



Effect of Neem on the Fate of Endosulfan Residue in Tomato

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Abstract

A study was conducted on tomato plants grown under field conditions to assess the effect of neem on the fate of endosulfan residues. Endosulfan was applied at the recommended dose and doubles the recommended dose (E1 and E2). Neem in the form of neem oil based biopesticide was applied @ 0.4% as a supplemental dose along with the selected test doses of endosulfan (E1+N and E2+N). A profound increase of 218.94% in total residue as well as increase in the contribution of high persisting forms of endosulfan viz. β endosulfan and endosulfan-sulphate was observed when applied pesticide concentration was increased from E1 to E2. However supplementation of neem along with E1 and E2 dose has resulted in 54.18% and 51.79% reduction in total residue respectively, besides reducing the high persisting forms of endosulfan viz. β endosulfan and endosulfan-sulphate in higher percentages, thereby showing two way benefits.

Keywords: Accumulation; biopesticide; persistence; pesticide; reduction.

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1. Introduction

The use of pesticides, in modern day agricultural practices, provides unquestionable benefits by increasing the production of crops. However, one of its drawbacks is pesticide residues which remain on the vegetables, constituting potential health risks to consumers. On the one hand this leads to the establishment of legal directives to control pesticide levels through the MRLs (maximum residue levels) and on the other to continue search for pesticides, which are less persistent and less toxic for human beings [8].

Pesticides are known pollutants of the environment and their improper use under exploitative agriculture has created havoc. Fruits and vegetables generally contain pesticide residues even after being washed or peeled off [13]. The transfer of these organic chemicals into plants occurs via two major pathways: (i) by root uptake from soil solution and (ii) transfer from air through wet and dry deposition of particles on plant surfaces that is followed by desorption into the inner parts of the plant [4]. Uptake, translocation and persistence of pesticides particularly in plants may lead to high toxic substance levels that are hazard to both human health as well as ecosystems, and there is considerable research interest in the prediction of these residue amounts [12]. Through food, water and environment these pesticide residues find their way into the human body. Analysis of pesticide residues in food as well as other environmental commodities like soil, water, fruits, and vegetables therefore has become essential for consumers, producers and food quality control authorities [3].

Different manufacturers propose different pre-harvest intervals ranging between 7 to 21 days on vegetables. Residues and pre-harvest intervals depend on various factors including the climatic conditions and methods used for the application of pesticide [7]. However as per general practices in the field, farmers even do not wait till the prescribed pre harvest time frame and supply the vegetables or fruits to the market at the earliest for monetary requirements.

Pesticides that are being actively used to boost agriculture to meet the demand of the growing population have both positive and negative effects [13]. Wherever pesticides are used in excessive quantities, monitoring and assessment of contamination caused by them is very difficult. Therefore, pesticide residue has become a major food safety concern for consumers and governments [11].

Tomato is one of the most important components of the human diet in different countries, where it is consumed in its raw form, home cooked or processed as juice or paste. It is a functional food as it contains antioxidant molecules such as Ascorbic Acid, Carotenoids, Flavonoids, Vitamin E and Phenolic Acids which contribute to human health [6].

Endosulfan is an organochlorine insecticide. It is applied on various crops, fruits and vegetables including tomato and is used to control a wide range of sucking and chewing insects. It is also included in the list of top 50 pesticides used on Tomato for processing in California in 2012 with application rate 0.89 pounds per acre [16]. According to PAN data, Endosulfan is still in use in many countries including Australia, Bangladesh, Brazil, India, Pakistan, Russia, South Africa and United States [15]. It is considered as a persistent organic pollutant (POP), is volatile and has the potential for long-range atmospheric transport. Thus it contaminates environment

far from where it is used. It is stored in the fatty tissues of animals and humans thus accumulating up the food chain, including in mothers' milk [15].

The replacement of endosulfan by another hazardous pesticide could cause additional environmental and health problems. The removal of endosulfan, therefore, should be complemented by a general reduction in pesticide use, and shift toward use of less toxic methods and products including its use in combination with other pesticides or biopesticides which can help in reducing its residue levels apart from pest control. In this context Neem and its products, which have emerged as a potential biopesticide, may proved to be beneficial. They have not been reported to cause any environmental problems and hold considerable promise for further development commercially.

The present study was thus aimed to assess the effect of neem on the fate of endosulfan residues in tomato.

2. Materials and Methods

2.1. Chemicals

All solvents like hexane and acetone were purchased from Sigma-Aldrich. Co. USA, Spectrochem Pvt. Ltd. India Sodium chloride (NaCl) and sodium sulphate (Na_2SO_4) were procured from Himedia Pvt. Ltd. India. Commercial formulation of Endosulfan 35% EC and Neem in the form of neem oil based biopesticide (300 ppm azadirachtin) was used for the experimental study.

2.2. Field work

Tomato plants, cultivar '*Lakshmi NP 5005*', were cultivated in plot size of 2 m^2 for each replicate. Endosulfan was applied at the recommended concentration @ 0.04% and double the recommended dose @ 0.08% (treatments E1 and E2) with a consumption of 0.1 L m^{-2} . Neem was applied @ 0.4% as a supplemental dose along with the selected test doses of endosulfan (treatments E1+N and E2+N). Spraying was carried out, using a portable sprayer equipped with a gun nozzle, 3 times at an interval of 15 days starting from 2 months old plants. The experiment was performed in triplicate and a control plot was kept aside in which no pesticide was applied.

2.3. Extraction and Cleanup

Extraction was done according to modified method of [9]. 100 gm samples were chopped and homogenized with 200 ml acetone for 2 min at high speed and filtered through a fast flow-rate filter paper in a Buchner funnel. An aliquot of 80 ml was taken in a 1 litre separatory funnel and was extracted with 200 ml mixture of hexane: dichloromethane (1:1, v/v) by vigorous shaking for 1 min. The lower aqueous phase was then transferred to another 1 litre separatory funnel. The organic phase of the first separatory funnel was dried by passing through approximately 1.5" sodium sulphate supported on pre-washed cotton in 4" funnel. To the separatory funnel containing aqueous phase, 10ml saturated sodium chloride solution was added and shaken vigorously for 30 sec. To this, 100 ml dichloromethane was added, shaken vigorously and lower organic phase was dried through the same sodium sulphate that was used for drying organic extract of the first separatory funnel. Extraction was repeated once more with 100 ml dichloromethane and was near to dried as above.

Sodium sulphate was rinsed with about 50 ml dichloromethane and extract was concentrated using Vacuum rotary evaporator. Concentration step was repeated in the presence of hexane to remove all traces of dichloromethane, and then repeated again to produce final extract in acetone solution. Solution was not allowed to go to dryness during any of the concentration steps. Volume of extract was adjusted to 5 ml with acetone.

50 ml hexane was added to a sintered chromatographic column (22mm i.d.), and 4.0 g of activated florisil was then poured slowly, followed by 2.0 g of sodium sulphate. One ml of the extract was then diluted to 10 ml with 10% acetone in hexane. Solution was then transferred to the florisil column. Container was rinsed with 2x3 ml portions of hexane. Column was eluted at about 5 ml/min with 50 ml eluant (50% dichloromethane: 1.5% acetonitrile: 48.5% hexane v/v/v). Florisil eluate was concentrated to 5 ml for GC.

2.4. Analysis

The final extracts were analyzed on (Perkin Elmer Clares-500) GC equipped with fused silica capillary column PE-5 equivalent to DB-5 (30 m x 0.25 mm x .25µm) coated with 1% phenyl-methylpolysiloxane (0.25 µm film thickness) using ⁶³Ni electron-capture detector (ECD). General operating condition were as follows: Column temperature program: initially 170°C for 2 min, increase at 5°C/min up to 270°C hold for 10 min. Injection volume: 1 µl nitrogen flow rate 0.79 ml/min and makeup 30 ml/min with split ratio 1:5; using carrier gas (N₂) 99.5%; Injector port temperature 250°C; detector temperature 350°C.

2.5. Calculations

Equivalent sample weight in final solution was calculated as:

$$\text{g sample weight equivalent} = \frac{100 \times 80}{200 + W - 10}$$

where 100 g sample analyzed, 80 ml filtered extract taken for hydromatrix partition, W, amount of water present in 100 g sample, 200 ml acetone used for blending, 10, adjustment for water/acetone volume contractions.

As water content of most of fresh fruits and vegetables may be assumed to be 85%, [10], total equivalent sample weight in final solution was taken as

$$= \frac{100 \times 80}{200 + 85 - 10} = 29.09 \text{ g sample}$$

Residue (µg/g or mg/kg) was calculated as:

$$\frac{\text{Sample area}}{\text{Std area}} \times \frac{\text{Std vol injected}}{\text{sample wt}} \times \text{Std conc } (\mu\text{g/ml}) \times \frac{\text{Final vol}}{\text{sample vol injected}} \times \text{Dilution factor (if any)}$$

2.6. Recovery Test

Pesticide standards @ 0.01 mg/kg and .05 mg/kg a.i. were spiked into 100gm test sample.

The preparation of sample solution was done as described above. Recoveries for pesticide were calculated after GC analysis. Recovery % for spiking varied from 75.87% to 91.07% with SD 4.61 to 15.36 (% SD: 6.07% to 16.87%). These findings are in line with [5], who found that recoveries ranged between 70-109.95% and relative SD ranged between 3-20% when samples of untreated tomato fruits were spiked with 0.02 mg/kg endosulfan.

3. Results and Discussion

Table 1: Effect of endosulfan and its combined doses with neem on residue accumulation in tomato.

Treatments	Avg residue α endosulfan (mg/kg)	Avg residue β endosulfan (mg/kg)	Avg residue endosulfan-sulfate (mg/kg)	Avg total residue (mg/kg)
E1	0.383±0.075	0.100±0.049	0.008±0.013	0.491±0.082
E1+N	0.187±0.022	0.038±0.004	0.000±0.000	0.225±0.019
E2	1.082±0.177	0.456±0.283	0.028±0.013	1.566±0.367
E2+N	0.563±0.083	0.189±0.060	0.003±0.005	0.755±0.064

Values are Means±SD of 3 replicates

Table 2: % share of three forms of endosulfan in total residue at different treatments in tomato.

	α endosulfan	β endosulfan	endosulfan-sulfate	total residue
E1	78.00	20.37	1.63	100.00
E1+N	83.11	16.89	0.00	100.00
E2	69.09	29.12	1.79	100.00
E2+N	74.57	25.03	0.40	100.00

Table 3: % reduction in endosulfan residues in tomato due to supplementation of neem.

	α endosulfan	β endosulfan	endosulfan-sulfate	total residue
From E1 to E1+N	51.17	62.00	100.00	54.18
From E2 to E2+N	47.97	58.55	89.29	51.79

Table 4: Increase in residue at higher pesticide dose from lower pesticide dose, in the absence and presence of neem and % share of three forms of endosulfan in this increase in residue.

	α endosulfan	β endosulfan	endosulfan-sulfate	total residue
Absolute increase from E1 to E2 (in mg/kg)	0.699	0.356	0.020	1.075
% share in increase from E1 to E2	65.02	33.12	1.86	100.00
Absolute increase from E1+N to E2+N (in mg/kg)	0.376	0.151	0.003	0.530
% share in increase from E1+N to E2+N	70.94	28.49	0.57	100.00

On applying lower test dose E1, total endosulfan residue in tomato fruits was found to be 0.491 mg/kg which was well below the MRL level of 1.00 mg/kg (Table 1, Fig. 1). Endosulfan contamination has been widely detected in tomatoes grown for the market, up to the level of 0.510 mg/kg [2]. Endosulfan residues were reported to be in the range of 0.03 to 0.85 mg/kg in field trials in USA and Southern Europe [14].

However, on application of higher test dose E2 total residue increased to 1.566 mg/kg which was found to be higher than the considered MRL level (Table 1, Fig. 1). Thus a profound increase of 218.94% in total residue was observed when applied pesticide concentration was increased by 100% from lower dose E1 to higher dose E2. Literature reveals that vegetables contain the pesticide residues above their respective maximum residue limit (MRL) [1].

Half life periods for various forms of endosulfan were in the range of 12-39 days for α endosulfan, 58-264 days for β endosulfan and about 150 days for endosulfan-sulphate in soil analysed in laboratory, however in field respective half lives were observed as 6-11 days, 19-36 days and 75-161 days [14]. As per these reported half life periods the order of persistence of these forms of endosulfan from higher to lower persisting order comes out to be endosulfan-sulphate (highest), then β endosulfan and then α endosulfan (least).

α endosulfan, β endosulfan and endosulfan-sulphate contributed 78.00%, 20.37% and 1.63% in total residue of 0.491 mg/kg at E1 dose and 69.09%, 29.12% and 1.79% in total residue of 1.566 mg/kg at E2 dose (Table 2). Thus on applying higher concentration of pesticide (E2) contribution of high persisting forms of endosulfan viz. β endosulfan and endosulfan-sulphate, in total residue, increased in comparison to those found at lower concentration of pesticide (from 20.37% at E1 to 29.12% at E2 for β endosulfan and 1.63% at E1 to 1.79% at E2 for endosulfan-sulphate respectively). Hence application of higher concentration of pesticide not only increased the total residue but also increased the contribution of higher persisting forms of endosulfan thus causing harm in both ways.

Total residue at E1+N and E2+N dose was found to be 0.225 mg/kg and 0.755 mg/kg respectively in comparison to 0.491 mg/kg and 1.566 mg/kg at E1 and E2 dose respectively (Table 1, Fig. 1). Thus 54.18% and

51.79% reduction in total residue was observed at E1 and E2 dose respectively due to supplemental dose of neem (Table 3). Further, reduction in α endosulfan, β endosulfan and endosulfan-sulphate residues were found to be 51.17%, 62.00% and 100.00% when neem was supplied with lower test dose of pesticide E1 and 47.97%, 58.55% and 89.29% when neem was supplied with higher dose of pesticide E2 (Table 3.). Thus application of neem was found to be beneficial not only in reducing the total residue but also helped significantly in reducing the high persisting forms, viz. β endosulfan and endosulfan-sulphate, in higher percentages thereby providing benefit in both ways. However this supplementation of neem was found to be more effective when combined with lower dose of pesticide than with higher dose of pesticide both in terms of total residue reduction and reduction in residue in various forms of endosulfan. Contribution of α endosulfan, β endosulfan and endosulfan-sulphate in total residue of 0.225 mg/kg at E1+N dose was 83.11%, 16.89% and 0.00% as against 78.00%, 20.37% and 1.63% in total residue of 0.491 mg/kg at E1 dose (Table 2). Thus application of neem as a supplemental dose has also changed the percentage share of these forms in total residue and shifted the share favourably towards the low persisting form of endosulfan i.e. α endosulfan. Similar trend was observed when neem was applied with E2 dose and contribution of α endosulfan, β endosulfan and endosulfan-sulphate was found to be 74.57%, 25.03% and 0.40% in total residue of 0.755 mg/kg at E2+N dose as against 69.09%, 29.12% and 1.79% in total residue of 1.566 mg/kg at E2 dose (Table 2). Further, contribution of α endosulfan, β endosulfan and endosulfan-sulphate in the increase of 1.075 mg/kg in total residue at E2 dose (from 0.491 mg/kg at E1 dose to 1.566 mg/kg at E2 dose) was 65.02%, 33.12% and 1.86%, whereas these contributions were 70.94%, 28.49% and 0.57% in the increase of 0.530 mg/kg in total residue (from 0.225 mg/kg to 0.755 mg/kg) at E2+N dose in comparison to E1+N dose (Table 4). Thus when applied pesticide concentration was increased from E1 to E2, i.e. in the absence of neem, the increase in high persisting forms of endosulfan was found to be higher in comparison to the corresponding increases when applied pesticide concentration was increased in the presence of neem, i.e. from E1+N to E2+N. This suggests that even if higher concentration of pesticides is to be used anywhere, if it is used in combination of neem the persistence of the pesticide would be lesser.

4. Figure

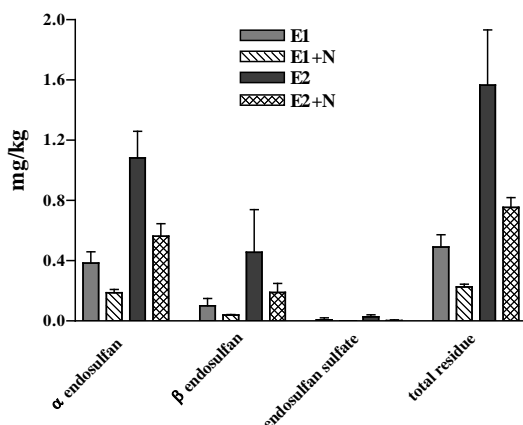


Figure 1: Bar graph along with error bars of SD showing the effect of endosulfan and its combined doses with neem on residue accumulation in tomato.

5. Conclusion

Endosulfan is highly persistent in its forms β endosulfan and endosulfan-sulphate and to a lesser level in the form of α endosulfan. A high concentration of pesticide applied to plants resulted in two adverse changes in comparison to the low concentration; first, there was a disproportionate increase in total residue and second, the proportional composition of endosulfan forms shifted slightly toward the more persistent forms. It was encouraging to find that neem reduced the total residue by more than 50% when supplemented with any of the test concentrations of the pesticide and that too by reducing the share of high persisting forms of endosulfan to a greater extent.

Endosulfan residues are cause of worry all over the world but the use of neem alongwith endosulfan can reduce the intensity of this concern. This combination also may prove to be helpful during the process of phasing out endosulfan entirely. Further as pesticide residue is a major global concern, neem should be evaluated for possible beneficial effects on residues of other pesticides.

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