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Research Article

Evaluation of bio-agents, synthetic insecticides and organic amendment against the root-knot nematode, *Meloidogyne* spp. in cardamom [*Elettaria cardamomum* (L.) Maton]

T. SATHYAN[1], M. K. DHANYA[1*], M. MURUGAN[1], K. ASHOKKUMAR[1], T. S. ASWATHY[1], R. NARAYANA[2] and K.B. DEEPTHY[3]

[1]*Cardamom Research Station, Pampadumpara, KAU, Idukki – 685553, Kerala, India*
[2]*Department of Nematology, CoA, Vellayani, Thiruvanathapuram – 695522, Kerala, India*
[3]*Department of Agrl. Entomology, CoA, Vellanikkara, Thrissur – 680656, Kerala, India*
**Corresponding author E-mail:* dhanya_mk2000@yahoo.co.in

ABSTRACT: Investigations were made to study the effects of three nemato-pathogenic fungi viz. *Purpureocillium lilacinum*, *P. lilacinum* + neem cake, *Pochonia chlamydosporia* and *Trichoderma* sp. as well as two synthetic insecticides (cartap hydrochloride and carbosulfan) against the Root-knot Nematodes (RKN) of cardamom at the farmers' fields in three locations of Idukki district, Kerala, India during 2016-2019. Results revealed that the combined application of *P. lilacinum* with neem cake gave maximum reduction of typical symptoms of infestation like the leaf narrowing (6.42%) and root knot formation (1.32 nos.) followed by *P. chlamydosporia* with 8.04% narrowed leaves and 1.83 root knots. The application of *P. lilacinum* and neem cake in combination (0.82 kg/plant and 2.99) followed by *P. chlamydosporia* (0.71 kg/plant and 2.71 recorded highest yield and Benefit Cost Ratio, respectively). Bio-control agents are therefore efficient and environmentally safer for managing the RKN in cardamom. Future studies can be directed to focus on the compatibility and efficiency enhancement of these bio-agents with organic amendments.

KEY WORDS: Bio-agents, cardamom, Root Knot Nematode, insecticides and organic amendments, management

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INTRODUCTION

Plant-parasitic Nematodes (PPNs) are believed surreptitious foe of the farmers as they dwell in subterranean habitats, so that growers become unaware about the losses caused by them. Nematodes harm the plants by injuring and devouring on the root hairs, epidermal cells, cortical and stealer cells (Khan, 2008). In most of the cases, their damages are undescribed or are often puzzled with other problems such as pathogen infection, moisture stress or physiological disorders. By the moment the malady is detected, the loss to crops has already been escalated by these minute creatures. Abd-Elgawad and Askary (2018) reported huge losses to crops by nematodes in quantitative, qualitative and monetary terms. Many genera and species of nematodes could be economically important in crop production. In many occasions diverse population of nematodes might be present in a field, rather than a single species occurring alone (Usman and Siddiqui, 2012).

In general, the most widespread and economically significant important nematode species include Root-knot Nematode (RKN), lesion nematode, burrowing nematode, cyst nematode etc and of them, the Root-knot Nematodes often occur in crops. Root-knot Nematodes (RKN), *Meloidogyne* spp. are sedentary obligate endoparasites distributed worldwide than any other group of PPN and always out-number the other nematode species (Jones and Goto, 2011; Queneherve et al., 1995). The genus comprises of more than 100 species, but only four of them are considered as the most damaging PPN's due to its wide range of plant hosts, worldwide distribution and high reproductive capacity (Jones et al., 2013). Their feeding on plant roots result in formation of large galls or "knots" throughout the root system of the crop plants. Damage include root malformation (Wieczorek, 2015), association with other plant-parasitic micro-organisms (Shahbaz et al., 2015) and plant parasitic fungi (Francl and Wheeler, 1993), alteration in nutrient and water uptake and its translocation from roots (Williamson and Hussey, 1996) and inhibition of nodulation (Wasson et al., 2009) which in turn indirectly affect photosynthesis and respiration.

Small cardamom [*Elettaria cardamomum* (L.) Maton], proudly known as the "Queen of spices" prefers high rainfall environment and is being cultivated in medium to higher altitudes of the Western Ghats in the Indian states of Kerala, Tamil Nadu and Karnataka. Its cultivation is challenged by abiotic (higher air temperature, lower moisture, speedy surface wind etc.) and biotic stresses (pests and diseases). Among various pests of cardamom, the damage caused by PPN is somewhat severe in the recent past couple of decades (Eapen et al*.,* 2005). Root knots and leaf narrowing caused by *Meloidogyne* spp. is a major constraint against the successful cultivation of cardamom (Pervez, 2018) and extensive occurrence of *M. incognita* and *M. Javanica* has been reported both in cardamom nurseries and plantations of India (Ali and Koshy, 1982; Ali, 1986; Raut and Pande, 1986). Severe RKN infection in mature plants causes stunting, reduced tillering, yellowing, premature drying of leaf tips and margins, narrowing of leaf blades, delay inflowering, immature fruit drop and reduction in yield. Though the application of synthetic insecticides gives better control, they pose ill effects to the mountain environment as well as non-target organisms, which necessitate searching for alternative management strategies. Chemical insecticides and cultural practices have been adopted to mitigate pest populations, but they never provide a long-term suppression at economically feasible costs (Gomes et al*.,* 2010). Utilization of bio-agents and organic amendments had great scope owing to its eco-friendly nature and sustainable control. Biological control is considered as an environment friendly strategy against RKN (Eapen et al*.,* 2005; Dong and Zhang, 2006; Sun et al*.,* 2006; Silva et al*.,* 2017). The egg-parasitic fungi *Pochonia chlamydosporia* (formerly *Verticillium chlamydosporium*) and *Purpureocillium lilacinum* (formerly *Purpureocillium lilacinum*) are among the most studied biological agents aiming at nematode management across agroecosystem (Moosavi et al*.,* 2010; Carneiro et al*.,* 2011). In this context, the present investigation was formulated to evaluate the potential of select bio-agents, synthetic insecticides and organic amendment against the RKN in small cardamom.

MATERIALS AND METHODS

The study was carried out at Cardamom Research Station, KAU, Pampadumpara, Idukki district, Kerala, India during 2016-2019.

Survey and sampling

Soil and root samples were taken from the rhizosphere of select cardamom plantations from Nedumkandam and Kattappana blocks of Idukki district, Kerala. The top 3–5 cm soil and litter layer were removed and about 500 g soil and 5 g feeder roots were collected in polythene bags, wrapped and taken to the laboratory for examination of nematode population and symptoms.

Site selection for trial

The experiments were conducted in cardamom plantations affected with Root-knot Nematodes in three different locations *viz.* Vandanmedu (2016-17), Anakuthi (2017-18) and Mundiyeruma (2018-19) of Idukki district, Kerala, India.

Mobilization of bio-agents

Talc based formulation of bio-agents viz. *P. lilacinum* (NBAII isolate) and *Trichoderma* sp. (KAU isolate) and *P. chlamydosporia* (IISR isolate)each with a cfu of 10^8/g were used for the study. Neem cake and synthetic insecticides *viz.* cartap hydrochloride 50% WP (Radan) and carbosulfan 25% EC (Marshal) were purchased locally from Pampadumpara, Idukki district and applied as per the treatments protocol.

Treatment details

The experiments were carried out in five year old cardamom plantations (Variety: Green Gold) under randomized block design with seven treatments (Table 1) and three replications. Five plants were maintained in each replication as observational clumps.

Specific observations on damage and yield

Pre and post-count of nematodes in soil was enumerated by processing 200 cm^3 soils by standard Cobb's modified decanting and sieving techniques. Similarly the aerial (leaf narrowing) and root knot symptoms were recorded prior to treatment application and 30 days after that. The percent leaf narrowing was worked out to assess the extent of damage caused by the nematodes. It was calculated as number of narrowed leaves to the total number of leaves in a plant and expressed as percentage. The root knots were counted from 5 g root sample taken at random from each treatment plants. The yield in terms of dry capsule weight was taken from each plant (kg/plant) and Benefit-cost Ratio (BCR) was also worked out. Similarly the percent reduction over control was arrived by following formula,

$$P = (C-T) / C \times 100$$

Where, P = percent reduction; C = leaf narrowing in control plants; T = leaf narrowing in treated plants.

Statistical analysis

The data gathered were subjected to the Analysis of Variance (ANOVA) and later scrutinized with Duncan's

Table 1. List of bio-agents, chemical insecticides and organic amendment used in the study

S. No.	Treatment	Dose	Application interval
1.	*P. lilacinum*	10 g/lit (10 lit/plant)	Bimonthly
2.	Neem cake + *P. lilacinum*	1 kg/plant + 10 g/lit (10 lit/plant)	Twice the year (before SW and NE monsoon) + Bimonthly
3.	*Trichoderma* sp.	10 g/lit (10 lit/plant)	Bimonthly (three times)
4.	*P. chlamydosporia*	50 g/clump	Bimonthly (three times)
5.	Cartap hydrochloride 4G	50 g/clump	Twice in an year
6.	Carbosulfan 25 EC	1 ml/l (10 lit/plant)	Twice in an year
7.	Control	-	-

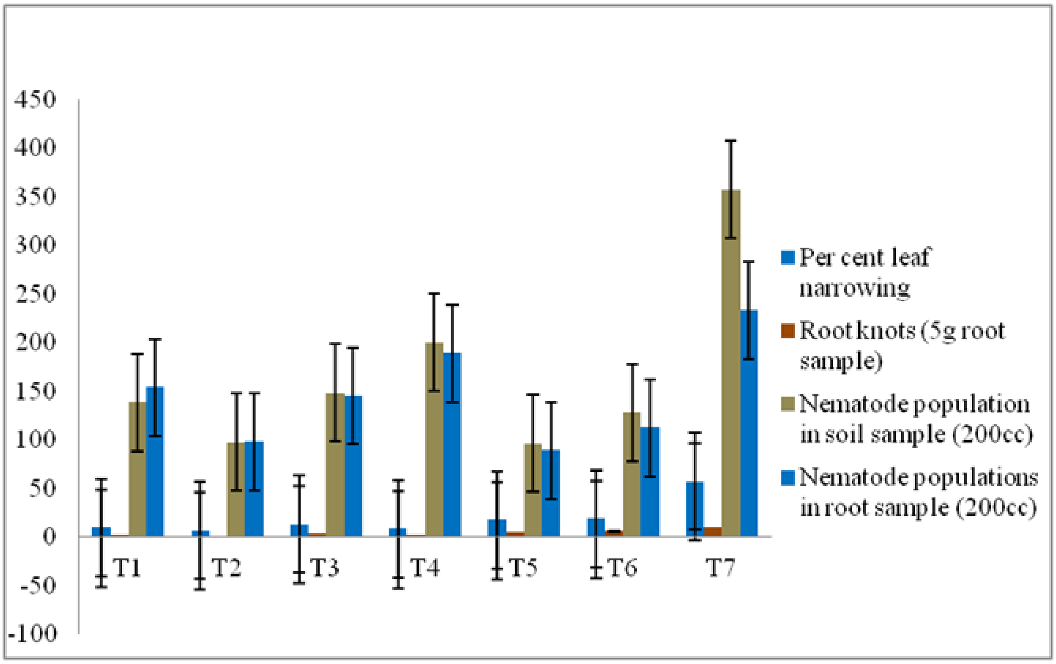

Fig. 1. Influence of bio-agents, synthetic insecticides and organic amendment on RKN in cardamom

Table 2. Effect of bio-agents, insecticides and organic amendment on aerial and root damages caused by RKN in cardamom

Treatment	Percent leaf narrowing		Root knots (5 g root sample)	
	*Pooled	Percent reduction over control	*Pooled	Percent reduction over control
T1	9.29[de]	83.77	2.54[bc]	72.99
T2	6.42[e]	88.79	1.32[c]	85.99
T3	13.09[cd]	77.13	3.49[bc]	62.87
T4	8.04[e]	85.95	1.83[bc]	80.50
T5	17.17[bc]	69.99	4.50[bc]	52.10
T6	18.69[b]	67.34	5.42[b]	42.25
T7	57.21[a]	-	9.39[a]	-
CV (%)	10.20	-	39.65	-
CD (0.05%)	4.63	-	3.94	-

T1 = *P. lilacinum*; T2 =Neem cake + *P. lilacinum*;T3 = *Trichoderma* sp.; T4 = *P. chlamydosporia*; T5 = Cartap hydrochloride; T6 = Carbosulfan and T7 = Control
*Figures in parentheses are square root transformed values

Multiple Range Test (DMRT) to separate the treatment means to get useful information with respect to the efficacy of treatments (Gomez and Gomez, 1984). All statistical analyses were carried out using Web Agri Statistical Package (Analytical Software, ICAR, New Delhi, India).

RESULTS

The statistical analysis of pooled data combining three locations showed that all the treatments viz. sole application of *P. lilacinum*, combined application of neem cake plus *P. lilacinum*, *Trichoderma* sp., *P. chlamydosporia*, cartap hydrochloride and carbosulfan were found significantly effective than the untreated control with respect to RKN in cardamom (Figure 1, Table 2).

Effect on cardamom plants

The pooled data of aerial symptoms for three year experiment showed that *P. lilacinum* + neem cake (T2) was the most effective treatment with least percent leaf narrowing (6.42%) and highest reduction over control (88.79%). *P. chlamydosporia* (T4) had 8.04% narrowed leaves with 85.95% reduction over control. They were on par with each other in terms of efficacy. Sole application of *P. lilacinum* (T1) was the next best treatment with 9.29% narrowed leaves and 83.77% reduction over control. *Trichoderma* sp. (T3) treated plants also expressed considerably less symptoms (13.09%) with 77.13% reduction over control. On the other hand, chemical insecticides *viz.* cartap hydrochloride (T5) and carbosulfan (T6) applied plants recorded 17.17% and 18.69% narrowed leaves, respectively. Severity of leaf narrowing was the highest in untreated control plants (57.21%).

Similar trend was observed for the root zone also. The combined application of *P. lilacinum* + neem cake (T2) has resulted in the formation of least root knots (1.32 nos.) with 85.99% reduction over control. Sole application of treatments viz. *P. chlamydosporia* (T4), *P. lilacinum* (T1), *Trichoderma* sp. (T3) and cartap hydrochloride (T5) stood next with 1.83, 2.54, 3.49 and 4.50 nos. of root knots respectively. They were equal among themselves. The highest number of root knots was found in the untreated control (9.39 nos.).

Effect on nematode population

The pre-treatment observation of soil and root samples revealed that all the treatments were not significant with one another. The nematode count in 200 cc soil samples brought out that *P. lilacinum* + neem cake (T2) and *P. chlamydosporia* (T4) applied soil harboured less nematode population *viz.* 94.67 and 90.33 nos. correspondingly. *P. lilacinum* (T1) and *Trichoderma* sp. (T3) treated soils had 126.3 and 127.0 nematodes, respectively. They were on par with each other. In the untreated check, about 357 nematodes were found which was the highest among all.

Similarly analysis of the root samples treated with *P. lilacinum* + neem cake (T2) registered less nematode population (87.67 nos.) followed by *P. chlamydosporia* (T4) with 96.67 nematodes. *P. lilacinum* (T1) treated soil had 110.33 nematodes followed by *Trichoderma* sp. (T3) with 142.67 nematodes. In the untreated check, about 234 nematodes were enumerated which was the greatest among all treatments.

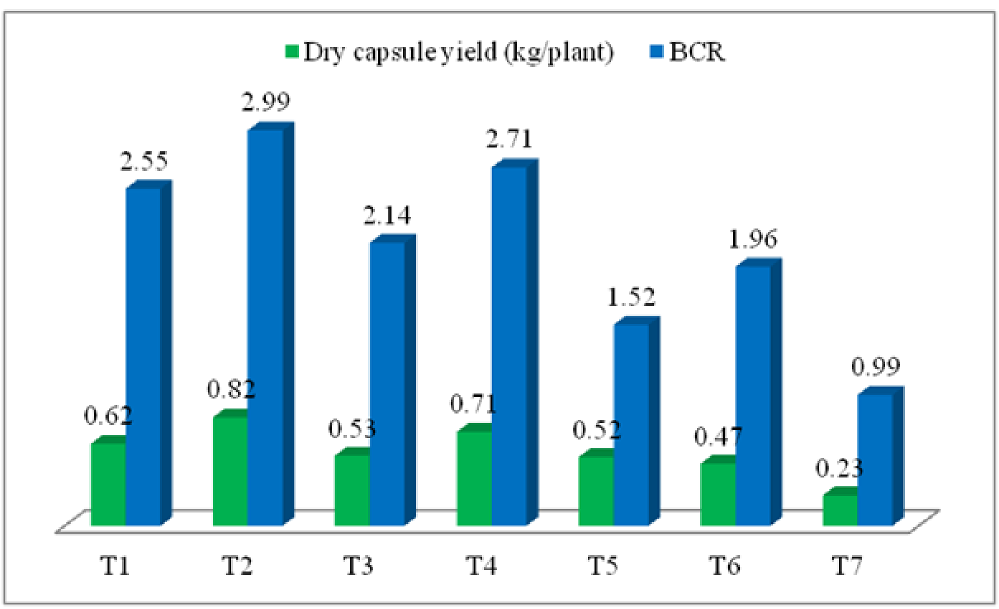

Fig. 2. Impact of bio-agents, synthetic insecticides and organic amendment on yield components in cardamom

Table 3. Effect of bio-agents, insecticides and organic amendment on RKN present in soil and roots of cardamom

Treatment	Nematode population (Nos.)				*Dry weight (kg/ plant)	BCR
	Soil sample (200 cc)		**Root sample (200 cc)**			
	Pre-count	**Post-count**	**Pre-count**	**Post-count**		
T1	122	126.3[d]	114	110.33[e]	0.62[abc]	2.55
T2	120	94.67[e]	121	87.67[g]	0.82[a]	2.99
T3	119	127.0[d]	126	142.67[d]	0.53[bc]	2.14
T4	123	90.33[de]	110	96.67[f]	0.71[ab]	2.71
T5	125	147.67[c]	120	156.0[c]	0.52[bc]	1.52
T6	122	202.33[b]	112	188.0[b]	0.47[c]	1.96
T7	113	357.0[a]	115	234.0[a]	0.23[d]	0.99
CV (%)	-	1.15	-	1.77	24.02	-
CD (0.05%)	-	3.36	-	4.56	0.24	-

T1 = *P. lilacinum*; T2 =Neem cake + *P. lilacinum*;T3 = *Trichoderma* sp.; T4 = *P. chlamydosporia*; T5 = Cartap hydrochloride; T6 = Carbosulfan and T7 = Control
*Figures in parentheses are square root transformed values

Effect on yield and BCR

With respect to the yield data, the dry capsule yield was the maximum in plants applied with *P. lilacinum* + neem cake (T2) with 0.82 kg/plant followed by application of *P. chlamydosporia* (T4) with 0.71 kg/plant. Sole application of *P. lilacinum* registered a yield of 0.62 kg/plant. In untreated check, plants yielded 0.23 kg/plant on dry capsule weight (Figure 2, Table 3).

Regarding the Benefit Cost Ratio, the highest BC ratio was observed in T2 (2.99) followed by T4 (2.71), T1 (2.55) and T3 (2.14), respectively. The chemical treatments viz. cartap hydrochloride (1.96) and carbosulfan (1.52) had consistently low BCR. The untreated check had very less BCR compared to all other treatments (0.99).

DISCUSSION

Root-knot Nematodes have been identified as a serious threat to cardamom. Though tiny worms, they have been perfectly adapted to all agro-environments. More works have been focused on alternative control methods to manage them. Special attention has also been given to the biological control agents and organic amendments which help effective suppression of RKNs. There are many reputed reports available for the management of RKNs with biological control agents in cardamom and other crops.

The nematicidal fungi, *P. lilacinum*, the notable soil-inhabiting, nematophagous fungi (widely used against RKN) also have the capacity to solubilize the semi available phosphorus presents in soil (Gaur, 1990). Through colonization of the root system they ensure phosphorus availability to plants. This might be the reason for formation of new roots and good recovery of plants in the present study. Increase in plant growth parameters and nematode egg parasitism was found when *P. lilacinum* was applied in combination with neem cake (Reddy et al., 1997). Its efficacy can be enhanced by the addition of organic amendments like neem cake, cowdung etc. (Gaur 1990). The application of *P. lilacinum* in combination with neem cake improved nematode control, plant growth and yield in nematode infested cardamom plantations (Ali, 1987; NRCS, 1993; Eapen and Venugopal, 1995; Mathew, 2007; Devasahayam et al., 2015). Sheela (2007) found that *P. lilacinum* reduced root knot nematodes by 48.5 to 57% in pot culture studies and by 19.7% in field studies. Enhancement of all plant growth characters and reduction in the root knot infestation with response to *P. lilacinum* was reported by Usman and Siddiqui (2012). This study results proved the efficiency enhancement of *P. lilacinum* when applied in combination with organic amendments like neem cake.

Pochonia chlamydosporia is also a fungal antagonist of RKN and cyst nematodes and acts directly by parasitizing eggs and indirectly by repelling, immobilizing and/or killing them by means of metabolites, and/or inducing plant response. This fungus endophytically colonizes the roots of several plants. Silva et al. (2017) acquired 34-44% reduction in nematode population with the application of *P. chlamydosporia* in tomato whereas in our study it was double (85.95% for leaf narrowing and 85.50% for root knot). According to Zakaria et al. (2013) the efficiency of *P. chlamydosporia* could be improved if applied in combination with other bio-control agents like *P. lilacinus, Trichoderma* sp., *Bacillus subtilis* and compost amendments.This study need to be duplicated in cardamom ecosystems for further confirmation.

In addition to the reduction of Root-knot Nematode problems, *Trichoderma* spp. was also found to improve plant growth and yield in cardamom (NRCS, 1993; Eapen and Venugopal, 1995; Sheela, 2007; Narayana et al., 2011). However they are not a regular candidate for biological control of nematodes. In the present study also *Trichoderma* sp. showed quite less efficacy compared to other antagonists viz. *P. lilacinum* and *P. chlamydosporia*.

With respect to chemical management practices, carbofuran and phorate are potent in secticides used against most of the nematodes, especially the RKN. In spite of high persistence and toxicity levels, they were categorized under red triangle insecticides. Therefore their usage has been banned or restricted in many states including Kerala (Narayana et al., 2018). However, a moderate to short persistence of insecticides in soil with a half life of 1-8 weeks in temperate (Meher et al., 2010) and 1-3 weeks in tropical environments (Meher and Sethi, 1992; Meher et al., 2010) is essential. Considering these into account, the insecticides such as cartap hydrochloride, carbosulfan, chlorpyriphos (Wiratno et al., 2009) etc. are being used for the management of nematodes wherein they suppress them and leave less persistence in soil.

Narayana et al. (2017) found that cartap hydrochloride 4%G was effective in controlling *Meloidogyne javanica* in cardamom with improved yield. In another study, Narayana et al. (2018) also revealed improved efficiency of carbosulfan 6%G @16.7kg/ha and cartap hydrochloride 4%G @ 25 kg/ha against RKN and galls in black pepper. They also recorded a considerable increased yield in response to these chemical management practices. In the present investigation also, these insecticides showed reduction in nematode population of soil along with improved yield and found superior over the control.

CONCLUSION

Management of RKN infecting cardamom using fungal biocontrol agents is a proficient and ecologically safer approach and is far superior to the chemical management. The climatic condition prevailing in the Cardamom Hill Reserves (CHR) is also highly acquiescent for the growth and multiplication of these nematophagous fungi. They act as surrogate of synthetic insecticides by creating a pollution free and sustainable agro-environment in the CHR. Furthermore, studies are required to understand efficiency enhancement, if any in *P. chlamydosporia* when applied along with organic amendments against RKN in cardamom as the sole application of former itself gave better results in the present study.

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DISCLOSURE STATEMENT

There is no financial interest or benefit that has arisen from the direct applications of this research and no potential conflict of interest was reported by the authors.

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