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Research Article

Bacterial Feeding Nematodes Use for Nitrogen Mineralization and Plant Production

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Abstract

An experiment was conducted to determine the ability of nematodes that they contribute to maintaining adequate levels of plant-available N in farming systems relying on organic sources of fertility. Four types of treatments with combination of control applied into soil (tomato pots) which have tomato seedlings. The laboratory cultured nematodes were inoculated into soil containing pre-germinated tomato (*Lycopersicon esculentum* Mill.) plants and C: N ratio in soil was observed. The present study provides the direct role of microbial feeding nematodes in enhancing soil nitrogen. Nitrogen enhancement rates was calculated by TKN method (Total Kjeldhal Nitrogen). Different treatments had differed in the amount of nitrogen fixed in the soil, Pak Nema-1 treated pot showed higher amount of nitrogen as compared to other treatments. Pak Nema 1 (*Oscheius* A. n. sp.) significantly ($P < 0.001$) increased the nitrogen level in soil as compared to Pak Nema-3 and Pak Nema-4. Pak Nema-2 is also significantly increased the nitrogen level. Root length, shoot length and number of forks significantly ($P < 0.01$) increased in Pak Nema-1 while only shoot length is significantly increased in Pak Nema-2 as compared to Pak Nema-3 and Pak Nema-4.

Keywords: Bio-fertilizer; Nematode; Oscheius; Nitrogen Mineralization; Plant Production

Abbreviations

TKN Method: Total Kjeldhal Nitrogen; C: Carbon; N: Nitrogen; PO_{4}: Phosphate; P: Probability

Introduction

Soil health is the capacity of a soil to function within ecosystem boundaries to sustain in biological productivity, maintain environmental quality and promote plant and animal health. A healthy soil should be able to support life processes such as plant anchorage and nutrient supply retain optimal water and soil properties, support soil food web, recycle nutrients, maintain microbial diversity, remediate pollutants and heavy metals. Increasing use of chemical fertilizers in agriculture make country self- dependent in food production but it deteriorate environment and cause harmful impacts on living beings. Due to insufficient uptake of these chemical fertilizers by plants, they reach into water bodies through rain water, cause eutrophication in water bodies and affect living beings including growth inhabiting microorganism. The excess uses of chemical fertilizers in agriculture are costly and also have various adverse effects on soils as depletion of water holding capacity, soil fertility and disparity in soil nutrients. It was felt from a long time to develop some low cost effective and eco- friendly biofertilizers which work without disturbing nature [17,18,20].

Saprophytic nematodes have been recognized as the part of agrarian fauna and they play a important role in the ecosystem.

Usually, 50 percent nematodes fauna present in the soil are saprophytic and the ratio reaches 80% at locations of high bacteriological population [8,9,11,15-17], these are useful pointer of soil health because of their marvelous variety and their role in many function of the soil food web. Many scientists have been given the techniques to measuring the status of soil health by calculating the numbers of nematodes in different families in addition to their variety; they are beneficial indicators because of their population in response to fluctuations in moisture and temperature. Soil Saprophytic nematodes preserved the levels of plant-absorbable nitrogen in organic farming systems. The process of recycling nutrients from organic to inorganic form is termed mineralization; Nematodes involved directly to nitrogen enhancement by their feeding interactions. For example, saprophytic nematodes ingest nitrogen in the form of proteins and other nitrogenous compounds and release excess amount of nitrogen in the form of ammonium, which is readily absorbable for plant use [11,16,17]. Indirectly, nematodes enhance decomposition and nutrient cycling by grazing and rejuvenating old, inactive bacterial and fungal colonies, and by spreading bacteria and fungi to newly available organic residues. In the absence of grazers, such as nematodes and protozoa, nutrients can remain immobilized and unavailable for plant uptake in bacterial and fungal biomass. The laboratory cultured nematodes were inoculated into soil containing pregerminated plants and carbon-nitrogen ratio in soil was observed.

Material and Methods

Nematode isolation: Pak Nema-1 (*Oscheius*) and Pak Nema-2 (*Acrobeles*), were extracted from soil samples by using *Galleria* baiting technique [2]. The collected nematode populations were maintained on *Galleria mellonella* larvae as well as nutrient agar media in the laboratory. The adults were obtained by dissecting infected *G. mellonella* larvae periodically in Ringer solution. Infective Juveniles (IJs) were collected by the Petri plate assay.

Soil analysis: Soil used in this experiment was a sandy loam collected from the experimental field of National Nematological Research Centre, University of Karachi. Soil analysis was performed in the Department of Environmental studies.

Host plant: Tomato plants (*Lycopersicon esculentum* Mill.)

Experimental design: Four pots contained 250 gm soil that were treated with Pak Nema-1 *Oscheius* spp., Pak Nema-2, (*Acrobeles*), Pak Nema-3 (1 and 2), and Pak Nema-4 (Manure), with control. The nematode inoculums, 200 ml nematode juveniles in per pot used in this experiment. Tomato seeds are incubated in moistened filter paper in a Petri dish for 3 days at 20 - 25°C. After the germination of seed baby plant were transferred into pots (5 in each) and then Pak Nema1 was inoculated at the rate of 200 Juveniles/ml. After every 15 days interval root length and shoot length was taken and compared.

Statistical analysis: Root and shoot data were analyzed with two way ANOVA (days and soil treatment), with the soil treatment broken down into controls (with or without heated soil). Root length and number of forks were transformed prior to analysis.

Results and Discussion

In this experiment sterilized soil used by providing heat treatment. Four types of treatments with combination of control applied into soil (tomato pots) which have tomato seedlings. Treatments of nematodes culture obtain from the culture lab of National Nematological Research Centre, University of Karachi. Nitrogen enhancement rates was calculated by TKN method (Figure 1 and table 1). Different treatments had differed in the amount of nitrogen fixed in the soil, Pak Nema-1 treated pot showed higher amount of nitrogen as compared to other treatments. The study reported herein were conducted in green house condition where different factors are involved, may be this experiment showed better result if it is conducted on lab condition.

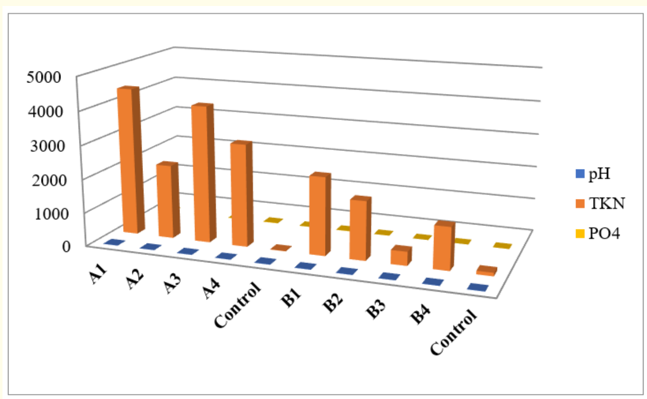

Figure 1: Enhancement of nitrogen (TKN method) by bio-fertilizer Pak Nema-1.

Treatment	Sample No	pH	TKN (mg/kg)	PO_4 (mg/kg)
After Application	Pak Nema-1A (*Oscheius* An. sp.)	5.6	4416	12.10
	Pak Nema-2 A (*Acrobeles*)	6.2	2211	9.84
	Pak Nema-3 A (1 and 2)	6.7	4068	7.39
	Pak Nema-4 A (Manure)	6.0	3050	9.17
	A5 (Control)	7.1	11.42	2.5
	Pak Nema-1 B (*Oscheius*)	8.1	2319	5.11
Before Application	Pak Nema-2 B (*Acrobeles*)	8.4	1744	3.16
	Pak NemaA-3 B (1 and 2)	6.8	413	-
	Pak Nema-4 B (Manure)	7.6	1261	-
	B5 (Control)	6.8	103	2.4

Table 1: Measured total nitrogen (By total kjeldhal nitrogen).

Soil analysis: The soil composed of 55% of sand, 27.4% silt and 16.5% clay and contained PO_4 2.5 mg/kg, total N: 11.42 mg/kg by TKN method (Total Kjeldhal Nitrogen) and the pH was 7.1. Fresh soil passed through a 2 mm mesh to remove stones, macro-fauna and discernible. The half of soil was sterilized, which contained PO_4 2.4 mg/kg, total N: 103 mg/kg by TKN method (Total Kjeldhal Nitrogen) and the pH was 6.8.

Nematode population in soil used for the enhancement of Nitrogen: Pak Nema-1 significantly ($P < 0.001$) increased the nitrogen level in soil as compare to Pak Nema-3 and -4. Pak Nema-2 is also significantly increase the nitrogen level (Figure 1).

Nematode used for the root growth development: Nematode abundance, in the soil or pot for the root growth experiment, was not significantly different between Pak nema1and Pak Nema-2 but was significantly greater ($P < 0.01$) than Pak Nema-3 and Pak Nema-4 (Figure 2).

Root and shoot growth: Root length, shoot length and number of forks significantly ($P < 0.01$) increased in Pak Nema-1 while only shoot length significantly increased in Pak Nema-2 as compare to Pak Nema-3 and 4 (Figure 2).

Figure 2: After one month root length and shoot length of: A= Pak Nema1 (*Oscheius*); B = Pak Nema2 (*Acrobelus*); C= Pak Nema1&2; D = Manure; E = Control.

Results showed that Pak Nema-1 is significantly enhanced the soil fertility or nitrogen in soil. The increase in nematode numbers, especially bacterial feeding, nematodes is directly related to the rate of decomposition of different organic amendments [8]. It is well known that bacterial feeders significantly increase soil nutrient concentrations and bacterial numbers [7].

Bacterial-feeding nematodes contribute to N mineralization by feeding on and dispersing bacteria, stimulating microbial activity and excreting mineral nitrogen. Inorganic nitrogen in the soil which supports plant growth originates mainly from biotic activities. Thus, information about the abundance, diversity and activities of different biotic groups responsible for nitrogen mineralization is of crucial importance in the management of soil productivity. Soil organic matter could represent the main source of inorganic nitrogen, even in the presence of fertilizer [4,6]. About 30% of the total inorganic nitrogen mineralized from soil organic matter is a result of microbial consumption by the soil fauna [16,17]. Microphagous nematodes, constitute important groups that influence micro-organism activity and are important regulators of decomposition and nutrient release processes [6,12]. Interactions between microbes and microphagous nematodes have mainly been studied under temperate soil conditions. The enhancing effect of bacterial-feeding nematodes on microbial population growth in soil microcosm had been reported by [12,16], they found that all the treatments containing nematodes and bacteria had higher bacterial densities than the treatments without nematodes. Many experiments have strong evidence that the bacterial feeding nematodes enhanced the nitrogen fixation [1,5,8,10] and stimulated the plant growth [3,11,13,14,16]. They significantly contribute to C mineralization and nutrient cycling, largely through feeding on bacteria and fungi. Plant secretes the food for bacteria and fungi which attracts to nematodes towards roots because they feed on bacteria and fungi. When nematodes graze on these microbes they give off CO_2 and NH_4 and increase soil fertility. Nematodes keep 1/6 of the nitrogen, they process and rest 5/6 is excreted to the soil for plant absorption. These nematodes excrete the maximum amount of nitrogen from ingested nitrogen mostly in the form ammonia that plant can use. Classical management practices along with bio-fertilizers are useful to increase soil conditions and crop productivity.

Conclusion

Above mentioned results supported this conclusion, suggesting that nematodes increased the bacterial densities, and populations of nematodes and bacteria simultaneously. There is need to use the bacterial feeding nematodes as bio-fertilizer for production of healthy plants or crops. Based on the previous studies the practical use of nematodes seems to be more appropriate as they are effective to enhance nitrogen and carbon level in soil. Nematodes use as a bio-fertilizer gave benefits in agriculture to raise productivity.

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Conflict of Interest

There is no conflict of Interest.

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