

Insecticide-Residue Indicator Drop (i-RID): A low-cost tool for on-site residue screening in brinjal

Susmitha S^{1*}, Yasodha P², Sheeba Joyce Roseleen S², Sabarinathan K³

¹ Department of Agricultural Entomology, Anbil Dharmalingam Agricultural College & Research Institute (ADAC&RI), Tamil Nadu Agricultural University (TNAU), Tamil Nadu, India

² Department of Agricultural Entomology, HCRI&W, TNAU, Tamil Nadu, India

³ Department of Microbiology, AC&RI, TNAU, Tamil Nadu, India

Corresponding Author: Susmitha S

Abstract

A simple and novel technique, the insecticide-residue indicator drop (i-RID) method, was developed to detect insecticide residues on-farm, at market, or before cooking, based on the surface tension of a coloured water drop. Eight insecticides such as Carbofuran, Chlorpyrifos, Dimethoate, Fipronil, Profenofos, Spiromesifen, Thiamethoxam and Triazophos were sprayed on brinjal in the field, and fruits were sampled at 1–10 days after treatment (DAT). A drop of water, coloured with Congo red dye for improved visibility was placed on the blossom end of each collected fruit, and the spread area (mm²) was measured through a transparent grid placed between glass lids. Insecticide residues lowered the surface tension of the drop, causing it to spread more than on residue-free control fruit, where drops remained small and globular (9.37 ± 0.03 mm²) throughout the 10-day observation period. On the first day after treatment, the maximum spread was recorded on triazophos-treated fruits (18.03 ± 0.43 mm²), followed by fipronil (17.59 ± 0.65 mm²) and thiamethoxam (17.21 ± 0.37 mm²), while the least spread among treated fruits was observed with carbofuran (12.48 ± 0.40 mm²). Residue detectability persisted up to 6 DAT in profenofos-treated fruits, whereas the remaining insecticides showed detectable residues only up to 3–5 DAT. These findings indicate that the i-RID method can effectively differentiate insecticide-treated fruits from untreated ones and estimate residue persistence over time. Being simple, rapid, and inexpensive, this technique holds promise as a practical on-site tool for farmers, traders, and consumers to screen insecticide residues on brinjal before harvest, sale, or consumption.

Keywords: Brinjal, i-RID method, insecticide residue detection, residue persistence, surface tension

Introduction

Pesticides are one of the indispensable components in integrated pest management (Stern et al. 1959) ^[15]. However, a pesticide should be effective against pests along with toxicologically acceptable residue on the food commodity (Singh et al. 2007) ^[14]. The farmers often use pesticides indiscriminately, leading to several environmental problems, one of them being residues (Aktar et al. 2009) ^[1]. In order to monitor the pesticide residues in food commodities, samples are drawn as per the standard procedure adopted all over world (FAO/WHO, 1982; FAO/WHO, 2003) ^[8]. The steps in such residue analysis involve collecting representative samples, extraction, cleanup, identification and quantitation, if necessary, confirmation by using hi-tech lab instruments and detectors. Finally, the results are presented as ‘ppm’ or ‘trace’ or below detectable level (BDL) (Roy et al. 1995) ^[13]. Although the first two results are worth the high-costs involved, the third result implies significant wastage of time, money and manpower. This investigation attempts to develop a method and a kit to detect the presence of residues on perishables at farm, market or household levels without spending much time and money. Brinjal, *Solanum melongena* L. (Solanaceae) was selected as the test crop to detect the residues both *in vivo* as it is one of the top ten vegetables in the world, infested by more than 100 pests (AVRDC 2003) ^[8], showing high levels of pesticide residues when collected from markets (Arora and Gopal 2002) ^[2]. The insecticide-Residue Indicator Drop (i-RID) method developed for rapid detection of pesticide residues

in crop plant samples has been granted an Indian patent (Patent No. 549336; Application No. 201841010373; granted on 30 September 2024).

Materials and Methods

The insecticides Carbofuran, Chlorpyrifos, Dimethoate, Fipronil, Profenofos, Spiromesifen, Thiamethoxam and Triazophos were sprayed at the recommended dosage by a hand atomizer over fruit-bearing brinjal plants (cv.CO 2) early in the morning under field conditions at Anbil Dharmalingam Agricultural College & Research Institute (ADAC & RI), Thiruchirappalli. Brinjal samples were collected from the treated plants from day 1 to day 10 to determine the rate of dissipation of the particular insecticide through the i-RID method. The control plants received no treatment.

The insecticide – Residue Indicator Drop (i-RID) kit includes a Borosil bread-making glass bowl (11.0 x 4.3 x 2.7” at top and 8.6 x 3.1 x 2.7” at bottom) and a 1 or 2-piece lid made up of two rectangular glass plates (11.0 x 4.3 x 2.7”) stacked with a transparent mm-grid (photocopy) in between. The glass bowl was one-third filled with moist river sand that acted as stage to place the sample brinjal fruits inverted as there is more chance for the pesticide residues to persist at the blossom end of the brinjal fruit. When a drop of water is placed on a pesticide-treated fruit surface, the drop either remains intact keeping its shape or tends to spread depending on the residue level on the crop surface as the water drop acquires or picks up the residue

again. It can now be called a residue indicator drop (RID) and the method insecticide - residue indicator drop (i-RID) method, wherein a water drop from a 2-20 μl micropipette (size: 15 μl) was carefully placed on the flat blossom end of the brinjal fruits. Congo red, a brick-red coloured laboratory stain, was added to distilled water as a colouring agent to improve its visibility and surface tension. The surface tension of any liquid can be measured in area spread by droplet (Lamour *et al.*2010). The spread in mm of the i-RID on brinjal fruit was assessed by placing a drop and counting the mm squares it covers in the transparent lid grid with the aid of a hand lens (10 x). Physical properties like shape, size and colour retention of the i-RID was also studied. In the presence of residue, the surface tension of i-RID gets reduced, causing the drop to spread more.

Statistical analysis

The experimental data on i-RID spread (mm^2), was statistically analysed in paired t-test after square root ($x+0.5$) transformation.

Results

According to the results, on the day of insecticide spray, the i-RID spread on the treated brinjal fruit was always wider, compared to that on untreated control fruits. The drop was also colourless on the first day, compared to the distinct red colour later when the surfactants fail to reduce the surface tension as the drop gets normal in spread.

1. Carbofuran 3 G

Though applied in soil, carbofuran residues were detected by i-RIDs. The i-RID placed on carbofuran treated brinjal fruits spread significantly wider at 1 DAT (days after treatment) ($12.48 \pm 0.40 \text{ mm}^2$), compared to that on control fruit ($9.37 \pm 0.03 \text{ mm}^2$) (Fig. 1). Statistically, the drop differed significantly in spread between the treated and control fruits for 3 days. The results also indicated that the i-RID was colourless when it was flat at 1 DAT. In contrast, on the last day at 10 DAT on treated fruits, it was reddish, globular and smaller in size similar to all 10 days in control fruits indicating the absence of residues.

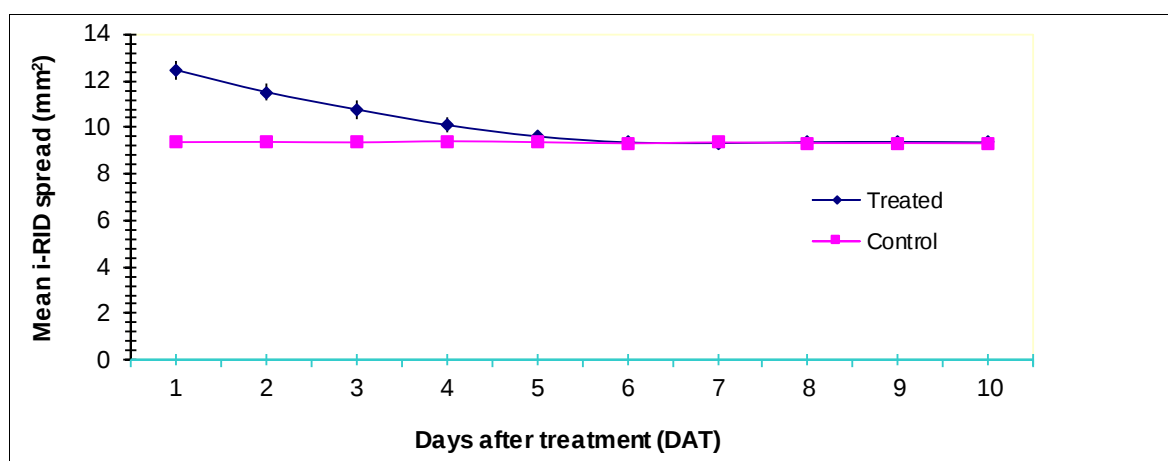


Fig 1: Dissipation of carbofuran 3 G residue on brinjal fruits detected by i-RID spread

2. Chlorpyrifos 20 EC

Significant difference was observed in i-RID spread on chlorpyrifos treated brinjal fruits at 1 DAT ($13.91 \pm 0.42 \text{ mm}^2$), compared to control drops ($9.36 \pm 0.03 \text{ mm}^2$) (Fig. 2). Statistically, the drops differed significantly in spread between treated and control fruits up to 4 DAT. The i-RIDs

on control fruits were smaller and globular on all 10 days, indicating the absence of residue. Results also indicated that the i-RID was colourless and flat at 1 DAT globular and prominently red at 10 DAT, indicating that the i-RID colour is also related to the residue level.

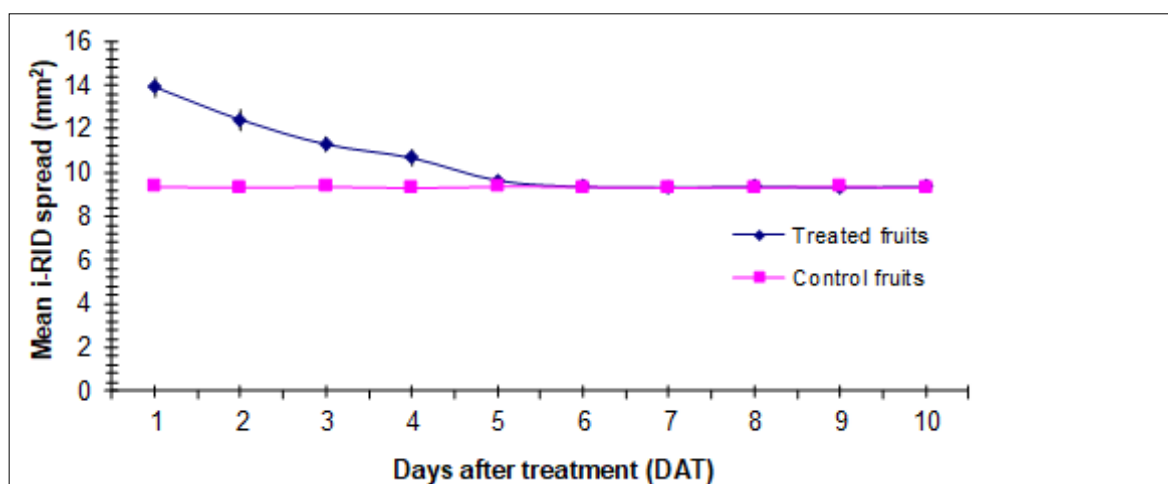


Fig 2: Dissipation of chlorpyrifos 20 EC residue on brinjal fruits detected by i-RID spread

3. Dimethoate 30 EC

Dimethoate residues were significantly variable in spread for 5 days (Fig. 3), the mean spread decreasing from $15.18 \pm 0.63 \text{ mm}^2$ at 1 DAT to $9.36 \pm 0.07 \text{ mm}^2$ at 10 DAT. The drop was wider and clear, on the first day indicating highest

residue level. They spread 10.62 mm^2 and 5.33 mm^2 on treated and control fruits, on average respectively. They were more transparent and wider, at 1 DAT, in contrast to prominently red and globular size at 10 DAT, indicating no residue.

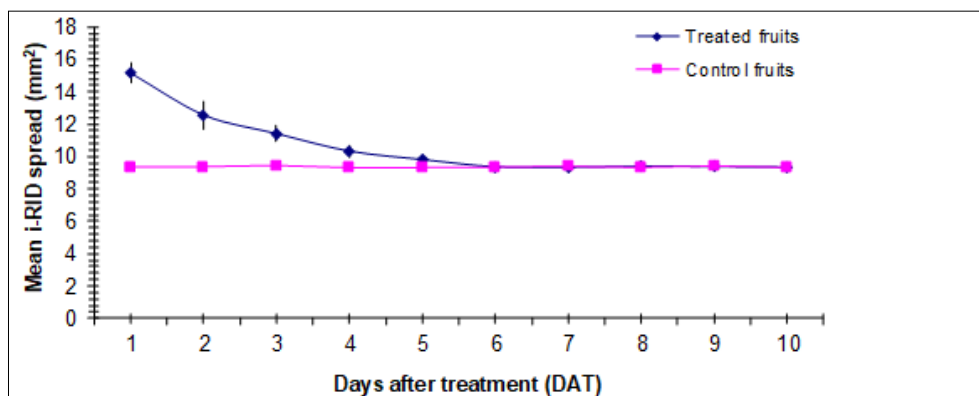


Fig 3: Dissipation of dimethoate 30 EC residue on brinjal fruits detected by i-RID spread

4. Fipronil 5 SC

Fipronil residue also persisted for 3 days with significant difference between the treated and control fruits in i-RID spread (Fig. 4). On the first day, the drop was widest in spread ($17.59 \pm 0.65 \text{ mm}^2$), compared to ($9.30 \pm 0.07 \text{ mm}^2$) control

fruits. The drop spread averaged $11.05 \pm 0.29 \text{ mm}^2$ on treated fruits and $9.36 \pm 0.06 \text{ mm}^2$ on control fruits over the 10 day period. As days passed, spread was less with increasing colour intensity, i.e., flat and clear at 1 DAT and red and globular at 10 DAT, indicating the absence of residue.

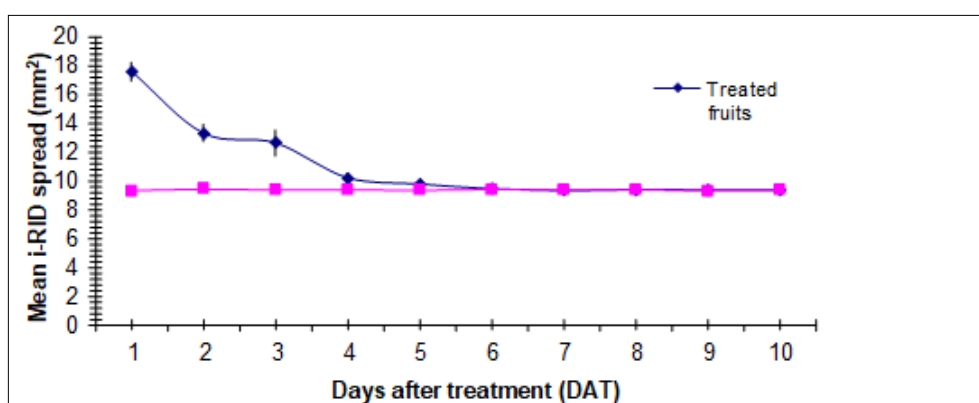


Fig 4: Dissipation of fipronil 5 SC residue on brinjal fruits detected by i-RID spread

5. Profenofos 50 EC

The drop placed on profenofos-treated brinjal fruits spread significantly wider at 1 DAT ($15.36 \pm 0.94 \text{ mm}^2$), than the control drops ($9.37 \pm 0.03 \text{ mm}^2$) (Fig. 5). Statistically, the difference was evident

up to 6 DAT. While the drops on control fruits were smaller and globular on all 10 days, in the absence of residue, They were colourless and flat at 1 DAT and globular and red at 10 DAT, indicating that the drop colour is also related to the residue level.

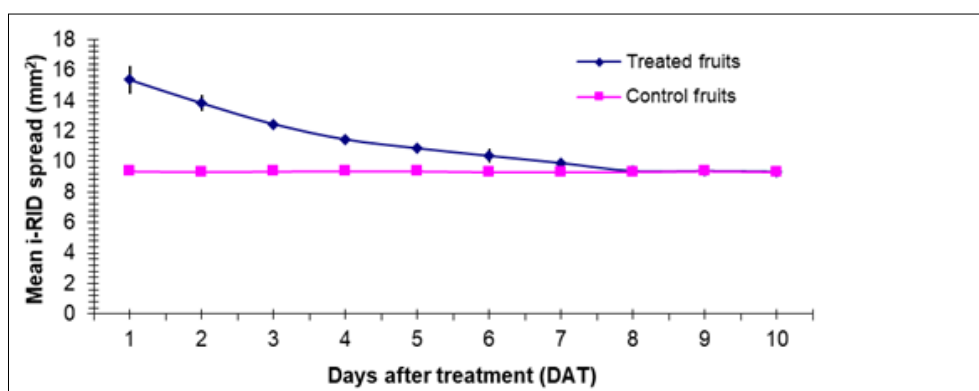


Fig 5: Dissipation of profenofos 50 EC residue on brinjal fruits detected by i-RID spread

6. Spiromesifen 22.9 SC

According to the i-RID method, spiromesifen residue persisted for 3 days with significant difference in its spread between the treated and control fruits (Fig. 6). On the first day, the drop spread was more on treated fruits ($13.85 \pm 0.41 \text{ mm}^2$),

than on control fruits ($9.37 \pm 0.03 \text{ mm}^2$). The drop spread averaged $10.10 \pm 0.15 \text{ mm}^2$ on treated fruits and $9.36 \pm 0.06 \text{ mm}^2$ on control fruits. As days passed, the i-RID spread significantly less but looked more reddish. It was flat and clear immediately after spray but globular and red at DAT 10, when there was no residue.

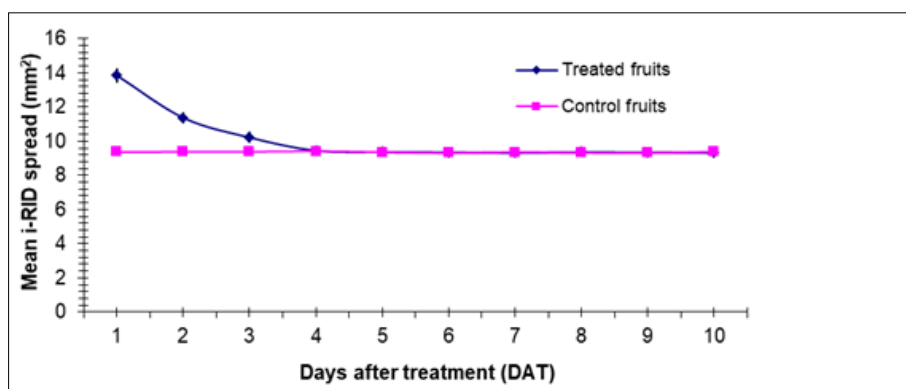


Fig 6: Dissipation of spiromesifen 22.9 SC residue on brinjal fruits detected by i-RID spread

7. Thiamethoxam 25 WG

The i-RID detected the presence of thiamethoxam residue for 3 days with significant difference in its spread between treated and control fruits (Fig. 7), spreading significantly more on the first day ($17.21 \pm 0.37 \text{ mm}^2$), than on control

fruits $9.37 \pm 0.03 \text{ mm}^2$. Its spread size averaged $10.87 \pm 0.27 \text{ mm}^2$ on treated fruits and $9.35 \pm 0.05 \text{ mm}^2$ on control fruits. With the passage of days, it appeared smaller and more colourful, flat and clear at DAT 1 and red coloured and spherical at DAT 10, indicating the absence of residue.

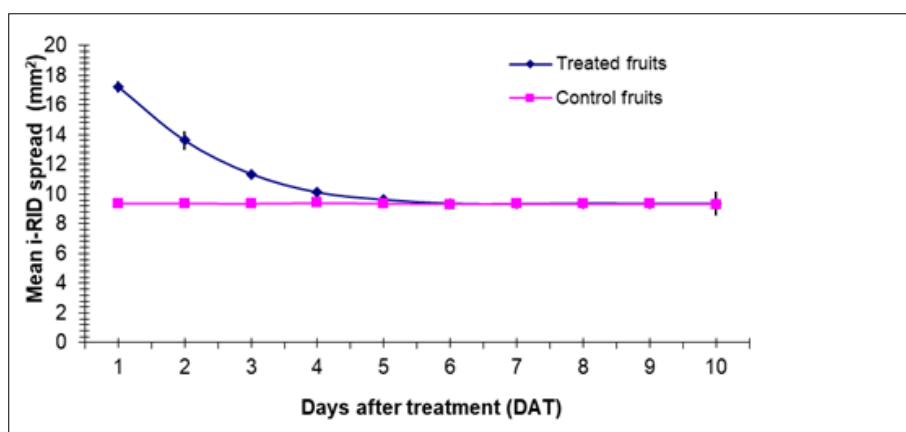


Fig 7: Dissipation of thiamethoxam 25 WG residue on brinjal fruits detected by i-RID spread

8. Triazophos 40 EC

The i-RID method detected triazophos residue for three days with significant difference between the treated and control fruits (Fig. 8). On the first day, the drop spread much more ($18.03 \pm 0.43 \text{ mm}^2$), than that on control fruits (9.37 ± 0.03

mm^2). The mean drop spread was ($11.48 \pm 0.33 \text{ mm}^2$) on treated fruits and ($9.35 \pm 0.05 \text{ mm}^2$) on control fruits. The i-RID appeared spherical with increase in red colour intensity as the days progressed, i.e. flat and clear at DAT 1, red and globular at DAT 10, after all the residue was dissipated.

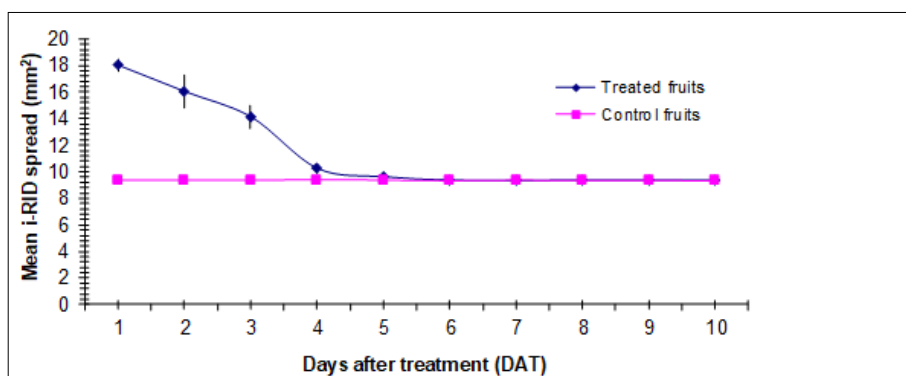


Fig 8: Dissipation of triazophos 40 EC residue on brinjal fruits detected by i-RID spread

Discussion

In this study the i- RID on control fruits were typically globular on all 10 days, indicating the absence of residues. If the residues (toxicant + adjuvants) are present on crop surface, the i-RID pick up the residue according to its level resulting in lower surface tension than that on a control surface. Pesticide formulations are mixed in water to prepare the spray fluids which is low in surface tension due to the action of adjuvants. When the tension is reduced to very low level, the drop begins to spread (Brumbaugh *et al.* 1995)^[4], forming a thin film over the fruit surface that dries up rapidly (Lo and Hopkinson, 1995)^[11]. Surface-active agents (surfactants) lower the free surface energy of the substrate (fruit) when the fruit gets wetted. A wetting agent allows a spray fluid to cover larger area than without a wetting agent (Hartley and Graham-Bryce, 1980)^[9]. Surfactants reduce the surface tension in the spray drop, which ensures that the formulation spreads out and covers plants with a thin film rather than beading up (Coret *et al.* 1993; Kirkwood, 1999)^[5, 10].

According to the results, on the day of insecticide spray, the i-RID spread on the treated brinjal fruit was always wider, compared to that on untreated control fruits. The drop was also colourless on the first day, compared to the distinct red colour later when the surfactants fail to reduce the surface tension as the drop gets normal in spread. The i-RID spread and colour reflected this even under field condition. Addition of Congo red (sodium salt) as a dyeing agent in textile industry yields a red colloidal solution (Elghetany *et al.* 1989)^[6]. Its colour varies from red, pink, light pink, bright pink, dull pink and blue colour on different substrates. Fipronil residue persisted for 3 days with significant difference between the treated and control fruits in i- RID spread. According to earlier reports the residue of fipronil takes 10-15 days to dissipate (Reddy *et al.*, 2014)^[12].

Conclusion

Pesticide residues on vegetables can be detected by the i-RID method as demonstrated by the results of this study. It can help at the field sampling stage to identify the samples meant for laboratory analysis. It can also be used by any lay man to differentiate between a treated brinjal sample and an untreated one. However, more research is needed in future with other vegetable samples for better results.

Acknowledgements

The authors sincerely acknowledge Tamil Nadu Agricultural University for providing the facilities and institutional support required to carry out this research work during the course of the study.

Conflict of Interests

The authors declare that the method described in this manuscript is protected by an Indian patent (Patent No. 549336; Application No. 201841010373), granted on 30 September 2024. One or more authors of this manuscript are inventors of the patented technology and the patent is assigned to Tamil Nadu Agricultural University (TNAU).

References

1. Aktar MW, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol*, 2009;2(1):1–12.

2. Arora S, Gopal M. Status of pesticide residues in brinjal (*Solanum melongena* L.): Indian scenario. *Ann Agric Res (India)*, 2002;3(2):12–15.
3. AVRDC. AVRDC report, 2000. AVRDC publication no. 00-503. Asian Vegetable Research and Development Center, Shanhua, Taiwan, 152p, 2003.
4. Brumbaugh EH, Roggenbuck FC, Penner D. Surface tension and spreading as indicators of spray adjuvant efficacy. In: *Proc Fourth Int Symp on Adjuvants for Agrochemicals*, 1995, 260–265.
5. Coret J, Bambonnet B, Brabet F, Chamel A. Diffusion of three ethoxylated octylphenols across isolated plant cuticles. *Pestic Sci*, 1993;38:201–209.
6. Elghetany MT, Saleem A, Barr K. The Congo red stain revisited. *Ann Clin Lab Sci*, 1989;19(3):190–195.
7. FAO/WHO. Pesticide residues in food – 1981. Report of the joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues, FAO Plant Production and Protection Paper 37, FAO, Rome, 1982.
8. FAO/WHO. Food Standard Programme. Codex Alimentarius Commission. Report of the thirty-fifth session of the Codex Committee on Pesticide Residues, Rotterdam, The Netherlands, 31 March–5 April 2003, 2003, 46–55.
9. Hartley GS, Graham-Bryce JJ. Coverage and spreading. In: *Physical properties of pesticide behaviour*. Academic Press, London, 1980, 402–469.
10. Kirkwood RC. Recent developments in our understanding of the plant cuticle as a barrier to the foliar uptake of pesticides. *Pestic Sci*, 1999;55:69–77.
11. Lo CC, Hopkinson M. Influence of adjuvants on droplet spreading. In: *Fourth Int Symp on Adjuvants Agrochem*, 1995, 144–149.
12. Reddy AA, Vemuri S, Cherukuri SR. Dissipation pattern of bifenthrin in cabbage (*Brassica oleracea* var. *capitata*). *Indian J. Adv. Pl. Res.*, 2014;1(3):51-56.
13. Roy RR, Albert RH, Wilson P, Laski RR, Roberts JJ, Hoffmann TJ, et al. U.S. Food and Drug Administration pesticide program: incidence/level monitoring of domestic and imported pears and tomatoes. *J AOAC Int*, 1995;78:930–940.
14. Singh G, Singh B, Battu R, Jyot G, Singh B, Joia B. Persistence of ethion residues on cucumber, *Cucumis sativus* (Linn.) using gas chromatography with nitrogen phosphorus detector. *Bull Environ Contam Toxicol*, 2007;79:437–439.
15. Stern VM, Smith RF, Van den Bosch R, Hagen KS. The integration of chemical and biological control of the spotted alfalfa aphid–1. The integrated control concept. *Hilgardia*, 1959;29:81–101.