Distribution of entomopathogenic nematodes and fungi in cashew ecosystem

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ABSTRACT

A Survey was conducted in the cashew plantations of the Directorate of Cashew Research and Karnataka Cashew Development Corporation at Puttur, Karnataka to observe the presence of entomopathogenic nematodes and fungi which could be potent natural enemies of cashew stem and root borer, *Plocaederus* species. Entomopathogenic nematodes were obtained by baiting them from soil samples using *Galleria mellonella* larvae. Out of the 110 soil samples collected from various cashew plantations, 10 soil samples indicated the existence of nematodes, which was noticed by the mortality of wax moth larvae due to infection. These entomopathogenic nematodes were identified by utilizing currently available molecular tools, four isolates matched with *Heterorhabditis bacteriophora* and two with *Steinernema abbasi.* The results revealed that, field collected fungus, was pathogenic to laboratory reared larvae of cashew stem and root borers and was identified as *Metarhizium anisopliae* (Metsh).

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INTRODUCTION

Cashew stem and root borers (CSRB) belong to a major family of insects, Cerambycidae which are generally known as long horn beetles and includes a highly diverse group of insects, larvae of which feed by tunneling into the stem and roots of host trees in agriculture, forestry and also structural timber. *Plocaederus ferrugineus* L. and *P.obesus* Gahan are recorded to be two major CSRB species while, *Batocera rufomaculata* De Geer is recorded to be a chief secondary borer of cashew trees in India (Sundararaju and Bakthavatsalam, 1990). Currently, management of CSRB is being done by adopting post extraction prophylaxis (PEP) wherein the pest stages are extracted from infested trees and treated cashew trees are protected from further egg laying by the pest, by swabbing and drenching with chlorpyriphos (NRCC, 1997). Several pest management techniques such as, stem injection, root feeding or stem padding of insecticides were evaluated (Jena, 1990; Samiayyan *et al.*,1991; Senguttuvan and Mahadevan, 1997; Rao,1998), but led to minimum success as systemic pesticides get accumulated in the xylem tissues and CSRB larvae are phloem feeders. The recommended practice for managing the CSRB incidence is removal of pest stages from infested trees followed by swabbing and drenching the infested portion of stem and roots with chlorpyriphos, as well as removal of dead and CSRB infested trees beyond recovery from the cashew plantations.

The efficacy of entomopathogenic nematodes (EPN) in managing a variety of subterranean root borers was reported earlier on cerambycid pests and lepidopteran borers. *Steinernematidae* and *Heterorhabditidae* (Rhabditida) were reported to be lethal insect parasites occurring in natural and agricultural soils. It was recommended that, while developing a durable management strategy for release of EPN against pest insects, indigenous nematodes were more suitable because of their adaptation to local climate and host population (Bedding, 1990). Thus, exploration of indigenous EPN is now getting attention; several surveys conducted around the world indicated widespread occurrence and provided wide information on the indigenous species and strains of EPN.

Use of EPN is an option that is environmentally friendly and does not affect the non-target

organisms. Vasanthi and Raviprasad (2012) reported the virulence of *Steinernema abbasi* and *Heterorhabditis indica* against CSRB species under laboratory conditions. However, indigenous EPN species from the cashew ecosystem were not isolated till date. Biological control by EPN could be a potentially effective component in integrated pest management schedule against the CSRB. Several fungal species were also reported to be pathogenic to *Plocaederus* species. Bhat and Raviprasad (1996) reported pathogenicity of three confirmed species of entomopathogenic fungi, *Beauveria bassiana*, *B.brogniartii*, and *Metarrhizium anisopliae* against CSRB. Ambethgar (2001) tested the pathogenicity of indigenous entomopathogenic fungi for their efficacy as biological control agents against CSRB larvae. The present work objective is to investigate the occurrence of indigenous species of entomopathogenic nematodes and fungi in the cashew ecosystem, which could be potential natural enemies of CSRB, *Plocaederus* species.

MATERIALS AND METHODS

Survey on indigenous EPN species

The soil samples were collected from the cashew ecosystem of Directorate of Cashew (DCR) Research 12º 45' N latitude, 75º40' E longitude; 90 m above MSL and nearby cashew plantations of Karnataka Cashew Development Corporation (KCDC) at Alankar, 12°77'N; 75°32'E and Ramakunja, 12° 79'N ;75° 32'E Dakshina Kannada Dist., Karnataka. The sampling was carried out after cessation of monsoon in the month of September to November 2012 on a random basis, at 10-15 cm distance from the tree base on the four directional quadrants. About 1kg soil from each tree base was taken for examination and such 110 soil samples were collected. Soil samples obtained from different cashew plantations were brought to the laboratory in plastic bags. They were later transferred to autoclaved glass troughs (30 x 15 x 5 cm). The soil pH, moisture content of each soil sample was recorded. Clean R.O water was sprayed onto the soil samples, to maintain minimum 50 percent humidity.

The final instar larvae of greater wax moth (*Galleria mellonella* L) were used as bait insect. Twenty individuals of the bait insect larvae were released to each of the soil samples. The troughs were later labelled, shielded to prevent escape of the bait insect larvae. The experimental set up was incubated at room temperature for 48 - 72 hrs. Further, observations were done on the mortality of wax moth larvae. The larval cadavers of wax moth were transferred from the soil surface to 'white traps' so as to facilitate the emergence of infective juveniles (IJs). Observations were recorded on the emergence of IJs, which were harvested using R.O. water. To ensure the presence of EPN, in the wax moth larval cadavers, the IJs which emerged were used to inoculate a fresh batch of host (*Galleria mellonella*) larvae and second generation of EPN were obtained. Adults of EPN as well as IJs were harvested as per the standardized method and whole mounts of the EPN were prepared using standardized techniques. The species level identification was done by molecular analysis at National Bureau Agriculturally Important Insects (NBAII), Bengaluru. The EPN species were tested for their virulence against laboratory reared larval stages of CSRB.

Entomopathogenic fungi against CSRB

The mortality of field collected CSRB larvae due to fungal infection was recorded in various months. Seasonal incidence of the fungus on CSRB was recorded for two years (2011-2012) in the cashew plantations of DCR. The larval stages were collected monthly interval and observed for infection due to the fungus. The temperature (ºC) and rainfall (mm) were recorded at the meteorological observatory and were correlated with per cent pathogenecity. The laboratory culture of the field collected strain of *M. anisopliae* maintained on potato dextrose medium was tested for their infectivity by two different methods of application, (at 10^5 spores/ml) on the laboratory reared larvae of CSRB. Topical application by smearing the suspension (1mL) onto the larvae and bark dipped in suspension (1mL) and provided as feed.

Regular observations were recorded on the infection of the fungus and further mycosis to the treated larvae. Date of first external symptom of infection *viz*., development of fungal mycelia on the larval cadaver was recorded. The development of greenish grey spores on the larval cadaver confirmed the

infection being due to *M. anisopliae*. Percentage mortality due to various methods of application of spores of *M. anisopliae* was recorded. The methods of application were compared with each other statistically by student T test using MS Excel.

RESULTS AND DISCUSSION

Among the 110 soil samples collected from various cashew plantations (pH ranged 5.7-5.9), 10 soil samples had indicated the presence of EPN as revealed by mortality of wax moth larvae due to EPN infection. However, of these ten samples, seven provided the second generation EPN IJs under laboratory conditions. This helped further isolation and characterization of the DNA for species level identification of the natural enemies of the CSRB in the soil of cashew ecosystem. The occurrence of *Steinernema abbasi* and four other species of *Steinernema was* reported in the agro-ecosystems of India (Hussani *et al.,* 2001).The present study is in support of this earlier record.

The DNA taken from six EPN species were sequenced and further blasted to locate any matching sequence from NCBI library. Two of the EPN isolates from DCR, gave the 98% matching nucleotide sequences with *Heterorhabditis bacteriophora* and two from DCR, Kemminje campus and one from KCDC, Alankar gave 98% matching with *Steinernema abbasi*, both deposited from NBAII, Bengaluru in 2008. Both the species of EPN were virulent to CSRB, *Plocaederus* species under laboratory conditions when applied 1000IJ/larva in Petri plate **(**with more than 75 percent mortality of the young (45-90 days) CSRB larvae with in three days of contact with the EPN isolates**.** In the current study, 6.38 per cent of the soil samples collected from various cashew plantations yielded both *Steinernema* and *Heterorhabditis*, which was lesser in comparison to the earlier report on the occurrence Heterorhabditidae and Steinernematidae in the 10.5 per cent of the soil samples collected from Nepal.(Khatri *et.al.,* 2010) Both these species of EPN were reported from cashew ecosystem for the first time.

Prabhu and John (2008) evaluated the efficacies of two native entomopathogenic nematodes *viz*., *Steinernema* sp. and *Heterorhabditis indica* under laboratory against red hairy caterpillar *Amsacta albistriga* Walk. in groundnut and reported 80 per cent and 42per cent of mortality within 24 hrs by *H. indica* and *Steinernema* sp. respectively. The present investigations exhibited a similar kind of trend in the percentage morality of CSRB larvae but with exposure to EPN up to 72-96 h. The susceptibility of cerambycid larvae to EPN was well established by previous workers on the red oak borer *Anoplophora glabripennis* (Fallon *et al*, 2004). In support of the earlier report by Vasanthi and Raviprasad (2012), the current study revealed the susceptibility of *S. abbasi* and *H. bacteriophora* isolated from the indigenous soil against CSRB under laboratory conditions. However, a detailed standardisation of technique for large scale production, storage and application of the ideal strain of EPN against CSRB needs to be worked out to achieve the level of management of the Cardamom root grub as reported to be successfully managed by using indigenous EPN in Kerala (Varadarasan *et al*., 2011).

Evaluation of entomopathogenic fungus

The field collected larvae developed cotton-like mycelia on the larval cadaver, followed by greenish grey spore development. The mean percentage incidence of *M. anisopliae* was positively correlated with rainfall (r = 0.6882) while negatively correlated with temperature (r = - 0.49180) (**Fig 1).**

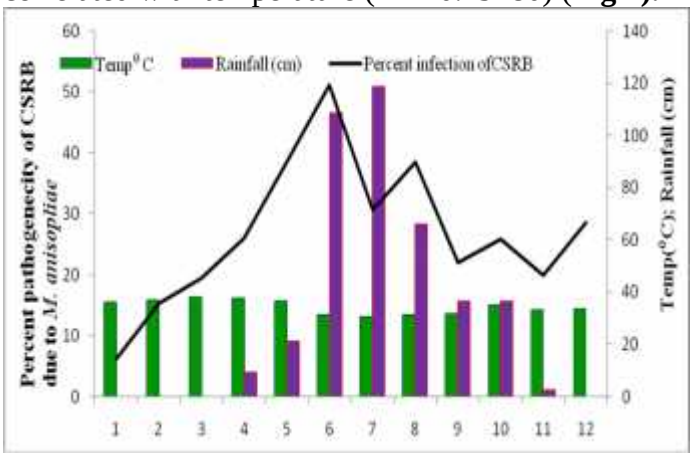

Fig 1. The incidence of *M. anisopliae* in relation to weather parameters.

The fungal spores when inoculated, were found to be pathogenic to CSRB under laboratory conditions. Mean percentage mortality of CSRB was significant when tested by topical application **(Table 1).**

The susceptibility of *Plocaederus* species to entomopathogenic fungi was reported by earlier workers. The results of present study were in confirmation with the earlier findings of Bhat and Raviprasad (1996), who reported pathogenecity of

Table 2. Mean percentage mortality of CSRB larvae by *M.anisopliae*

Method of application	**Mortality (%)**
Topical application	68.33 ± 1.2
Bark dipped in suspension and provided as feed.	25.00 ± 3.4

three commercially available species of entomopathogenic fungi, *Beauveria bassiana*, *B.brogniartii*, and *Metarrhizium anisopliae* against CSRB. *Beauveria bassiana* induced 90% mortality on direct topical application while, *Beauveria brogniartii* and *M. anisopliae* induced about 50 and 40 % mortality of the CSRB larvae. Ambethgar (2001) tested the pathogenicity of indigenous entomopathogenic fungi for their efficacy as biological control agents against CSRB larvae and recorded mortality to the tune of 23.7 - 86.6 percent in case of various entomopathogenic fungi such as *Aspergillus flavus, A. niger*, *B. bassiana* and *M. anisopliae*. Sahu and Sharma (2008) reported that application of *M. anisopliae* spawn 250 g/tree along with neem cake (500g/tree) was most effective treatment with least infested trees (7.40%) followed by application of *B. bassiana* spawn 250g/tree along with neem cake 500g/tree which led to 11.11% tree infestation. In the current study, *M. anisopliae* was recorded to occur in the cashew ecosystem which would infect the field population of these pest species. Meshram and Soni (2011) conducted field trials using *B. bassiana* and *M.anisopliae* against *Buchanania lanzan*, *Plocaederus obesus* Gahan (Coleoptera: Cerambycidae) which was also recorded to be a major CSRB species. The efficacy of *B. bassiana* and *M. anisopliae* were on par, but the delivery methods varied significantly. Pouring conidial suspension affected 16.0-25.0 % recovery of infested trees followed by swabbing conidial slurry with 17.0-20.0% and soil application with 13.0-14.0% recovered trees. The implementation of fungal application in integrated control of *P. obesus* was recommended. The present study also is in corroboration with the report. However, for utilization of these indigenous natural enemies of CSRB in IPM strategies, further investigations are needed.

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REFERENCE

Ambethgar, V. 2010. Field assessment of delivery methods for fungal pathogens and Insecticides against cashew stem and root Borer, *Plocaederus ferrugineus* L. (Cerambycidae: Coleoptera) *Journal of Biopesticides,* **3**(1):121 – 125.

Bedding, R.A. and Akhurst, R.J. 1975. A simple Technique for detection of insect parasitic Rhabditid nematodes in soil. *Nematologica,* **21**: 109-110.

Bhat, P.S. and Raviprasad, T. N. 1996. Pathogenecity of entomopathogenic fungi against CSRB *Plocaederus ferrugineus* L. *Journal of Plantation Crops,* **24** (Suppl.): 265-271.

Declan J. Fallon, Leellen F. Solter, Melody Keena, Michael Mc Manus, James R. Cate and Lawrence M. Hanks 2004. Susceptibility of Asian long horned beetle, *Anoplophora glabripennis* (Motchulsky) (Coleoptera: Cerambycidae) to entomopathogenic nematodes. *Biological Control*, **30**:430–438.

Hari Bahadur Khatri-Chhetri, Lieven Waeyenberge, Hira Kaji Manandhar, and Maurice Moens. 2010. Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Nepal. *Journal of Invertebrate Pathology,* **103**: 74–78.

Hussani S.S., Ansari, M.A. Ahmad, W. and Subbotin S.A. 2001. Identification of some Indian populations of *Steinernema* species (Nematoda) by RFLP analysis of the ITS Region

of r -DNA. *International Journal of Nematology,* **11**(1): 73-76.

Jena, B. C. 1991 Cashew Stem and root borers and their management. *The Cashew* Jan-Mar. pp: 5-7.

Meshram, P. B. and Soni, K. K. 2011 Application of Delivery Methods for Fungal Pathogens and Insecticides Against Chironji (*Buchanania lanzan*) Stem Borer, *Plocaederus obesus* Gahn. *Asian Journal of Experimental Sciences,* **2** (1): 53-57.

NRCC. 1997. Annual Report. National Research Centre for Cashew ISSN # 0972-2637 pp.50-51.

Prabhu, S. and John Sudheer, M. 2008. Evaluation of two native isolates of entomopathogenic nematodes *Steinernema* sp. and *Heterorhabditis indica* from Andhra Pradesh against *Amsacta albistriga* Walk. in groundnut. *Journal of Biopesticides,* **1**(2):140 – 142.

Rao, E.V.V. Bhaskara. 1998. Integrated production practices of cashew in India. RAP FAO Corporate Document Repository. Available on line at www. fao. org/ docrep/ 005/ ac451e/ ac451e04. htm. 12pp.

Samiayyan, K., Palaniswamy, K. P., Ahmed Shah, H. and Manivannan, K. 1991. Effect of prophylactic measures against cashew stem and root borer, *(= Plocaederus) ferrugenius* L. *The Cashew,* **5**(1): 16-17.

Senguttuvan, T. and Mahadevan, N.R. 1997. Integrated management of cashew stem borer *Plocaederus ferrugineus* L. *Pest management in Horticultural eco-system,* **3**(2): 79-84.

Sundararaju,D. and Bakthavatsalam, N. 1990 Cashew Pest Management for coastal Karnataka. *The Cashew*, **4** (3): 3-6.

Varadarasan, S., Hafitha, N.M., Sithara, L., Balamurugan, R., Chandrasekar, S.S., Ansar Ali, M.A. and Thomas, J. 2011. Entomopathogenic Nematodes - Science,Technology and Field outreach for biological control of cardamom root grub. *Journal of Plantation Crops,* **39**(1): 86-91.

Vasanthi, P. and Raviprasad, T. N. 2012**.** Relative susceptibility of cashew stem and root borers (CSRB), *Plocaederus* spp. and *Batocera rufomaculata* (De Geer) (Coleoptera: Cerambycidae) to entomopathogenic nematodes *Journal of Biological Control*, **26** (1): 23-28.

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