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Biosafety of hexanal as nanoemulsion on egg parasitoid *Trichogramma* Spp

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Abstract

Experiments were carried out under laboratory condition to assess the toxicity of nano emulsion of hexanal on the parasitoids *viz*., *Trichogramma chilonis* Ishii and *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) which are widely used to check many lepidopteran pest. Four days old *T. chilonis* and *T. pretiosum* parasitized *Corcyra* egg were treated with nano emulsion of hexanal. The treated parasitized *Corcyra* eggs were observed for the emergence of *Trichogramma* adults. Based on the emergence hole of adult, the percent parasitization was worked out at 72 hours after treatment. *Trichogramma* adults emerged from hexanal treated cards were assessed for parasitization and subsequent adult emergence from the parasitisized egg (II generation) was also noted. The results clearly indicated the safety of nanoemulsion of hexanal to *T. pretiosum* and *T. chilonis* with 98.53 and 97.88 percent parasitization and 97.57 and 96.60 adult emergence respectively. In addition, the respective II generation *T. pretiosum* and *T. chilonis* adults also showed 96.12 and 97.65 percent parasitization with 94. 29 and 96.40 percent adult emergence.

Keywords: *Trichogramma* spp, hexanal, biosafety, contact toxicity

Introduction

India ranks first in the world mango production with 19.27 million tonnes from 2.5 million hectares accounting 21 percent of the total fruit production in the country [1, 19]. The main constraints in export and marketing of mango fruit is post-harvest losses. Several scientific approaches and technologies have been developed with the objective of enhancing the shelf life and quality of harvested fruits. Many biologically active volatile compounds like hexanal is found to reduce the post-harvest losses by delaying the ripening process. Phospholipase D (PLD) is the key enzyme involved in the initiation of plasma membrane deterioration. Hexanal inhibits PLD in the fruit skin and delays the post-harvest deterioration in mango [2, 10] Hexanal is also found to delay ripening, increase firmness and enhanced shelf life in banana [11] and tomato [14, 15]. Nanoemulsion of hexanal would be more effective than the conventional form of treatment owing to uniform and extremely small droplet sizes, typically less than 100 nm range. In addition, high kinetic stability, low viscosity and optical transparency make them very attractive systems for product delivery [12]. Biosafety of nano product is one of most important prerequisite to be assessed against beneficial and non-target organisms. [12] In mango ecosystem is known for the rich biodiversity of insects viz., thrips, aphids, leafhoppers, mites, scales, mealy bugs and caterpillars along with natural enemies [17].

Trichogramma (Hymenoptera: Trichogrammatidae) is a microscopic egg parasitoid, which plays a vital role in biological control especially when inundatively released against many lepidopteran pests [20]. It is known to parasitize the eggs of over 400 lepidopteran species.[3] Newer molecules have to be evaluated for their safety against natural enemies before field recommendation. Hence, the investigation was undertaken to assess the safety of nanoemulsion of hexanal to parasitoids *T. pretiosum* and *T. chilonis* under laboratory conditions.

Materials and methods

Laboratory experiments were carried out at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2015-2016, to study the toxicity of nanoemulsion of hexanal to *T. chilonis* and *T. pretiosum*. Safety of nanoemulsion of hexanal (Enhanced Freshness Formulation) was compared with pure form of hexanal which is apatentable product of Canada.

The susceptibility of *Trichogramma* spp 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 [9, 12].

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%	

Studies on the safety of nanoemulsion of hexanal to egg parasitoid

The egg parasitoid *T. chilonis and T. pretiosum* were obtained from the biocontrol unit in the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

In the first experiment, the parasitized egg cards (1 cm^2 bits) were treated with respective hexanal formulation at different concentrations using hand atomizer and each treatment was replicated thrice. For untreated check, distilled water was used for treatment. The treated egg cards were shade dried for 10 min and kept in test tube of 10 x 1.5 cm size for observation. Observation made on the number of adult parasitoids emerged after 72 h of treatment and per cent adult emergence was worked out.

$$\text{Per cent adult emergence} = \frac{\text{Number of adult parasitoids emerged}}{\text{Total no. of parasitized eggs treated}} \times 100$$

In the second experiment, *Corcyra* egg card 6x1 cm^2 was sprayed with hexanal formulation at different concentrations and the treated egg cards were cut into bits of (1 cm^2 bits). One treated bit of *Corcyra* card and one bit of parasitized egg card (untreated) obtained from the nucleus culture of the biocontrol unit were kept in 10 x1.5 cm test tube in the ratio 6:1 and the mouth of the test tube was covered with muslin cloth. Each treatment was replicated three times. The number of parasitised eggs (eggs appearing black and plumpy) was recorded 72 h after treatment and per cent parasitisation was worked out using the formula,

$$\text{Per cent parasitisation} = \frac{\text{Number of parasitized eggs}}{\text{Total number of } Corcyra \text{ eggs provided}} \times 100$$

Impact of Nanoemulsion of hexanal on II generation of *Trichogramma* spp

The emerged adults (I generation) from the treated parasitized cards were provided with fresh UV treated *Corcyra* eggs for oviposition. After 48 hours of exposure the cards were removed and kept in separate test tube for adult emergence.

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

Results and discussion

Pest and their natural enemies usually occur in fields simultaneously and so natural enemies would be subjected to various agro chemical stress and acquire toxicant by direct contact. For successful implementation of an IPM program involving biological and chemical control, an understanding on the effect of newer agro chemical molecules effect on non-target organisms would be a inevitable. [5] The effects of newer molecules on non-target organisms can be categorized as harmless (<50% mortality), slightly harmful (50 to 79% mortality), moderately harmful (80 to 89% mortality) and harmful (>90% mortality) when tested at the field recommended dose [8, 14]

The effect of hexanal treatment on the adult emergence and parasitization of *T. chilonis* and *T. pretiosum* are summarized in the Table 1 & 2. The results indicated that all the treatments showed minimum impact on the emergence of adults. The mean adult emergence of *T. chilonis* and *T. pretiosum* ranged from 96.60 to 97.57 per cent in different treatments. The hexanal treatments also showed little impact on the parasitization of *T. chilonis* and *T. pretiosum*. The mean parasitization of *T. chilonis* and *T. pretiosum* ranged from 97.88 to 98.53 per cent, in different treatments. *Trichogramma* adults (II Generation) emerged from hexanal treated cards clearly indicated the safety of hexanal to the II generation adults of *T. chilonis* and *T. pretiosum* which respectively showed 96.12 and 97.65 percent parasitization and 94.29 and 96.40 percent adult emergence. The present study on the safety of hexanal nanoemulsion clearly revealed that the hexanal being a plant component was not toxic to the egg parasitoid *T. chilonis* and *T. pretiosum* even at 0.06 per cent which is evidenced by higher parasitisation and adult emergence.

Karthika *et al.* (2015) [12] 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 *Trichogramma* spp. showed higher sensitivity to pesticides at adult stage than the egg, larval and pupal stages which possess eggshell to protect the immature stage of parasitoid. [4, 21, 6, 22] Likewise, five antimoulting compounds when exposed at 4 days after parasitisation of *T. chilonis,* had drastically affected the development of the immature stages and the effects were more pronounced in triflumuron and HOE 607 [7].

Table 2: Effect of nanoemulsion of hexanal on *Trichogramma spp*

Treatment	*Trichogramma pretiosum*		*Trichogramma chilonis*	
	Adult emergence%	**Parasitization%**	**Adult emergence%**	**Parasitization%**
T_1- Nano emulsion of hexanal @ 0.02%	97.57 (81.04)^{ab}	98.53 (83.04)^{ab}	96.60 (79.38)^{b}	97.88 (81.62)^{ab}
T_2- Nano emulsion of hexanal @ 0.04%	96.47 (79.17)^{bc}	96.92 (79.90)^{bc}	94.92 (76.97)^{bc}	97.38 (80.68)^{b}
T_3 - Nano emulsion of hexanal @ 0.06%	95.72 (78.06)^{c}	96.01 (78.48)^{cd}	93.58 (75.32)^{cd}	95.85 (78.25)^{c}
T_4- Pure hexanal @ 0.02%	94.90 (76.94)^{cd}	95.87 (78.27)^{cde}	93.39 (75.11)^{cd}	93.72 (75.48)^{d}
T_5- Pure hexanal @ 0.04%	93.78 (75.56)^{de}	94.74 (76.74)^{de}	91.74 (73.30)^{de}	93.15 (74.83)^{d}
T_6- Pure hexanal @ 0.06%	92.88 (74.52)^{e}	94.00 (75.83)^{e}	90.41 (71.96)^{ef}	92.51 (74.12)^{de}
T_7- Tween 20 @ 0.02%	92.35 (73.94)^{e}	93.83 (75.62)^{e}	88.87 (70.51)^{f}	90.38 (71.93)^{f}
T_8- Ethanol @ 0.02%	92.86 (74.50)^{a}	94.62 (76.59)^{de}	89.28 (70.89)^{f}	91.21 (72.75)^{ef}
T_9- Control	98.97 (84.18)^{a}	99.31 (84.18)^{a}	99.05 (84.41)^{a}	98.93 (84.07)^{a}

*Mean of three replications; Figures in parentheses are *arc sine* transformed values;
In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05).

Table 3: Effect of nanoemulsion of hexanal on II generation *Trichogramma* spp

Treatment	*Trichogramma pretiosum*		*Trichogramma chilonis*	
	Adult emergence%	**Parasitization%**	**Adult emergence%**	**Parasitization%**
T_1- Nano emulsion of hexanal @ 0.02%	94.29 (76.17)^{ab}	96.12 (78.63)^{b}	96.40 (79.06)^{b}	97.65 (81.19)^{ab}
T_2- Nano emulsion of hexanal @ 0.04%	93.61 (75.35)^{ab}	94.50 (76.44)^{bc}	94.80 (76.82)^{b}	96.12 (78.64)^{bc}
T_3- Nano emulsion of hexanal @ 0.04%	92.40 (73.99)^{c}	94.83 (76.86)^{bc}	92.79 (74.43)^{c}	95.08 (77.19)^{cd}
T_4- Pure hexanal @ 0.02%	91.47 (73.02)^{b}	93.67 (75.43)^{cd}	92.78 (74.41)^{c}	94.85 (76.89)^{cd}
T_5- Pure hexanal @ 0.04%	90.17 (71.73)^{b}	92.44 (74.04)^{de}	90.82 (72.37)^{d}	93.77 (75.55)^{de}
T_6- Pure hexanal @ 0.06%	89.78 (71.36)^{b}	91.49 (73.04)^{ef}	88.79(70.44)^{e}	92.54 (74.15)^{ef}
T_7- Tween 20 @ 0.02%	89.69 (71.27)^{b}	90.66 (72.20)^{f}	86.46 (68.41)^{f}	90.84 (72.38)^{g}
T_8- Ethanol @ 0.02%	89.73 (71.31)^{b}	90.98 (72.52)^{ef}	87.50 (69.29)^{e}	91.08 (72.63)^{fg}
T_9- Control	97.40 (80.72)^{a}	99.08 (84.50)^{a}	98.98 (84.19)^{a}	98.71 (83.48)^{a}

*Mean of three replications; Figures in parentheses are *arc sine* transformed values;
In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05).

Conclusion
The finding obtained in the research study clearly indicated that hexanal being a naturally occurring alkyl aldehyde compound in plants in contrast unlike to synthetic chemical insecticides had no adverse effect on the natural enemies which can be attributed to their co- evolution along with plant species containing this naturally occurring compound.

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