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PERSISTENCE AND DEGRADATION PATTERN OF CHLOROTHALONIL IN SPINACH

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ABSTRACT

With a view to study the dissipation of chlorothalonil in/on spinach, a field experiment was conducted at Indian Institute of Horticultural Research, Bangalore, India. The foliar application of chlorothalonil (Kavach 75% WP) was carried out at 2 concentrations 50g and 100 g a.i. ha^{-1} in spinach at 45 days after sowing. Samples were drawn at 0 (1hour), 1, 3, 5, 7, 10, 15 and 20 days after spray. Soil samples were also collected on 20thday after application. The initial deposits of chlorothalonil on spinach beet were 111.98 and 192.93 at recommended dose and double the recommended dose of application, respectively. The residues gradually declined and persisted upto15th day at lower and higher dose, respectively. The residues reached below quantitation limit of 0.01 ug g^{-1}on 20thday at recommended and double the recommended dose, respectively. Half life of chlorothalonil on spinach beet was 1.27 and 1.28 days for standard dose and double the standard dose, respectively. Soil samples analysed on the 20thday after last spray revealed residues at below quantitation limit of 0.01 ug g^{-1} at either dose of application.

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INTRODUCTION

Bright, vibrant-looking spinach leaves are not only more appealing to the eye but more nourishing as well. Among the World's Healthiest vegetables, spinach comes out at the top of our ranking list for nutrient richness. Rich in vitamins and minerals, it is also concentrated in health-promoting phytonutrients such as carotenoids (beta-carotene, lutein, and zeaxanthin) and flavonoids to provide powerful antioxidant protection. Spinach is thought to have originated in ancient Persia (Iran).Spinach grows well in temperate climates. Today, the United States and the Netherlands are among the largest commercial producers of spinach. Leafy vegetables also contain antioxidants necessary for neutralizing free radicals which result in many diseases in humans (Ashok Kumar *et al*, 2013). Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is a common broad spectrum fungicide used for controlling various diseases of vegetable crops. It is non systemic, protectant fungicide effective against many diseases which damage vegetables viz. rusts, anthracnose, downymildews, leaf spots, soft rot, leaf blight, scab, early blight, late blight, pink rot, powdery mildew, etc. Chlorothalonil is commonly used in green onions mainly for control of purple blotch (Miller*et al*, 1986). Spinach-beet or Palak (Beta vulgaris L) can be infected by several fungi that cause leaf spot diseases (Correll *et al*, 1994). Fuentes-Davila (1988) reported that leaf spot caused by *C. variabile* can be controlled by chlorothalonil alone or in combination. Since leafy vegetables are grown with little or no spacing they grow densely and often have larger surface area and therefore are likely to contain higher initial deposit of any pesticide following its foliar spray. Thus, it is important that persistence of chlorothalonil residues in spinach be studied and waiting period determined. This is even more important due to the fact that there is no label claim for chlorothalonil use in any leafy vegetable in India, so the residue persistence data and risk assessment data can be used for fixing maximum residue limits of this fungicide in these crops. The present study therefore aimed at determining the persistence and degradation pattern of chlorothalonil residues in spinach beet.

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MATERIALS AND METHODS

Chemicals

Chlorothalonil (Kavach 75 WP) was obtained from local market.

Analytical standard of chlorothalonil was procured from M/S Sigma Chemical Co., USA. All chemicals and reagents used were AR grade, from E. Merck (India) Ltd.

Field Experiments

Field trials were conducted in the experimental field of the Indian Institute of Horticultural Research, Bangalore, India as per good agricultural practices (GAP).The field soil was sandy loam type and the average weather parameters during this study was temperature (maximum)– 29.7 ^{0}C , temperature (minimum)- 17.6 ^{0}C, relative humidity (maximum)- 73.9 %, relative humidity (minimum) – 45.6%, rainfall- 5.0mm. Spinach Beet (cv. Arka Anupama) was grown with chlorothalonil application. Three sets of 6 plots, each individual plot measuring 5m x 5m with randomized block design were directly sown with spinach beet seeds with a distance of 30 cm from row to row and 7.5 cm plant to plant(Sadashiva *et al*) at the same time. Each set of plots corresponded to one treatment, viz. recommended dose and double the recommended dose and control.

Chlorothalonil was sprayed using a Knapsack sprayer at the recommended dose of 2g/L and double the recommended dose of 4g/L. Spray volume used was 500L/ha. Spinach leaf samples were collected at 0(1hour), 1, 3, 5, 7, 10, 15 and 20 days after chlorothalonil application and analysed for its residues. Soil samples were collected on last date of sampling and analysed for residues. Extraction and clean-up of chlorothalonil in spinach was carried out as per standard protocol of Kurz *et al*. (2008).

Sample Preparation

Extraction: On every sampling day about 3 kg spinach beet was collected from each treated plot. The root was discarded and the shoot was taken in spinach, pooled together and cut into small pieces, homogenized in a Waring blender. A50g representative samples (3 replicates) were taken for residue analysis and extracted with 100mL hexane + acetone (60 +40) mixture and filtered under vacuum through a Buchner funnel.

The filter cake was re-extracted twice with 150mL of hexane + acetone (60 +40) mixture. The filtered extract was combined and concentrated under rotary vacuum evaporator (IKA Model RV10) at 40^{0}C to remove this organic mixture. Similarly soil samples were also extracted. The soil samples were collected from treatment plot at ten different sites at 0-15 cm depth , pooled together to form a sample size of approximately 5 kg, and air dried under shade (in the laboratory), powdered and passed through a 2 mm sieve. A representative 100g soil sample in triplicate was taken for residue analysis.

Clean up

The aqueous extract thus obtained was transferred into the separatory funnel and 200ml distilled water along with 12gm of sodium chloride was added and partitioned with hexane (3x50ml).The organic layer was passed through anhydrous sodium sulphate(20g)and combined. The combined hexane extract was concentrated to near dryness using rotary vacuum evaporator (IKA Model RV10) at 40^{0}C. The residues were re-dissolved in distilled acetone and final volume made up to 5ml in graduated test tube for analysis by gas liquid chromatography (GLC).

Estimation of recovery

A recovery experiment was carried out to ascertain the efficiency of the analytical method by spiking untreated samples (spinach and soil) with analytical grade chlorothalonil at the rate of 0.01, 0.05 and 0.1mg kg^{-1}.The spiked samples in five replicates were processed as per the analytical method described above to obtain the percent recovery.

Instrumental analysis

The determination of chlorothalonil residues was carried out using Varian gas chromatograph (GC) Model 3800 equipped with electron capture detector (ECD) and a DB-5 (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column. The standard split/splitless injector was used for splitless injection at 270 ºC with an injection volume of 1 µL. The ECD detector was maintained at 300 ºC, with the make-up gas nitrogen flow-rates at 30.0 mL min^{-1}. The oven temperature program was 80^{0}C (5 min) ramped to 150 ^{0}C (5 min) @ 15 ^{0}C/min ramp to 250^{0}C (0min) @4^{0}C/min again ramped to 280^{0}C (17min)@10^{0}C/min. Under these operating conditions, the retention time of chlorothalonil was 18.7 min.

RESULTS AND DISCUSSION

Method validation

The analytical method used for analysis of chlorothalonil in spinach beet and soil were suitable for the intended use. The performance of the method was evaluated according to the guidelines presented in SANCO (2013), where the satisfactory recoveries are within 70-120% with the relative standard deviation (RSD) <-20%(SANCO 2013).

Table 1. Recovery of chlorothalonil residues from spinach and soil at variousspiked levels

Fortification Level mgkg-1	Mean recovery* (%) ±SD Spinach	Soil
0.01	85.5 ± 0.66	91.2 ±3.05
0.05	87.5 ± 1.05	90.3 ± 0.76
0.1	90.7 ± 0.90	97.2± 2.04

*Average of five replicates

Table 2. Dissipation of Residues of Chlorothalonil in Spinach

Days after application	Chlorothalonil residues mg kg^{-1}	
	T1 2.0g/L	T2 4.0g/L
0	111.98±1.64	192.93±2.63
1	107.57±2.91 (3.94)#	161.06± 4.91 (16.52) #
3	98.86±0.49 (11.72) #	125.61±1.53 (34.89) #
5	86.22 ± 0.24 (23.01) #	103.66±0.25 (46.27) #
7	40.59±0.99 (63.75) #	44.08±0.99 (77.15) #
10	4.82±0.28 (95.70) #	5.51±0.09 (97.14) #
15	0.02	0.04 ± 0.00 (99.98) #
20	BDL##	BDL##
$t_{1/2}$ (days)	1.27	1.28
T_{tol}(days)	19.46	20.21

##BDL - Below Quantifiable Limit, Limit of Quantification = 0.01mgkg-1

#Figures in Parenthesis represent percent dissipation of residues from 0 day values.

Recoveries of chlorothalonil residues from fortified samples of spinach and soil using the analytical protocol described is presented in Table1.Recovery of chlorothalonil residues from spinach and soil was in the range of 85.5 -90.7 %, and 90.3 - 97.2% respectively indicating high efficiency of analytical method used. The limit of quantification (LOQ) of chlorothalonil in spinach and soil using the above method was 0.01mgkg^{-1}.

Residues of chlorothalonil in spinach

Determination of chlorothalonil residues in spinach was carried out in the year 2014.The spray application of chlorothalonil was given to spinach at the recommended and double the recommended doses of 50 and 100 g a. i. ha^{-1} respectively. The initial residue deposit of chlorothalonil at recommended dose in spinach was 111.98 mg kg^{-1} and at double the recommended dose was 192.93mg kg^{-1} (Table 2).These dissipated to below LOQ level of 0.01 mg kg^{-1} on 20th day of chlorothalonil application in treated spinach in both doses . No residue of chlorothalonil was detected in untreated spinach and soil.

Half-lives and Waiting period

The residue dissipation of the chlorothalonil followed the first order kinetics, which could be expressed in the form, Ct – Coe-kt .Chlorothalonil dissipation pattern on spinach is presented in Table2. The regression analysis, half-lives, and PHI are presented in Table 2. The half -life period ($t_{1/2}$) of chlorothalonil residues in recommended dose of spinach was 1.27 days and the residues dissipated with a half-life of 1.28 days in double the recommended dose respectively. On the basis of the dissipation pattern and Maximum residue limit (MRL) prescribed by European Union, chlorothalonil in spinach (0.01mgkg^{-1}) which is fixed at the level of quantification ,the waiting period recommended for safe harvest of spinach crop treated with chlorothalonil was 19.5 to 20.2days crop respectively. Chlorothalonil residues in spring cabbage have been shown to depend on application schedule, dosage and weather (Zhang *et al*. 2007). Balasubramanian (1994) reported the persistence and dissipation of chlorothalonil in tomato and chillies and residues dissipated at half life value of 4.80 days in tomatoes and 5.57 in chillies depending upon spray concentration and suggested a waiting period 6.28 and 6.55 days for the harvest of tomato and chilli fruits. Agnihotri *et al*. (1995) investigated the persistence of chlorothalonil in potatoes, tomatoes and soil following foliar application of chlorothalonil, and could not detect chlorothalonil residues in soils and potato tubers at harvest and in tomato it persisted upto 20 days and residues were below detectable in soil and tomato after 20 days.

Residues in Soil

Residues of chlorothalonil were not detected in the field soil collected at 20 days after the spray treatment. Degradation of pesticides in soil is dependent on pH, organic matter, microbial community, etc. (Shahgoli and Ahangar 2014).Persistence of pesticides in soils has been defined by Hill and Mc Carty (1967), as the length of the time that a pesticide takes to decompose 75 to 100 per cent of the original compound. Soils are known to have the greatest capacity to degrade pesticides (Hamarkar, 1972).

Conclusions

The method used for analysis of chlorothalonil in spinach and soil gave satisfactory results. The residues remained for15 days in treated spinach at the both doses. The spinach sample and field soil collected at 20th day after spray treatment was free from chlorothalonil residues in both doses. Abiotic dissipation of chlorothalonil has been reported to be accelerated in a soil amended with a high rate of FYM by Katayama et al. (1995). The waiting period recommended for safe harvest of spinach crop treated with chlorothalonil was 19.5 to 20.2days crop respectively.

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