DOI: 10.54083/PHA/1.2.2023/34-36

Plant Health Arch., 2023, 1(2):34-36

Plant Health Archives

e-ISSN: 2583-9659
July-September, 2023

Article ID: PHA010 **Research Article**

Development of Low Cost Artificial Diet for Mass Production of Entomopathogenic Nematode, *Heterorhabditis indica* a Strain ICRI EPN-18

P. Thiyagarajan*, M. Varna, M.A. Ansar Ali and A.B. Rema Shree

Indian Cardamom Research Institute, Spices Board India, Myladumpara, Idukki, Kerala (685 553), India

Corresponding Author

Thiyagarajan, P.

: thiyainsect@gmail.com

Conflict of interests: The author has declared that no conflict of interest exists.

How to cite this article?

Thiyagarajan *et al*., 2023. Development of Low Cost Artificial Diet for Mass Production of Entomopathogenic Nematode, *Heterorhabditis indica* a Strain ICRI EPN-18. *Plant Health Archives* 1(2), 34-36. DOI: 10.54083/PHA/1.2.2023/34-36.

Abstract

Eight different diets were compared for maximum production of entomopathogenic nematode infected *Galleria* cadavers with cheaper/ low cost in the laboratory. Among eight artificial diet composition tried, low grade milk powder 100 g, low grade glycerin 100 ml, honey 100 ml, corn flour 200 g, wheat bran 100 g, wheat flour 100 g and yeast 50 g produced almost maximum *Galleria* larvae with EPN infected cadavers and EPN infective juveniles (IJs) with cheaper cost while, the diet containing, ragi powder 100 g instead of milk powder, honey 100 ml, high grade glycerin 100 ml, corn flour 200 g, wheat bran 100 g, wheat flour 100 g and yeast 50 g produced least number of EPN infected *Galleria* cadavers and EPN IJs. Among the diet compositions, sugar solution 100 ml instead of honey along with other ingredients used, did not emerge or produce larvae from the *Galleria* eggs.

Keywords: Artificial diet, *Elettaria cardamomum*, Entomopathogenic nematode, Small cardamom

Introduction

Small cardamom (*Elettaria cardamomum* Maton) is said to be the "Queen of Spices" and in our country, it is known as the "Home of Cardamom". In India, small cardamom is cultivating in Kerala, Karnataka and Tamil Nadu and nearly 60 species of pests are found to infect cardamom plants. The cardamom root grub, scientifically known as *Bsailepta fulvicorne* Jacoby, is a highly destructive pest among them; and in the fight against this pest, an indigenous strain of entomopathogenic nematode (EPN) called *Heterorhabditis indica* strain ICRI-EPN-18, originally discovered in the soil of the cardamom growing region within the Cardamom Hill Reserve (CHR), emerges as a crucial player in the biological control of *B. fulvicorne* (Varadarasan and Nagarajan, 2014; Varadarasan *et al*., 2011; Varadarasan *et al.*, 2006). This strain is mass multiplied using the greater wax moth. *Galleria mellonella* L., recognized as a significant threat to bee hives and stored bee wax (Metwally *et al.*, 2012), and involved in the substantial expenses associated with rearing *Galleria* larvae in laboratory settings. Consequently, an experiment was undertaken with the aim of formulating an optimal and cost-effective artificial diet composition to achieve maximum mass production of *Galleria* cadavers infected with entomopathogenic nematodes (EPN) in laboratory conditions for the effective management of cardamom root grub.

Materials and Methods

The present study was conducted at the Division of Entomology, Indian Cardamom Research Institute (ICRI), Spices Board India, Myladumpara, Idukki district, Kerala (685 553), India during 2017. Eight different artificial diets were prepared (Table 1), utilized for growth of *Galleria* larvae and compared to determine the highest yield of *Galleria* cadavers infected with entomopathogenic nematodes (EPN). In this study, 200 eggs of *Galleria* were taken, placed in all eight diets and replicated thrice. The containers are kept at room temperature and maintain all culture. Biological parameter *viz*., number of matured and EPN infected *Galleria* cadavers

Article History

RECEIVED on 15th May 2023 RECEIVED in revised form 31st July 2023 ACCEPTED in final form 07th August 2023

was recorded and compared. Fully grown uniform aged *Galleria* larvae are used for *in vivo* multiplication of EPN. The *Galleria* larvae collected from all artificial diets are made to infect with infective juveniles (IJs) of EPN by placing collected larvae from eight diets on filter paper in petriplates and releasing a suspension of EPN IJs of *H. indica* strain ICRI-18 which was extracted by white trap (White, 1927). Within three days of inoculation, the *Galleria* larvae are killed and dead larvae are placed in BOD incubator (at 25±2 °C) for about 7-8 days and counted EPN infected *Galleria* cadaver and EPN IJs from per cadaver and analyzed.

Table 1: The ingredients of eight artificial diets for rearing of Galleria larvae for EPN production

Ingredients/ Diets	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8
Corn flour	200 g	200 g	200 g	200 g	200 g	200 g	200 g	200 g
Wheat flour	100 g	100 g	100 g	100 g	100 g	100 g	100 g	100 g
Wheat bran	100 g	100 g	100 g	100 g	100 g	100 g	100 g	100 g
Milk powder (high grade)	100 g	100 g	100 g	-	-	-	-	-
Milk powder (low grade)	-	-	-	100 g	100 g	100 g	-	-
Ragi powder	-	-	-	-	-	-	100 g	100 g
Yeast	50 g	50 g	50 g	50 g	50 g	50 g	50 g	50 g
Honey	100 ml	-	100 ml	100 ml	-	100 ml	100 ml	-
Sugar solution	-	100 ml	-	-	100 ml	-	-	100 ml
Glycerin (High grade)	100 ml	100 ml	-	100 ml	100 ml	-	100 ml	100 ml
Glycerin (Low grade)	-	-	100 ml	-	-	100 ml	-	-

Results and Discussion

Relative cost of production cadaver^{-1} for different diets were D_1 (Rs. 0.95), D_3 (Rs. 0.72), D_4 (Rs. 0.82), D_6 (Rs. 0.62) and D_7 (Rs. 2.61). Effect of different diet formulae on the number of EPN infected *Galleria* cadaver and the production of nematodes was summarized and presented in table 2. The average number of cadavers produced from the various recommended diets ranged from 0.00 to 180.33. Diet D_4 (180.33 nos.) was on par with diet D_1 (179.66 nos.), D_3 (179.66 nos.) and diet D_6 (177.66 nos.). Diet D_2, D_5 and D_8 did not produce *Galleria* larvae. The nematode production was ranged from 0.00 to 86,000.33 IJs cadaver^{-1} and diet D_1 (86,000.33 IJs cadaver^{-1}) was on par with diet D_6 (84,333.99 IJs cadaver^{-1}) and diet D_6 (84,333.99 IJs cadaver^{-1}) at on par with D_4 (83,667.33 IJs cadaver^{-1}). Among eight different diets were examined, low grade milk powder 100 g, low grade glycerin 100 ml, honey 100 ml, corn flour 200 g, wheat bran 100 g, wheat flour 100 g and yeast 50 g, produced nearly the highest number of EPN infected *Galleria* cadaver (177.66 nos.) and EPN IJs (84,333.99 IJs cadaver^{-1}) with cheaper cost (Rs. 0.62 cadaver^{-1}) and diet containing ragi powder (finger millet) 100 g instead of milk powder, honey 100 ml, high cost glycerin 100 ml, corn flour 200 g, wheat bran 100 g, wheat flour 100 g and yeast 50 g, produced least number of EPN infected *Galleria* cadaver (54.33 nos.) and EPN IJs (47, 333.66 IJs cadaver^{-1}). Diet containing sugar solution 100 ml instead of honey along with other ingredients used, did not produce or emerge larvae from the *Galleria* eggs (Varna, 2017).

Table 2: Effect of suggested artificial diets on number of produced EPN infected cadavers and IJs cadaver^{-1}

Diet Compositions	EPN infected Galleria Cadaver (nos.)	EPN IJs Cadaver^{-1} (nos.)
D_1	179.66^a	86,000.33^a
D_2	0.00^c	0.00^e
D_3	179.66^a	81,000.33^c
D_4	180.33^a	83,667.33^b
D_5	0.00^c	0.00^e
D_6	177.66^a	84,333.99^{ab}
D_7	54.33^b	47,333.66^d
D_8	0.00^c	0.00^e

ICRI (2008) and Varadarasan *et al*. (2011) reported that the progeny production in cardamom root grub showed more yield with ICRI strains (35,000-95,000 IJs grub^{-1}) than Project Directorate of Biological Control (PDBC) strain (50,000-75,000 IJs grub^{-1}). Among ICRI strains progeny production was higher with *Heterorhabditis* (49,000-95,000 IJs grub^{-1}) than *Steinernema* (35,000-64,000 IJs grub^{-1}). Liu *et al.* (1998) reported that there was no significant difference observed in the biological parameters of *Galleria mellonella* when comparing bee wax to artificial diets composed mainly of bran, wheat flour and corn meal, and Metwally *et al.* (2012) also stated the same. The data further showed that the diet compositions where milk powder and honey used had the maximum production of *Galleria* larvae under laboratory conditions. The reason is that, diet used milk powder and honey was made so soft of diet and ragi powder and sugar solution used diets become solid within a few days after preparation. Due to the softness of the diet, the larvae were able to move more freely within it, resulting in improved feeding efficiency, and ultimately led to increased larval length and weight (Desai and Siddhapara, 2020).

Conclusion

Small cardamom is cultivating under forest ecosystem and root grub is destructive one among them and an indigenous entomopathogenic nematode (EPN) strain *Heterorhabditis indica* strain ICRI-EPN-18, emerges as a crucial player in the biological control of cardamom root grub, *B. fulvicorne* (Varadarasan and Nagarajan, 2014). Cardamom growers drenching insecticides in soil to control root grub, pollutes soil, microbes and environment. Hence, in this study, a cheaper artificial diet composition was developed for the rearing of *Galleria* larvae, aiming to achieve maximum mass production of Galleria cadavers infected with entomopathogenic nematodes (EPN) for the management of cardamom root grub, and also further study is required for developing cheaper diet for EPN mass production.

References

Desai, A., Siddhapara, M.R., 2020. *Galleria mellonella* L.: An important factitious host. In: *ResearchGate*. pp. 1-79. DOI: 10.13140/RG.2.2.21027.60963.

ICRI, 2008. Management of cardamom root grub, *Basilepta fulvicorne* (Jacoby) with entomopathogenic nematodes. Final report submitted to Department of Biotechnology, Ministry of Science & Technology, New Delhi, India. p. 215.

Liu, C.M., Shen, W.Y., Chen, K.Y., 1998. Comparison of growth and development of the greater wax moth *Galleria mellonella* reared on natural diets and an ar tificial diet. *Wuyi Science Journal* 14, 136-138.

Metwally, H.M.S., Hafez, G.A., Hussein, M.A., Hussein, M.A., Salem, H.A., Saleh, M.M.E., 2012. Low cost artificial diet for rearing the Greater Wax Moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a host for entomopathogenic nematodes. *Egyptian Journal of Biological Pest Control* 22(1), 15-17.

Varadarasan, S., Nagarajan, K., 2014. Studies on suitable formulation of entomopathogenic nematode for the management of cardamom root grub, *Basilepta fulvicorne* (Jacoby). *Journal of Plantation Crops* 42(2), 262-264.

Varadarasan, S., Hafitha, N.M., Sithara, L., Balamurugan, R., Chandrasekhar, S.S., Ansar Ali, M.A., Thomas, J., 2011. Entomopathogenic nematodes-science, technology and field outreach for biocontrol of cardamom root grub. *Journal of Plantation Crops* 39(1), 86-91.

Varadarasan, S., Sooravan, T., Chandrasekar, S.S., Ansar Ali, M.A., Thomas, J., 2006. Survey for Entomopathogenic Nematode in cardamom growing areas of Kerala and Tamil Nadu. *Journal of Plantation Crops* 34(3), 398-400.

Varna, M., 2017. Development of low cost artificial diet for rearing the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a host for entomopathogenic nematode, the strain ICRI EPN-18. M.Sc. Dissertation, Mahatma Gandhi University, Kottayam, Kerala, India. p. 30.

White, G.F., 1927. A method for obtaining infective nematode larvae from cultures. *Science* 66(1709), 302-303.