



Insecticide-Residue indicator Drop (i-RID): A capillary-based approach for rapid on-site screening of insecticide residues in brinjal

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Abstract

The indicator-based residue detection (i-RID) technique was evaluated on brinjal fruits treated with eight insecticides: carbofuran 3 G, chlorpyrifos 20 EC, dimethoate 30 EC, fipronil 5 SC, profenofos 50 EC, spiromesifen 22.9 SC, thiamethoxam 25 WG and triazophos 40 EC. The capillary rise of the i-RID on the treated fruits was compared with that on untreated control fruits at successive days after treatment (DAT). On the day of spray, the i-RID spread wider on treated fruits than on controls. The drop was colourless at 1 DAT and turned distinctly red on later days, when the surfactants no longer reduced surface tension and the drop's spread returned to normal. The time taken for the drop to cover the microslides differed significantly between treated and control fruits at 1 DAT for every insecticide tested. Treated fruits ranged from 15.12 ± 0.58 min (spiromesifen) to 16.48 ± 0.79 min (thiamethoxam), against controls of 13.22 to 15.30 min. Most treatments then showed no significant difference from the control. Exceptions were carbofuran at 10 DAT, chlorpyrifos at 5 DAT, dimethoate at 3 DAT, spiromesifen at 2 DAT, and triazophos, which differed at 1, 3, 7 and 10 DAT. For carbofuran, chlorpyrifos, dimethoate, spiromesifen and thiamethoxam, the difference was significant only at 1 DAT and the first few days afterwards. The difference was strongest in the first days after application, when the surfactant load on the fruit surface was highest, and it disappeared as the residues weathered. The i-RID can therefore give a quick, low-cost indication of recent insecticide application on brinjal fruits, mainly within the first days after spraying.

Keywords: i-RID, brinjal, capillary rise, insecticide residues

Introduction

Pesticides are one of the indispensable components of integrated pest management (Stern 1959) [15]. However, a pesticide should be effective against pests and, at the same time, leave a toxicologically acceptable residue on the food commodity (Singh *et al.* 2007) [14]. Farmers often use pesticides indiscriminately, which leads to several environmental problems, one of them being pesticide residues (Aktar *et al.* 2009) [1]. To monitor pesticide residues in food commodities, samples are drawn according to the standard procedures adopted all over the world (FAO/WHO 1982; FAO/WHO 2003) [7, 8]. Residue analysis involves collecting representative samples, extraction, cleanup, identification and quantitation, followed, if necessary, by confirmation using sophisticated laboratory instruments and detectors. The results are finally presented as ppm, as "trace", or as below detectable level (BDL) (Roy *et al.* 1995) [13]. The first two results are worth the high cost involved. The third implies a significant wastage of time, money and manpower.

This investigation therefore attempts to develop a method and a kit for detecting residues on perishables at the farm, market or household level, without spending much time and money. Brinjal, *Solanum melongena* L. (Solanaceae), was selected as the test crop. It is one of the top ten vegetables in the world and is infested by more than 100 pests (AVRDC 2003) [3]. Brinjal fruits collected from markets have also shown high levels of pesticide residues (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

1. Insecticide treatment

Carbofuran, chlorpyrifos, dimethoate, fipronil, profenofos, spiromesifen, thiamethoxam and triazophos were applied at their recommended dosages with a hand atomizer. The sprays were made early in the morning on fruit-bearing brinjal plants (cv. CO 2) under field conditions at Anbil Dharmalingam Agricultural College and Research Institute (ADAC & RI), Thiruchirappalli. Fruits were harvested from the treated plants daily from day 1 to day 10 after spraying, and the dissipation of each insecticide was assessed with the i-RID method. Control plants were left unsprayed.

2. i-RID kit and procedure

The insecticide-Residue Indicator Drop (i-RID) kit consisted of a Borosil bread-making glass bowl ($11.0 \times 4.3 \times 2.7$ " at the top and $8.6 \times 3.1 \times 2.7$ " at the bottom) and a lid of one or two rectangular glass plates ($11.0 \times 4.3 \times 2.7$ ") with a transparent mm-grid (photocopy) sandwiched between them. The bowl was filled to about one-third with moist river sand, which served as a platform on which the sample fruits were placed upside down, since residues are more likely to persist at the blossom end of the brinjal fruit.

When a water drop is placed on a pesticide-treated fruit surface, it either keeps its shape or spreads, depending on the residue level on the surface, because the drop picks up the residue. The drop then acts as a residue indicator drop (RID), and the technique is called the insecticide-residue indicator drop (i-RID) method. A 15 µl drop was carefully placed on the flat blossom end of each fruit with a micropipette (2-20 µl range). Congo red, a brick-red laboratory stain, was added to distilled water to make the drop more visible and to modify its surface tension.

3. Measuring capillary rise

The surface tension of a liquid can be assessed from the area covered by a droplet (Lamour *et al.* 2010). Capillary rise is another property influenced by surface tension. In a capillary tube or between glass plates, the rise is greater when the surface tension is higher and smaller when it is lower. In this study, a pair of micro slides was placed vertically over the i-RID, and the time (min) taken by the drop to cover the entire slide was recorded as the capillary rise rate.

4. Statistical analysis

The capillary rise data (min) were subjected to a square-root ($x + 0.5$) transformation and analysed using a paired t-test.

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

The i-RID on brinjal fruits following carbofuran treatment, the capillary rise rate was significantly higher at 1 DAT (16.32 ± 0.51 min.), compared to control drops (15.30 ± 0.38 min.) (Fig. 1). Statistically, the capillary rise had no significant difference between the treated and control fruits up to 9 DAT, except at 10 DAT when they differed significantly. The capillary rise of the drop on control fruit was found comparatively on par with treated fruit.

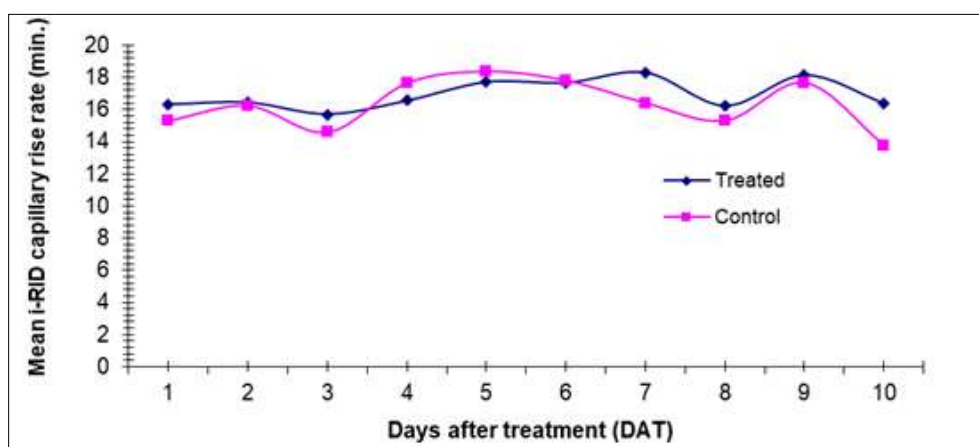


Fig 1: Dissipation of carbofuran 3 G residue on brinjal fruit as detected by the i-RID capillary rise rate

2. Chlorpyrifos 20 EC

The capillary rise rate was significantly higher at 1 DAT on chlorpyrifos treated brinjal fruits (15.67 ± 0.64 min.) than that on control fruits (14.86 ± 0.35 min.) (Fig. 2) and they were on par each other on control fruits up to 4 days, except at day 5.

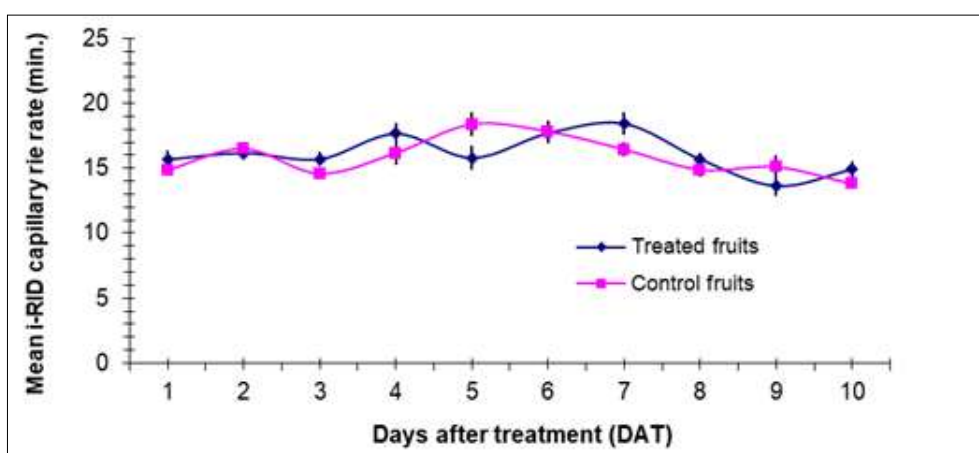


Fig 2: Dissipation of chlorpyrifos 20 EC residue on brinjal fruit as detected by the i-RID capillary rise rate

3. Dimethoate 30 EC

The capillary rise rate of i-RID was significantly higher on dimethoate treated fruits at 1 DAT (16.16 ± 0.80 min.) than on control drops (15.30 ± 0.38 min.) and at 3 DAT fruit (5.78 ± 0.56 min.) (Fig. 3) with no significant difference on all other days.

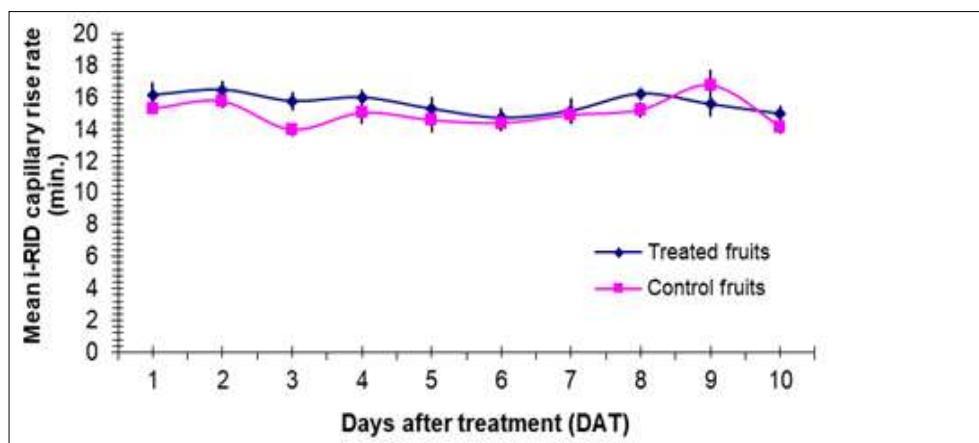


Fig 3: Dissipation of dimethoate 30 EC residue on brinjal fruits detected by i-RID capillary rise rate

4. Fipronil 5 SC

The capillary rise rate was significantly faster on fipronil treated brinjal fruits at 1 DAT (15.94 ± 0.54 min.) than on control i-RIDs (14.85 ± 0.44 min.) (Fig. 4).

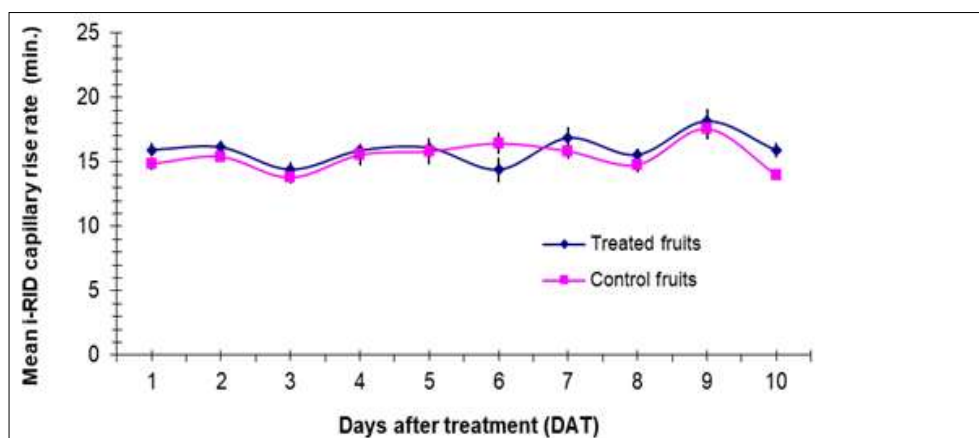


Fig 4: Dissipation of fipronil 5 SC residue on brinjal fruit as detected by the i-RID capillary rise rate

5. Profenofos 50 EC

The i-RID on profenofos treated brinjal fruits had capillary rise rate significantly higher at 1 DAT (16.32 ± 0.59 min.), than that on the control fruits (15.30 ± 0.38 min.) (Fig. 5).

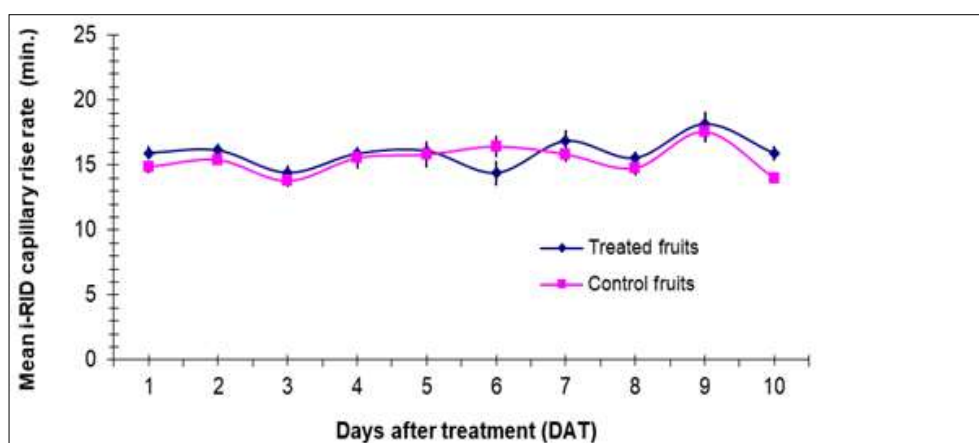


Fig 5: Dissipation of profenofos 50 EC residue on brinjal fruit as detected by the i-RID capillary rise rate

6. Spiromesifen 22.9 SC

The i-RID when placed on spiromesifen treated brinjal fruits took significantly 15.12 ± 0.58 min. to cover the microslides at 1 DAT compared to control drop which took 14.55 ± 0.47 min. (Fig. 6). The capillary rise did not differ significantly between the treated and control fruits up to 10 DAT, except day 2 and were on par with each other.

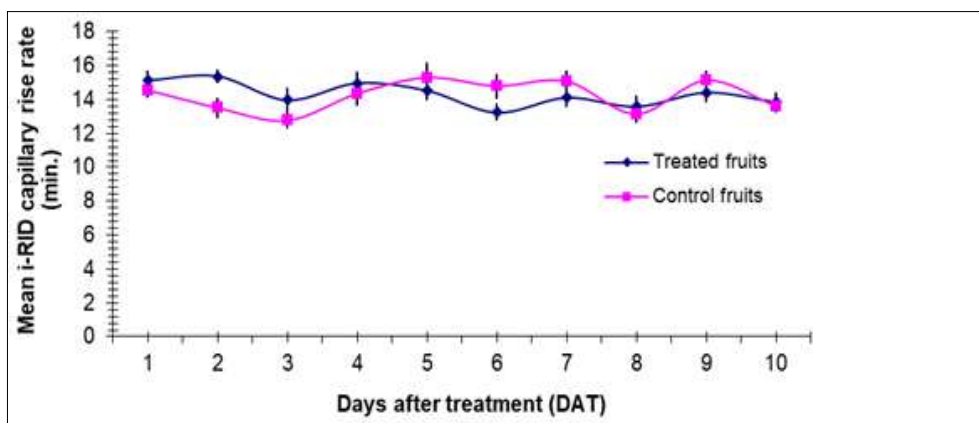


Fig 6: Dissipation of spiromesifen 22.9 SC residue on brinjal fruit as detected by the i-RID capillary rise rate

7. Thiamethoxam 25 WG

The capillarity of i-RID on thiamethoxam treated brinjal fruits was significantly faster at 1 DAT (16.48 ± 0.79 min.), compared to that on control fruits (13.22 ± 0.62 min.) with no significant difference later (Fig. 7).

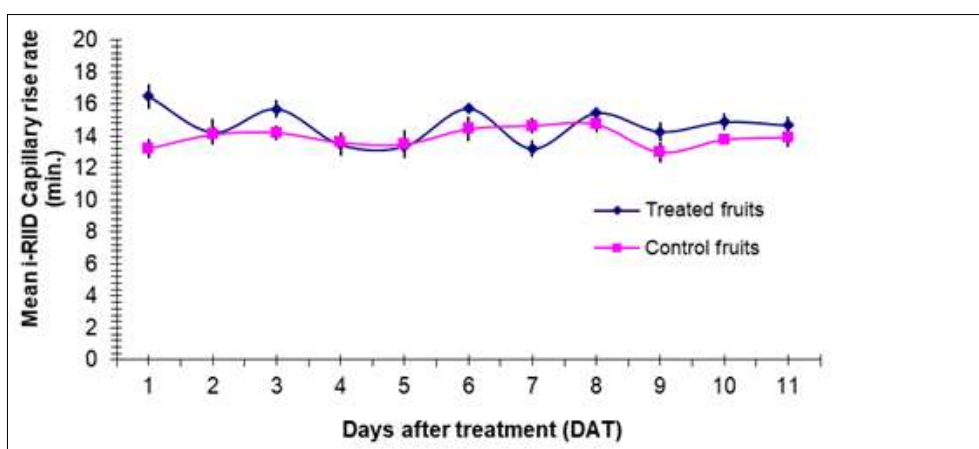


Fig 7: Dissipation of thiamethoxam 25 WG residue on brinjal fruit as detected by the i-RID capillary rise rate

8. Triazophos 40 EC

There was only a marginal difference in capillary rise rate between triazophos treated and control fruits although significant at 1,3,7 and 10 DAT it was equal on other days (Fig. 8).

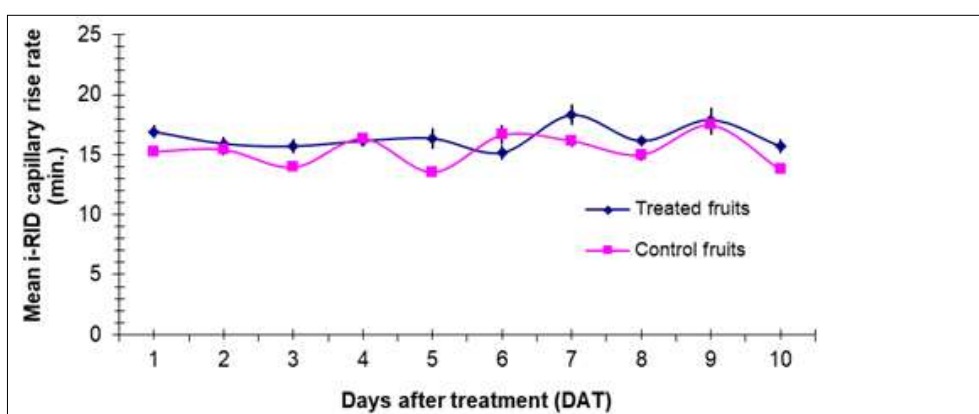


Fig 8: Dissipation of triazophos 40 EC residue on brinjal fruit as detected by the i-RID capillary rise rate

Discussion

Surface-active agents (surfactants) lower the free surface energy of the substrate, here the fruit, when it is wetted. A wetting agent allows a spray fluid to cover a larger area than it would without one (Hartley and Graham-Bryce 1980)^[9]. Surfactants reduce the surface tension of the spray drop, so the formulation spreads out and covers the plant with a thin

film instead of beading up (Coret *et al.* 1993; Kirkwood 1999)^[5, 10]. This explains the main finding of the study. On the day of insecticide spray, the i-RID spread on treated brinjal fruits was always wider than on untreated control fruits, and the difference in capillary rise between treated and control fruits was significant at 1 DAT for all eight insecticides.

The colour of the drop supports the same explanation. The drop was colourless on the first day, when the surfactants were active and the drop spread widely. On later days it turned a distinct red, as the surfactants no longer reduced the surface tension and the drop returned to its normal spread. Both the spread and the colour reflected this even under field conditions. The colour change is due to Congo red (sodium salt), which is used as a dyeing agent in the textile industry and yields a red colloidal solution (Elghetany *et al.* 1989)^[6]. Its colour varies from red through pink, light pink, bright pink and dull pink to blue on different substrates.

The i-RID spread also helped track how long a residue persisted. For fipronil, the residue persisted for 3 days, with a significant difference in i-RID spread between treated and control fruits. According to earlier reports, fipronil residues take 10 to 15 days to dissipate (Reddy *et al.* 2014)^[12]. The i-RID therefore mainly detects the surfactant and adjuvant component of the residue in the first days after spraying, rather than the total toxicant load. Where this component is the indicator, the method is useful as a quick, low-cost screening tool before laboratory analysis.

Conclusion

The capillary rise of the i-RID on brinjal fruits treated with the eight insecticides differed significantly from that on control fruits at 1 DAT. Thereafter the difference was mostly non-significant, except on a few days for some insecticides. The i-RID is therefore a rapid, simple and inexpensive method for screening recent insecticide residues on brinjal.

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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, 2003, 152p.
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 IJ. 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, Bong RL. 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.