhizosphere population, the non rhizosphere bacteria were also found to have recovered from the inhibitory effect of the insecticide treatment.

Table 2: Rhizospheric effect of the fungal, bacterial and actinomycetes population in the soil rhizosphere treated with Imidacloprid

	Rhizospheric effect in the tea soil treated with Imidacloprid					
	Fungi		Actinomycetes		Bacteria	
Days	Control Soil (Without Treatment)	Imidacloprid Treated Soil	Control Soil (Without Treatment)	Imidacloprid Treated Soil	Control Soil (Without Treatment)	Imidacloprid Treated Soil
5	2.26	1.62	1.17	2.09	0.80	0.78
20	1.41	1.26	1.36	0.78	0.76	0.71
35	1.32	1.19	1.11	1.09	1.41	1.02
50	1.5	1.25	1.51	1.32	1.55	1.22
65	1.61	1.5	1.48	0.97	1.68	0.89

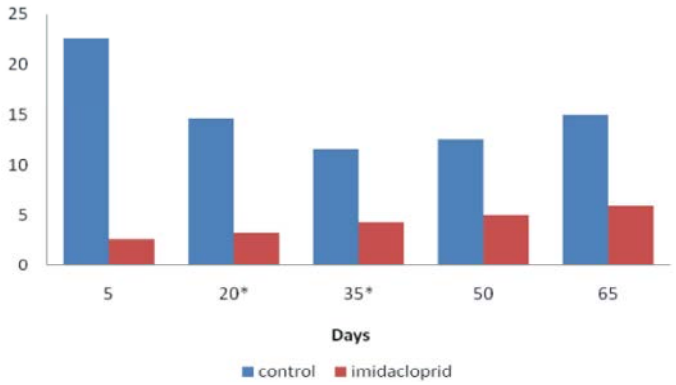

Fig. 1: The effect of Imidacloprid on the rhizosphere soil fungal population
*significant difference from the control (P<0.05)

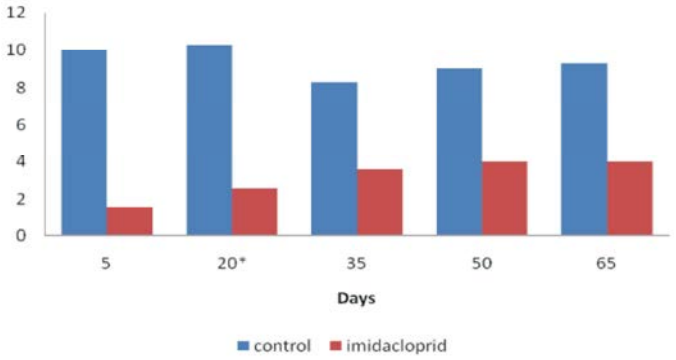

Fig. 2: The effect of Imidacloprid on the non rhizosphere soil fungal population
*significant difference from the control (P<0.05)

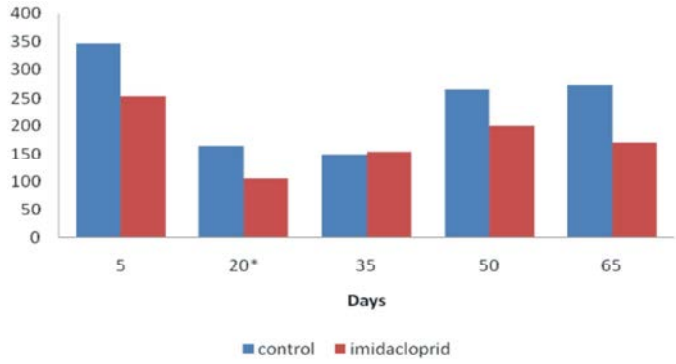

Fig. 3: The effect of Imidacloprid on rhizosphere soil actinomycetes population
*significant difference from the control (P<0.05)

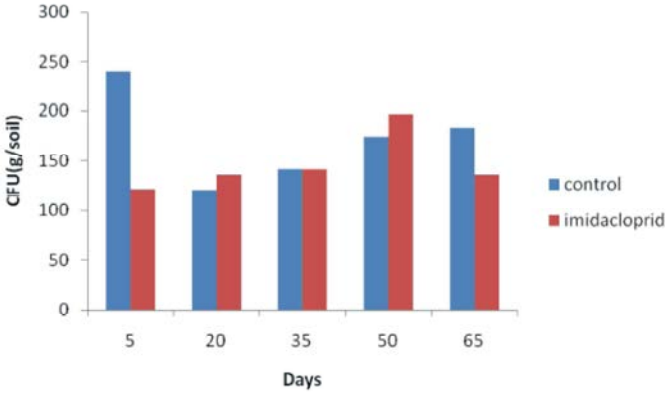

Fig. 4: The effect of Imidacloprid on the non rhizosphere soil actinomycetes population

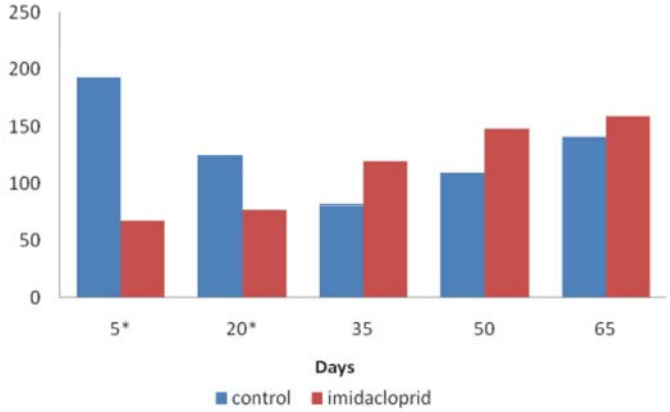

Fig. 5: The effect of Imidacloprid on the rhizosphere soil bacterial population
*significant difference from the control (P<0.05)

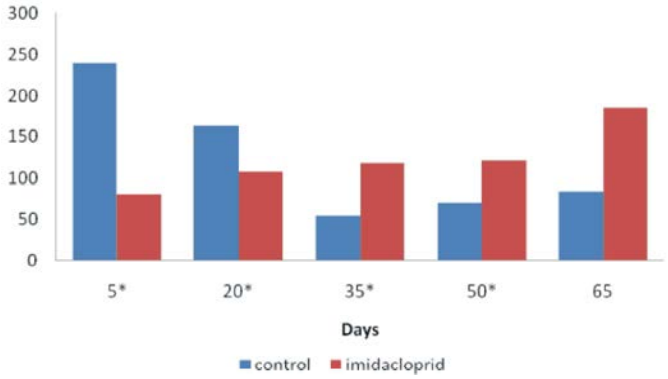

Fig. 6: The effect of Imidacloprid on non rhizosphere soil bacterial population
*significant difference from the control (P<0.05)

Assessment on the Rhizospheric Effect (R/S Ratio): A change in the rhizospheric effect was observed on the fungal population (Table 4). Here, it can be compared that the pesticide effect showed a marked stimulatory effect on the fungal population compared to the R/S ratio of the control soil. In case of the R/S ratio of actinomycetes population, the treatment showed a stimulatory effect following the first application, however during the second observation, it was drastically inhibited which again recovers but ultimately decreased on the 65th day (Table 5). The R/S ratio for the bacterial population is given in the Table 6. Here, the rhizospheric effect in the control soil was observed to be the least in the first two observations (0.80, 0.76) but then it gradually increased in

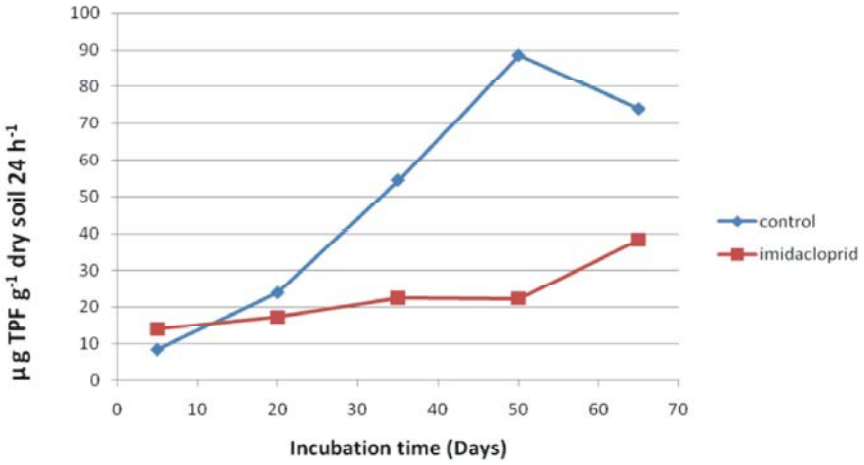

Fig. 7: Effect of Imidacloprid on dehydrogenase activity in tea soil. Dehydrogenase activity is expressed as µg TPF g^{-1} dry soil 24 h^{-1}

the subsequent observations (1.41, 1.55 and 1.68). In the treated soil, the rhizospheric effect on the bacterial population was less during the first two observations (0.78 and 0.71) than in the subsequent observations.

Effect of Imidacloprid on Soil Dehydrogenase Activity of Tea Soil: Soil dehydrogenase activity was observed under the influence of the insecticide Imidacloprid. It was observed that compared to the control the activity of the dehydrogenase enzyme was initially lower, subsequently it increased gradually with the passage of time (Fig. 7). The highest dehydrogenase activity was observed on the 65th day after the application of the insecticide (38.3 µg TPF g^{-1} dry soil 24 h^{-1}). According to Jastrzebska *et al*. [13], the activity of all the enzymes increased with time of fungicide action. Similar results were also reported by Sukul [14]. Glyphosate was also reported to have inhibited dehydrogenase activity in a sandy loam soil [15].

DISCUSSION

It is an established fact that the number of microorganisms is higher in the rhizosphere than in the non rhizosphere soil [16]. The above study shows that Imidacloprid shows a significant influence on the fungal population of the rhizosphere compared to the non rhizosphere soil. Similar results were also reported by Chauhan [17]. A study of response of soil microflora to three carbamate pesticides showed that after 35-49 days of treatment, the population of fungi and actinomycetes decreased and thereafter an increase in population was observed [18]. Omar *et al*. [6] also studied the effect of soil treatment with Brominal and Secleron on the population counts of bacteria, actinomycetes and cellulolytic fungi in soil and were tested during ten weeks of incubation. He reported that the two pesticides significantly decreased the total number of cellulolytic fungi and most fungal species. Adebayo [19] applied two insecticides separately to the soil. It was observed to have reduced the fungal and actinomycetes population, while the bacterial population was found to have significantly increased. The bacterial and actinomycetes population in the soil treated with the pesticides were stimulated at the field application rates in a study by Omar [6].

The present study concludes that the effect of the insecticide Imidacloprid was insignificant on the non rhizosphere and rhizosphere microorganisms, however the bacterial group was found to be the most affected, while the actinomycetes being comparatively resistant followed by the fungal population. It was also observed that the rhizosphere population of both the fungal and actinomycetes population were more affected compared to the non rhizosphere soil with the treatment of Imidacloprid. The dehydrogenase activity was also indicative of the physiologically active microorganisms which recovered from the initial inhibitory effects of the insecticide treatment.

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