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# Insecticide-residue indicator drop (i-RID): Contact angle measurement as a low-cost field-screening approach for brinjal

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## Abstract
Rapid and simple detection of pesticide residues on agricultural commodities is essential for ensuring food safety and monitoring compliance with maximum residue limits. The present study evaluated the efficacy of an insecticide–Residue Indicator Drop (i-RID) method based on changes in contact angle for rapid detection of insecticide residues on brinjal fruits. The i-RID response was assessed on fruits treated with different insecticides representing various formulations, including carbofuran 3 G, chlorpyriphos 20 EC, dimethoate 30 EC, fipronil 5 SC, profenofos 50 EC, spiromesifen 22.9 SC, thiamethoxam 25 WG and triazophos 40 EC. In the absence of detectable residue, the indicator drops remained relatively spherical with an obtuse contact angle, whereas in the presence of insecticide residues, wetting of the fruit surface resulted in spreading and a significantly more acute contact angle. On the day of insecticide application, contact angles on treated fruits ranged from 54.75° ± 4.95° for profenofos 50 EC to 92.50° ± 4.29° for carbofuran 3 G, compared with approximately 133° on untreated control fruits. Significant differences in contact angle between treated and control fruits persisted for 5 days for carbofuran 3 G, chlorpyriphos 20 EC, dimethoate 30 EC, spiromesifen 22.9 SC, thiamethoxam 25 WG and triazophos 40 EC, while the response persisted up to 6 days for fipronil 5 SC and 7 days for profenofos 50 EC. The results demonstrated a clear and visually measurable difference between residue-bearing and untreated fruit surfaces. Thus, the i-RID method provides a rapid, simple and potentially field-applicable approach for preliminary screening of insecticide residues on brinjal fruits without requiring sophisticated analytical instrumentation. Further validation against conventional chromatographic residue-analysis methods would strengthen its applicability as a rapid residue-screening tool.

**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) [16]. However, a pesticide should be effective against pests along with toxicologically acceptable residue on the food commodity (Singh *et al*. 2007) [15]. 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) [7, 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) [14]. 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* and *in vitro* as it is one of the top ten vegetables in the world, infested by more than 100 pests (AVRDC 2003) [3], 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, Chlorpyriphos, 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: 15µl) 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 contact angle (Lamour *et al*.2010) [12]. In the presence of residue, the surface tension of i-RID gets reduced, causing the drop to spread more and the contact angle gets acute (<90°). With no residue, the contact angle of the drop gets obtuse (>90°). These angles were measured by keeping a protractor against the drop by the opposite side of the glass bowl.

### Statistical analysis

The experimental data on Contact angles (°) were log transformed and statistically analysed in paired t-test.

### Results

According to the results, on the day of insecticide spray, In the absence of residue, wetting allows an i-RID to become less spherical and tends to flatten at the point of substrate contact, yielding a lower contact angle (acute) on the substrate. When measured using protractor the contact angle of an i-RID placed on a treated fruit was found acute (< 90°), compared to control which was obtuse (> 90°), with significant difference between them for 5 days with many samples.

#### 1. Carbofuran 3 G

Though applied in soil, the contact angle of the i-RID on treated fruits was 92.50° ± 4.29° and 133.25° ± 0.97° on control fruits (Fig. 1). Statistically, the i-RID contact angle differed significantly between the treated and control fruits up to 5 DAT.

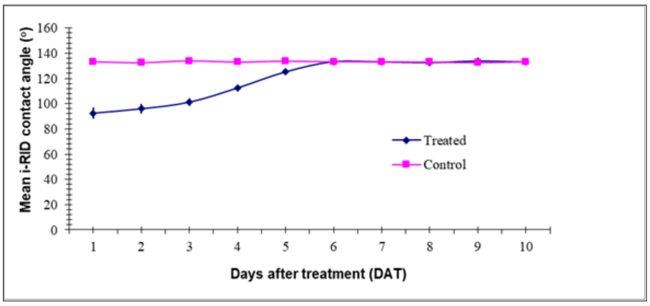


**Fig 1:** Dissipation of carbofuran 3 G residue on brinjal fruits detected by i-RID contact angle

#### 2. Chlorpyriphos 20 EC

Contact angle of the i-RID was more acute (87.50° ± 4.36°) on treated fruits while it was (133.25° ± 0.97°) on control fruits (Fig. 2). Statistically, the i-RID contact angle differed significantly between the treated and control fruits up to 5 DAT.

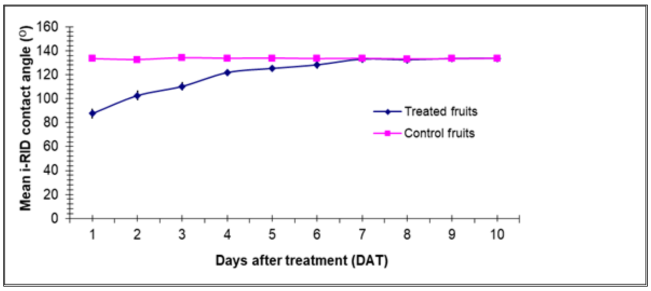


**Fig 2:** Dissipation of chlorpryiphos 20 EC residue on brinjal fruits detected by i-RID contact angle

#### 3. Dimethoate 30 EC

On the day of spraying the contact angle of the i-RID was more acute (74.00° ± 5.43°) on treated fruits and more obtuse (133.25° ± 0.97°) on control fruits (Fig. 3). Significant difference between treated and control fruits up to 5 DAT. The contact angle averaged 113.45° ± 2.62° and 132.97° ± 0.96 ° on treated and control fruits, respectively.

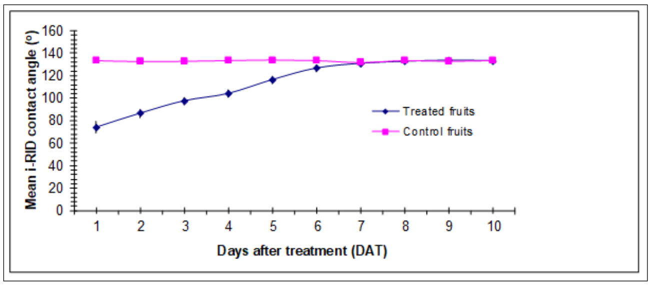


**Fig 3:** Dissipation of dimethoate 30 EC residue on brinjal fruits detected by i-RID contact angle

#### 4. Fipronil 5 SC

The contact angle of the i-RID placed on treated fruits was significantly more acute (65.75° ± 5.54°) at 1 DAT while it was more obtuse (133.25° ± 1.10°) on control fruits (Fig. 4). The contact angles differed significantly between the treated and control fruits up to 6 DAT, averaging 111.90° ± 2.97° and 133.15° ± 0.95° over the 10-day period.

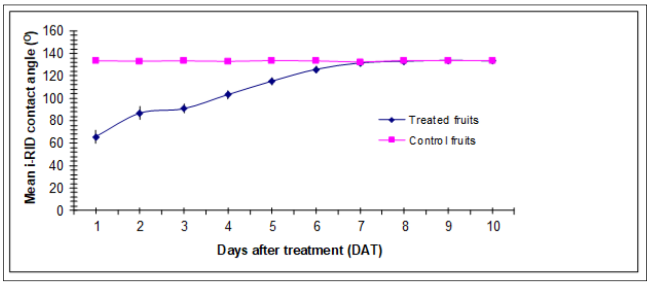


**Fig 4:** Dissipation of fipronil 5 SC residue on brinjal fruits detected by i-RID contact angle

#### 5. Profenofos 50 EC

Contact angle of the drop on the day of spraying was significantly acute on treated fruit (54.75° ± 4.95°) as against obtuse angles on control fruits (132.75° ± 0.92°) (Fig. 5).

The drop contact angle differed significantly between the treated and control fruits up to 7 DAT.

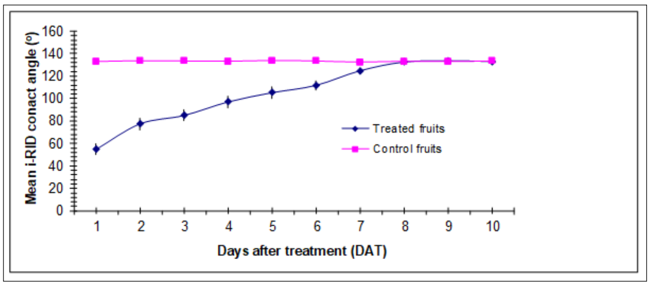


**Fig 5:** Dissipation of profenofos 50 EC residue on brinjal fruits detected by i-RID contact angle

### 6. Spiromesifen 22.9 SC

Contact angle of the i-RID on treated fruits was acute after its application (89.50º ± 4.21º) as against an obtuse angle on control fruits (133.25º ± 0.97º) (Fig. 6). The angle differed significantly between the treated and control fruits up to 5 DAT with a mean of 120.57º ± 1.96º and 132.25º ± 0.92º on treated and control fruits, respectively, over the experimental period.

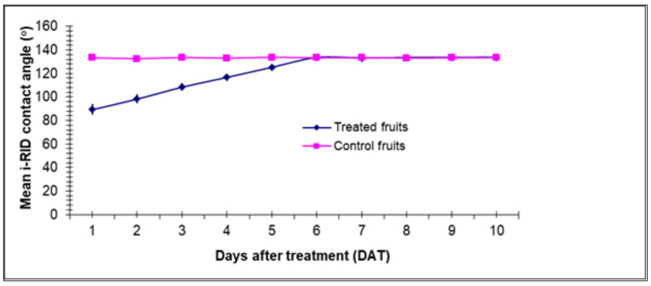


**Fig 6:** Dissipation of spiromesifen 22.9 SC residue on brinjal fruits detected by i-RID contact angle

### 7. Thiamethoxam 25 WG

Contact angle-wise, the i-RID looked significantly more acute on treated fruits (63.75º ± 6.03º) but obtuse on control fruits (133.25º ± 0.97º) (Fig. 7). Statistically, the contact angle differed significantly between the treated and control fruits for 5 days, with a 10-day means of 114.05º ± 2.51º and 132.92º ± 0.92º on treated and control fruits, respectively.

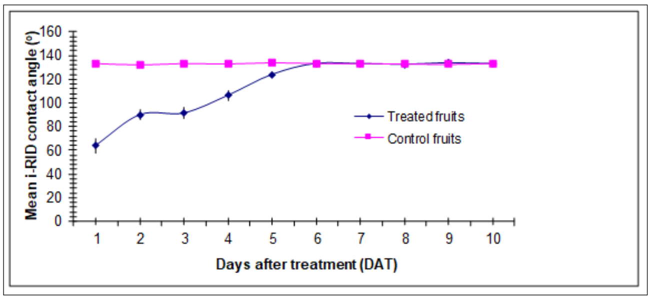


**Fig 7:** Dissipation of thiamethoxam 25 WG residue on brinjal fruits detected by i-RID contact angle

### 8. Triazophos 40 EC

The contact angle of the drop on treated fruits was narrower (58.75º ± 4.91º) on the day of spray and wider on control fruits (132.75º ± 0.92º) with significant difference between them up to 5 DAT (Fig. 8). The contact angle was 114.02º ± 2.07º and 132.85º ± 1.04º on treated and control fruits, respectively on average.

Fig. 8. Dissipation of triazophos 40 EC residue on brinjal fruits detected by i-RID
contact angle

## 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) [13]. 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) 19891. 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. In the absence of residue, wetting allows an i-RID to become less spherical and tends to flatten at the point of substrate contact, yielding a lower contact angle (acute) on the substrate. When measured using protractor the contact angle of an i-RID placed on a treated fruit was found acute (< 90º), compared to control which was obtuse (> 90º), with significant difference between them for 5 days with many samples.

The contact angle is defined as the angle formed by the intersection of the liquid-solid interface (Lamour *et al.* 2010) [12]A small (acute) contact angle is observed when the liquid spreads on the solid surface, while a large (obtuse) contact angle is observed when the liquid spreads (globular) on the surface. For super hydrophobic surfaces, water contact angles are usually greater than 150°, showing almost no contact between the liquid drop and the surface, which can rationalize the “lotus effect” (Lafuma and Quere, 2003) [11]. The contact angle was around 130-135° on brinjal fruits in the absence of any residue in control.

## 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.

The insecticide-residue indicator drop (i-RID) method was test verified to detect the presence of pesticide residues from brinjal fruit samples treated with insecticides for 10 days to study the dissipation of 10 insecticides. Among them, profenofos 50 EC residue lasted longer for 6 days on treated fruits as detected by the i-RID and the contact angle. Other insecticides persisted for 3 - 6 days. According to Katroju *et al.* (2014) profenofos residues dissipate by 42.24 % in tomato on the 1$^{st}$ day and 100 % on the 7$^{th}$ day. Interestingly, profenofos treated fruits had widest spread (15.36 $mm^2$) and lowest contact angle (54.75$^o$) on the first day, compared to all other compounds. Imidacloprid 17.8 SL lasted only for 3 days as detected by the i-RID spread and 5 days as detected by i-RID contact angle. Sahoo *et al*. (2012) reported that residues of imidacloprid took 5 to 7 days to reach limit of quantification. Normally, imidacloprid gets dissipated below the detectable level of 0.01 mg $kg^{-1}$ after 10 days (Mandal *et al.,* 2010). Its systemic nature may be the reason for this.In the laboratory analysis with HPLC, residues of imidacloprid 17.8 SL and flubendiamide 20 WDG were detected for 10 days. Earlier, (Karmakar and Kulshrestha, 2009) reported that thiamethoxam residues dissipated in 6-10 days. The half-life of flubendiamide in/on okra fruits ranged from 4.7 to 5.1 days at standard and double dose, respectively (Buddidathi *et al*., 2016). Chlorpyriphos residue was dissipated in 7 days on okra as per the reports published (Samriti *et al.,* 2012; Gupta *et al.*, 2011). The i- RID method detected its residue for 4 days by its spread and 5 days by its contact angle with significant difference between treated and control fruits. In this investigation, the method detected the residues of dimethoate for 5 days, by its spread and contact angle. Pandey *et al.* (2004) reported that dimethoate residues persist up to 10 days, reaching below tolerance limit within 2.19 days in okra.

Fipronil residue persisted for 3 days with significant difference between the treated and control fruits in i- RID spread and contact angle. According to earlier reports the residue of fipronil takes 10-15 days to dissipate (Reddy *et al.,* 2014).

The spiromesifen residues reached below quantification limit of 0.01 µg $g^{-1}$ on the 5$^{th}$ day on chilli (Sharma *et al.,* 2007). The i-RID method detected a 3-days persistence of spiromesifen residue with significant difference between the treated and control fruits in spread and contact angle.

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**Conflict of Interest**

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).

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