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## A method to optimize the pesticide dose considering the combined influence of plant, pest, pesticide, and spray equipment on bioefficacy

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#### Abstract

Aim of the study: To develop a method to optimize the pesticide dose considering the combined influence of plant, pest, pesticide, and spray equipment on bioefficacy.

Area of study: Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India.

*Material and methods:* A controlled droplet applicator generated droplets from 200 to 50 µm. The target leaf's deposition density of a preset droplet size can be controlled by timing the spray. A sequence of bioassays was performed at various droplet densities at each pesticide (imidachloprid 17.8% SL) dose and droplet size to determine the mortality of cotton aphids (*Aphis gossypii*) and jassids (*Amrasca biguttula*) feeding on immature cotton plants. Calculating the number of droplets per target area needed to cause 50% and 90% mortality (LN50 and LN90) yielded a series of model curves. Field tests were done on the computed optimal doses of the pesticide for a spray apparatus (electrostatically charged spray) to assess the spray's bioefficacy against *A. gossypii* and *A. biguttula*.

*Main results:* In comparison to uncharged mist blower spray, which had a bioefficacy of 91% for an LN90 dose of 110 g a.i. L<sup>-1</sup>, the spray had an 89% bioefficacy on *A. gossypii*. Using the electrostatic spray, it was 91% effective against *A. biguttula* and 98% effective against an uncharged mist blower at a dose of 110 g a.i. L<sup>-1</sup> of LN90.

*Research highlights:* This generalized method of modelling could effectively compute the optimal pesticide dose for any plant, pest, pesticide, and spray equipment combination.

Additional key words: bioassay; electrostatic spray; controlled droplet applicator; optimal dose; imidachloprid.

**Abbreviations used:** CDA (controlled droplet applicator); LC (lethal concentration); LD (lethal dosage); LN (lethal number of droplets per unit area); SL (soluble liquid); VMD (volume median diameter).

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## Introduction

Plant protection measures ensure the safety of crops from insects, weeds, pests, and various diseases, ultimately

enhancing the production and productivity of agricultural produce (Agoramoorthy, 2008; Abhilash & Singh, 2009). However, excessive use of these chemicals can be a threat to the environment, food, and human health.

Conventional crop protection equipment atomizes the spray liquid into fine droplets so that they can be uniformly deposited on the plant's leaves. However, only a small portion of the chemical reaches the targeted plant canopy. A large portion of the chemicals sprayed end up on the ground and are wasted. Another substantial part is also lost due to off-target drift and deposits elsewhere, chemically polluting the soil and water bodies through surface runoff (Salyani & Cromwell, 1992; Barba-Brioso et al., 2010; Hermosín et al., 2013; Robles-Molina et al., 2014). The minimal volume of spray fluid thus deposited on the leaves is expected to cause the desired insect mortality. It is hence crucial to apply a spray at an optimal pesticide concentration with the right equipment. The quantity of chemical substance ingested by the insect depends on many factors, such as the volume of droplets deposited and the density of such droplets per unit area of the leaf, apart from the concentration or dose of chemical per litre of water. It is possible to significantly boost the effectiveness of a pesticide by properly formulating it to increase the spread of pesticide droplets so as to increase the uptake of the pesticide by the crop or the pest. Importantly, the droplet size and flow rate from the sprayer have to be altered to achieve these results (Zabkiewicz, 2007). Most of the pesticides that are now used are systemic chemicals, which are absorbed by the plant through the stomata of the leaves and mix with the plant sap. When an insect pest ingests the plant leaf along with the sap, it causes the desired mortality. The pathway by which the potent chemical reaches the insect target is hence complex and will depend heavily on the factors mentioned earlier. The optimal droplet size and droplet density on the target leaf are crucial in maximizing the absorption of the pesticide into the plant system. This implies that the bioefficacy of the pesticide application process is influenced by the spraying equipment's depositional performance in providing the right quantity at the right droplet density on the leaves, the plant's characteristics on how well they can absorb the deposited fluid, and importantly, the dose that the insect ingests to cause the required mortality.

A variety of models for dose expression coexist: pesticide mass or volume unit (kg or L), related to a particular reference unit such as crop ground area, spray volume (concentration), leaf wall area tree row volume or plant row vol-

**Table 1.** Droplet sizes at different con-trolled droplet applicator (CDA) speeds.

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Speed (rpm)	Droplet size (µm)
2000	200
6000	135
8000	75
20000	50

ume (Doruchowski, 2017). Even when doses are applied according to the recommendations, the targeted pests frequently receive doses that are significantly lower than those required for pest mortality or even for toxicity symptoms (Velini et al., 2017). The kinetics of dose exposure to both targeted and non-targeted insect species is to be altered by the methods of application of pesticides used for those pesticides. The dose expression depends on several pest molecular target site factors for effectiveness (Duke, 2017).

It is generally known that the lethal dose (LD) values are more accurate for contact pesticides and lethal concentration values (LC) are more appropriate for the action of systemic pesticides on insect pests. But these bioassays do not consider the process of leaf absorbing the chemical into the plant system and factors such as the optimal droplet size and density of deposition on the leaf. The LD and LC give a guideline value of application rate, without duly considering the droplet spectrum and application efficiency of the pesticide application equipment.

Yet another approach is to use  $LN_{50}$  and  $LN_{90}$  values, which are the lethal number of droplets deposited per unit area of the plant surface to cause 50 and 90% mortality, respectively (Hovde, 1989). The current work uses the LN values in the perspective of combining the influence of pest, plant, chemical, and equipment characteristics. Once these values are known for a particular pest, pesticide, applicator, and plant combination, they can be used to compute the exact dose and volume of spray fluid to be applied. And one can fine-tune the application process to target insect pests more precisely with minimal use of pesticide. Using the simulated creation of droplet densities at various droplet sizes, the developed procedure attempts to determine the lethal number of droplets per unit area of

**Table 2.** Average droplet sizes and densities at different controlled droplet applicator (CDA) speeds and times of exposure.

Rotational speed (rpm)	Droplet size (µm)	Droplet density cm <sup>-2</sup>				
		<b>1</b> s	2 s	3 s	4 s	5 s
2000	200	15	25	35	45	60
6000	135	30	50	70	90	100
8000	75	90	150	240	300	400
20000	50	160	200	270	400	550



Figure 1. Procedural sequence for arriving at the optimal dose based on lethal number of droplets (LN) values.

the target surface that will produce the desired mortality. The generalized model thus developed can optimize the pesticide dosage by taking into account the combined influence of plants, pests, pesticides, and spray equipment through a systematic bioassay. The model does not use either the LC or LD values, which are deemed constant for a pest infesting a crop species. But rather uses the LN values that can combine the influence of plant, pest, pesticide concentration and spray characteristics on the insect mortality. Moreover, the use of equipment such as an electrostatic sprayer can reduce the pesticide requirement even further, because these sprayers can deposit spray on the abaxial surface of leaves, causing better insect mortality with less chemical use. The suggested method was assayed on a specific plant, pest, pesticide, and sprayer combo to prove that it is workable on any pesticide sprayed on a crop with any sprayer to bring about the desired insect mortality. The finalized dosage based on the model was field-tested to assess and verify the offered bioefficacy.

## Material and methods

The methodology explained (Fig. 1) develops a whole set of LN models that help arrive at an optimized pesticide dose for the desired plant, pest, pesticide, and spray applicator. The sequence of steps explained in sections 2.1 and 2.2 (Fig. 1) is marked on each of the flow elements (Fig. 1) to bring out clarity. Computation of LN value models is experimentally rigorous, since the bioassay is done systematically on different levels of (i) spray droplet sizes, (ii) droplet densities of deposition on the leaf and (iii) chemical concentration (pesticide dose).

#### Bioassay for assessment of lethal number of droplets (LN) deposited per unit leaf area for a specific pesticide dose applied on the selected pest and plant

Toward implementing the bioassay, the following methods were used to generate controlled levels of droplet sizes and droplet deposition densities.

— (i) The best method for generating spray droplets of predefined sizes is to use a centrifugal disc sprayer, known as CDA. A high-speed micro-drilling machine was used to rotate a standard CDA disc of 100 mm diameter at speeds up to 20,000 rpm to acquire droplet sizes to a minimum of 50  $\mu$ m. Preliminary trials provided data on the required



Figure 2. Equipment for altering the droplet density of deposition.

speed of rotation to generate the range of required droplet sizes (Table 1). Based on the data, the speed of rotation was regulated to generate a range of uniform droplet sizes required for the bioassay. A photo micrographic test setup consisting of a digital microscopic device of 10X magnification was used to capture digital images of droplet deposition dyed with methylene blue on photo paper. These digital images were input into a custom-made MATLAB code that finds the droplet sizes. The digital image processing code is also capable of computing the complete depositional spectrum including, the number median diameter, volume median diameter (VMD), droplet density, and uniformity ratio.

- (ii) The procedure for varying the droplet density was to expose the target (leaf used for the bioassay) to the spray at a variable time. An exclusive solenoid-actuated device with a microcontroller-based timer was developed to implement this procedure on real two-leaved cotton plants (Fig. 2). The timer module was built around an 8-bit PIC16F84A microcontroller operating at 20 MHz. The controller had 1024 words of program memory and was serially loaded with a simple program that outputs a timed 'high' to the solenoid through a driver transistor. The 'high' time is programmable by simple button presses and corresponds to the time of exposure required for the experiment. The solenoid in turn operated a swinging flap that exposed the target leaf to the spray for a predefined time interval to acquire the required droplet density. Preliminary trials were conducted to assess the relationship between the time of exposure and the droplet densities obtained (Table 2). Here again, digital images of the deposition were acquired to assess the droplet densities deposited on photo paper. The results of these trials were used to generate the required droplet density at a specific droplet size.

A bioassay on the action of imidachloprid on cotton aphids (*A. gossypii*) was attempted by varying the droplet densities at each droplet size and pesticide dose. Week old (two-leaved) cotton plants were grown in a protected environment for the bioassay. The developed solenoid-actuated device with the microcontroller-based timing device was used to expose the spray fluid on these young leaves at the chosen droplet size and varied droplet densities.

Leaf samples were sprayed with the first level of droplet sizes, namely 200  $\mu$ m, and the first level of pesticide dose, namely 5 $\mu$ L L<sup>-1</sup> of imidachloprid in water. The relationships resulting from experiments on the CDA were used to set the speed and exposure time to arrive at the five levels of droplet densities at that droplet size. For instance, to generate a droplet size of 200  $\mu$ m at five levels of droplet density (15, 25, 35, 45, and 60 droplets cm<sup>-2</sup>), the CDA was run at 2000 rpm and the target leaf exposed for 1, 2, 3, 4, and 5 s respectively (Table 2). The leaf samples were sprayed at these five densities, and each was replicated four times randomly.

The exposed leaves were cut and prepared for bioassay under laboratory conditions. A standard double cup method (Insect Resistance Action Committee, Method 8) was used, where two cups were inserted one into another holding a moistened cotton swab in the bottom cup (Fig. 3). The young cotton leaf cut after retaining a petiole of 4 cm length was kept in the top cup with the petiole protruding into the bottom cup through a hole in the top cup. The moistening of the petiole keeps the leaf green for about two days.

The leaf in the cup was then populated with 5 to 7 insects, and the top of the cup was covered with a fine muslin cloth. Insect mortality counts were recorded after 24 hours.



Figure 3. Double cupped bioassay.

# Modeling of the bioassay results to find the lethal number (LN) values

— (i) The GNU-based statistical platform R (R Core Team, 2018) was used on the generated experimental data to model and compute the  $LN_{50}$  and  $LN_{90}$  values. The generalized linear model of the binomial family and probit link was invoked to relate mortality (binomial; dead or alive insects) to the droplet densities (number of droplets deposited per unit leaf area). The general logistic regression model is of the form:

#### *ln* (*dead/alive*) = $\alpha + \beta$ (*dropletdensity*)

where the dead/alive ratio is the proportionality ratio of the dead vs alive insects in the bioassay cup. In the R language, the generalized linear model is invoked with a command,

#### $model = glm(y \sim log(dropletdensity), binomial(probit))$

where the link function is 'probit'.

The  $LN_{50}$  or  $LN_{90}$  values were found from this regressed model using the R function:

dose.p (model, p=0.5) or dose.p (model, p=0.9), respectively.

Each such regressed binomial model gave the lethal droplet density yielding 50 or 90% mortality ( $LN_{50}$  and  $LN_{90}$  respectively) on one droplet size at the selected pesticide dose.

— (ii) Similar bioassays as explained in section 2.1.i (Fig. 1) were implemented at three levels of pesticide doses, namely 0.005, 0.05, and 0.5 mL  $L^{-1}$  of imidachloprid in water. These computed  $LN_{50}$  (or  $LN_{90}$ ) values were plotted against the three levels of pesticide doses for the selected droplet size.

— (iii) The bioassays explained in sections 2.1.i and 2.1.ii (Fig. 1) were repeated for four levels of droplet sizes (200, 135, 75, and 50  $\mu$ m), and a complete set of model curves relating LN values to the pesticide doses for each droplet size was drawn. Fig. 4 illustrates a sample plot of LN<sub>50</sub> values against the three selected pesticide doses at four droplet sizes.

— (iv) In the plot thus made, the relationship between  $LN_{50}$  and pesticide dose for any desired droplet size can be

interpolated and drawn (red line). The droplet size (VMD) of the crop protection equipment being studied can now be used to interpolate such a model curve.

— (v) A horizontal line (blue line) was drawn corresponding to the actual droplet density generated by the crop protection equipment under study. This horizontal line intersects the interpolated plot at the point of optimal dose for the selected spray equipment. This implies that this dose will yield 50% mortality on the selected insect when using the selected crop protection equipment yielding a particular droplet size and deposition density. For instance, if the selected spray equipment gives a droplet size of 96  $\mu$ m (VMD) at a droplet density of 276 cm<sup>-2</sup>, the sample LN<sub>50</sub> plot (Fig. 4), indicates that a dose of 0.030 mL L<sup>-1</sup> would give the desired bioefficacy of 50%. The same procedure using this method could be followed to find the LN<sub>90</sub> values for any insect-pesticide-protection equipment combo.

# Bioassay on *A. gossypii* and *A. biguttula* for electrostatic spraying equipment

The above method was used in this study to ascertain the optimal pesticide dose for an electrostatically charged spray. It is already known that electrostatic charging of spray droplets is a viable option to transport the spray chemical to the plant's canopy and precisely distribute the droplets on the leaf surface. This spray application technique has already been proven to operate at a lower volume rate than conventional air-assisted sprayers, significantly reducing the loss of pesticides (Law & Lane, 1981; Wolf et al., 1996; Neto et al., 2015). The electrostatic spray charging system used in this study was developed in previous work (Maski & Durairaj, 2010a), which uses an air-assisted induction nozzle to impregnate electrical charge



**Figure 4**. An example of computing the  $LN_{50}$  based optimal dose.

Insect pest	Spray applicator	Optimal pesticide dose	Optimal dose	<b>Bioefficacy %, after</b>		
	(mist blower)	(mL L <sup>-1</sup> )	(g a.i. ha <sup>-1</sup> )	24h	48h	
A. gossypii	Electrostatic	0.025 (LN <sub>50</sub> )	2.2	43	46	
A. gossypii	Electrostatic	0.15 (LN <sub>90</sub> )	13.2	72	89	
A. gossypii	Uncharged	0.50 (LN <sub>90</sub> )	110	88	91	
A. biguttula	Electrostatic	0.20 (LN <sub>90</sub> )	22	79	91	
A. biguttula	Uncharged	0.50 (LN <sub>90</sub> )	110	90	98	

Table 3. Results of the field trials on bioefficacy of pesticide sprays at predicted doses.

into the atomized droplets. The used spray-charging system goes as an attachment to the existing knapsack mist blower. It has an induction charging nozzle powered by a high voltage DC module (5 kV at 1.5 W). In place of the air shear nozzle, the induction charging nozzle can be mounted on the conventional spray lance of the air-assisted mist blower.

Here in our study, the explained bioassay method was attempted to optimize the dose of imidachloprid for yielding the maximum bioefficacy (mortality) on cotton aphids (Aphis gossypii (Glover), Hemiptera: Aphididae) while using the electrostatically charged spray. The typical droplet sizes of the used electrostatic sprayer have been reported to be in the range of 70 to 100 µm VMD and the charge acquired by these droplets from the induction charging system was about 1.0 mC kg<sup>-1</sup> at a flow rate of 400 mL min<sup>-1</sup> (Maski & Durairaj, 2010b). The droplet density was about 200 cm<sup>-2</sup> on the adaxial surface and 70 cm<sup>-2</sup> on the abaxial surface. These data were used on the bioassav made on A. gossypii and the optimal doses of imidachloprid 17.8% SL for 50% and 90% mortality were found to be 0.025 and 0.15 mL L<sup>-1</sup> respectively. The same were used on the bioassay made on jassids (Amrasca biguttula (Ishida), Hemiptera: Cicadellidae) and the optimal doses of imidachloprid for 50% and 90% mortality were found to be 0.035 and 0.2 mL L<sup>-1</sup> respectively. Similarly, the optimal L90 dose for the uncharged mist blower spray was found to be 0.5 mL L<sup>-1</sup> for both A. gossypii and A. biguttula.

#### Field experiments to assess bioefficacy

Based on the results of the procedure, a field experiment was planned to have four treatment plots of cotton, namely the control plot without any spray applied to the plants, a plot sprayed by a conventional mist blower at 0.5 mL L<sup>-1</sup> imidachloprid and the rest with one charged spray each at 0.025 and 0.15 mL L<sup>-1</sup> doses, respectively, which are the doses optimized through the bioassay. The application rates of spray fluid were 1000 L ha<sup>-1</sup> for conventional spray and 400 L ha<sup>-1</sup> for charged spray, which correspond to 1.0 mL min<sup>-1</sup> and 0.4 mL min<sup>-1</sup> of discharges from the sprayer at the walking speed of spraying, respectively. The halving of the application rate for charged spray was deliberate, since it has already been proven in many studies that charged spray will double the spray deposition on the leaves. The experiment was conducted to assess the effect of these treatment sprays of imidachloprid on the mortality of *A. gossypii*.

An aphid-infested field cotton plot of 0.25 ha was selected in the eastern farm area of the Tamil Nadu Agricultural University, Coimbatore, India. It was divided into four blocks (subplots), one for each treatment, including the control plot left without any spraying. A buffer band of about 2 m was left on the inter-boundaries of these plots to prevent any interaction over the subplot boundaries. Samples were drawn from plants within these buffered subplots. Randomly selected plants in these treatment subplots were labelled for identity. The pre and post-spray counts of A. gossypii after 24 and 48 h were collected on the selected plants in each treatment subplot. The counts were drawn mainly from the terminal leaves of the selected plants. Statistical procedures were used to compute the bioefficacy based on insect mortality. Since the insect population could not be uniform all over the field plot. Henderson & Tilton (1955) formula furnished below, was used to calculate the corrected bioefficacy:

Corrected bioefficacy (%) =  $1 - \frac{n \text{ in } C \text{ before treatment} \times n \text{ in } T \text{ after treatment}}{n \text{ in } C \text{ after treatment} \times n \text{ in } T \text{ before treatment}} \times 100$ 

where n = insect population, T = treated, C = control (unsprayed).

The second field experiment was attempted on *A. biguttula*, a highly motile pest of cotton. Since they are motile, unlike in the case of *A. gossypii* they do not remain in the same plant after the spray is given. Hence, microcages were fabricated and used to retain a predefined number of *A. biguttula* on the plant's leaf after spraying. The microcages were simple perforated polythene covers with two reinforcement rings inside to keep them out of contact with the leaf when they were tied over a leaf (Fig. 5). *A. biguttula* nymphs were used for both the bioassay and the field experiment since the collection of adults is very cumbersome. The field was laid out to have three treatment plots to receive uncharged conventional spray at 1000 L ha<sup>-1</sup>, charged spray with the developed nozzle at 400 L ha<sup>-1</sup>, and no spray (control), respec-

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Reference	Insect pest	Dose used (g a.i. ha <sup>-1</sup> )	Mortality (%)	
Kumar et al., 2012	A. biguttula	27	56	
Naik et al., 2017	A. biguttula	20	22	
Nemade et al., 2017	A. gossypii	100	90	
	A. gossypii	50	82	
	A. biguttula	100	85	
	A. biguttula	50	72	
Ramalakshmi et al., 2020	A. gossypii	21	58	
Nihal & Bala, 2020	A. gossypii	22	61	
	A. biguttula	22	79	

Table 4. Earlier works on bioefficacy in relation to the applied pesticide dose.

tively. The application rate was meticulously controlled by monitoring the speed of the application. The optimal doses of imidacloprid for causing 90% mortality were 0.5 mL L<sup>-1</sup> for the conventional spray and 0.2 mL L<sup>-1</sup> for the charged spray. After spraying, the microcages were installed on the top and middle leaves of selected plants at random in each plot. Five nymphs of the same size were collected and released into the microcages. The insects were retained in the cages for 48 h and the mortality count was taken every 24 h. The corrected bioefficacy was calculated using the same mortality counts as before.

### Results

## Trials on the CDA equipment generating the desired droplet sizes

The CDA equipment explained in 2.1.i (Fig. 1) generated the droplet sizes at four levels of speeds (Table 1). The CDA provided a narrow spectrum of droplet sizes at each rotary speed of the disc, which was used in the experiments to generate the desired droplet sizes. As anticipated, the droplet sizes decreased from 200 to 50  $\mu$ m when the rotary speeds of the spinning disc were increased from 2000 to 20000 rpm.

# Trials on the equipment for varying the droplet density of deposition

The equipment explained in 2.1.ii (Fig. 1) was used to experiment with the droplet densities generated at each droplet density and time of exposure (Table 2). At each rotary speed of the spinning disc, the deposited droplet density increased with the time the leaf sample is exposed to the spray.

#### **Results of the field experiments**

In the first field experiment on *A. gossypii*, when charged spray was applied at 400 L ha<sup>-1</sup> at the LN<sub>50</sub> dose of 0.22 g ai L<sup>-1</sup> (Table 3), the bioefficacy of imidachloprid was 46% on the second day after spraying. The LN<sub>90</sub> dose of 0.15 mL L<sup>-1</sup> (13.2 g of ai ha<sup>-1</sup>) for charged spray of imidachloprid to control *A. gossypii* provided a bioefficacy of 89% on the second day after spraying. It was 91% when an uncharged spray of imidachloprid was applied at 1000 L ha<sup>-1</sup> with the LN<sub>90</sub> dose of 110 g of ai ha<sup>-1</sup>. The corresponding values of bioefficacy after 48 h of observation were 46 and 89% respectively for charged sprays, and 91% for uncharged spray.

In the second field experiment on cotton, when charged spray of imidachloprid was applied at 400 L ha<sup>-1</sup> and at a dose of 22 g a.i L<sup>-1</sup> to control *A. biguttula* nymphs, the bioefficacy after 24h was observed to be 79% (Table 3), whereas it was 90% when uncharged spray was applied at110 g a.i L<sup>-1</sup>. The corresponding values of bioefficacy after 48 h of observation were 91 and 98%, respectively.

### Discussion

In the experiments on droplet generation (Table 1), the increased speeds rendered smaller- droplet sizes because the centrifugal force is greater at higher speeds, imparting more atomization (Heijne, 1981; Salyani, 1998). The droplet densities (Table 2) as influenced by the exposure time, were consistently increasing with time (Table 1). Heijne (1981) explained that the droplet density of spray can be altered by changing the volume flow rate of fluid fed to the rotating centrifugal disc. But in this study, the droplet density was varied by changing the volume rate exposed to the target leaf.

The modelled bioassay provided a  $LN_{50}$  dose that gave closer to 50% mortality of insects (46%), when charged



Figure 5. A field experiment on cotton jassids.

spray was applied at 400 L ha<sup>-1</sup> at that dose of 0.22 g ai  $L^{-1}$  (Table 3). Similarly, the systematic computation of the  $LN_{90}$  dose to cause 90% mortality was effective at 89% in charged spray at the same flow rate. Here, the dose of 13.2 g a.i ha<sup>-1</sup> (Table 3) suggested by the model for A. gossypii is half of that suggested by the pesticide manufacturers (25-30 g a.i ha<sup>-1</sup>). A charged spray at less than half the flow rate of an uncharged spray was able to provide substantial (89%) mortality for A. gossypii at the predicted LN<sub>90</sub> dose. The enhanced deposition of charged spray on the abaxial surface of the leaves had helped in attaining this result (Law & Lane, 1981; Maski & Durairaj, 2010a). The uncharged spray applied at 1000 L ha<sup>-1</sup> on A. gossypii was 91% efficient only at a much higher dose of 110 g a.i ha<sup>-1</sup>, which corresponds to the LN<sub>90</sub> dose predicted by the bioassay. The bioefficacy reported in earlier works for uncharged spray on A. gossypii (Nihal & Bala, 2020; Ramalakshmi et al., 2020) ranged from 58 to 61% (Table 4) while using the pesticide manufacturer's recommendation of 25-30 g a.i ha<sup>-1</sup>. However, Nemade et al. (2017) reported that 82 and 90% mortality were achievable only at higher doses of 50 and 100 g a.i ha<sup>-1</sup>, respectively (Table 4). This corresponds to the  $LN_{90}$  dose predicted by the bioassay for uncharged spray on A. gossypii (Table 4).

The optimal LN doses computed by the bioassay for both charged and uncharged sprays were effective on *A. biguttula* too. The bioefficacy on *A. biguttula* nymphs after 48 h was 91% (Table 3) in the charged spray applied at 22 g a.i L<sup>-1</sup> and 98% in the uncharged spray applied at 110 g a.i L<sup>-1</sup>. In earlier works, bioefficacy for uncharged spray on *A. biguttula* was quite variable, with a range of 22 to 85% (Table 4) while using the pesticide manufacturer's recommendation of 25-30 g a.i ha<sup>-1</sup>. Nemade et al. (2017) reported that 72 and 85% mortality were achievable at higher doses of 50 and 100 g a.i ha<sup>-1</sup>, respectively (Table 4).

On both pests, a charged spray at less than half the application rate and dose of an uncharged spray gave the same level of bioefficacy. This implies that a 25% saving

in pesticide use is obtained by charging the spray, which has a direct impact on the environmental health of the cropped field (Giles & Blewett, 1991).

Although the results of this study are from an experiment on a specific combo of pesticide applicator, pesticide, and pest, the optimal pesticide dose computed using this method of systematic bioassay offers a promising pathway for addressing the issue of pesticide usage in the field. So far, only the volume-based indices of bioassay such as lethal concentration and lethal dose, are prevalently used to get a guideline value of dose. The pesticide manufacturers use these guideline values to fix workable levels through field experiments. The doses that arrive are frequently not tailored to the exact needs of the plant, pest, and pesticide applicator. The described method effectively computed the optimal lethal dose, saving unnecessary pesticide use. Such bioassays can easily create ready reckoners of optimal doses for each case of spray equipment, pest, and plant, which can encourage optimal pesticide use. The method can very well accommodate even sub-lethal pesticide concentrations corresponding to LC5 or LC10 values that are used as threshold concentrations while implementing integrated pest management concepts.

### Authors' contributions

Conceptualization: C. D. Durairaj Data curation: B. Jyoti Formal analysis: C. D. Durairaj, B. Jyoti Funding acquisition: C. D. Durairaj Investigation: C. D. Durairaj Methodology: C. D. Durairaj Project administration: C. D. Durairaj Resources: C. D. Durairaj Software: B. Jyoti Supervision: C. D. Durairaj Validation: B. Jyoti Visualization: B. Jyoti Writing – original draft: C. D. Durairaj, B. Jyoti Writing – review & editing: C. D. Durairaj, B. Jyoti

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