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Effect of Chlorpyrifos on Survival and Virulence of Native Entomopathogenic Nematode

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ABSTRACT: Combining compatible insecticides with entomopathogenic nematodes may constitute an effective alternative to conventional chemical control as efficient tool for pest and disease control in areca nut and coconut. Hence, *in vitro* a compatibility test was conducted to evaluate the effect of chlorpyrifos which is widely used against root grubs on native entomopathogenic nematode isolate, *Steinernema carpocapsae* (CPCRI-SC1). Results indicated that different concentrations of chorpyrifos significantly influenced the infective juveniles (IJs) mortality at 96 h of exposure. The maximum percent mortality of infective juveniles was recorded at higher concentration of insecticide 3200 ppm (31.5%) followed by 1600ppm (26.2%), whereas minimum percent mortality (18.2%) was recorded at lower concentration (200 ppm) followed by 400 ppm (21.3%) at 96 h of exposure. Increase in the mortality of IJs was noticed with increase in concentration (200 - 3200 ppm) along with their exposure periods (24 - 96 h). No significant difference was recorded in mass production and infectivity of IJs exposed to different concentrations of insecticide on third instar *G. mellonella*. The results showed tolerance between native EPN isolate *S. carpocapsae* and chlorpyrifos insecticide at lower concentration as it does not affect the biological parameters of the native EPN isolate.

Key words: Chlorpyrifos, Greater wax moth, *Steinernema carpocapsae*

Entomopathogenic nematodes (EPNs) have potential for biological control of cryptic insect pests because of their different forms of action, specificity and environmental safety. In addition, EPNs can show synergy with other entomopathogenic agents. Among EPNs, *Steinernema* Travassos and *Heterorhabditis* Poinar are of great relevance due to their symbiotic association with bacteria, *Xenorhabdus* Thomas & Poinar, and *Photorhabdus* Boemare, Louis & Kuhl by causing rapid insect death. Combining EPNs with compatible insecticides has contributed to the suppression of a number of economically important crop pests (Koppenhofer & Grewal, 2005). If there is compatibility when both nematodes and insecticides are implemented together they reduced the environmental risk and management costs. Insecticides may impact entomopathogenic nematodes by affecting longevity (survival), host acceptance, reproduction (fecundity), percent emergence and development time. However, some of the insecticides found synergistic effect for combined application with EPNs such as imidacloprid, cypermethrin, malathion, thiamehoxam *etc.* in contrast, bisultap, emamectin benzoate and rotenone proved harmful to *S. carpocpasae* (Xun Yan *et al*., 2012). The lack of compatibility information of native EPN isolates collected from coconut palms is a major impediment in further expansion of their use. Hence, the generated compatibility information could aid in development of IPM programme against key palm pests. Therefore, the study was focussed to evaluate the effect of chlorpyrifos on survival of IJs of *S. carpocpasae* exposed to different concentrations and their infectivity to larvae of the greater wax moth, *G. mellonella* (Lepidoptera : Pyralidae) under laboratory.

MATERIALS AND METHODS

The greater wax moth, *G. mellonella*, is used as a model insect for EPN bait trapping and for evaluation of EPN infectivity. Larvae of *G mellonella* were collected from the nearby beehives in and around Kasaragod and it was reared on artificial diet consisting of wheat and

maize flour, milk powder, rice bran, glycerol, yeast extract and honey in room temperature 28±2°C, relative humidity 85±5% . The adults were fed with honey.

The native entomopathogenic nematode isolate, *S. carpocapsae* (CPCRI-SC1) was used in this study. Prior to the assays, *S. carpocapsae* was mass multiplied in *in vivo* on greater wax moth, *G. mellonella*. Freshly emerged infective juveniles were used in the experiment. A serial dilution of the nematode stock solution was prepared to achieve the necessary concentration of nematodes.

Chlorpyrifos 20 EC (*O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate) was chosen for this study as it is being commonly used for management of soil pests such as root grub, *Leucopholis* spp. in palms ecosystem in southern region of India.

Effect of chlorpyrifos on nematodes

To determine the effect of chlorpyrifos on nematodes survival, infectivity and reproduction, a methodology suggested by (Negrisoli Jr. *et al*., 2008) was adopted. Suspension of *S. carpocapsae* (1000 IJs ml^{-1}) was added in Microtitter plate containing different concentrations of chlorpyrifos *viz.,* 0.32, 0.16, 0.08, 0.04 and 0.02 % and control (water alone). There were 5 replicates per treatment. The insecticide treated IJs were incubated at room temperature (28 to 30^{0}C). Nematode mortality was determined at 24, 48, 72 and 96 h after their exposure. A 200 µl samples were drawn from each treatment and placed in a 100-mm petri dish to assess the effect of insecticide on nematodes by counting the number of live and dead nematodes under stereo-microscope (Stepanka Radova, 2011). The nematodes that did not react to stimulation by a poultry feather pick were considered as dead. Nematode infectivity was tested on wax moth (*G. mellonella*) larvae. The IJs that survived in the presence of insecticide exposure were washed three times with distilled water and nematodes were allowed to settle at bottom and then top supernatant solution was discarded. After the last wash one ml suspension containing approximately 200 IJs was mixed to 1 ml of water, which was later applied to four filter papers in petri dishes (9 cm diameter) on which seven wax moth larvae were added. Dishes were then kept in a room temperature (28±1^{0}C) for three days. The larval mortality was observed at every 24 h up to 48 h. The resultant wax moth larval mortality was expressed as per cent mortality (Connick *et al*., 1994). The collected dead larvae were washed with distilled water and transferred to white traps for nematodes emergence. The total number of nematodes produced per larva was counted (Woodring and Kaya, 1988) for assessing the reproduction ability of nematodes exposed to insecticide.

Statistical analysis

Nematode and insect mortality data were expressed in percentages. All the data were arcsin and square root transformed before analysis. Transformed data were *subjected to analysis of variance (ANOVA) followed by DMRT.* All statistical analysis was performed using PROC. GLM (SAS software, version 9.3, SAS institute).

RESULTS AND DISCUSSION

Significant difference in the mortality of *S. carpocapsae* following direct exposure to the different concentrations of chlorpyrifos and control was observed. Nematode mortality to chlorpyrifos solutions ranged between 9.8 to 17.5 %. 12.3 to 21.0 %, 15.9 to 24.8 % and 18.2 to 31.5 % after 24, 48, 72 and 96 h, respectively. These had nil or negligible effects on survival of IJs at different concentrations of the insecticide tested as shown in Table 1. Similarly, there was no significant difference in infectivity of chlorpyrifos treated IJs as compare to non treated IJs in *G. mellonella,* highest percent mortality of *G. mellonella* larvae was observed in insecticide treated treatments compare to control at 48 hours of exposure (Fig. 1). This is consistent with the reports of Stepanka Radova (2011) and Paul Henrique Siqueira Sabino and co-workers (2014) who found that chlorpyrifos has no negative effects on nematode survival and reproduction in *G. mellonella*. Some chemical pesticides are toxic to EPNs (e.g., abamectin, acephate, aldicarb, dodine, fenamiphos, methomyl, parathion, and Teflubenuron), whereas others tend to be compatible and synergistic when applied with EPNs (e.g., carbaryl, chlorpyrifos, dimethoate, endosulfan, fonofos, tefluthrin, imidicloprid) (Alumai and Grewal, 2004; Koppenhofer and Grewal, 2005; Koppenhofer and Fuzy, 2008; Shapiro-Ilan *et al.*, 2011b). Significantly higher production of

Table 1. Effect of chlorpyrifos on survival of IJs of *S. carpocpasae*

Chlorpyrifos (ppm)	% mortality of IJs at different intervals (N=1000)			
	24 h	48 h	72 h	96 h
3200	9.2 (17.5)^{a}	12.9 (21.0)^{a}	17.6 (24.8)^{a}	27.3 (31.5)^{a}
1600	7.0 (15.2)^{ab}	9.5 (17.9)^{ab}	13.1 (21.9)^{b}	19.5 (26.2)^{b}
800	5.6 (13.4)^{cab}	8.1 (16.4)^{b}	10.7 (19.0)^{bc}	16.2 (23.7)^{c}
400	4.8 (12.3)^{cb}	7.6 (15.8)^{b}	9.6 (18.0)^{dc}	13.2 (21.3)^{d}
200	3.0 (9.8)^{c}	4.7 (12.3)^{c}	7.6 (15.9)^{d}	9.8 (18.2)^{e}
Control (sterile water)	0.0^{d}	0.0^{d}	0.0^{e}	0.0^{f}

Values in the parenthesis are Arc sine transformation. Mean followed by different letters indicate significant difference (P=0.05)

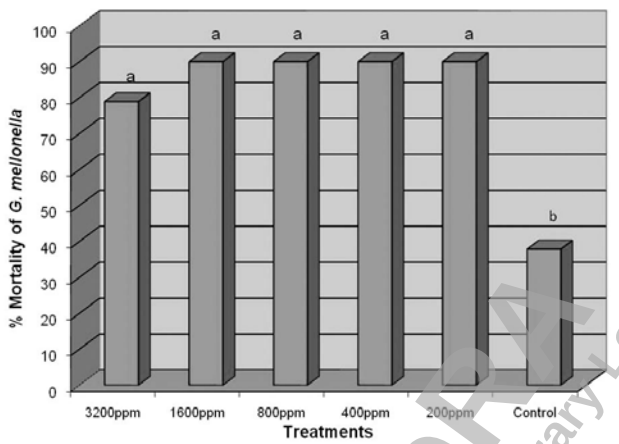

Fig. 1. Effect of chlorpyrifos on the infectivity of IJs of *S. carpocapsae* on 3rd instar *G. mellonella*

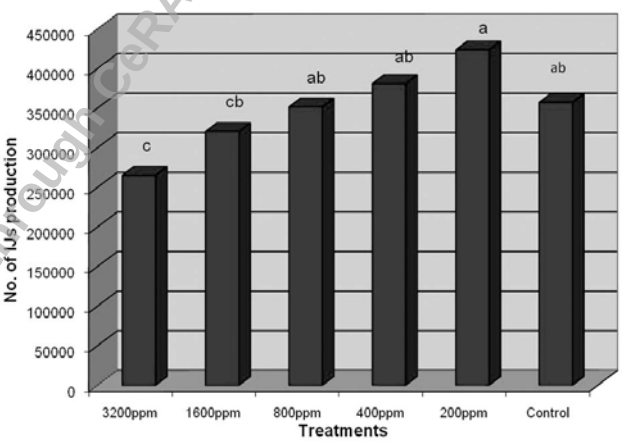

Fig. 2. Effects of chloropyrifos on reproduction of nematodes in 3rd instar *G. mellonella*

infective juveniles on 3rd instar *G. mellonella* was observed in nematodes exposed to insecticides compares to control (Fig. 2). According to Ishibashi and Takii (1993) some insecticides were found to enhance *S. carpocpasae* activity.

Studies carried out by other authors also showed low IJ mortality for *S. carpocapsae* when exposed to chlorpyrifos(Zimmerman and Crashaw, 1990; Gutierrez *et al*., 2008). One hypothesis that may explain this insensitivity in the EPNs involves the presence of butyrylcholinesterase in the synapse of parasitic nematodes, protecting the acetylcholinesterase, and thus acting as a frontline defence against such compounds (Selkirk *et al*., 2001)

The oriental beetle, *Exomala orientalis* is an important pest of turfgrass in Korean golf courses, *Steinernema longicaudum* combination with a one-half rate of chlorpyrifos-methyl caused 96.8 % mortality, found synergistic than a full rate of *S. longicaudum* (45.9% mortality) or a full rate of chlorpyrifos-methyl (28.7 % mortality) (Lee *et al.*, 2002). The combination of tefluthrin with *S. carpocapsae* resulted in a synergistic response and a average(24%) increase in expected mortality of corn rootworm (Nishimatsu and Jackson, 1998).

Combining the use of biological control agents like EPN with insecticides is the central theme to IPM programmes against many agricultural pests

(Koppenhofer and Grewal 2005). This strategy not only facilitate the use of EPN, increase the control efficiency of programme including EPN and reduce the cost of EPN application, however it may also reduce the dependence on chemical insecticides and thus contribute to slow down the development of insecticide resistance and preventing the adverse effects with the environment. The present laboratory study explored the effects of common insecticide (chlorpyrifos) on EPN which are being used as bio-control agents in palms ecosystem.

Phytosanitary strategy based on combined entomopathogenic nematode and insecticide treatment are promising strategies for plant protection within the Integrated Pest Management programs. However, field evaluation of their effect on targeted pest is needed to be established.

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