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Root Knot Nematode Management Using Chitin Rich Fish Industry by Product in Organic Brinjal Cultivation

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Abstract: Root-knot nematode, *Meloidogyne incognita* is a major silent enemy in organic brinjal cultivation. Root-knot nematodes are sedentary vascular endoparasites that feed on plant roots and form galls that interfere with the uptake of water and nutrients in brinjal. This led to stunted plant growth and economical damages up to 80% on it. Effective management of root-knot nematode by using synthetic nematicide is detrimental to non-target organisms and the environment. Therefore, this research was conducted to explore the nematicidal effect of chitin rich shrimp and crab exoskeleton powder against *M. incognita*. Aquatic industry waste of shrimp and crab exoskeleton were selected and cleaned. Sun-dried exoskeletons were ground into fine powder by mortar and pestle separately. Five grams of each exoskeleton powder were mixed with potting media and allowed to decompose for a week. Two weeks old brinjal plants were planted and inoculated with 5 mature *M. incognita* females per pot. Experiments were carried out under Complete Randomized Design. Root-knot index scale as 0–5, plant shoot and root fresh, as well as dry weight, were taken. Data were analyzed using SAS 9.1 software and DMRT was performed to find out the best treatment combination among the treatment at $p < 0.05$. The findings revealed that all the chitin amendments treated plants exhibited a significant reduction in the extent of galls ($p < 0.05$) which indicates that chitin amendments have ability to suppress the infestation of *M. incognita*. The knot-index was 2 in crab exoskeleton powder treated plant and highly significant in comparison to untreated control (root-knot index 5). The growth rate of brinjal was significant in crab and shrimp exoskeleton powder treated plants ($p < 0.05$). Maximum dry shoot weight was achieved in crab exoskeleton powder amended brinjal (4.46 ± 0.35 g) followed by shrimp exoskeleton powder (2.66 ± 0.31 g) over untreated control (1.39 ± 0.48 g). Crab exoskeleton powder treated plant dry root weight (0.02 ± 0.01 g) lower than shrimp exoskeleton powder (0.05 ± 0.01 g) and control (0.14 ± 0.05 g) treatment but not significant among them. Based on the findings, it could be confirmed that crab and shrimp exoskeleton powder amendments effectively suppress the *M. incognita* infestation. This finding suggests that the application of chitin is a compatible and low cost ecofriendly tactic of root-knot management in organic brinjal cultivation.

Keywords: brinjal; chitin amendment; *Meloidogyne incognita*; root gall; root knot nematode

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1. Introduction

Solanaceae Family member Brinjal (*Solanum melongena* L.) is an economical important vegetable crop provide diversified nutrients to low income people in Asia and Africa [23]. It ranked among top ten vegetables those have high antioxidants such as phenols and flavonoids [10]. Brinjal is cultivated more than 1800,000 ha around the world annually and yields 50 million tons [8]. Biotic stress are majour hurdles to organic brinjal cultivation, and soil borne pathogens and pest can inflict 78% of damage on brinjal production if not monitored and managed properly [19].

Obligate endo-parasite root-knot nematode, *Meloidogyne* spp. is most dangerous polyphagous pathogen, and more than 70 species of root knot nematodes have been identified. It has been estimated that 95% of the root-knot nematode infestation are cause by four important species of genous *Meloidogyne* such as *M. incognita, M. arenaria, M. hapla* and *M. javanica* are at [11,12,21,27]. Tylenchid family member *M. incognita*'s existence is high in tropical regions and causes economical loss to more than 2000 plant species [4,6].

Root knot nematodes present in soil and affect below ground plant. Therefore, management is difficult task to the nematodes [6]. Large amount of fertilizer and pesticides are applied in brinjal cultivation [25]. Chemical management of root knot nematodes effective method [1] but which leads to hazards effects on living environment such as human, predators, pollinators and parasites like beneficial organisms. Chemical pesticides usage restricted by European Legislation (Reg. CE 396/2005 and 1095/2007) [29]. Hence entomologists found new way to manage the pest with eco-friendly manner [2].

Utilization of organic amendments are alternatives for the effective management of root knot nematode. Particularly botanicals replace the synthetic nematicides. Taye and Tefera (2013) reported that the Mexican marigold leaf, bitter leaf, lantana leaf extracts and neem (*Azadirachta indica*) and thulasi (*Ocimum sanctum*) aqueous extracts inhibit the egg hatching [20]. Castor leaf and seed (*Ricinus communis*) as well as moringa leaf and seed (*Moringa oleifera*) extracts reduce the root gall index [17]. *Syzygium aromaticum* L., *Nicotiana tobacum* L. and *Nerium oleander* leaves extracts have the ability of direct immobilising effect on second stage juveniles of root knot nematode [22,28].

Some organic amedments derive from sewage wastage, livestock manure, poultry refuse, rice bran, tea waste, dry and fresh saw dust act againt root knot nematode [7]. Different composts such as, goat manure, poultry manure and vermicompost which inhibit the extend of gall [18]. Refuge dump, rice husk and saw dust amended plants soil exhibited the 70–88% of root knot nematodes population reduction [9].

Chitin is polysaccharide with polymer of unbranched chains of β-(1→4) linked 2-acetamido-2-deoxy-D-glucose residues, and It possesses nematicidal property. Chitin amendments can be used instead of synthetic nematicides. It has been proofed that chitin induce conducive rhizosphere environment for bebeficial microbial excretions which hazard to *M. incognita* [14,24]. Moreover, chitin and chitin-urea amendments release the ammonical nitrogen which act as nematicide and combine with chitinolytic microorganism, therefore, it is good to use when fallowing period practiced [5].

2. Materials and Methodology

2.1. Inoculum Maintenance and Extraction

Root knot nematode infected plants and soil samples were collected from Thirunelvelly, Thanduvan and Omanthai by random sampling method. The *M. incognita* pure population was maintained on Thirunelvelly purple eggplant in Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna. Collected plant's root was washed under running tap water to remove soil and adhered particles. Galls on root were cut and peeled. Pear shape female *Meloidogyne* sp. was observed under stereomicroscope and isolated by fine brush and scalpel carefully by direct examination method.

2.2. Preparation of Natural Chitinous Amendment

The exoskeleton of shrimp and crab were collected and removed the inner contents of shrimp as well as crab. Cleaned exoskeleton was washed with tap water followed by distilled water. The exoskeletons were allowed to dry under sun light for 3 days. Dried exoskeletons were ground and made into fine powder by mortar and pestle separately.

2.3. Pot Preparation

This pot experiments were undertaken in seven kilogram of black polybags. Soil was sterilized and allowed to cool for two days before sowing. Each five grams of exoskeleton powder was mixed with potting media. It was stay to decomposition for one week. Watering was done during the decomposition period.

2.4. Nematode Inoculation

After one week of decomposition of amendments, three weeks old Thirunelvelly purple brinjal seedlings were transplanted into the polybags. Two days later, seedlings were inoculated with fifteen matured pear shape female nematodes. The inoculation was done around the seedling root zone by making holes. The control pots were not given any chitin amendments treatment. Seedlings were allowed to reach growth performance for a month.

2.5. Observation and Data Collection

Daily mean temperature and rainfall were recorded. One month after the inoculation brinjal plants were uprooted carefully to avoid the damage and washed to remove adhere particles on root. Data on plant growth parameters such as fresh and dry weight of shoot and root (g), shoot height (cm) and root length (cm) as well as pathological parameters of root knot index (RKI = 0–5 scale) were taken. Root knot index (Table 1) scaled according to [3] where 0 = No galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls and 5 => 100 galls.

Table 1. Effect of chitin amendments on Meloidogyne incognita.

Treatment	Length (cm)		Fresh Weight (g)		Dry Weight (g)	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Control	12.50 [c]	4.68 [b]	2.84 [c]	1.53 [a]	1.4 [c]	0.14 [a]
Shrimp	13.67 [b]	5.60 [a]	3.78 [b]	0.9 [b]	2.66 [b]	0.05 [a]
Crab	14.90 [a]	3.48 [c]	5.39 [a]	0.49 [b]	4.46 [a]	0.02 [a]

2.6. Experimental Design and Statistical Analysis

The experiment was designed in Complete Randomized Design with four replicates. Results were analyzed by SAS 9.1 software.

3. Results and Discussion

Exoskeleton powder amendments were evaluated for their pathogenicity on root knot nematode and growth of brinjal.

3.1. Influence of Chitinous Amendments on Number of Root Knot Nematode Galls

Nematicidal activity of chitinous amedments was analyzed by number and size of galls on root. Amendments of exoskeleton on chitin rich fish industry by-product to M. incognita inoculated brinjal significantly ($p < 0.05$) suppressed the root galls than control. Figure 1 illustrates that both shrimp and crab exoskeleton powder have the ability to suppress the root galling by M. incognita which demonstrated that shrimp and crab exoskeletion have efficient nematicidal activity against M. incognita. Exoskeleton powder of crab (15 ± 0.75) significantly proved most effectively suppressed the root gall formation than control (35 ± 2.0). Size of galls in shrimp and crab exoskeleton treated treatment was smaller over untreated control.

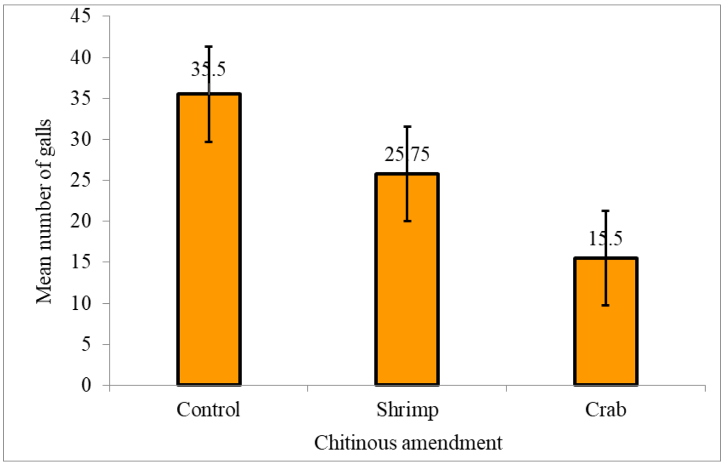

Figure 1. Root-knot variation in different chitin ammendments treatment.

3.2. Influence of Chitinous Amendments on Plant Growth

Results revealed that application of exoskeleton amendments was statistically significant ($p < 0.05$) in brinjal shoot height to improve in shrimp and crab exoskeleton treated plants over untreated control where chitin amendments treated plants exhibited highest results in taller over control. Crab exoskeleton powder treated plant achieved maximum shoot weight over shrimp exoskeleton powder treated plant and control. Meanwhile root length was higher in shrimp shell than control but root length of brinjal in crab exoskeleton amendment was lesser than control (Table 1). The results were discovered that Thirunelvelly purple brinjal sensitive to crab exoskeleton chitin amendment.

Crab exoskeleton powder amendend brinjal plant attained maximum fresh shoot weight (5.39 ±0.36 g) followed by shrimp (3.78 ±0.29 g) over untreated control (2.84 ±0.32 g). The dry shoot weight of brinjal was found highest as 4.46 ±0.36 g when plants treated with crab exoskeleton powder followed by shrimp (2.66 ±0.31 g) as compared to (1.4 ± 0.48 g) control. The highest root weight was detected when plants were treated with shrimp exoskeleton powder than crab exoskeleton treated plants in both fresh and dry basis.

This study revealed that the exoskeleton of shrimp and crab has a significant effect on M. incognita in eggplants. The exoskeleton used in our study showed a different level of nematicidal effect. Among the exoskeleton amendments, crab exoskeleton highly suppressed the extend of gall formation over shrimp exoskeleton and untreated control and the sizes of formed galls were smaller. Chitin induced the soil microorganism population which are actinomycetes, bacteria and limited number of fungus species contains chitinolytic properties since nematode's body has chitin materials. The chitinolytic properties degrade the nematode eggshell chitin, chitinase enzyme activity increase and defense mechanism activate. Chitinolytic fungi, pressure within egg cuticle with chitinase activity which cause penetration and degradation of egg [19,24]. This microbial environment pitfall to M. incognita [14], beacause significant reduction in number of galls and nematode eggs were occurred [13].

These exoskeleton amendments not only inhibit the gall formation but also induce the plant growth performance. Crab and shrimp exoskeleton powder were stimulated the brinjal shoot height as well as shoot weight in fresh and dry basis. Shoot dry matter contents increased when chitosan applied [15,19]. Within that crab powder amended brinjal exhibited maximum shoot growth. In case of root growth of brinjal, shrimp exoskeleton treated plant achieved highest root length meanwhile that was attained root weight was lesser compared as control after one month. Crab exoskeleton powder induced the shoot growth while highly suppressed the root growth performance. Hence crab exoskeleton powder treated plants root weights were significantly lower over other treatments. Previous studies reported that the ammonia release through chitin decomposition. Ammonia is toxic to roots and nematodes [24].

4. Conclusions

Shrimp shell and crab chitin materials reduced the number and size of root-knot formation in brinjal by M. incognita, and induced the plant growth and biomass accumilation. The aboundance availability of chitin materials in Sri Lnaka, farmers can use as an eco-friendly alternate to synthetic hazardus nematicieds in organic agriculture, and as an organic ammendments to increase the production and reduce the cost of production.

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