Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(2): 504–508

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

Leaf-Dip and Diet Incorporation Bioassays for Determining the Lethal Concentrations of a Chemical Pesticide and a Biopesticide against *Hyposidra talaca* Walker (Lepidoptera: Geometridae), a Major Defoliating Pest of Tea, from Darjeeling-Terai, West Bengal

Saugata Ghosh*[1]

Received: 12 Jan 2021 | Revised accepted: 14 Mar 2021 | Published online: 22 Mar 2021

A B S T R A C T

Hyposidra talaca Walker is a major tea pest in the plantations of Darjeeling-Terai region. To control this looper pest, emamectin benzoate, a chemical pesticide is widely used. The commercial formulation of *Bacillus thuringiensis* subsp. *Kurstaki* has also been proved to be a potential biopesticide in lepidopteran pest management system. To study the effect of these pesticides on this black inch looper pest, it is necessary to assay their cellular, biochemical, enzymatic response at the sub-lethal doses of those pesticides. In this study, lethal concentrations of these pesticides were determined by two standard bioassay methods- leaf-dip method, using tea leaves as their natural diet and diet-incorporation method, using synthetic diet. In leaf-dip method the LC values were found to be higher than the other method for both pesticides. As the population of this Geometrid moth caterpillars had not been exposed to this biopesticide, so this looper pest was expected to be more susceptible to it. The LC values of *Bt* for one day exposure were observed lower than that of the emamectin benzoate in both methods of bioassays according to the expectation however, for prolonged exposure the LC values of the biopesticide were found to be higher than the chemical pesticide.

***Key words*:** Tea, Bioassay, Pesticide, Tea pest, *Hyposidra talaca,* Lethal concentration, Leaf-dip, Diet-incorporation, *Bacillus thuringiensis,* Emamectin benzoate

Tea, *Camellia sinensis* (L.) O. Kuntze, the most popular beverage in India, is the economic life line of a large section of people from the northern part of West Bengal and the north-east India. But it is regularly attacked by huge number of tea pests that are injurious or potentially injurious to economy of this region. Tea plantations are infested by about 167 insect species in the north-eastern tea growing regions of India [1-2]. Of these, six species have attained major pest status causing 11-55% crop loss [3]. Recently the geometrid looper, *Hyposidra talaca* (Walker) emerge as major defoliator of tea in the Sub-Himalayan West Bengal [4]. Tea pests are largely controlled by the broad-spectrum chemical pesticides. However, in spite of adopting control measures with synthetic insecticides, considerable amount of crop loss is incurred every year due to the attack of looper stage of *H. talaca*. Moreover, large scale and injudicious application of these synthetic pesticides over the years created problems of pesticide residues in made tea as well as pesticide resistance of this geometrid moth against certain pesticides. The effective pest management strategies against *H. talaca* demands the insight knowledge of the defense mechanisms of this lepidopteran pest against the chemical pesticides that are used in the sub-Himalayan tea plantations in large scale, as well as, against the potential bio-pesticides to provide a non-chemical alternative for insect pest management. To study the defense system of this destructive defoliating pest, it is necessary to assess the immune response against the pesticide challenge at the sub-lethal concentration of the respective pesticide.

To study the response of the pesticide on phytophagous pest, *Hyposidra talaca* at the sub-lethal concentration of the respective pesticides, leaf-dip method for bioassay is the most common procedure if tea leaves are used as diet during the experiment [5]. But due to the unavailability of fresh tea leaves throughout the year, continuous cultures of this black inch loopers for testing new chemicals, microbial production or biocontrol agents is not possible. Moreover, the allelochemicals present in the tea leaves may interfere the effect of pesticides on the pest. To overcome this problem a synthetic diet has been formulated by [6]. It was also found, that the loopers, reared on this artificial diet have higher hemocytic load in comparison to the larvae cultured on natural diet [7]. As the cellular defense system depends on the number and types of the hemocytes [8], so the formulated synthetic diet is suitable for disease free continuous rearing of the

*Saugata Ghosh
sgwbes@gmail.com

[1]Department of Zoology, (Supported by BOOST programme of Department of Biotechnology, GOWB), A.P.C. Roy Govt. College, Matigara, Siliguri - 734 010, Darjeeling, West Bengal, India

looper caterpillars. To assay the response of the pesticide on the loopers reared on artificial diet, diet incorporation method of bioassay seems to be the best method for comparison the dose-mortality relationship [9]. In this present study, the response of laboratory population of *Hyposidra talaca* against two pesticides- one widely used chemical pesticide, Emamectin Benzoate and the other most potential bio-pesticide, *Bacillus thuringiensis*, was evaluated by leaf-dip and diet incorporation bioassays to determine the LC values of these pesticides.

MATERIALS AND METHODS

Insects

The moths of *Hyposidra talaca* were collected from the tea plantations of Terai region of Darjeeling District. These were allowed to mate and lay eggs in rearing containers made of transparent plastic (11 cm height × 12 cm diameter) in 2:2 or 2:3 female to male ratio in the laboratory. After hatching, different batches of neonates (about 1-12 hours old) were reared in a BOD incubator (CHM-10 PLUS, REMI, India: Make) at a temperature of 27 ± 2°C, L:D 13:11 h and RH 75 ± 5% on natural diet (tea) or on artificial diet following the method of Prasad and Mukhopadhyay (2015). At this temperature, the growth of geometrids was optimal, and the diet did not undergo fast desiccation or drying.

Diet

Fresh tea leaves, the natural diet, was collected from the tea twigs of TV-26 (Tocklai variant) clone in the experimental plot of the organically maintained Tea Garden in University of North Bengal, Siliguri-13 (26° 71' N and 88° 35' E). Leaves were provided as food after surface sterilization with 1% sodium hypochlorite by dipping the leaves for 3 seconds and then thoroughly washing them in distilled water. The larvae reared on natural diet were used for dip-leaf method of bioassay.

The artificial diet was prepared by mixing different ingredients such as wheat germ (16.675 g), cellulose powder (10 g), casein (11.675 g), potassium sorbate (2.075 g), dextrose (11.675 g), sodium alginate (1.675 g), sucrose (0.9 g), Wesson's salt mixture (3.35 g), choline chloride (0.35 g),a vitamin mixture (thiamine mono nitrate 30 mg, vitamin $B_2$30 mg, vitamin B_6 9 g, nicotinamide 300 mg, calcium pantothenate 150 mg, folic acid 4500 mcg, vitamin B_{12} 45 mcg, vitamin C 450 mg, biotin 300 mcg, tocopheryl acetate 400 mg), cholesterol (1 g), linoleic acid (1 g) and wheat germ oil (0.9 ml) in autoclaved 3% agar medium (300 ml) in the laminar hood following the method of Prasad and Mukhopadhyay (2015). Artificial food was used to rear the larvae for diet-incorporation bioassay.

Insect rearing

On natural diet

After hatching, neonates were released in a plastic container (11 cm height X 11 cm diameter) containing single tea twig in water filled micro-centrifuge tube for the development of the first two instars. From the third instar onward, they were reared in a large cylindrical bucket (45 cm height X 30 cm diameter) whose mouth was covered by autoclaved cotton cloths and secured by rubber bands, to avoid the escape of larvae. The bucket was provided with 7-8 tea leaf twigs immersed in the water-filled100 ml conical flask. The twigs being provided ad-libitum, were replaced daily or alternate days, before being consumed totally by the larvae or before getting wilted [7].

On artificial diet

After hatching, the first instar larvae were transferred to 100 ml sterile plastic container (Tarson-510010) having 1 cc. of diet cubes suspended freely with the help of toothpick. The artificial diets were provided ad-libitum before getting dried or before getting consumed. To avoid high rate of mortality due to moisture accumulation, the containers were covered in such a way that proper aeration, as well as the proper moisture, could be maintained [10]. As the larvae grew bigger these were segregated into smaller groups to new containers provided with diet to allow sufficient space for their free movement and to avoid mortality due to possible injury to larvae while competing for food.

Insecticides

The bioassay was done with a chemical pesticide, Emamectin Benzoate 5% SG (Brand name: Missile, manufactured and marketed by Crystal Crop Protection Pvt. Ltd.). It is most widely used pesticide with field dose 400 mg/ litre as per PPF in Plant Protection Code, 2014 to control looper caterpillars in Sub-Himalayan tea plantations [11]. It is a second generation avermectin insecticide and act as neurotoxin interfering with the normal function of GABA by binding to its receptors and increasing the permeability of the chloride ions in nerve and muscle membranes by opening chloride channels [12].

The bioassay was also done with a commercial formulation of *Bacillus thuringiensis* (Brand name: DiPel-8L, manufactured and marketed by Sumitomo Chemicals India Pvt. Ltd.). It is the most potential biopesticide for controlling a variety of lepidopterans with field dose 2.5 ml/ litre as per TRA recommendation [13]. Genes producing the following ICPs are present in *B. thuringiensis* subsp. *Kurstaki*: Cry IA (a), Cry IA (b), Cry IA (c), Cry IIA and Cry IIB [14].

Bioassay

Leaf-dip method

Laboratory based leaf-dip method of bioassay was performed by the standard method (Method No.7) recommended by Insecticide Resistance Action Committee (IRAC) [15] to determine the LC values of pesticide. According to IRAC method 7, the leaves were dipped in graded concentrations of pesticide for 5 s with gentle agitation and air-dried. For the bioassay of the chemical pesticide graded concentrations of Emamectin Benzoate (0.75 mg/l, 1.5 mg/l, 3.00 mg/l, 6.00 mg/l, 12.00 mg/l, 24.00 mg/l, 48 mg/l and 96 mg/l) and for biopesticide graded concentration commercial formulation of *Bacillus thuringiensis* (2.5 ml/l, 3.0 ml/l, 3.5 ml/l, 4.0 ml/l, 4.5 ml/l, 5.0 ml/l, 5.5 ml/l and 6 ml/l) were prepared in distilled water. For control, the leaves were dipped in double distilled water. For each concentration as well as for the control, healthy second generation one–day old 5th instar larvae (n=30) of *H. talaca* with three replicates, each of 10, were used. The larvae were released separately into each bucket (45 cm height X 30 cm diameter) each containing 15 pesticide-exposed tea twigs with two leaves and a bud immersed in the water-filled 100 ml conical flask [16]. Before release the larvae were starved for 4 hours. The mouth of the bucket was covered by autoclaved cotton cloths and secured by rubber bands, to avoid the escape of larvae.

Diet incorporation method

Laboratory based diet incorporation method of bioassay was performed by the standard method (Perez *et al.*, 1997). The artificial diet was supplemented with graded

concentrations of pesticide dissolved in distilled water, at the ratio of 9:1 (9 parts diet and 1 part aqueous solution of pesticide) during the preparation of diet when the temperature of the artificial diet dropped to 40°C (the lowest temperature at which the insecticides does not loss integrity) [17]. Final concentrations of the pesticide in artificial diet were 0.5 mg/l, 1.0 mg/l, 1.5 mg/l and 2.00 mg/l for emamectin benzoate and 1.0 ml/l, 1.5 ml/l, 2.0 ml/l and 2.5 ml/l. For control experiment, double distilled water was used to the un-modified diet instead of the pesticide. For each concentration as well as for the control, healthy second generation one–day old 5th instar larvae (n=30) of *H. talaca* with three replicates, each of 10, were used. The artificial diet was provided ad-libitum for individual larva by using 1 cm^3 of diet-cube suspending freely with the help of toothpick in each of the 100 ml sterile plastic container (Tarson-510010). Before release the larvae were starved for 4 hours. The mouth of the container was covered by autoclaved cotton cloths and secured by rubber bands, to avoid the escape of larvae.

Observations of larval mortality were recorded in each concentration of the pesticide for three days at 24 hours interval after treatment. Moribund insects were counted as dead [18].

Statistical analysis

A concentration-probit mortality curves for different concentrations of the pesticides were obtained using LdP Line software. The mortality data was converted to corrected percent mortality by using Abbott's formula [19] and subjected to probit analysis [20] to obtain different LC values.

RESULTS AND DISCUSSION

Leaf-dip bioassay

The 1-day old fifth instar loopers (n=30) showed gradual response along with the concentration gradient against both chemical pesticide and bio-pesticide. At the concentrations of 0.75 mg/l, 1.5 mg/l, 3.00 mg/l, 6.00 mg/l, 12.00 mg/l, 24.00 mg/l, 48 mg/l and 96 mg/l of emamectin benzoate the mortality was recorded as 2, 3, 5, 10, 13, 18, 19, 25 and 3, 8, 19, 24, 25, 28, 30, 30 after 24 hours and 48 hours exposure respectively. For 72 hours exposure of the pesticide at the concentrations of 0.75 mg/l, 1.5 mg/l, 3.00 mg/l, 6.00 mg/l, 12.00 mg/l and 24.00 mg/l the mortality was recorded as 11, 22, 27, 28, 30 and 30 respectively. No mortality was, however, found in control experiment. From the concentration dependent mortality data, using LdP line software the LC50 of Emamectin Benzoate (EB), was determined as 17.03 mg/l, 2.80 mg/l and 0.93 mg/l when the black inch looper were exposed to the pesticide for 24, 48 and 72 hours respectively (Table 1).

At the graded concentrations of biopesticide (2.5 ml/l, 3.0 ml/l, 3.5 ml/l, 4.0 ml/l, 4.5 ml/l, 5.0 ml/l, 5.5 ml/l and 6 ml/l) the mortality was recorded as 0, 1, 3, 4, 7, 7, 8 10; 1, 3, 7, 9, 10, 11, 16, 17 and 2, 6, 10, 11, 17, 19, 21, 23 respectively after three successive days of pesticide application. No mortality was recorded in control experiment. From the concentration dependent mortality data, the LC50 of *Bacillus thuringiensis*, was determined as 7.56 ml/l, 5.47 ml/l and 4.37 ml/l for the exposure of 24, 48 and 72 days exposure respectively (Table 1). Mortality of the 5th instar looper stage of *H. talaca* was determined as high as 94.32 % for only 24 hours exposure of Emamectin Benzoate applied on tea leaves at field concentration (400 mg/l), as per PPF in Plant Protection Code, 2017 [21]. But at the field concentration of *Bacillus thuringiensis* (2.5 ml/l), as per TRA recommendation [22] mortality of larvae was determined as low as 2.76% for 24 hours exposure and was increased slightly up to 8.96% when the larvae were exposed for 72 hours to the treated leaves.

Diet incorporation bioassay

The 1-day old fifth instar loopers (n=30) showed gradual response along with the concentration gradient of the pesticide. At the concentration of 0.5 mg/l, 1.0 mg/l, 1.5 mg/l and 2.00 mg/l of the chemical pesticide the mortality was recorded as 2, 4, 6, 13; 3, 10, 16, 22 and 4, 19, 23, 29 for the successive three days after providing modified synthetic diet to the looper caterpillars respectively. No mortality was found when unmodified diet was provided to the larvae. From the concentration dependent mortality data, the LC50 of Emamectin Benzoate (EB) supplemented to the artificial diet to which the larvae were exposed for 24, 48 and 72 hours, were determined as 2.86 mg/l, 1.34 mg/l and 0.89 mg/l respectively using LdP line software (Table 1).

Table 1Result from two bioassay methods on the population of *Hyposidra talaca*

Method of bioassay	Insecticide	Exposure time (in hours)	LC_{50} (in PPM)	LC_{90} (in PPM)	Slope ± SE
Leaf-dip method	Emamectin benzoate	24	17.03 (11.91-25.75)	219.19 (111.48-640.28)	1.1550 ± 0.1472
		48	2.80 (2.08-3.66)	13.70 (9.40-24.48)	1.8577 ± 0.2472
		72	0.93 (0.56-1.25)	3.53 (2.52-6.80)	2.2164 ± 0.4597
	Bacillus thuringiensis	24	7.56 (6.22-14.04)	15.80 (10.14-71.99)	4.0014 ± 1.1286
		48	5.47 (4.97-6.38)	10.25 (8.15-16.15)	4.6929 ± 0.8214
		72	4.37 (4.10-4.72)	7.44 (6.48-9.39)	5.5423 ± 0.7829
Diet-incorporation method	Emamectin benzoate	24	2.86 (1.97-1.67)	10.84 (4.67-97.53)	2.2147 ± 0.07107
		48	1.34 (1.11-1.67)	3.45 (2.48-7.05)	3.1214 ± 0.6284
		72	0.89 (0.75-1.03)	1.76 (1.48-2.32)	4.3186 ± 0.6549
	Bacillus thuringiensis	24	3.20 (2.50-7.72)	7.28 (4.28-62.62)	3.5926 ± 1.1228
		48	2.62 (2.20-3.98)	5.50 (3.73-17.48)	3.9841 ± 1.0405
		72	2.22 (1.92-2.88)	5.71 (3.89-15.70)	4.0126 ± 0.9454

In diet incorporation bioassay of biopesticide, the mortality of 1-day old fifth instar loopers (n=30) also showed gradual response along with the concentration gradient (1.0 ml/l, 1.5 ml/l, 2.0 ml/l and 2.5 ml/l). The mortality was recorded as 1,4,6, 11; 2, 5, 9, 15 and 3, 7, 11, 19 at different concentrations of the biopesticide supplemented to the artificial diet to which the larvae were exposed for 24 hours, 48 hours and 72 hours respectively. No mortality was recorded when unmodified diet was provided to the larvae. From the concentration dependent mortality data, the LC50 of *Bacillus thuringiensis*, was determined by software analysis as 3.20 mg/l, 2.62 mg/l and 2.22 mg/l for three successive days after providing supplemented diet to the larvae (Table 1).

Leaf-dip as well as diet-incorporation method for bioassay to determine the median lethal concentration of emamectin benzoate against the fifth instar larvae of *H. talaca* showed sharp decrease with the change of the exposure time, for which the larvae are exposed to the pesticide, from 24 hours to 48 hours. It indicates that this chemical pesticide is a fast-acting pesticide as it acts on the nervous system of the caterpillar within few hours after the application of the pesticide [23-24]. But gradual decrease of LC_{50} value of the biopesticide along with the increase of exposure time indicate the slow activity of the insecticidal crystal proteins produced by *Bacillus thuringiensis* that have to be ingested by the pests to be toxic. The field dose of emamectin benzoate as per PPF was found to be sufficient to kill about 95% of the 5^{th} instar larvae, the major defoliating phase of this geometrid pest, within 24 hours. However, for biopesticide (*Bt)* the TRA recommended field concentration was found to kill less than 10% even after 3 days of exposure. This large scale, injudicious application of the chemical pesticide over the years not only upset the natural ecosystem by enhancing secondary pest outbreak, pest resurgence, and variation in susceptibility but also created problems of pesticide residue in tea [25-26]. The less susceptibility of the looper stage of *H. talaca* against emamectin benzoate in comparison to *Bt* was also indicated by very high LC_{50} values for 24 hours exposure of the synthetic pesticide in comparison to that for biopesticide, determined by both bioassay methods. The LC_{50} values obtained from the incorporation method for both pesticides were lower than the other method. Because leaf-dip is a residue bioassay in which the pesticide is spread evenly on the leaf surface only, but in diet incorporation assay the pesticide is homogeneously mixed with the diet. So, even same concentration of the pesticide is used for these two bioassays, dose of the pesticide will be more in case of incorporation method.

CONCLUSIONS

From these studies the LC values for the most widely used chemical pesticide and the most potential biopesticide against the major defoliating tea pest, *Hyposidra talaca* reared on natural and artificial diet, have been revealed. This knowledge will certainly opens up new research opportunities regarding the effect of these pesticides at the sublethal dose to assay the defense response of this Geometrid tea pest against these pesticides.

Acknowledgement

The author would like to extend his sincere appreciation to the Department of Science & Technology and Biotechnology (West Bengal) for its funding of this research work through the R & D project [821 (Sanc)/ ST/P/S & T/2G-13/2018 dated 18/06/2020]. The author is also thankful to the Department of Biotechnology (West Bengal) for infrastructural up gradation of the laboratory in the Department of Zoology, APC Roy Govt. College, Siliguri, where the whole research work was carried out, through the BOOST programme [118/4/BT(Estt)/1P-4/2013 dated 15.02.2017].

LITERATURE CITED

1. Das GM. 1965. Pest of Tea in North-East India and their Control. Tocklai Experimental station, Tea Research Association, Jorhat, Assam, India.
2. Mukhopadhyay A, Roy S. 2009. Changing dimensions of IPM in the tea plantations of the North Eastern sub-Himalayan region. *In*: (Eds) Ramamurthy VV, Gupta GP. Proceedings of National Symposium on IPM Strategies to Combat Emerging Pest in the Current Scenario of Climate change; 28-30 January; Arunachal Pradesh (India): Central Agricultural University Pasighat. pp 290-302.
3. Gurusuvramanian G, Rahman A, Sharmah M, Roy S, Bora S. 2008. Pesticide usage pattern in tea ecosystem, there retrospect and alternative measures. *Journal of Environment Biology* 29(6): 813-826.
4. Basu MA, Ghosh P. 2004. *Hyposidra talaca* (Walker) a destructive pest of tea in Dooars tea plantations. *Two and a Bud* 51: 49-51.
5. Perez CJ, Tang JD, Shelton AM. 1997. Comparison of leaf-dip and diet bioassays for monitoring *Bacillus thuringiensis* resistance in field populations of diamondback moth (Lepidoptera: Plutellidae). *Insecticide Resistance and Resistance Management* 90: 94-101.
6. Prasad AK, Mukhopadhyay A. 2015. Fitness traits of the tea defoliator, *Hyposidra talaca* (Walker, 1860) (Lepidoptera: Geometridae) on natural and artificial diets in relation to gut enzymes and nutritional efficiencies. *Annales de la Société Entomologique de France* 51(2): 145-152.
7. Ghosh S, Prasad AK, Mukhopadhyay A. 2018. Effects of feeding regimes on hemocyte counts in two congeners of *Hyposidra* (Lepidoptera: Geometridae). *Entomologia Generalis* 38(1): 73-82.
8. Russo J, Brehelin M, Carton Y. 2001. Hemocyte changes in resistant and susceptible strains of *D. melanogaster* caused by virulent and avirulent strains of the parasitic wasp *Leptopilina bouladi*. *Journal of Insect Physiology* 47: 167-172.
9. Roy S, Muraleedharan N, Mukhopadhyay A. 2014. The red spider mite, *Oligonychus coffeae* (Acari:Tetranychidae): its status, biology, ecology and management in tea plantations. *Experimental and Applied Acarology* 64(1): 431-463.
10. Roy S, Muraleedharan N, Mukhopadhyay A, Handique G. 2015. The tea mosquito bug, *Helopeltis theivora* waterhouse, (Heteroptera: Miridae): its status, biology, ecology and management in tea plantations. *International Journal of Pest Management*. DOI org/10.1080/09670874.2015.1030002.
11. Anonymous. 2017. Plant protection code-policy on usage of plant protection formulations in tea plantations of India. Tea Board of India, Kolkata. pp 1-57.

12. Grant AN. 2002. Medicines for sea lice. *Pest Management Science* 58(6): 521-527.
13. Basu MA, Pathak SK, Hath TK. 2012. Evaluation of some bio-rational insecticides against the looper complex, Hyposidra spp. in tea plantations of Dooars, West Bengal. *Biopest* 5(1): 91-95.
14. Koziel MG, Carozzi NB, Currier TC, Warren GW, Evola SV. 1993. The insecticidal crystal proteins of *Bacillus thuringiensis*: past, present and future uses. *Biotechnol. Genet. Eng. Rev*. 11: 171-228.
15. Kranthi KR. 2005. Insecticide Resistance Monitoring, Mechanisms and Management Manual. Central Institute of Cotton Research, ICAR, Nagpur. pp 1-153
16. Das S, Mukhopadhyay A, Roy S, Biswa R. 2010. Emerging looper pests of tea crop from sub-Himalayan West Bengal, India. *Resistant Pest Management Newsletter* 20(1): 8-13.
17. Durmusoglu E, Hatipoglu A, Gurkan MO, Moores G. 2015. Comparison of different bioassay methods for determining insecticide resistance in European Grapevine Moth, *Lobesia botrana* (Denis and Schiffermuller) (Lepidoptera: Tortricidae). *Turk. Entomol. Derg*. 39(3): 271-276.
18. Gurusuvramanian G, Bora S. 2007. Relative toxicity of some commonly used insecticides against adults of *Helopeltis theivora* (Miridae: Hemiptera) collected from Jorhat area tea plantations, South Assam, India. *Resistant Pest Management Newsletter* 17(1): 8-12.
19. Abbott WS. 1925. A method computing the effectiveness of an insecticide. *Journal of Economical Entomology* 18: 265-267.
20. Finney DT. 1971. *Probit Analysis*. The Cambridge University Press, London. pp 333.
21. Hazarika LK, Puzari KC, Wahab S. 2001. Biological control of tea pests. *In*: (Eds) Upadhyay R.K., Mukerji K.G., Chamola B.P. Biocontrol Potential and its Exploitation in Sustainable Agriculture. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-1377-3_11
22. Angelica PR, Quintero HA, Serrão JE, Martínez. LC. 2020. Insecticidal activity of *Bacillus thuringiensis* strains on the nettle caterpillar, *Euprosterna elaeasa* (Lepidoptera: Limacodidae). *Insects* 11: 310; doi:10.3390/insects11050310
23. Ghatak SS, Reza MW, Poi SC. 2008. Bio-efficacy of some bio-pesticides and modern synthetic pesticides against tea mosquito bug, *Helopeltis theivora* Waterhouse (Hemiptera:Miridae). *Res. Crops* 9: 165-171.
24. Isman MB. 2006. Botanical insecticides, deterrents, and repel-lents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomology* 51: 45-66.
25. Devi KD, Nishikanta K, Varatharajan R. 2016. Diversity and density of tea pests in the tea gardens of Manipur. *Journal of Plantation Crops* 44(1): 47-51.
26. Hazarika LK, Bhuyan M, Hazarika BN. 2009. Insect pests of tea and their management. *Annu. Rev. Entomology* 54: 267-284.