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Shifa
Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

FA Zaki
Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Malik Mukhtar
Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Ashaq Pandit
Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Imtiyaz Murtaza
Division of Biochemistry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Nagina Nazir
Division of Agri-statistics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Khurshid A Hakeem
Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Correspondence
Shifa
Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

A critical study of reduced pesticide application rates of nano-deltamethrin in comparison to its conventional analogue against *Trialeurodes vaporariorum*

Shifa, FA Zaki, Malik Mukhtar, Ashaq Pandit, Imtiyaz Murtaza, Nagina Nazir and Khurshid A Hakeem

Abstract
Bioefficacy of nano-formulation of deltamethrin (average particle size of 90 nm) and its conventional commercial analogue were evaluated under *in vitro* conditions during the year 2016-2018 against Greenhouse whitefly, *Trialeurodes vaporariorum* through contact or residual bioassay. Both the insecticide treatments were tested at a range of dose concentrations starting from the recommended concentration of the commercial formulation (0.01%). At this dose conc. (0.01%), mean mortality of 82.95% was obtained in case of nano-formulation which was very high as compared to the mean mortality of 38.77% caused by its commercial analogue. This clearly indicated superior insecticidal activity of nano-formulation and therefore, lower doses for nano-formulation were tested to establish those dose concentrations which invoked equivalent mortality response against *T. vaporariorum* as the commercial formulation. Nine lower dose concentrations were tested (0.009, 0.008, 0.007, 0.006, 0.005, 0.004, 0.003, 0.002 and 0.001) which caused 77.32%, 72.00%, 64.01%, 58.32%, 55.68%, 49.36%, 44.21%, 39.87% and 37.20% mean mortality respectively. It was thus established that the mortality percentage of 38.77% caused by the commercial formulation of deltamethrin at the recommended dose (0.01%) was equated by the equivalent mortality response range of 37.20% to 39.87% in case of nano-deltamethrin, caused at very low concentrations of 0.001% to 0.002%. Therefore, decreasing the recommended concentration (0.01%) of commercial formulation by a factor of 5 and even up to 10 yielded the concentration of nano-deltamethrin (0.001% to 0.002%) which induced equivalent quantitative mortality.

Keywords: Deltamethrin, nano-deltamethrin, *Trialeurodes vaporariorum,* bioassay, mortality

1. Introduction
There are approximately 1200 species of whiteflies worldwide; out of which many are considered as pests which feed and damage many vegetable and field crops; greenhouse crops, nursery crops and house plants [4]. One important whitefly specie of many fruits, vegetables, and ornamental crops is Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). This whitefly specie is distributed in all continents except Antarctica [24]. It is a polyphagous specie, colonizing more than 250 host plants [15]. Under greenhouse conditions this pest can multiply quickly into many generations [24].
The larval and adult forms feed on plants through direct feeding, inserting their stylet into leaf veins and withdrawing nourishment from the phloem sap. Serious infestations cause a decline of plant vitality, stunting, yellowing of foliage and untimely leaf drop. As a by-product of feeding, sticky honeydew is excreted that undervalues the appearance of the plant and allows gray sooty mould fungi to grow on the foliage. One of the most damaging characteristics of *T. vaporariorum* is the ability of adults to transmit numerous plant viruses. It spreads several viruses like begomoviruses, criniviruses, ipomoviruses. torradoviruses, and some carlaviruses [17]. Often, neighbouring vegetable crops are inundated by the dispersing *T. vaporariorum* adults following the senescence or decline of an infested planting [12]. Management of this pest below economic threshold level is not easy. Many growers routinely apply pesticides to suppress *T. vaporariorum* populations but these repeated applications can lead to a population increase of this insect pest. Its control is difficult and complex, as this insect pest rapidly gains resistance to chemical pesticides [11].

The continuous use of conventional insecticides has led the development of insecticide resistance, which emphasizes the need for new pest management alternatives [14].

Nano-pesticides are an efficient solution to this problem as properties of nanoparticles can be exploited in the production of new insecticides [20]. Researchers, worldwide have developed different types of nanopesticides like nanocapsulated formulations, nanoemulsion, nanogel, nanospheres, and metal and metal oxide nanoparticles. Detailed review on the development of nanopesticides has been done [13]. Nano-formulations of pesticides have many advantages over commercial formulations like improved efficiency, stability and decrease of effective pesticide concentration. A number of nano-formulations of pesticides have been exploited in the field of plant protection like pyridalyl [22], imidacloprid [5], thiacloprid [23], thiamethoxam [26], thiram [8] and β-cyfluthrin [16] The potential of nano-pesticides to reduce toxic impact of a conventional chemical pesticide and provide target specific control of crop pest can be helpful in development of intelligent nano systems for minimization of unfavourable problems to agriculture like environmental imbalance, food security and food productivity [19]. This sums up some excellent properties of nano-pesticides making them a matter of elevated significance in the field of pest management and with their added advantages over the conventional formulations; these can be called "the future of pesticides". Hence, it was imperative to conduct this study to compare the efficiencies of commercial and nano-formulation of deltamethrin against *T. vaporariorum* and thereby establishing suitable doses of nano-deltamethrin which were at par with the recommended dose of commercial formulation.

2. Materials and methods

2.1 Experimental site: This research was carried out from the year 2016-2018 in the flower and vegetable nurseries of Shalimar, Nishat and adjoining areas of Srinagar, experimental field of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) at Shalimar campus, Srinagar and in the experimental laboratories at the Division of Entomology, SKUAST-K, Shalimar campus, Srinagar, J&K. These experimental localities were situated at an altitude of 1535 meters and the experimental site recorded maximum temperature of 35 ºC to minimum of 16 ºC with a relative humidity of 39- 46%.

2.2 Host plants: A number of flower and vegetable green houses were examined for the presence of *T. vaporariorum* infestation and the pest load was found to be very high in these nurseries. Gerbera plants (*Gerbera jamesonii*) followed by brinjal (*Solanum melongena*) and tomato (*Solanum lycopersicum*) were found to carry a heavy load of this insect pest and therefore were used for collection of the test insect as well as for carrying out bioassay.

2.3 Test insect: *T. vaporariorum* populations were collected from the green house nurseries of Shalimar, Nishat and adjoining areas. Only the adult forms were exposed to the pesticides for bioassay.

2.4 Treatment details: The commercial formulation of deltamethrin was evaluated at only one concentration i.e. its recommended concentration (0.01%) throughout this experiment while the nano-formulation was initially evaluated at this concentration (0.01%); followed by other lower concentrations in the rest of the experiment (Table 1). At 0.01 percent dose concentration, bioassay for both the pesticides was performed, followed by the comparison of mortalities. Thereafter, nano-formulation was tested at a wide range of other lower concentrations selected by retrogression (0.01%, 0.009%, 0.008%, 0.007%, 0.006%, 0.005%, 0.004%, 0.003%, 0.002%, and 0.001%) (Table 1). These concentrations were prepared by serial dilution. Each treatment was replicated five times along with the control where only water was used.

Table 1: Details of the pesticide treatments used in this study.

Treatment	Treatment code	Concentration (%)	Dose (μlL^{-1})
Deltamethrin-Commercial formulation (D)	D1	0.01	3571.42
Nano-deltamethrin ND	ND1	0.01	3571.42
	ND2	0.009	3214.28
	ND3	0.008	2857.14
	ND4	0.007	2500.00
	ND5	0.006	2142.85
	ND6	0.005	1785.71
	ND7	0.004	1428.57
	ND8	0.003	1071.42
	ND9	0.002	714.28
	ND10	0.001	357.14

2.5 Bioassay: The bioassay was performed for both the pesticides at the recommended conc. of commercial formulation (0.01%) and the mortalities obtained were compared after transforming these to corrected percent mortality. After comparison of mortalities, the bioassay for nano-formulation of deltamethrin at lower concentrations was again performed. As whiteflies are winged and fragile insects, these had to be collected using an aspirator, and the bioassay was performed in the aspirator itself. This was because the mobility of whiteflies does not allow transferring them into a *Petri* plate or some other container. However, as the aspirator surface area was large enough, treating only the leaf surface would not help because the whiteflies could escape to the untreated walls of the aspirator and not be exposed to the pesticide at all, which would eventually yield misleading results. Because of this reason, contact or residual bioassay was carried out i.e. the aspirator was coated with the pesticide solution from inside. However, in contact bioassay, acetone is used for dilution because volatile solvents evaporate quickly, leaving behind the pesticide on the walls of the container. Contact or residual bioassay was carried out on the leaves of gerbera against the adult stage of *T. vaporariorum*. Unsprayed gerbera plant leaves were taken, and washed. These leaves were dipped in pesticide solution for 20 seconds with gentle agitation and were placed on filter paper for drying. Consequently, the aspirator was also coated with the pesticide solution from inside. The solvent was allowed to evaporate by rotating the aspirator so that the insecticide was spread evenly over the entire surface leaving a residual film. The dried leaf was then introduced in the aspirator and the insects were sucked in through it. For each treatment thirty adult T. *vaporariorum* were blown through the aspirator. The experiment was conducted in the laboratory with a temperature of 32 ± 1 °C and relative humidity of 40 ± 5%. Mortality at different concentration levels was assessed after 24 hours of the exposure to the pesticide.

2.6 Statistical analysis of data: The mortality data was transformed to percent mortality and consequently corrected according to the formula given by Abbott [1]. The mortality was corrected because often a few insects die during an experiment from natural causes which are not concerned with the insecticide use. The magnitude of this mortality should also be estimated. This can be done by exposing the batch of insects in control (where no insecticide is applied) exactly in the same manner as is done in exposure to insecticide. When there is natural mortality among the controls, the mortalities have to be corrected by Abbott's formula [1]:

$$\frac{X - Y}{X} \times 100$$

Where, X = percent survival of insects in check
Y= percent survival of insects in treated
The control mortality should be less than 20%; otherwise, the corrected mortality will not be reliable. The mortality in control so obtained will affect the precision of the result.

Additionally, the research data was subjected to the analysis of variance and difference was compared at 5 percent level of significance. Some other statistical measures were computed by using OPSTAT software. Simple correlation between dose concentration of pesticides and corrected percent mortality of Green house whitefly (*T. vaporariorum*) was worked out.

3. Results and Discussion

The bioassay of commercial and nano-formulation of deltamethrin revealed the mortalities in both cases and this mortality data was transformed to percent mortality which was afterwards changed to corrected percent mortality [1]. The corrected percent mortalities were compared and the comparison showed nano-deltamethrin to be more potent than the commercial formulation at the dose of 0.01 percent. It was established that the nano-formulation of deltamethrin caused two times more mortality than the commercial formulation of deltamethrin at the same concentration of 0.01 percent (Table 2).

Table 2: Comparison of the corrected percent mortality of *T. vaporariorum* caused by commercial formulation and nano-formulation of deltamethrin at 0.01 percent dose concentration

Chemical	Concentration (%)	Treatment code	Corrected percent mortality	Relative fold (in terms of mortality)
Deltamethrin- Commercial formulation (D)	0.01 (Recommended)	D1	38.77*	1
Nano-deltamethrin (ND)	0.01	ND1	82.95*	2.13

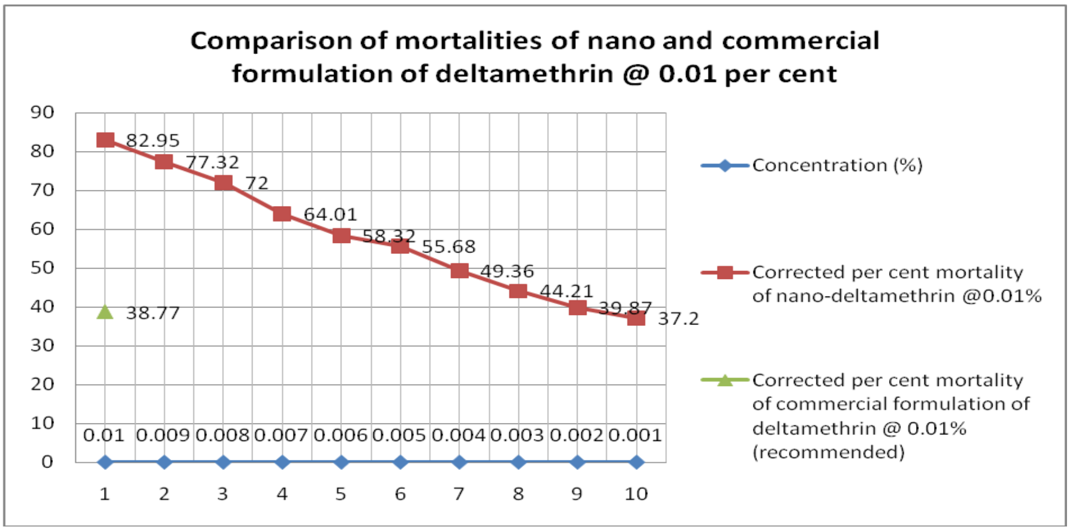
* Each figure is a mean of five replications.

This clearly indicated that the efficiency of nano-formulation was more than the commercial formulation and therefore, for computing the suitable dose of nano-deltamethrin, which invoked the same degree of quantitative response (mortality) in *T. vaporariorum* as the recommended dose of commercial formulation; lower doses of nano-deltamethrin were evaluated against the pest till the desired mortality range was reached (Table 3).
Bioassay with lower doses of nano- deltamethrin put forward the corrected mortality percentages which are presented in Table 3. At the concentration 0.01 percent (3571.42 µl/ L) of commercial formulation of deltamethrin, the corrected percent mortality caused was 38.77 percent and this was the required response mortality for nano-deltamethrin as well. Amongst all the lower concentrations tested, the concentrations between 0.001 to 0.002 percent (35.71 to 71.42 µl/ L) caused approximately the same required response mortality in *T. vaporariorum* i.e. 37.20 to 39.87 percent (Table 3 and Graph 1).

Table 3: Corrected percent mortality of *T. vaporariorum* at lower concentrations

Treatment	Concentration (%)	Dose (µlL^{-1})	Treatment code	Corrected percent mortality
Deltamethrin-Commercial formulation (D)	**0.01**	**3571.42**	**D1**	**38.77***
Nano-deltamethrin ND	0.01	3571.42	ND1	82.95*
	0.009	3214.28	ND2	77.32*
	0.008	2857.14	ND3	72.00*
	0.007	2500.00	ND4	64.01*
	0.006	2142.85	ND5	58.32*
	0.005	1785.71	ND6	55.68*
	0.004	1428.57	ND7	49.36*
	0.003	1071.42	ND8	44.21*
	0.002	714.28	ND9	39.87*
	0.001	357.14	ND10	37.20*

*Each figure is a mean of five replications



Graph 1: Comparison of the corrected percent mortalities of nano-deltamethrin and commercial deltamethrin @ 0.01 percent

Therefore, decreasing the recommended concentration (0.01%) of commercial formulation by any factor between 5 to 10 yielded the concentration of nano deltamethrin which induced almost equivalent quantitative mortality as recommended concentration of deltamethrin (Table 4).

Table 4: Relative concentration fold of nano-deltamethrin with respect to commercial formulation at equivalent corrected percent mortality

Pesticide	Conc. (%)	Corrected percent mortality	Relative fold (in terms of concentration)
Deltamethrin- (Commercial formulation)- D	0.01	38.77	1
Nanodeltamethrin- ND	0.001-0.002	37.20-39.87	5-10

*Each figure is a mean of five replications

Dose concentration had significant and positive correlation with corrected percent mortality of *T. vaporariorum* with r value of 0.99 at 5 percent level of significance. Coefficient of determination (R^2) was worked out to be 0.98 and coefficient of non- determination ($1-R^2$) was 1-0.98 (Table 5).

Therefore, these findings can be expressed as:

Corrected percent mortality at D1 << Corrected percent mortality at NDC1

Corrected percent mortality at D1 = Corrected percent mortality at NDC9 to NDC10

It was established that all the possible concentrations between 0.001 to 0.002 percent gave us the required mortality results.

Table 5: Correlation coefficient and coefficient of determination between dose concentration and percent mortality of nano-deltamethrin in Greenhouse whitefly, *Trialeurodes vaporariorum*

Chemical	Concentration (%)	Corrected percent mortality (%)	Correlation coefficient (r)	Coefficient of determination ($1-R^2$)
Nano-deltamethrin	0.01	82.95	0.99*	0.98*
	0.009	77.32		
	0.008	72.00		
	0.007	64.01		
	0.006	58.32		
	0.005	55.68		
	0.004	49.36		
	0.003	44.21		
	0.002	39.87		
	0.001	37.20		

*Significant at 5 percent

The results drawn from this research clearly established the fact that nano-formulation performed much better than the commercial formulation. This study is supported by the research of various authors who reported superior activity of nano-pesticides against different pests. Chin [6] reported that the nanosuspension of carbofuran controlled diamond back moth (*Plutella xylostella*) efficiently while Elek *et al.* [7] put forward that *Spodoptera littoralis* population could be managed at lower doses of Novaluron nanoparticles. Better control of aphids was demonstrated by polymer nanoparticles i.e. encapsulation of commercial pesticides, than regular commercial pesticide as demonstrated by Boehm [3]. Our findings that nano-deltamethrin is almost two times efficient than commercial formulation (Table 2) is in conformity with the research done by Gopal *et al.* [9] who reported bio-efficacy of nanohexaconazole to be 2-6 times higher than commercial hexaconazole against fungal pathogens. The present investigation clearly put forward the superior control of nanodeltamethrin and this finding is analogous to the finding of Guan *et al.* [10] who prepared nano-imidacloprid by encapsulation of imidacloprid and found it to be more effective than commercial imidacloprid against the adult stage of *Martianus dermestoides*. Our results suggest some intrinsic merits of nanoformulations that are responsible for their efficient performance and such distinct properties were proposed by many authors e.g. possible higher efficacy [2, 28], reduced hydrolysis [25], and reduced volatilization of the a.i [28]. Recent work has indicated higher uptake of a.i. as well [2]. This perusal of the data of our research demands enabling smaller quantities of the pesticides to be used (Table 3 and Graph 1) as it can be proposed that nanopesticides resist the severe environmental processes that act to eliminate conventionally applied pesticides, i.e. leaching, evaporation and photolytic, hydrolytic and microbial degradation. This observation is backed by a research suggesting excellent potential of nanoformulation to protect deltamethrin from photodegradation [18]. Both direct and indirect photodegradation were reduced for the formulated a.i. relative to the pure a.i. of deltamethrin. The interpretation of this research concludes that only 10% to 20% of the recommended concentration (0.01%) of commercial formulation yields the mortality that is obtained at recommended conc. This is supported by a study done with Green house whitefly where nano-thiamethoxam was efficient against whitefly at 50% of the recommended dosage for the pure a.i. [27]. However, no comparison with commercial formulations or with the pure a.i. was performed in this study and it was therefore not possible to draw any conclusions on possible reductions in application rates with this research. However, our current research is a complete study of whitefly against the comparison of mortalities in both nano and commercial formulation and the reduction in application rates are very much evident. The present investigation is hence quite imperative and vital for novel techniques of pest management.

4. Conclusion

Nanotechnology and nano-particle engineering have been instrumental in providing breakthroughs in the field of pharmaceuticals and medicine since the 1970s [21], but it haven't had such an impact on agriculture and the agro-chemical industry. However, it is understood that an analogous transformation and paradigm shift might occur in the agricultural sector with the introduction of nano-technology. Through this study, it is quite evident that nano-pesticides; at very low dose concentrations, provide us with the results that conventional formulations give at high concentrations. If the desired control of pests is achieved by using very less product, then there is no use of spraying higher concentrations as it will only cause contamination of environment, build up of pesticide residues and affect non-target fauna. Nano-formulations of pesticides are more effective and selective over conventional formulations and which is why this period of pesticides can be called as the "rise of new era of pesticides". Apart from all the benefits, what is imperative is an evaluation of the expected quantities and concentrations of nano-pesticides in the environmental systems and cycles after its usage.

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