Chemical Science Review and Letters ISSN 2278-6783

Research Article

Persistence of Chlorothalonil in Spinach Cultivated under Open and Protected Conditions

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Abstract

Persistence of chlorothalonil in spinach by following application of commercial formulation of chlorothalonil (Kavach 75% WP)@50 g a.i.ha^{-1} in field and polyhouse with and without bio-fertiliser is reported. Samples of spinach beet drawn periodically after application of chlorothalonil were analysed by GC-ECD. The residues of chlorothalonil in spinach dissipated by almost 80% in 5 days with a half life of 2.03 days in field grown bio-fertiliser treated crop and 2.12days in polyhouse grown biofertiliser treated crop whereas in case of bio-fertiliser untreated crop the half life was 2.40 days and 2.76 days in field and polyhouse respectively. The pesticide persisted little more in biofertilizer untreated crop than in treated crop. Also, the residues persisted longer in polyhouse than in open field. The bio-fertilizer used in this study helped increase yield without harming the environment and also resulted in lower residue levels.

Keywords: Chlorothalonil, bio-fertilizer, spinach, open field, polyhouse, residues, half-life.

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Introduction

Agricultural practices often include the use of pesticides to enhance crop yields. The use of pesticides in agriculture has become necessary and is essential in the modern concept of scientific farming. In recent years however due to increase in consumer demand and increasing pest incidence, some pesticides are used even on low value leafy vegetable crops. Incidence of pesticide residues is more on leafy vegetables as these crops are harvested frequently and consumed fresh and sometimes raw. Also surface area of these crops is often more hence initial deposit of pesticide spray is higher. There is an increasing demand for good quality, safe vegetables in the market. To reduce pesticide residue load on fresh produce pre-harvest interval (PHI) or safe waiting periods have been recommended for vegetables. Also, for every pesticide maximum residue limits (MRLs) are fixed. To achieve these levels a time gap has to be maintained between the last spray of pesticide and the first harvest of crop. This time gap is known as the pre harvest interval (PHI) or waiting period. To calculate PHI, persistence study of pesticides on crops are conducted in the field by following good agricultural practices.

Chlorothalonil, 2,4,5,6-tetrachloro-1,3 benzene dicarbonitrile, is a broad spectrum polychlorinated contact fungicide, first registered in U.S.A. for use on potatoes for the control of early blight and late blight in 1969. Registration for use on seventeen additional economically important vegetable and field crops was accepted in 1971 [1]. The compound is extensively used for control of wide variety of fungal diseases in different crops. It is effective against all four classes of fungi namely Oomycetes, Ascomycetes, Basidiomycetes, Fungi Imperfecti. It controls leaf spots, rust, blights, fruit rot, mildews and scab [2, 3, 4]. Chlorothalonil is used for control of leaf spot diseases in spinach. Fate of chlorothalonil needs to be evaluated in leafy vegetables like spinach so that their safe use can be recommended. This study was therefore carried out to determine safe waiting period for chlorothalonil in spinach and also to explore ways to accelerate residue dissipation in spinach after the pests have been controlled. A microbial consortia developed at IIHR [5] has shown excellent results in increasing vegetative growth of vegetables, thus we

worked on the hypothesis that the same can be used to reduce the residues of pesticides in leafy vegetables by residue 'dilution'. Similarly extent of reduction of residues on spinach by domestic methods e.g. washing and cooking was also determined.

Materials and Methods

Analytical reference material of chlorothalonil (purity 99.3%) was obtained from Sigma Aldrich. All organic solvents were of analytical grade procured locally.

A field experiment was conducted during 2013 at research farm and polyhouse, located at Indian Institute of Horticultural Research, Bangalore with and without bio-ferilizer viz. Arka microbial consortium (AMC) treatment and chlorothalonil application on spinach variety Arka Anupama. The plot size was 5mx5m with randomized block design. The seeds sown germinated after 5-6days and AMC (biofertilizer) treatment was given @ 2g/L as foliar spray. Exactly after one month of the first spray the second spray of AMC was given. After two days of second biofertilizer spray the chlorothalonil @2g/L spray was given using a knapsack sprayer. Control plots were also maintained along with treatment plots. Samples were collected at 0(1h), 1, 3, 5, 7, 10, 15 and 20 days of treatment and analysed for residues of chlorothalonil. Soil samples were collected on last date of sampling (20^{th} day) from 10 cm depth and analysed for residues. Extraction and clean-up of chlorothalonil residues in spinach was carried out as per standardized protocol [6]. A 50g portion of representative spinach sample was homogenized in a Waring blender with 100mL of 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 &concentrated under rotary vacuum evaporator (40^0C). The remaining aqueous extract was transferred into a separatory funnel, 200ml distilled water and 12gm of sodium chloride added and the extract partitioned with hexane (3x50ml).The organic layer was passed through anhydrous sodium sulphate and combined hexane extract was concentrated to near dryness using rotary vacuum evaporator. 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). Soil samples were collected from treatment plot at ten different sites, pooled together, air dried and passed through a 2 mm sieve. A representative 100 g soil sample in triplicate was taken for analysis. Soil samples were processed in similar manner for chlorothalonil residue analysis as in case of spinach samples.

A recovery experiment was conducted by spiking untreated samples of spinach and soil with analytical grade chlorothalonil at fortification levels of 0.01, 0.05 and 0.1 mg/kg. The spiked samples in five replicates were processed as per the analytical method described above to obtain the percent recovery. A gas liquid chromatography (Varian 3800) equipped with Electron Capture Detector (ECD) was used for analysis of chlorothalonil residues. A capillary column, fused silica 30m X 0.53mm i.d. was used. Ultra pure nitrogen was used as carrier gas with a flow rate of 1.0 ml/min.

Results and Discussion

Results of recovery study carried out by spiking spinach and soil with chlorothalonil is presented in **Table 1**. In spinach recovery of chlorothalonil was in the range of 85.50 - 90.70 percent while in soil it ranged from 90.30 - 97.20 percent. The limit of quantification (LOQ) of chlorothalonil in spinach and soil for the above method was 0.01mg/kg. Residues of chlorothalonil were analyzed in spinach over aperiod 20 days. Residue deposit of chlorothalonil after the foliar spray at recommended dose in untreated spinach was 111.98 mg/kg in field and 110.23 mg/kg in polyhouse, whereas in treated spinach it was 116.83 mg/kg in field and 114.73 mg/kg in polyhouse respectively. The level declined quickly and reached below determination level of 0.01 mg/kgon 15^{th} day from treatment in untreated spinach of open field and polyhouse where as in treated crop, the residues reached below determination level on 10^{th}day. The half life period ($t_{1/2}$) of chlorothalonil in untreated spinach was 2.4 days in open field and 2.76 days in polyhouse, whereas in consortia treated spinach it was 2.03days in open field and 2.12days in polyhouse. No residues of chlorothalonil were detected in control spinach and soil.

In the present study it was observed that the residues of chlorothalonil dissipated faster in bio-fertiliser treated crop than the untreated crop. Thus the biofertiliser simultaneously increase the yield without harming the environment while reducing the pesticide residues to some extent. Also residues persisted much longer in polyhouse than in the open field, probably due to the UV component of sunlight which degrades pesticides is reduced by at least 40% in polyhouse.

Table 1 Recovery of chlorothalonil residues on spinach and soil at various spiked levels.

Spiked concentration mg/kg	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, SD= standard deviation

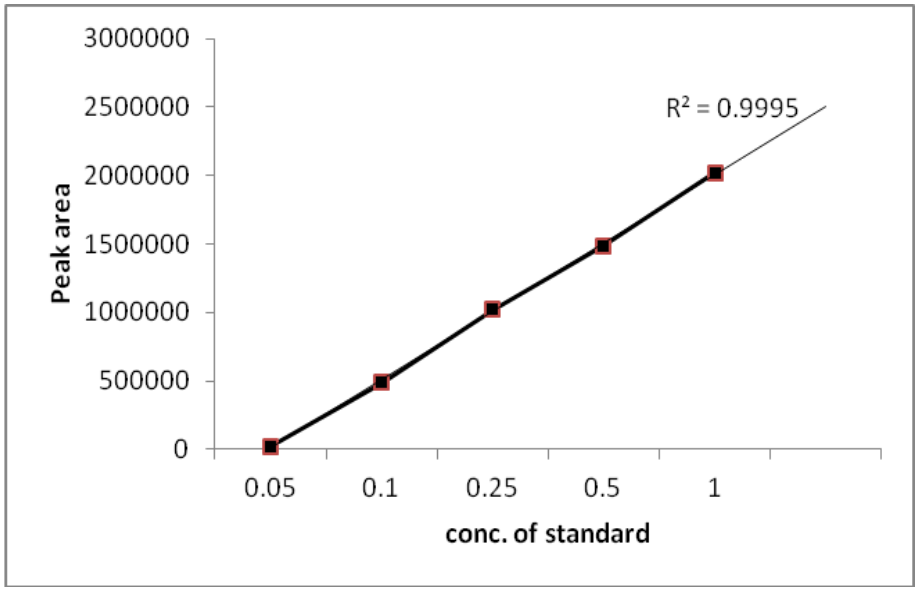

Figure 1 Calibration curve showing response of chlorothalonil.

Table 2 Residues of chlorothalonil in/on spinach.

Days after application	Chlorothalonil residues (ppm)			
	Open field		Polyhouse	
	W/O BF	WBF	W/O BF	WBF
	2.0g/L	2.0g/L	2.0g/L	2.0g/L
0	111.98±1.64	116.84±3.38	110.23± 1.99	114.74±1.50
1	107.57±2.91 (3.94)	95.95±1.55 (17.88)	108.42 ±1.73 (1.64)	96.34±0.68 (16.03)
3	98.86±0.49 (11.72)	60.33±2.02 (48.37)	99.84 ± 0.61 (7.91)	65.51 ± 2.93 (31.95)
5	86.22 ± 0.24 (23.01)	45.27±3.29 (61.25)	89.21 ± 89.22 (10.65)	47.04 ± 2.49 (28.24)
7	40.59±0.99 (63.75)	8.31± 0.03 (92.89)	46.84 ± 0.16 (47.50)	9.22± 0.41 (80.40)
10	4.82±0.28 (95.70)	BDL	6.99 ± 0.28 (85.07)	BDL
15	BDL	BDL	BDL	BDL
20	BDL	BDL	BDL	BDL
Soil samples collected at 20thday after last spray	BDL	BDL	BDL	BDL

W/O BF - without biofertilizer application, WBF - with biofertilizer application, BDL - Below Quantifiable Limit, i.e. less than 0.01ppm, Figures in Parenthesis represent Percent Dissipationof Residues

Acknowledgements

The authors thank the Director, Indian Institute of Horticultural Research, Bengaluru for providing the facilities for carrying out this study.

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Publication History
Received 20th May 2016
Accepted 10th June 2016
Online 05th Oct 2016