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Biosafety of Nanoemulsion of Hexanal on *Chrysoperla zastrowi* sillemi Stephens (Chrysopidae: Neuroptera)

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A B S T R A C T

Experiments were carried out under laboratory condition to assess the toxicity of nano emulsion of hexanal on egg and grub stage of green lacewing, *Chrysoperla zasrowi sillemi* Stephens (Chrysopidae: Neuroptera) through laboratory bioassay. The results showed that, nanoemulsion of hexanal was found to cause egg hatchability up to 88.50 percent in higher concentrations of 0.06 percent. Egg hatchability at field recommended concentration of 0.02% was found maximum of 91.44 percent respectively. In addition nano emulsion of hexanal at field recommended concentration 0.02% tested against grubs of *C. zastrowi sillemi* results revealed that zero percent grub mortality in poison food technique and 6.25 percent in diet contamination method at 48 and 24 h after exposure it was least toxic to grub of *C. zastrowi sillemi*. The maximum percent pupation and adult emergence was recorded in nanoemulsion of hexanal @ 0.02% showed 90 and 100 percent, respectively. In addition the effect of nanoemulsion of hexanal on biological parameters of *C. zastrowi sillemi* showed that 14.80 days adult longevity and 323 egg are laid by five female. The results clearly indicated that all the concentration of nano formulation of hexanal was harmless to *C. zastrowi sillemi* which recorded a mortality of < 30 percent as per the threshold prescribed by IOBC.

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). One of the major constraints in the production and marketing of mango is the post harvest losses. Many biologically active volatile compounds like hexanal is 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 and Subramanian, 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). There are several natural enemies in mango ecosystem among them the *C. zastrowi sillemi* 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 larvae 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 hexanal on *C. zastrowi sillemi* 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 *C. zastrowi sillemi.* Safety of nanoemulsion of hexanal was compared with pure form of hexanal which is a patentable product of Canada. The susceptibility of *Chrysoperla* to nano emulsion of hexanal at different concentrations (Table 1) were 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 (Jafari and 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)

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

Biosafety test to *Chrysopid* predators

Effect of hexanal formulation on the eggs of *C. zastrwi sillemi*

Laboratory studies were conducted to assess the effect of nano emulsion of hexanal formulation on the eggs of *C. zastrowi sillemi* 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 by the formula as given below:

$$\text{Hatchability (\%)} = \frac{\text{No. of grubs hatched}}{\text{Total number of eggs treated}} \times 100$$

Effect of hexanal formulation on grubs of *C. zastrowi sillemi* – 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 *C. zastrowi sillemi* 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.

Grub mortality (%) =

$$\frac{\text{No. of grubs dead}}{\text{Total number of grubs released}} \times 100$$

Effects of hexanal formulation on the adults of *C. zastrowi sillemi* – Diet contamination method

Five pairs of freshly emerged adults of *C.zastrowi sillemi* were allowed in separate plastic container. The adults were fed with 10 per cent sucrose solution containing hexanal formulation in different concentrations of as mentioned in 2.1. In the untreated check, the adults were fed with 10 per cent sucrose solution alone. The eggs laid on brown sheets kept on the inner wall of inside the galvanized iron (GI) troughs (30 cm dia x 12 cm ht), in each treatment were collected daily from each treatment. Observation on the adult longevity and fecundity were recorded for per five females.

Results and Discussion

Effect of hexanal on eggs of *C. zastrowi sillemi*

The effect of nano emulsion of hexanal on eggs of *C. zastrowi sillemi* was studied under laboratory condition. The data in table 1 represents the hatching of *Chrysoperla* eggs in different concentrations of nano emulsion of hexanal. The results indicated that treatment of nano emulsion of hexanal at 0.02, 0.04 and 0.06 percent had little effect on the hatching of eggs with 91.4, 89.4 and 88.5 percent hatching respectively. The eggs treated with pure hexanal inhibited hatching up to 16.67 percent over control in 0.06 percent concentration. The other standard checks viz., Tween 20 and ethanol at 0.2 percent did not inhibit the hatching significantly. The untreated check showed cent percent hatching.

Effect of hexanal formulation on biological parameters of adult of *C. zastrowi sillemi*

The effect of adult treatment with nano emulsion of hexanal at different concentrations on the longevity and fecundity are presented in table 2. The results indicated that the longevity of female adult was maximum in untreated control (15.5 days) which was on par with Tween 20 @ 0.2 percent (14.8 days). Among the concentrations of nano emulsion of hexanal tested 0.02 percent had higher longevity of

14.00 days as against 13.5 days noted in both 0.04 and 0.06 percent. The longevity was reduced (11.03 days) in 0.06 percent pure hexanal treatment. The adults exposed to nano emulsion of hexanal treatment laid maximum eggs of 302 in 0.02 percent concentrations as against 471 noted in untreated control. The number of eggs laid in both 0.04 and 0.06 percent of pure hexanal was significantly low 268 and inferior to all the concentration of nano emulsion of hexanal tested.

Effect of nanoemulsion of hexanal formulation on II instar grub of *C.zastrowi*-Poison food technique

Nano emulsion of hexanal treatments had not affected the grub mortality, pupation and adult emergence (Table 3). There was no grub mortality recorded the all the dose of nano emulsion of hexanal at 24, 48 and 72 HAT (0.00), which was on par with the untreated control. The standard check, pure hexanal showed maximum mortality of 4.40, 5.60 and 6.70 per cent, respectively in 0.02, 0.04 and 0.06 per cent concentration at 72 hours after treatment (HAT). All the nano emulsion of hexanal treated treatments recorded cent percent pupation which was on par with the untreated control (Table 3). The adult emergence recorded in the nano emulsion of hexanal treatments @ 0.02, 0.04, and 0.06 percent were 90, 88.4 and 87 percent respectively. The standard check pure hexanal @ 0.02, 0.04 and 0.06 per cent showed lesser adult emergence of 86.67, 84.30 and 84.0 percent, respectively (Table 3).The surfactant Tween 20 and solvent absolute ethanol @ 0.2% recorded the adult emergence of 95.0 and 90.30 per cent respectively, were next to best untreated control which showed cent percent adult emergence.

The highest number of eggs laid by a single female was recorded in the untreated control (110 eggs) followed by 0.2 percent of the surfactant Tween 20 and absolute ethanol which recorded 85.0 and 76.0 eggs respectively. The eggs laid in the nano emulsion of hexanal treatments @ 0.02, 0.04, and 0.06 percent concentrations were 75, 74 and 71 eggs as against the remaining treatments which showed a range from 71 – 50 eggs per female. The adult longevity recorded in all the nano emulsion of hexanal (0.02, 0.04 and 0.06 percent) treatments were 14.25, 13.33 and 13.00 days respectively. The untreated control recorded the highest longevity of 20.00 days, while the standard checks pure hexanal @ 0.2, 0.4 and 0.06 percent recorded lowest adult longevity of 12.68, 12.50 and 11.45 days respectively. (Table 3). The surfactant Tween 20 and absolute ethanol @ 0.2% recorded adult longevity of 15.00 days .

Effect of nanoemulsion of hexanal formulation on adult of *C. zastrowi sillemi*

The percent mortality in adults of *C. zastrowi sillemi* fed with all the three concentration of nano emulsion of hexanal through contaminated adult diet is presented in table 4. At 24 hours after treatment (HAT), the adult mortality was maximum (16.16) percent in 0.06 percent pure hexanal followed by mortality of 11.64 percent noted in 0.04 percent. The nano emulsion of hexanal at 0.02 and 0.04 percent showed statistical parity with adult mortality of 6.25 and 7.00 percent respectively. The surfactant Tween 20 and solvent absolute ethanol @ 0.2% recorded adult mortality 3.33 percent at same period of observation. There was no adult mortality recorded in untreated control.

Table.1 Treatments tested for biosafety

T_1-Nanoemulsion of hexanal @ 0.02%	**T_6- Pure hexanal @ 0.06%**
T_2-Nanoemulsion of hexanal @ 0.04%	T_7- Ethanol @ 0.02%
T_3- Nanoemulsion of hexanal @ 0.06%	T_8- Tween 20 @ 0.02%
T_4- Pure hexanal @ 0.02%	T_9- Control
T_5- Pure hexanal @ 0.04%	

Table.1 Ovicidal effect of nanoemusion of hexanal on the eggs of green lacewing bug, *Chrysoperla zastrowi sillemi*

Treatments	Per cent egg hatchability*	Percent reduction over control
T_1- Nanoemusion of hexanal @ 0.02%	91.44 (76.37)[d]	8.56
T_2- Nanoemusion of hexanal @ 0.04%	89.46 (71.06)[e]	10.54
T_3 - Nanoemusion of hexanal @ 0.06%	88.50 (70.18)[f]	11.50
T_4- Pure hexanal @ 0.02%	86.32 (68.29)[g]	13.68
T_5- Pure hexanal @ 0.04%	85.56 (67.66)[h]	14.44
T_6- Pure hexanal @ 0.06%	83.33 (65.91)[i]	16.67
T_7- Tween 20 @ 0.02%	99.32 (85.27)[b]	0.68
T_8- Ethanol @ 0.02%	94.44 (76.37)[c]	5.56
T_9- Control	100 (90.00)[a]	0.00
Mean	**90.90**	**7.95**
S.ED	**1.979**	**0.088**
CD (0.05%)	**3.961**	**0.182**

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 values

Table.2 Effect of nanoemusion of hexanal on the development of II instar grub of green lace wing, *C. zastrowi sillemi* (Poison food method)

Treatments	Mortality (%)			Pupation (%)	Adult emergence (%)	Number of eggs laid/ female	Adult longevity (days)
	24 HAT	48 HAT	72 HAT				
T$_{1}$-anoemusion of hexanal @ 0.02%	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	100 (90.00)^{a}	90.00 (71.57)^{c}	75.0 (8.66)^{cd}	14.25 (3.77)^{bc}
T$_{2}$- Nanoemusion of hexanal @ 0.04%	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	100 (90.00)^{a}	88.43 (70.12)^{cd}	74.0 (8.60)^{cd}	13.33 (3.65)^{c}
T$_{3}$- Nanoemusion of hexanal @ 0.06%	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	100 (90.00)^{a}	87.00 (69.73)^{cd}	71.0 (8.43)^{d}	13.00 (3.61)^{c}
T$_{4}$- Pure hexanal @ 0.02%	1.10 (6.02)^{b}	2.20 (8.54)^{b}	4.40 (12.11)^{b}	95.33 (77.52)^{b}	86.67 (69.44)^{d}	71.0 (8.43)^{d}	12.68 (3.56)^{cd}
T$_{5}$- Pure hexanal @ 0.04%	3.30 (10.47)^{d}	4.40 (12.11)^{d}	5.6 (13.69)^{b}	92.13 (76.61)^{b}	84.30 (67.46)^{e}	64.0 (8.00)^{e}	12.50 (3.54)^{d}
T$_{6}$- Pure hexanal @ 0.06%	2.20 (8.53)^{c}	3.30 (10.47)^{c}	6.70 (14.96)^{b}	64.99 (53.72)^{c}	84.00 (67.21)^{e}	50 (7.07)^{f}	11.45 (3.38)^{e}
T$_{7}$- Tween 20 @ 0.2%	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	100 (90.00)^{a}	95.00 (77.17)^{b}	85.0 (9.22)^{b}	15.00 (3.87)^{b}
T$_{8}$- Ethanol @ 0.2%	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	100 (90.00)^{a}	90.30 (72.85)^{b}	76.0 (8.72)^{c}	15.00 (3.87)^{b}
T$_{9}$- Control	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	100 (90.00)^{a}	100.00 (90.00)^{a}	110 (10.49)^{a}	20.00 (4.47)^{a}
Mean	**0.73**	**1.10**	**1.85**	**94.71**	**89.52**	**75.11**	**14.13**
S.ED	**0.024**	**0.031**	**0.041**	**0.914**	**0.655**	**0.036**	**0.115**
CD (0.05%)	**0.049**	**0.062**	**0.082**	**1.876**	**1.325**	**0.073**	**0.234**

HAT- Hours after treatment

*Values in the parentheses are (x+1) 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

Table.3 Effect of nanoemusion of hexanalon the mortality of adult green lacewing bug, *C. zastrowi sillemi*– Diet contamination method)

Treatments	Mortality (%)			Mean
	6 HAT	**12 HAT**	**24 HAT**	
T_1- Nanoemusion of hexanal @ 0.02%	4.40 (12.11)^{c}	5.6 (13.69)^{c}	6.25 (14.48)^{c}	5.42
T_2- Nanoemusion of hexanal @ 0.04%	5.00 (12.92)^{c}	6.75 (15.06)^{cd}	7.00 (15.34)^{cd}	6.25
T_3- Nanoemusion of hexanal @ 0.06%	5.12 (13.08)^{cd}	7.12 (15.48)^{cd}	8.00 (16.43)^{d}	6.75
T_4- Pure hexanal @ 0.02%	6.67 (14.95)^{d}	8.62 (17.07)^{d}	9.71 (18.16)^{e}	8.33
T_5- Pure hexanal @ 0.04%	7.78 (16.20)^{e}	9.71 (18.16)^{de}	11.64 (19.95)^{f}	9.71
T_6- Pure hexanal @ 0.06%	10.00 (18.43)^{f}	13.25 (21.35)^{e}	16.16 (23.70)^{g}	13.14
T_7- Tween 20 @ 0.2%	1.51 (7.06)^{b}	2.81 (9.65)^{b}	3.33 (10.31)^{b}	2.55
T_8- Ethanol @ 0.2%	1.78 (7.67)^{b}	2.87 (9.75)^{b}	3.33 (10.31)^{b}	2.66
T_9- Control	0.00 (0.19)^{a}	0.00 (0.19)^{a}	0.00 (0.19)^{a}	0.00
Mean	**4.69**	**5.68**	**7.26**	**6.09**
S.ED	**0.061**	**0.073**	**0.079**	
CD (0.05%)	**0.126**	**0.148**	**0.162**	

In a column means followed by a common letter are not significantly different at p= 0.05 by DMRT

Figures in parentheses are arcsine $\sqrt{P}$ transformed values

HAT- Hours after treatment

Table.4 Effect of nanoemusion of hexanal on biological parameters of adult green lace wing bug, *C. zastrowi sillemii*

Treatments	Total insects (No.)	Adult longevity	Total no. of eggs laid per five females
T_1- Nanoemusion of hexanal @ 0.02%	30	14.80 (3.85)[ab]	323 (17.97)[b]
T_2- Nanoemusion of hexanal @ 0.04%	30	14.20 (3.77)[b]	304 (17.44)[c]
T_3 - Nanoemusion of hexanal @ 0.06%	30	14.00 (3.74)[bc]	302 (17.38)[c]
T_{4-} Pure hexanal @ 0.02%	30	12.98 (3.67)[c]	294 (17.15)[d]
T_{5-} Pure hexanal @ 0.04%	30	11.34 (3.44)[d]	268 (16.37)[f]
T_{6-} Pure hexanal @ 0.06%	30	11.03 (3.40)[d]	268 (16.37)[f]
T_{7-} Tween 20 @ 0.2%	30	13.50 (3.67)[bc]	285 (16.88)[g]
T_{8-} Ethanol @ 0.2%	30	13.50 (3.67)[bc]	279 (16.70)[e]
T_{9-} Control	30	15.50 (3.94)[a]	471 (21.70)[a]
Mean	30	**13.42**	**310.44**
S.ED		**0.015**	**0.032**
CD (0.05%)		**0.074**	**0.152**

[*] Values in the parentheses are $\sqrt{x+0.5}$ transformed values

In a column means followed by a common letter(s) are not significantly different by DMRT (*p* =0.05)

[*] Number of larvae per ten plants and mean of three replications

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%) [5, 6]. The mortality of honey bees 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). Karthika *et al.*, (2015) who reported the lesser toxicity of nanoemulsion of hexanal @ 0.02% to the immature stages of *T. japonicum* Ashmead was in line with present findings. But several authors who have tested chemical pesticides reported that *C. zastrowi sillemi* 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. Pathan *et al.,* (2008) reported that the LC 50 of lambda-cyhalothrin against the larvae of *C. zastrowi sillemi* was 359.08 ppm. The combination of insecticides profenophos 40 per cent + cypermethrin 4 per cent were tested against the grubs of *C. zastrwi sillemi* recorded significantly highest mortality (93.01%) after 48 HAT (Shinde *et al.,* 2009). Nasreen *et al.,* (2007) reported fenpropathrin and cyfluthrin caused 57.00 and 40.00 per cent adult mortality, respectively. In the present study it was concluded that the different concentrations of nanoemulsion of hexanal tested on *C. zastrowi sillemi* 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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