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Original Research Paper

Effect of Salinity on an Entomopathogenic Biocontrol Nematode, *Hetrorhabditis indica*

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ABSTRACT

Entomopathogenic nematodes, especially ICRI-18, a strain of *Heterorhabditis indica*, are being used as efficient biological control agent for a sub-terranean pest called *Basilepta fulvicorne* affecting cardamom plant roots. The cardamom plantation has been spread over the western ghats of south India where *H. indica* (strain ICRI-18) is used for the control of root grub. Salinity plays a significant role in microbial interactions and all inhabitants of soil. As the method of irrigation varies from place to place so does the salinity of water and soil. As salinity plays a key role in survival of soil organisms, the aim behind the present study was to investigate the compatibility of the bio-control agents with salinity. The effect of sodium chloride over *H. indica* at various concentrations (0.1M to 0.5M) was evaluated. The observations revealed that salinity did cause a proportional mortality to that of salt concentration. However, more than 80% of nematodes survived up to 0.3M concentration, after which there was a drastic reduction of the nematode population. The nematode population reduced at 0.4 M considerably, which can be concluded that salinity of the soil at cardamom plantations did not cause any adverse effect on the nematodes.

INTRODUCTION

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae have considerable potential for biological control of insect pests. The non feeding infective juvenile (IJ), carrying cells of the symbiotic bacteria, *Xenorhabdus* in gut, migrate through soil, enter a susceptible host and release the symbiont into the haemocoel. Proliferation of the bacteria leads to death of the insect within a few days, followed by nematode growth and reproduction (Gaugler & Kaya 1990). Cardamom is a shade loving plant growing in hilly slopes of Western Ghats of south India under shade of forest trees. It requires moisture in the soil all round the year. Only certain regions of cardamom plantations receive rainfall all round the year, while the other regions depend on the ground water irrigation. Sustainable agricultural practices depend on soil quality which enriches in turn the soil organisms. The soil, and the chemical composition of the soil solution, directly affect plants and nematodes, as well as nematode eggs in the soil (Herald & Heilman 1971).

Entomopathogenic nematodes have been recovered from soil in many parts of the world**.** These nematodes occur more commonly in sandy to loamy soils than in clay soils (Hara et al. 1991). Soil has a varying range of salinity from one place to another which affects the habitat of the soil ecosystem. Microbial interactions and coexistence of plants in soil will vary depending on the several abiotic factors, and salinity is one among them. Rietz & Haynes (2003) have shown that the increasing salinity and sodicity caused by irrigation has resulted in a progressively smaller, more stressed microbial community which was less metabolically efficient. Most chemical stressor-nematode combinations caused high host mortality and low infectivity rates. However, infective juvenile stimulation by the cation Mn^{2+} did enhance infectivity compared to the control (Brown et al. 2006, Shahina et al. 2005) has screened several isolates for its survival and infectivity at elevated temperature in saline solution. The exponential relationships with electrical conductivity demonstrated a highly detrimental effect that small increase in salinity had made impact on the microbial community. Plant parasitic nematodes were shown to be important modifying influence within the plant environment, either accentuating or ameliorating salinity stress effects (Maggenti & Adnan 1973). Studies conducted by Nischwitz et al. (2002) have indicated that salinity may be a factor that increased incidence of charcoal rot of melon, but not influenced the infectivity of root-knot nematode. Infective juveniles (Ijs) of six European isolates of *Herterorhabditis* were stored for up to 19 weeks at 20°C in either seawater or distilled water. The Irish and NWE isolates survived better and remained infective for longer when stored in seawater then in distilled water (Christine et al. 1993).

The aim of this study was to determine experimentally whether the sensitivity of nematodes to salinity affects the infectivity on the grub. Our intent was to check the response

of nematodes that have been little studied to those well characterized physiologically, and ecological assessment of the respective influence of specific ions and osmotic tension.

MATERIALS AND METHODS

Culture of nematode strain: The nematodes used were the local isolates of Indian Cardamom Research Institute, which are found to be effective biocontrol agent against root grubs. The strains were collected from EPN culture laboratory of ICRI and maintained in the laboratory at 25°C using greater wax moth, *Galleria melonella* larvae. Infective juveniles, used for the treatment, were freshly collected from EPN infected *Galleria* cadavers using white trap method.

Salinity tolerance: The influence of salt concentration on the nematode was analysed with freshly harvested nematodes and all experiments were conducted with five replicates.

A varying concentration of sodium chloride was prepared where salt solutions of 0.1M to 0.5M were made for treatment. Stock solution was freshly prepared at a concentration of 10M using sterile water and diluted to required concentration for treatment. Since, the local strain of *H. indica* is sensitive to the available oxygen, two modified methods of treatment were adopted. First, 25 mL of each 1X solutions were taken in separate 250 mL conical flasks and nematodes were added to make it to a concentration of 2000 nematodes per mL. In its alter, shallow water Petri dish method was used where 10mL of each treatment was taken in 15 mm diameter Petri dish with 2000 nematodes per mL. Sterile distilled water was taken as control in both the methods.

All flasks were kept at 150 rpm in rotating shaker. All treatments were incubated at room temperature for 72 h. Three samples of 50µL each were taken from each treatment and checked for number of nematodes survived, and its mean was calculated for each observation. Nematodes which did not move even after prodding were considered dead. The percentage of nematode survival was calculated for each treatment. Infectivity for all treated nematodes was analysed by infecting *Galleria melonella* larvae added in Petri plates. Mortality of larvae was checked after 2 days of incubation at room temperature.

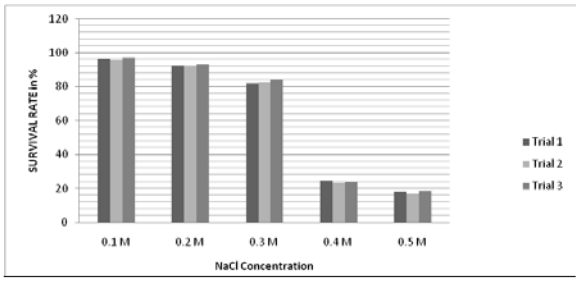

Fig. 1: Effect of salinity on survival of *Heterorhabditis indica.*

Table 1: Effect of salinity (0.1 to 0.5 Molar NaCl) on survival of the nematode *Heterorhabditis indica.*

S. No	Salinity-NaCl in Molar conc.	Survival rate of *H. Indica* in %		
		Trial 1	Trial 2	Trial 3
1	0.1	96.6	95.7	97.1
2	0.2	92.2	91.8	93.3
3	0.3	81.9	82.3	84.1
4	0.4	24.6	23.5	23.9
5	0.5	18.0	16.7	18.4

RESULTS AND DISCUSSION

The results of the study are presented in Table 1 and Fig. 1. The survival of *H. indica* was affected in a proportional way to that of saline concentration from 0.1M to 0.3M, where the survival was more than 80%. When the saline concentration was further increased to 0.4M there was a drastic reduction in the survival rate (< 25%). Infectivity was found to be unaltered at a saline concentration of 0.3M, above which the infectivity was also reduced. This observation adds evidence to the results obtained by Graham et al. 1994), where they found NaCl to be toxic and affecting the host-finding behaviour of *H. bacteriophora*. Although moderate concentrations of salts including potassium chloride and calcium chloride enhanced *H. bacteriophora* virulence, high concentrations of the salts inhibited its ability to move through a soil column and locate and infect a susceptible host (Graham et al. 1994). Other factors like pH have also been found to affect the survival of infective juveniles of *Steinernema carpocapsae* and *Steinernema glaseri.* As the tested soil pH decreased from pH 8 to pH 4, a gradual decline of infective juvenile survival was observed by 16 weeks.

Although the nematode survival rate and its infectivity has reduced at 0.4M saline concentration, we can conclude that salinity of the soil in cardamom plantations will not cause any adverse effect over nematode as it has not exceeded unfavourable limit.

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