ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Foliar Application of Native Bio-Formulated Entomopathogenic Nematodes against Diamondback Moth in Aquaponic Agriculture.

Mona A Hussein[1*], Hala M Metwally[1] and M Abd Elraouf[1&2]

[1]Pests and Plant Protection Department, National Research Centre, Dokki, Giza, 12311, Egypt
[2]Federal Research Center for Cultivated Plants, Julius Kühn-Institut, Institute for Biological Control, Heinrichstraße 243, 64287 Darmstadt, Germany.

ABSTRACT

Field studies were conducted on diamondback moth (DBM) larvae, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), a foliar pest infesting watercress planted under aquaponic agriculture system. The work was targeted to improve efficiency of two native entomopathogenic nematodes (EPNs), (*Steinernema carpocapsae* BA2 and *Heterorhabditis bacteriophora* BA1) against DBM. The infective juveniles (IJs) of tested EPNs were formulated with calcium alginate adjuvants and sprayed at rate of $2X10^4$ IJs/ Plant. The population density of *P. xylostella* was significantly decreased from 23.4 to 8.3 DBM/ Plant after spraying the watercress with formulated BA2 causing 64.4% reduction compared to non-formulated nematodes which caused decrease in *P. xyllostella* from 17.6 to 11.1 DBM/ Plant causing 36.9% reduction. At the same time, formulated *H. bacteriophora* BA1 decreased the no. of DBM from 24.3 to 4.9 DBM/ Plant with 79.8% reduction compared to non-formulated BA1 which caused reduced the no. of DBM from 20 to 4.6 DBM/ Plant with 77% reduction. This work considers first record on the application of EPNs against foliar pests in aquaponic agriculture system.

Keywords: Foliar application; formulation; entomopathogenic nematodes; diamondback moth; aquaponic agriculture

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Egypt's agricultural landscape only comprises 5-7 % of the total area of the country and the rest is desert unable to blossom with conventional farming techniques [1]. Aquaponic Farm is a new technique to improve agriculture in Egypt. Water that results from agriculture is generally rich in nutrients especially in nitrogen and phosphorus compounds, possessing great potential for use in hydroponic vegetables production and the integration of the freshwater culture with hydroponic systems was sufficient to meet the demand for nutrients in the case of watercress [2].

Watercress (*Nasturtium officinal* R.Br.), is an aquatic, perennial herb [3]. Watercress provides a plentiful amount of compounds that may help fight wide range of cancers as well as lutein and zeaxanthin, carotenoids that are essential for macular (eye) and cardiovascular health, as well as essential vitamins [4, 5].

Plutella xylostella, is a serious pest worldwide, causes 60% loss in watercress production [6, 7]. Synthetic chemical insecticides have been used for many years to control this pest but the lack of effective natural enemies and insecticide resistance caused the outbreak for DBM [8]. Considering restrictions on the use of chemical pesticides in the fields of natural resources, it is necessary to find and to use one of the promising eco-friendly bio-control agents [7, 9].

Entomopathogenic nematodes (EPNs) are an alternative to control DBM as they had great success in controlling many insect pests worldwide [10,11]. Application and formulation technology has strengthened the position of nematode-based products in the market place [12,13]. The major limiting factor of foliar application of EPNs to leaf surfaces is the rapid desiccation of the IJs [10,14,15].

In Egypt, no records for application of EPNs against foliar pests in aquaponic agriculture were done. Therefore, the aim of this study is to evaluate the efficiency of two formulated EPNs against the diamond back moth, *P. xylostella* attacking watercress in aquaponic agriculture.

MATERIALS AND METHODS

Nematodes and Adjuvant

Heterorhabditis bacteriophora (BA1) and *Steinernema carpocapsae* (BA2) are two Egyptian isolates of EPNs [16]. Nematodes were *in vivo* produced using larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) according to Metwally, *et al.,* [17] and Woodring & Kaya, [18]. Nematodes were stored at 7°C in a cold room until used (<1 mo). The IJs were formulated using Calcium alginate according to Hussein & Abd El-Aty [19] and their viability was tested before each application by dissolving a pinch (~10 mg) of each formulated nematode into water and observing nematode shape and mobility under a light microscope.

Insects and field plots

This experiment was conducted at Mid August 2014-2015 at "El-Bustan Farm", the first commercial aquaponics farm in Egypt**,** outside Cairo (Smart Village). Water circulates from tanks hosting schools of fleshy Nile tilapia through hydroponic trays which grow vegetables including cucumber, basil, lettuce, kale, peppers, tomatoes and watercress on floating foam beds with run-off flushed out to irrigate olive trees planted around the farm (Figure 1).

The experiment was done inside the greenhouse on watercress plants heavily infested with diamondback moth larvae. The population density of larvae of DBM was estimated before and after spraying with a concentration of $2X10^4$ IJs/Plant of both bio-formulated EPNs. For each nematode species 6 plates were sprayed. Control plates sprayed with EPNs suspension in only water. The temperature reached 45±2 ^{o}C inside the experiment plot. A week later, no. of *P. xylostella* larvae in each plate was counted and % mortality was calculated.

ISSN: 0975-8585

Figure 1: Experiment plots inside the greenhouse at "El-Bustan" farm. A: water tank covered with foams B: Watercress planted Hydroponically

Insects and field plots

Statistical analysis

Data were analyzed by SPSS version 17 using Paired Sample T-Test for each single treatment and one way ANOVA test.

RESULTS

This work considers first record on the application of bio-formulated EPNs against foliar pests in aquaponic agriculture. From data shown in Figs. (2 and 3) and Table (1), EPNs significantly reduced DBM population. It was noticed that the formulated *S. carpocapsae* BA2 had significantly decreased the population density of DBM larvae compared to the non- formulated nematodes of the same species (control) although the high temperature in the green house (Temp.45±2 ^{o}C).

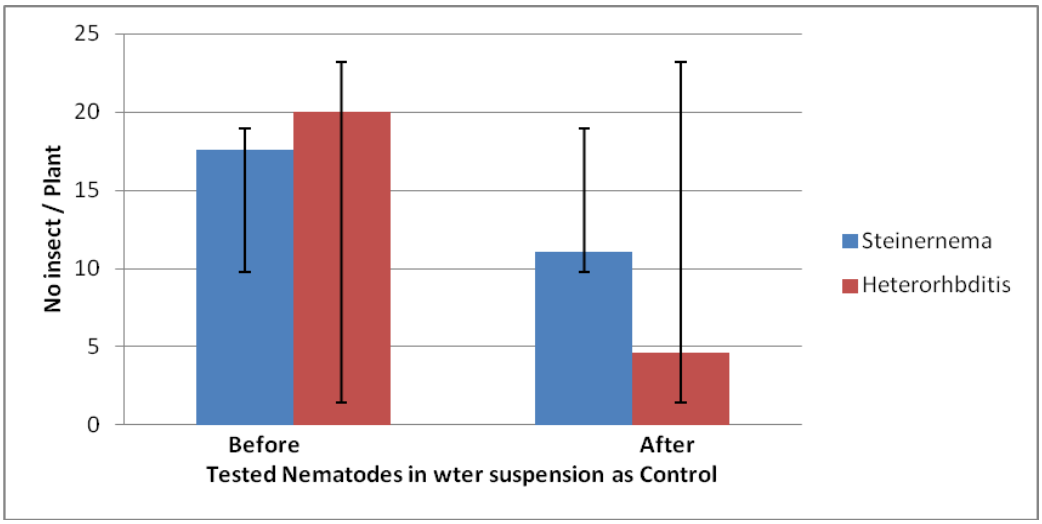

Figure 2: Mean number of Diamond back moth attacking watercress before and after spraying of non-formulated entomopathogenic nematodes.

ISSN: 0975-8585

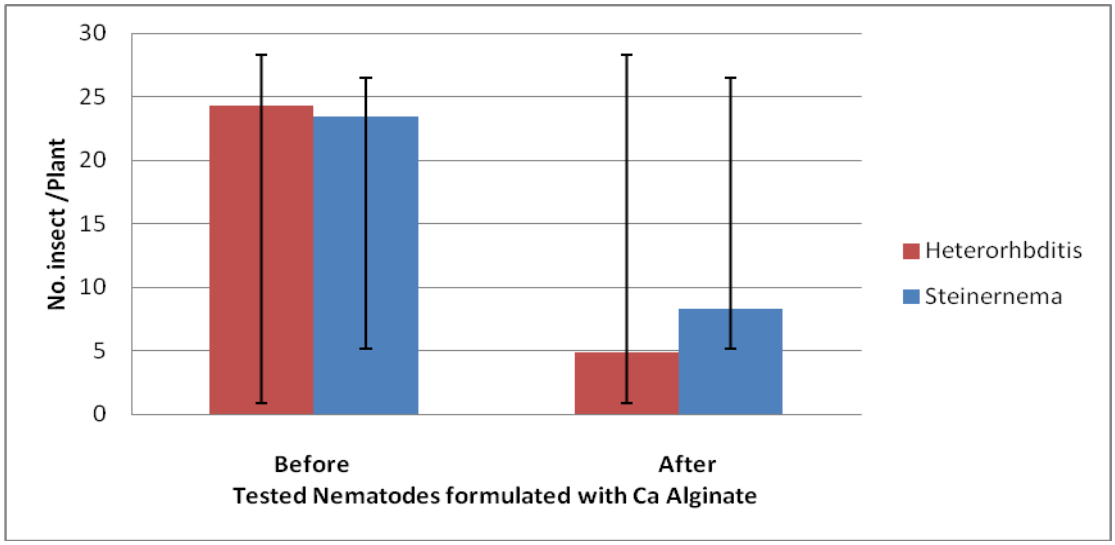

Figure 3: Mean number of Diamond back moth attacking watercress before and after spraying of bio-formulated entomopthogenic nematodes.

From Fig. (2) and Table (1),the control plot which was sprayed with *Heterorhabditis* suspension without additive, the no. of infestation of watercress with *P. xylostella* significantly decreased from 20 to 4.6 DBM/ Plant causing a highly significant reduction of 77% in DBM population where P< 0.05. Meanwhile, the nematode, *S. carpocapsae* BA2 decreased DPM population attacking watercress from 17.6 to 11.1 DBM/ Plant with a highly significant reduction reached 36.9% (P< 0.05). It was noticed from the data represented in Fig. (2), that the efficiency of *Heterorhabditis* in controlling *P. xylostella* in green house under high temperature and without additive was superior (77%) to that of *Steinernema* (36.9%).

Table 1: Statistical analysis of treatments using Paired Samples Test.

Treatments		Paired Differences			t	df	Sig. (2-tailed)*
		Mean	Std. Deviation	Std. Error Mean			
Steinernema	**before - after**	6.50000	4.62481	1.46249	4.444	9	.002
Formulated *Steinernema*		15.30000	4.44847	1.40673	10.876	9	.000
Heterorhabditis		15.40000	5.62139	1.777	8.663	9	.000
Formulated *Heterorhabditis*		19.40000	4.27395	1.35154	14.354	9	.000

*Treatments are significant at P< 0.05.

Data shown in Fig. (3) and Table (1), represent the efficiency of bio-formulated EPNs against DBM larvae attacking watercress. Addition of alginate polymer to the EPNs enhanced the efficiency of both nematode strains in controlling a serious foliage pest, *P. xylostella*. It was noticed that the no. of DBM remarkably decreased after spraying the watercress with formulated *S. carpocapsae* BA2 from 23.4 to 8.3 DBM/ Plant, causing a highly significant reduction reached 64.4% where P< 0.05. At the same time, formulated *H. bacteriophora* BA1 reduced the rate of watercress infestation with DBM from 24.3 to 4.9 DBM/ Plant causing 79.8% reduction. It was noticed that the effect of alginate polymer on the efficiency of *Heterorhabditis* is mild compared to its effect on *Steinernema*, where addition of alginate polymer to the *Steinernema* significantly increased its efficiency from 36.9% to 64.4%, while the efficiency of *Heterorhabditis* was slightly increased its efficiency from 77% to 79.8%.

DISCUSSION

The major limiting factor of foliar application of EPNs to leaf surfaces is the rapid desiccation of the IJs. Arthurs, *et al.,* [20]; Baur, *et al.,* [21] and Hussein, *et al.*, [22] showed that additives generally improved

EPNs persistence and efficacy on watercress and cabbage, but the improvement was probably not sufficient to increase the feasibility of foliar applications of EPNs against *P. xylostella* [10]; [14] and [15]**.** Prior to this investigation several adjuvants used in formulation technology [23] were tested for their feasibility to be used with EPNs and the results indicate that with few exceptions all compounds can be used for the development of new formulation to enhance EPNs performance on the leaf. The addition of polymers seems to be the key factor for improvement of EPNs efficacy [14].

Although results showed that *Heterorhabditis* wasn't affected by high temperature (45± 2°C) when applied in water suspension or in formulated polymer as did *Steinernema* which was greatly affected, formulated steinernematids in general had significantly better efficiency than the control ($P < 0.05$) under high temperature conditions. Significant differences between virulence of the two EPNs formulated isolates against DBM and those in aqueous suspensions were noticed after one week at 45± 2°C. These findings are in agreement with those workers who have also reported the efficacy of different *Steinernema* spp. and *Heterorhabditis* spp. against DBM [24-30]. Efficiency of *S. carpocapsae* BA2 was noticeably less comparing to *H. bacteriophora* BA1 and these results disagree with Metwally [31]and Saleh, *et al.* [32] who revealed that *S. carpocapsae* BA2 was more virulent against *Spodoptera littoralis* in pots and field studies than *H. bacteriophora*.

In agreement with our finding, Schroer, *et al.* [14] and [15] reported that the addition of alginate increased efficacy to 90% against larvae of DBM, *P. xylostella* infesting cabbage leaves. They indicated that formulation decreased mobility of DBM larvae and at the same time provided conditions enhanced nematode host seeking and invasion of the target insect [33].

ACKNOWLEDGEMENT

We would like to thank Dr. Samah M. M. Abd El Aziz, for helping in statistical analysis. This work has been conducted through the framework of the 10th research plan of the National Research Centre, Internal Project no. 10120611.

REFERENCES

[1] FAO. 2011. The state of food and agriculture 2010-2011. ISBN 978-92-5-106768.
[2] Castellani, et al. Bioikos 2009; 23(2): 67-75.
[3] Seelig, R. A. 1974. Fruit and vegetable facts and pointers. United Fresh Fruit and Vegetable Association Report. 7 pp.Washington, D.C.
[4] Higdon, et al. Pharmacol Res 2007; 55(3):224-236.
[5] Juge, et al. Cell Mol Life Sci 2007; 64(9):1105-27.
[6] Obeng-Ofori, et al. Cabbage and cauliflower. In Vegetable and spice crop production in West-Africa. (K. Ofori, ed.), 2007; pp. 119-122. City Publishers Ltd. Accra, Ghana.
[7] Pratissoli, et al. Horticultura Bras 2008; 26: 194-196.
[8] Nakahara, et al. 1986. Integrated control of Diamondback Moth and other insect pests using an overhead sprinkler system, an insecticide, and biological control agents, on a watercress farm in Hawaii. In NS Takelar and TC Griggs (eds). Diamondback Moth Management: Proceedings of the First International Workshop, 403-4. Asian Vegetable Research & Development Centre, Shanhua, Taiwan.
[9] Hill TA and Foster RE. J Econ Entomol 2000; 93 (3): 763-768.
[10] Schroer S and Ehlers RU. Biol Control 2005; 33:81–86.
[11] Shapiro-Ilan DI and Cottrell TE. J Invertebr Pathol 2005; 89:150–156.
[12] Gaugler R and Kaya HK. (eds). Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, Florida, 1990; 365 pp.
[13] Georgis R and Gaugler R. J Econ Entomol 1991; 84: 713-720.
[14] Schroer S. et al. Nematol 2005; 7(1): 37-44.
[15] Schroer et al. Biocont Sci Technol 2005;15(6): 601-613.
[16] Hussein MA and Abou El-Soud AB. Int J Nematol 2006; 16(1): 7-12.
[17] Metwally, Hala MS, et al. J Biol Pest Cont 2012; 22(1): 15-21.
[18] Woodring JL and Kaya HK. Steinernematid and heterorhabditid nematodes: A handbook of techniques. 1988; Arkansas Agricultural Experiment Station Southern Cooperative Bulletin, 331: 430.
[19] Hussein, Mona A and Abdel Aty MA. J Biopest 2012;5: 23-27.

[20] Arthurs, et al. Bull Entomol Res 2004; 94(4): 297-306.
[21] Baur, et al. Biocont Sci Technol 1997; 7: 513-525.
[22] Hussein, et al. Int J Nematol 2009; 19(1):103-107.
[23] Bernhardt, et al. A catalogue of formulation additives: function, nomenclature, properties and suppliers. In: Burges, H.d. (Ed.). Formulation of microbial biopesticides: Beneficial microorganism, nematodes and seed treatments. 1998; Dordrecht, The Netherlands, Kluwer Academic Publishers, pp. 333-366.
[24] Ratansinghe, G. and Hauge, N. G. M. Afro Asian J Nematol 1995; 5: 20-23.
[25] Lello, et al. Crop Protection 1996; 15: 567-574.
[26] Mason, et al. Crop Protection 1998; 17: 453-461.
[27] Mason, et al. J Invert Pathol 1999; 73: 282-288.
[28] Belair, et al. J Nematol 2003; 35: 259-265.
[29] Mahar, et al. Univ Sci 2004; 5: 1183-1190.
[30] Somvanshi, et al. Bio Control 2006; 37(1) 9–15.
[31] Metwally HM. Improving production and potency of bio-insecticides based on entomopathogenic nematodes. 2013; PhD. Thesis, Faculty of Science, Univ. Ain Shams. Pp. 142.
[32] Saleh, et al. Adv in Appl Agric Sci 2015; 3: 51-61.
[33] Navaneethan T and Strauch O. Bio Control 2010; 55: 777-788.