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Biosafety assessment of nanemulsion of hexanal on *Mallada boninensis* Okamoto (Chrysopdae: Neuroptera)

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ABSTRACT: Experiments were carried out under laboratory conditions to assess the effect of nano emulsion of hexanal on egg and grub stage of green lacewing, *Mallada boninensis* Okamoto (Neuroptera: Chrysopidae) through laboratory bioassay. The results showed that, nanoemulsion of hexanal was found to cause egg hatchability up to 90.04 percent in higher concentrations of 0.06 percent. Egg hatchability at field recommended concentration of 0.02% was found maximum of 94.18 percent respectively. In addition nanoemulsion of hexanal at field recommended concentration 0.02% tested against grubs of *M. boninensis* results revealed that 6.67 percent grub mortality in dry film method at 48h after exposure it was least toxic to grub of *M. boninensis*. The maximum percent pupation and adult emergence was recorded in nanoemulsion of hexanal @ 0.02% showed 95.33 and 95.0 percent, respectively. The results clearly indicated that all the concentration of nano formulation of hexanal was harmless to *M. boninensis* which recorded a mortality of < 30 percent as per the threshold prescribed by IOBC.

Keywords: Mango, post-harvest losses, hexanal, nanoemulsion, biosafety

INTRODUCTION

India ranks first in the world production of mango with the production of 19.27 million tonnes from 2.5 million hectares which is the largest in the world and it accounts for 21 percent of the total fruit production in the country (FAOSTAT. 2011). Post harvest losses are one of major constraints in the production and marketing of mango. Many biologically active volatile compounds like hexanal are found to reduce the post harvest losses by checking the over ripening. The key enzyme involved in the ripening of fruits is Phosholipase D (PLD). The use of hexanal by inhibits PLD production in fruit skin which turn delays post harvest deterioration. (Paliyath, G. and Subramanian, J. 2008). Formulation of hexanal as nanoemulsion would be more effective for fruit preservation, which shall be uniform droplet sizes (<100 nm), high kinetic stability and optical transparency. Biosafety of nano product is one of most important prerequisite to be assessed against beneficial and non-target organisms. (Karthika*et al.*, 2015; Mohan *et al.*, 2017) There are several natural enemies in mango ecosystem among them the *Mallada boninensis*is a most important generalist predator of many soft bodied insect pests of several crops (Geetha and Swamiappan, 1998) and it can be easily mass cultured in laboratory (Ridgway *et al.,* 1970). The grubs are voracious and efficient predators for various phytophagous arthropods which include whiteflies, aphids, scales, mealy bugs, leafhoppers, psyllids, thrips, mites, small caterpillars, eggs of moths and other soft bodied insects (McWen *et al.,* 2001). The biosafety of nanoemulsion of hexanal on the natural enemies associated with mango cropping system need to be studied and Hence, the present studies was undertaken on safety of nanoformulation of hexanal on *M. boninensis* under laboratory condition.

MATERIALS AND METHODS

Laboratory experiments were carried out at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2015-2017, to study the toxicity of nanoemulsion of hexanal to *M. boninensis.* Safety of nanoemulsion of hexanal was compared with pure form of hexanal which is a patentable product of Canada. The susceptibility of *M. boninensis* to nanoemulsion of hexanal at different concentrations was evaluated along with control in a Completely Randomized Design (CRD) replicated three times.

Preparation of nanoemulsion of hexanal

Preparation of hexanal nanoemulsion involves mixing of hexanal:Tween 20 and ethanol in the ratio of 1:10:10 v/v basis and were sonicated using sonicator at 20 kHz for 15 min for good emulsion as per the standard method. (Jafariand Bhandari, 2006).

Statistical analysis

The data on percentage values were transformed in to arcsine values and the population recorded as numbers were transformed into $\sqrt{x+0.5}$ before statistical analysis. The data obtained from laboratory experiments were analyzed in completely randomized design. The

mean values were separated using Duncan's Multiple Range Test (DMRT). The corrected percent mortality was worked out using the formula (Abott, 1925)

$$\text{Corrected per cent mortality} = \frac{P_0 - P_c}{100 - P_c} \times 100$$

P_0 - Observed mortality in treatment

P_c - Observed mortality in untreated check

Effect of hexanal formulation on the eggs of *M. boninensis*

Laboratory studies were conducted to assess the effect of nano emulsion of hexanal formulation on the eggs of *M. boninensis*as per the method described by Krishnamoorthy (1985). The eggs along with stalks on brown paper strips were sprayed with hand atomizer at different concentrations separately as mentioned in table 1. Each treatment was replicated three times with 100 eggs per treatment. Untreated check was maintained by spraying distilled water. The number of grubs hatched from each treatment was recorded and per cent hatchability worked out.

Effect of hexanal formulation on grubs of *M. boninensis*– Poison food techniques

The UV treated *Corcyra* eggs was sprayed different concentrations of hexanal formulation separately using hand atomizer as mentioned in table 1. The treated eggs were shade dried for 30 minutes and then transferred to rectangular multi cavity tray @ 2 cc per tray. The untreated check was maintained by spraying distilled water. Second instar *M. boninensis* grubs were transferred to these cavity trays @ one per cavity. After the grubs completely fed the hexanal treated eggs, the grubs were provided with untreated *Corcyra* eggs till pupation. Observation on mortality of grub at 12, 24 and 48 h after treatment were recorded. In addition, the percent pupation and percent adult emergence of live grub were also noted.

RESULTS AND DISCUSSION

Effect of hexanal on eggs of *M. boninensis*

The effect of nanoemulsion of hexanal on eggs of *M. boninensis* was studied under laboratory condition. The data in table 2 represents the hatching of *Mallada* eggs

Table 1. Ovicidal effect of nanoemusion of hexanal on the eggs of *Mallada boninensis*

Treatment	Egg hatchability (%)	Percent reduction over control
T_1- Nanoemusion of hexanal @ 0.02%	94.18 (76.86)^c	4.82
T_2- Nanoemusion of hexanal @ 0.04%	94.26 (76.49)^c	5.74
T_3- Nanoemusion of hexanal @ 0.06%	90.04 (71.65)^d	9.95
T_4- Pure hexanal @ 0.02%	88.74 (70.52)^{de}	11.26
T_5- Pure hexanal @ 0.04%	88.47 (70.18)^{de}	11.53
T_6- Pure hexanal @ 0.06%	87.32 (69.19)^e	12.68
T_7- Tween 20 @ 0.2%	99.32 (85.27)^b	0.68
T_8- Ethanol @ 0.2%	94.44 (76.37)^c	5.56
T_9- Control	100 (90.00)^a	
Mean	**92.97**	**7.90**
S.ED	**7.83**	
CD (0.05%)	**16.46**	

Mean of three observations ; In a column means followed by a common letter are not significantly different at p = 0.05 by DMRT;

Figures in parentheses are arcsine √ P transformed value

Table 2. Effect of nanoemusion of hexanal on the development of II instar grub of *Malladaboninensis*

	Grub mortality (%)						Pupation (%)	Adult emergence (%)
	12 HAT	Corrected mortality	24HAT	Corrected mortality	48HAT	Corrected mortality		
T_1- Nanoemusion of hexanal @ 0.02%	3.33 (10.51)[a]	0.00	6.67 (14.97)[a]	0.00	6.67 (14.96)[a]	0.00	95.33 (77.52)[ab]	95.00 (77.17)[b]
T_2- Nanoemusion of hexanal @ 0.04%	3.33 (10.51)[a]	0.00	6.67 (14.97)[a]	0.00	6.67 (14.96)[a]	0.00	95.33 (77.52)[ab]	95.00 (77.17)[b]
T_3- Nanoemusion of hexanal @ 0.06%	3.33 (10.51)[a]	0.00	6.67 (14.97)[a]	0.00	13.33 (14.96)[a]	7.14	94.44 (76.37)[bc]	92.13 (76.61)[bc]
T_4- Pure hexanal @ 0.02%	6.67 (14.97)[b]	3.34	10.0 (18.43)[a]	3.57	16.66 (24.09)[a]	10.70	92.00 (73.57)[c]	91.11 (76.37)[bc]
T_5- Pure hexanal @ 0.04%	10.0 (18.43)[c]	6.67	13.33 (21.41)[c]	7.14	16.67 (24.10)[c]	10.71	90.70 (72.25)[cd]	90.00 (71.57)[c]
T_6- Pure hexanal @ 0.06%	13.33 (21.41)[d]	10.0	20.0 (26.57)[d]	14.28	26.67 (31.09)[d]	20.00	90.00 (71.57)[d]	88.89 (70.53)[d]
T_7- Tween 20 @ 0.2%	6.67 (14.97)[b]	3.34	10.0 (18.43)[b]	3.57	6.67 (14.97)[b]	0.00	95.65 (78.17)[a]	100.00 (90.00)[a]
T_8- Ethanol @ 0.2%	3.33 (10.51)[a]	3.33	6.67 (14.97)[a]	0.00	6.67 (14.97)[c]	0.00	95.33 (77.52)[ab]	100.00 (90.00)[a]
T_9- Control	3.33 (10.51)[a]	-	6.67 (14.97)[a]	-	6.67 (14.96)[a]	-	96.29 (78.94)[a]	100.00 (90.00)[a]
Mean	**5.98**	**3.35**	**9.26**	**3.74**	**11.25**	**5.71**	**93.71**	**94.68**
S.ED	**0.57**	**0.37**	**0.86**	**0.46**	**1.145**	**0.70**	**7.90**	**7.97**
CD (0.05%)	**1.21**	**0.78**	**1.81**	**0.98**	**2.40**	**1.47**	**16.61**	**16.76**

*Values in the parentheses are arcsine transformed values; In a column means followed by a common letter(s) are not significantly different by DMRT (P=0.05);

*Mean of three replications; HAT- Hours after treatment

in different concentrations of nano emulsion of hexanal. The results indicated that Cent per cent egg hatchability was observed in untreated check 48 hours after treatment (HAT) followed by the surfactant Tween 20 and solvent absolute ethanol @0.2% which recorded 99.32 and 94.44 percent egg hatchability respectively. The nanoemulsion of hexanal @ 0.02, 0.04 and 0.06 percent showed 94.18, 94.26 and90.40 percent egg hatchability at the same period of observation. The standard check purehexanal @ 0.02, 0.04 and 0.06 % percent showed 88.74, 88.47 and 87.32 percent egg hatchability, respectively (Table 1).

Effect of nanoemulsion of hexanal formulation on II instar grub of *M. boninensis*– Poison food techniques

The data presented in table 3 represents the mortality of grubs, percent pupation and adult emergence. The results of the study indicated that nano emulsion of hexanal at 0.02 percent was on par with 0.2 percent of Tween 20 and ethanol, 0.02 percent pure hexanal and untreated check with the grub mortality of 6.67 percent. The percent grub pupated was maximum (96.29) in untreated control which was on par with nano emulsion of hexanal at 0.02 and 0.04 percent (95.33%) and standard checks Tween 20 (95.65) and ethanol at 0.2 percent (95.33). The adult emergence recorded in all the concentrations (0.02, 0.04 and 0.06%) of nano emulsion of hexanal ranged 92.2- 95 percent were next best to untreated control, Tween 20 and ethanol at 0.2 percent which recorded cent percent adult emergence.

Based on the toxicity levels of the newer molecules tested against beneficial organisms, they are classified as harmless (mortality <30%), slightly harmful (> 30% and <79%), moderately harmful (> 80% and <99%), and harmful (>99%). The mortality of *M.boninensis*

recorded in nano emulsion of hexanal 0.02% at field recommended dose being significantly lesser than the standard check and control with mortality < 30 percent very close to the threshold prescribed by IOBC for the test product shall be claimed as harmless (Hassan *et al.,* 1992). The results indicated maximum hatchability of predator eggs at all concentrations of nanoemulsion of hexanal. The findings of Karthika et al. (2015) who showed the safety of nanoemulsion of hexanal to *C. zastrowi* eggs can also be related to the present finding. But several authors who have tested chemical pesticides reported that *M.boninensis* showed higher sensitivity to newer molecules at adult stage than the egg, larval and pupal stages which possess eggshell to protect the immature stage of bio control agents.

Kulkarni and Patil (2012) reported that egg hatchability and adult emergence of C. zastrowi were higher, when treated with castor leaf extract (5%), garlic bulb extract (2%), neem (1%) and flufenoyuron (0.5%). Vasanthakumar *et al.* (2012) observed that the neem formulation azter (Azadiractin 0.15%) and neem kernel aqueous extract (NKAE) were safer to predator M. boninensis with no adverse effect on the predatory efficiency. The above findings reported on the biosafet of plant products to Chrysopids were similar to the present finding.Uthamasamy *et al.* (2003) reported that acetamiprid concentrations tested on *C. carnea* showed less than 10 percent mortality in contact toxicity method against 22.5 percent mortality in feeding toxicity method. This has clearly indicated the poison on ingestion will have more adverse effect than physical contact. The above factor can be attributed to the safety of nanoemulsion of hexanal which was evaluated through contact toxicity showing least adverse effect. Likewise, the studies on the attraction of *C. zastrowisillemi* and *M. boninensis* using the synthetic herbivore induced plant volatile (methyl salicylate) indicating no adverse effect can be related to the plant derived hexanal showing no adverse effect on *M. boninensis*.

In the present study it was concluded that the different concentrations of nanoemulsion of hexanal tested on *M. boninensis* was least toxic to eggs and grubs. This information can be used in the development and improvement of IPM programmes to reduce harm to beneficial insects from hexanal applications.

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REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology,* **18:** 265-267.

FAOSTAT. 2011. (Available at http://www.faostat.fao.org).

Chelladurai, M. 1999. Studies on bioefficacy of betacyfluthrin against *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera) on groundnut (*Arachis hypogaea* L.) and determination of residues. M.Sc. (Ag.) Thesis, Tamil Nadu Agricutural University, Coimbatore, India, 126 pp.

Geetha, B. and Swamiappan, M. 1998. Improved adult rearing cages for the predator, *Chrysoperla carnea*. *Madras Agriculture Journal,* **85** (5-6): 333-334.

Hassan, S. A. Guidelines for testing the effects of pesticides on beneficial organisms: description of test methods. IOBC/ WPRS Bulletin.1992; **15** (3):18-39.

Jafari, S. M., Y. H. He. and B, Bhandari. 2006. Nano-emulsion production by sonication and microfluidization - A comparison. *International Journal of Food Processing,* **9** (3): 475- 485.

Karthika, S., Nandakumar, N. B., Gunasekaran, K. and Subramanian, K. S. Biosafety of nanoemulsion of hexanal to honey bees and natural enemies. J *Sci* and *Tech*. 2015; 8(30).

Kulkarni, N. S. and A. B. Patil. 2012. Field efficacy of thiamethoxam 25 WG (Actara 25 WG) against flea beetle, jassids and thrips in grapes. *Karnataka Journal of Agricutural Sciences,* **25** (1): 146-147

Krishnamoorthy, A. 1985. Effect of several pesticides on eggs, larvae and adults of the green lacewing, *Chrysopa scelestes,* Banks. *Entomon,* **10** (1): 21-28.

McCutchen, B. F. and Plapp, F.W. 1988. Monitoring procedure for resistance to synthetic pyrethroids in tobacco bollworm larvae. In: Proc. Beltwide Cotton Prod. Res. Conf. New Orelans, 3-8, January. National Cotton Council of America, Memphis. 356-358.

McWen, P. K., New, T.R.R. and Whitington. A. 2001. Lace wings in the Crop Management. Cambridge University Press.

Mohan, C., Sridharan, S., Gunasekaran, K., Subramanian K. S. and Natarajan, N., 2017. Biosafety of hexanal as nanoemulsion on egg parasitoid *Trichogramma* spp. *Journal of Entomology and Zoology Studies*, **5** (2): 1541-1544.

Nasreen, A., Ashfaq, M., Mustafa, G. and Khan, R. 2007. Mortality rates of five commercial insecticides on *Chrysoperla carnea* (Stephens) (Chrysopidae: Neuroptera) *Pakistan Journal Agriculture Science*, **44** (2): 266-271.

Paliyath, G. and Subramanian, J. 2008. In: Phospholipase D inhibition technology for enhancing shelf life and quality. *Postharvest Biology and Technology of Fruits, Vegetables and Flowers,* 240-245.

Pathan, A. K., Sayyed, A. H., Aslam, M., Razaq, M., Jilani, G. and Saleem, M. A. 2008. Evidence of field-evolved resistance to organophosphates and pyrethroids in *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Journal of Economic Entomology*, **101** (5): 1676-1684.

Ridgway, R. L., Morrison, R. K. and Bagdley, M. 1970. Mass rearing of green lacewing. *Journal of Economic Entomology,* **63**: 834-836.

Shinde, C. U., Patel, M. B. and Mehendale, S. K. 2009. Relative toxicity of different insecticides to the larvae of *chrysoperla carnea* (Stephens) under laboratory conditions. *Pest Management in Horticultural Ecosystems,* **15** (2): 161-164.

Uthamasamy, S., S. Raguraman, R., Srinivasan, P., Tanoon, C. R., Ballal, S. K., Jalali and Rabindra. 2003. Biosafety of acetamiprid 20 sp and Spinosad 48 sc to the green lace wing, *Chrysoperla Carnea* (Stephens) (Neuroptera : Chrysopidae). Procedings of the symposium of Biological Control of Lepidoteran Pests. July 17 – 18 2002, Bangalore. 43-46p.

Vasanthakumar., Azariah Babu., Raja gopalan Shanmuga priyan and Subramaniam. 2012. Impact of Azter (azadirachtin 0.15% EC), a neem-based pesticide, against tea red spider mite, *Oligonychus coffeae* Neitner (Acarina: Tetranychidae) and its natural enemies. *International Journal of Acarology,* **39**: 140-145.

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