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Research article

Dissipation pattern of phosalone on chilli in polyhouse and open fields and decontamination methods for removal of phosalone residues from chilli for food safety

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ABSTRACT

Phosalone is a broad spectrum foliar insecticide widely used on chilli in India for the management of sap sucking insects, thrips and mites. Use of phosalone in poly house and open fields is very common.As per the ICAR recent suggestions phosalone was applied twice @450 g a.i. ha-1, first spray at fruit initiation followed by second spray at 10 days interval as per the farmers practice to compare the dissipation dynamics of pesticide residues in open field and poly house situations so as to suggest PHIs for addressing the food safety issues. Phosalone residues were quantified through regular sampling till the residues fell below determination level (BDL) of 0.25 mg kg-1 following the validated QuEChERS method. The qualitative and quantitative analysis of phosalone was performed on GC-FPD and GC-MSMS (TQD).Initial deposits of 5.35 mg kg^{-1} detected in chilli samples collected from poly house, dissipated to BDL by 15th day with a half-life of 1.66 days. In open fields, deposits of 4.21 mg kg-1 dissipated to BDL by 10th day with half-life of 1.44 days, indicating that dissipation is slow in poly house compared to open fields due to various factors, There are no maximum residue limit for phosalone in chilli as per Codex Alimentarius Commission (CAC) while Food Safety and Standards Authority of India (FSSAI) suggested 1 mg kg-1 for vegetables and hence based on the present study, in poly house and open field PHI of 10 days and 7 days can be recommended as the residues degraded to BDL by 15th day and 10^{th} day. Among various decontamination methods tested, veggywash found to be very effective in removing phosalone residues to an extent of 74.77% which can be recommended as risk mitigation method for food safety, followed by 4% acetic acid solution (67.87%) and tap water wash which was least effective (29.80%) in removing phosalone residues from chilli.

Key Words: chilli, food,

Introduction

Chilli (*Capsicum annum* L.), also called "red pepper," is an important cash crop in India and is used throughout the world as a spice, an important ingredient in day to day curries, pickles and chutnies. It is a rich source of A, C, E and P and an alkaloid capsacin, which has high medicinal value and is used in many pharmaceutical preparations. India is the world's largest producer, consumer and exporter of chilli with an area 794.12 Thousand ha and production of 1304.38 m t, respectively. Andhra Pradesh ranks first both in area (210.02 thousand ha) and production (685.15Thousand m t) (NHB, 2013). In recent years, due to better cost benefit ratio, farmers are growing chillies in controlled atmosphere conditions, majorly in poly house, besides regular open fields during crop seasons. Chilli suffers major quantitative and qualitative loss in production due to chilli thrips, *Scirtothripsdorsalis* Hood and yellow mite, *Polyphagotarsonemuslatus*(Banks) and fruit borers *Helicoverpaarmigera* (Hubner). The overall reduction in fruit yield of chilli due to thrips and mites damage is up to 34% (Thania *et al*., 2011).These pests not only cause reduction in yield, but also act as vectors for several viral diseases and cause complete failure of crop. A number of pesticides are being frequently used, to combat these pests. However, some of these insecticides leave residues on pods and these residues may persist up to harvest. Presence of pesticide residues in the harvested chillies is posing problem at the time of export and in recent times importing countries have rejected few consignments. Pesticide use has increased rapidly over the last two decades at the rate of 12 % per year (Thacker et al., 2005). Many farm gate chilli samples showed presence of insecticide residues (Singh etal.,1999).Phosalone(6-chloro-3-(diethoxy phosphinothioylsulfanylmethyl)-1,3-benzoxazol-2-one) an organo phosphate insecticide and acaricide, is the most commonly used against both sap sucking and chewing insects and mites due to its systemic, contact and acaricidal action. Since chilli is an important component of daily food, food safety issues are very essential.In recent years due to the support of the Government under National Horticulture Mission (NHM), chilli is widely cultivated under poly house conditions. As per Central Insecticides Board and Registration Committee (CIBRC), phosalone is recommended for use on chilli but no MRLs are set. Since the persistence and dissipation of insecticides in poly houses will be different from normal conditions, the present study is proposed to monitor the usage on chilli, dissipation studies of phosalone both in poly house and open field conditions and also evaluation of decontamination methods for removal of phosalone residues, so as to recommend the safe waiting periods based on the Maximum Residue Limits (MRLs) calculated, as it helps in recommending risk mitigation protocols for food safety.

Materials and Methods

Chemicals and Reagents: Certified Reference Materials (CRM) ofPhosalone (96.9% purity) were procured from M/S Sigma Aldrich, Germany, and primary, intermediary and working standards were prepared from the CRMs using GC PR grade acetone and hexane as solvents. Working standards of were prepared in the range of 0.01 ppm to 0.5 ppm in 10 mL calibrated graduated volumetric flask using distilled n-hexane as solvent. Primary Secondary Amine (Agilent), magnesium sulfate anhydrous (Emsure grade of Merck), sodium sulfate anhydrous (Emparta ACS grade of Merck), acetonitrile (HPLC gradient grade of Merck), acetic acid glacial (HPLC grade of Merck), acetone (Emplure grade of Merck), n-hexane (HPLC grade of Merck) were used during the study for sample preparation. Phosalone 50% EC was procured from local market.

Analytical Instruments and Limits of Detection

Working standards were injected in Gas Chromatograph (Agilent 7890 B) with Electron Capture Detector (ECD) and Thermionic Specific Detector (TSD) with injector split ratio of 1:10 using VF-5ms Capillary Column) and confirmatory analysis was done on Bruker Scion 436 GC-MS/MS Triple Quadrupole Detector (EI) using Multiple Reaction Monitoring (MRM) method (Qualifier ions: 339>188, 339>251,

339>269, 139>97; Quantifier Ions: 139>97). It was found that the limit of detection for phosalone is 0.05 ng in GC-TSD with linearity range of 0.05 ng to 5 ng.

Method validation: Prior to field experiments, QuEChERS (Quick Easy Cheap Effective Rugged Safe) method for extraction and clean up was validated as per SANCO/12571/2013 guidelines. Chilli fruits (5 kg) collected from control plots were homogenized with high volume homogenizer (Robot Coupe Blixer 7L) and 15 g was taken in to 50 mL centrifuge tubes. The required quantity of phosalone intermediary standards is added to each 15 g sample to get fortification levels of 0.50 mg kg-1 and 0.25 mg kg-1 in three replications each. 30±0.1 mL acetonitrile was added to the tube, and sample was homogenized for 2-3 min using Heidolph silent crusher (low volume homogeniser). Then 3±0.1g sodium chloride was added to tube and mixed by shaking gently, and centrifuged for 3 min at 2500-3000 xg with Remi R-238 to separate the organic layer. The top organic layer of about 16 mL was taken into the 50 mL centrifuge tube to which 9±0.1 g anhydrous sodium sulphate was added to remove the moisture content. 8 mL of extract was taken in to 15 mL tube containing 0.4±0.01g PSA sorbent (for dispersive solid phase d-SPE cleanup) and 1.2±0.01 g anhydrous magnesium sulphate, and the sample tube was vortexed for 30 sec followed by centrifugation for 5 min at 2500-3000 xg. The extract of (2mL) was transferred into test tubes and evaporated to dryness using concentration work station (Turbovap LV of Caliper life sciences) with nitrogen gas and reconstituted with 1mL n-Hexane: Acetone (9:1) for dimethoate analysis. Chilli samples fortified with phosalone at 0.25 mg kg-1 and 0.5 mg kg-1 were analyzed and the mean recovery of the residues calculated for applying recovery factor while calculating the residues in samples. Fortification and recovery test results are presented in Table 1 and the method followed for qualitative and quantitative estimation of phosalone is suitable up to 0.25 mg kg-1 levels as the recoveries obtained are 90.86% and 98.61 0.25 and 0.50 mg kg-1 fortification level. The residues detected below 0.05 mg kg-1 were mentioned as levels Below Determination Level (BDL) in all cases.

Table 1. Recovery of phosalone residues in chilli

Details	**Recoveries of phosalone from fortified chilli samples**			
	Fortified level (mg kg^{-1})			
	0.25 mg kg^{-1}		0.50 mg kg^{-1}	
	Residues recovered (mg kg^{-1})	Recovery %	Residues recovered (mg kg^{-1})	Recovery %
R1	0.218	87.36	0.526	105.16
R2	0.223	89.39	0.481	96.17
R3	0.240	95.83	0.473	94.51
Mean		90.86		98.61
SD		4.422		5.733
RSD		4.867		5.814

Field experiments and sample collections: Chilli crop (Popular hybrid pusajwala) was raised in both poly house and open field in Randomized Block Design at spacing of 60×45 cm with plot size of 20 m^2 and all Good Agricultural Practices (GAPs) recommended by University were followed. Phosalone35% EC procured from local market was sprayed @ 450 g a.i. ha-1 twice; first spray at fruit initiation stage followed by second spray at

10 days after first spray, using high volume knapsack sprayer with a spray solution of 500 L ha-1. Pest damage free and crack free chilli fruits of 5 kg were collected from each plot in separate polythene bags and brought to laboratory. Samples were collected at regular intervals i.e. 0, 1, 3, 5, 7, 10, 15, 20 days after last spray for dissipation studies. For evaluation of risk mitigation / decontamination methods, zero day samples were collected separately in large quantities and made into 6 sets, each in 4 replications. One set of sample is analyzed for initial deposits of phosalone.

The remaining sets of samples were subjected to various decontamination methods(Table 1A) separately and the residues were calculated to know the efficiency of the various decontamination methods in removal of pesticide residues from the chilli samples. After decontamination treatments, the samples were shade dried for 10 min placing on clean blotting papers and analysed for residues remaining on chilli.

Table-1 A decontamination / risk mitigation methods for removal of phosalone residues from chilli

T_1	Tap water wash	Four litres of tap water was taken into the plastic tub of 7 litres capacity and 2 kg of chilli fruits were dipped in the tub for 10 min, followed by the tap water wash for 30 sec, further the fruits were kept for air drying on tissue paper for 5 min.
T_2	Soaking in 2% salt solution for 10 min followed by tap water wash	Four litres of 2 % salt solution was prepared by mixing 80 g of table salt in 4 litres of water in plastic tub of 7 litres capacity and 2 kg chilli fruits were dipped in the tub for 10 min, followed by the tap water wash for 30 sec, further the fruits were kept for air drying on tissue paper for 5 min, followed by analysis.
T_3	Dipping in 0.1% baking soda ($NaHCo_3$)	Four litres of 0.1% baking soda solution was prepared by mixing 4 g of baking soda in 4 litres of water in plastic tub of 7 litres capacity and 2 kg chilli fruits were dipped in the tub for 10 min, followed by the tap water wash for 30 sec, further the fruits were kept for air drying on tissue paper for 5 min, followed by analysis.
T_4:	Soaking in 4% acetic acid solution for 10 min followed by tap water wash	Four litres of 4% acetic acid soltion was prepared by mixing 160 ml of glacial acetic acid 100% in 4 litres of water in plastic tub of 7 litres capacity, mixture was kept for 1 min and 2 kg of chilli fruits were dipped in the tub for 10 min, followed by the tap water wash for 30 sec, further the fruits were kept for air drying on tissue paper for 5 min, followed by analysis.
T_5	(Veggy wash):	Four litres of veggy wash was prepared by mixing 160 ml of glacial acetic acid 100%, 4 g of baking soda and lemon juice of 4 lemons in 4 litres of water in plastic tub of 7 litres capacity, mixture was kept for 1 min and 2 kg chilli fruits were dipped in the tub for 10 min, followed by the tap water wash for 30 sec, further the fruits were kept for air drying on tissue paper for 5 min, followed by analysis.

Results and discussions

The dissipation kinetics of phosalone on chilli in open field and poly house situation was studied collecting fruit samples at 0, 1, 3, 5, 7, 10, 15 and 20 days after second spray of phosalone @ 450 g a.i. $ha^{-1}$and presented in Tables 2 and Figs.1and 2.

In open field studies, initial deposits of 4.21 mg $kg^{-1}$of phosalone detected at 2 hours after last spray, dissipated to Below Detectable Level (BDL) of 0.25 mg kg^{-1} by 10^{th} day after last spray. The initial deposits dissipated to 2.44, 1.15, 0.81and 0.48 mg kg^{-1} by 1, 3, 5 and 7 days after last spray, respectively. The residues dissipated by 42.04, 72.68, 80.76 and 88.59% at 1, 3, 5 and 7 days, respectively. Phosalone is recommended for use on chilli against fruit borer as per Insecticide Act, 1968 @ 450 g a.i. ha^{-1} and Food Safety and Standards Authority of India (FSSAI) fixed MRL as 1 mg kg^{-1} for vegetables. As per the Act, PHIs are not recommended as Codex, MRLs are not fixed for phosalone on chilli. Based on the present studies, it is found that the half-life of phosalone on chilli is 1.44 days in open field, and hence a safe waiting period (PHI) of 7 days can be recommended as the residues dissipated to LOQ of 0.25 mg kg^{-1} by 7^{th} day.The chilli fruit samples collected from phosalone treated plots @ 450 g a.i. ha^{-1} in poly house recorded an initial deposit of 5.35 mg kg^{-1} at 2 hours after last spray and dissipated to 2.92, 1.96, 1.39, 0.81 and 0.50 mg kg^{-1} by 1, 3, 5, 7and 10 days, respectively, and BDL of 0.25 mg kg^{-1} by 15^{th} day. The residues dissipated by 45.42, 63.36, 74.01, 84.8 and 90.6 % at 1, 3, 5, 7 and 10 days, respectively. There are no maximum residue limit for phosalone in chilli as per Codex Alimentarius Commission (CAC) while Food Safety and Standards Authority of India (FSSAI) suggests 1 mg kg^{-1} for vegetables and hence based on the present study, PHI of 10 days can be recommended as the residues degraded to BDL by 15^{th} day. From Tables 2 and 3, it is evident that there is a clear difference in dissipation pattern of phosalone in poly house and open fields. Initial deposit of 5.35 mg kg^{-1} was recorded in poly house, where as in open fields it was 4.21mg kg^{-1}, which dissipated to BDL of 0.25 mg kg^{-1} by 10^{th} day and 15^{th} day, respectively. Half-life value of 1.44 days were recorded in poly house and 1.66 days in open field trial. In general, the studies conducted by various scientists (Rajukannu*et al*,1979 and Narasimha Rao *et al*,1986) on dissipation of phosalone on tomato showed that the initial deposit of 7.40-7.80 mg kg^{-1} dissipated to BDL by 7-10 days depending up on the stage of application at a dose of 700 g a.i. ha^{-1}, and in the present study, an initial deposit of 4.21 mg kg^{-1} in open field, and 5.35 mg kg^{-1} in poly house from 450 g a.i. ha^{-1} applied plots suggests that the results are in full agreement with the general trend.

The findings of present investigation are in agreement with results of Pareek*et al.* (1990) who reported that initial deposits of phosalone 4.74 mg kg^{-1} dissipated and persisted up to 7^{th} day with half-life values of 2.96 days when applied @ 310 g a.i. ha^{-1} on round gourd. Rajukannu*et al.* (1980) reported that phosalone when applied @ 700 g a.i. ha^{-1} on brinjal reached Below Determination Level (BDL) by 5^{th} day with half-life value of 1.56 and suggested waiting period of 1.55 days. Based on the available literature on dissipation of phosalone on various vegetable crops, it is clear that in most cases, the initial deposits are in the range of 7 to 10 mg kg^{-1} at recommended doses which dissipated to BDL in 7-15 days, depending up on the season and crop. In the present

study on chilli, phosalone dissipation pattern is in agreement with all available literature clearly indicating that phosalone dissipates to BDL in 10-15 days depending up on the crop management practices. In the existing circumstances where no MRLs are available, PHI of 10 days for poly house and 7 days for open field situations can be safety suggested for food safety.

The efficiency of various risk mitigation methods for removal of phosalone residues from chilli is presented in Table 3 and Fig 3. The percentage removal of phosalone residues from chilli when subjected to different decontamination solutions at 2 hours after spraying showed that dipping in veggy wash solution for 10 min was found to be most effective removing 74.77% residues, than other treatments. The next promising treatment was 4% Acetic acid solution (67.87 %), followed by 2% salt solution (65.05 %), 0.1% Baking soda solution (55.75 %) and tap water (29.80 %). Based on the percentage removal of residues, it was statistically proved that there is significant difference in the efficiency of decontaminating solutions in removing residues of above mentioned pesticides. In the present study, veggy wash, a formulation prepared by AINP on Pesticide Residues proved to be the most efficient in removing various pesticides.

Many workers (Radwan*et. al.* 2004b; Jayakrishnan*et. al.* 2005; Zhang *et. al*. 2006; Klinhom, 2008; Liang *et. al.*, 2012) suggested that washing with 1% acetic acid solution, 0.1% NaHCO3 solution removes various pesticide residues in different vegetables, and the extent of removal varies from type of pesticide and vegetables. Bhushan *et al.* (2012) reported that dipping of chilli fruits in 2%salt solution and water removed 78.95 and 77.36% of acephate, 32.56 and 16.28% of triazophos, 90.56 and 28.37% of cypermethrin. Studies conducted by Cherukuri et al (2014) and Shashi et al (2014) revealed that washing of brinjal and chilli with 2% salt solution is effective in removing various pesticides. Mergnat*et al*. (1995) who reported that washing of apples with water removed 30-50 % of phosalone residues.

Based on the results, it can be concluded that phosalone can be removed from chilli for food safety with simple house processing methods, and out of all methods, washing with AINP formulation i.e. veggy wash proved to be the best, and also economical.

Table 2. **Dissipation of phosalone in chilli**

	in poly house						**in open fields**					
Days after last spray	**Residues of phosalone (mg kg^{-1})**					**Dissipation %**	**Residues of phosalone (mg kg^{-1})**					**Dissipation %**
	R1	**R2**	**R3**	**R4**	**Average**		**R1**	**R2**	**R3**	**R4**	**Average**	
0	5.31	5.35	5.38	5.34	5.35	0	3.99	4.14	4.24	4.48	4.21	0
1	2.95	2.96	2.86	2.90	2.92	45.42	2.40	2.40	2.53	2.43	2.44	42.04
3	1.99	1.93	1.98	1.94	1.96	63.36	1.13	1.14	1.16	1.16	1.15	72.68

5	1.32	1.40	1.41	1.44	1.39	74.01	0.71	0.82	0.90	0.80	0.81	80.76
7	0.71	0.90	0.80	0.81	0.81	84.8	0.48	0.49	0.48	0.46	0.48	88.59
10	0.50	0.50	0.50	0.50	0.50	90.6	BDL	BDL	BDL	BDL	BDL	100
15	BDL	BDL	BDL	BDL	BDL	100	BDL	BDL	BDL	BDL	BDL	100
20	BDL	BDL	BDL	BDL	BDL	100	BDL	BDL	BDL	BDL	BDL	100
Regression equation	Y = 3.952 + (-0.415) X						Y = 3.36 + (-0.481) X					
R^2	0.770						0.811					
Half-life	1.66 days						1.44 days					
Safe waiting period : 10 days Codex MRL : NA FSSAI MRL : 2 mg kg-1 for Fruits and Vegetables							Safe waiting period : 7 days Codex MRL : NA FSSAI MRL 2 mg kg-1 for Fruits and Vegetables					
BDL : Below Determination Level (< 0.25 mg kg^{-1}) NA : Not available							BDL : Below Determination Level (< 0.25 mg kg^{-1}) NA : Not available					

Table.3: Removal of phosalone residues from chilli fruits with different decontamination methods

Treatments	Mean of phosalone detected (mg kg^{-1})	Mean percent removal of insecticide **(%) ±SD**
Tap water	2.96	29.80 ± 0.10
2% salt solution	1.47	65.05±1.07
0.1% Baking soda solution	1.86	55.75 ± 0.64
4% Acetic acid solution	1.35	67.87 ± 0.79
Veggy wash	0.91	74.77 ± 6.34

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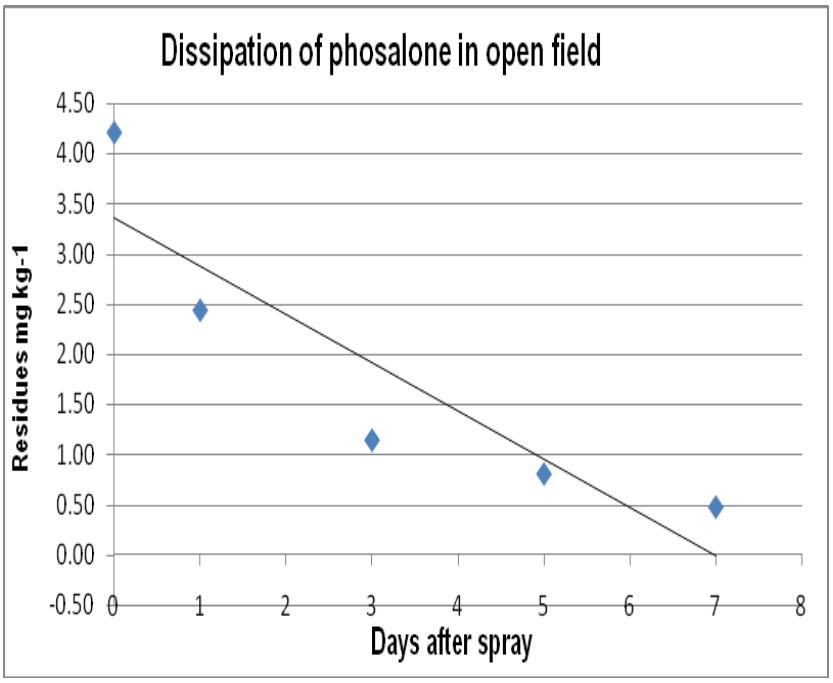

Fig1 Dissipation of phosalone in chilli in open field

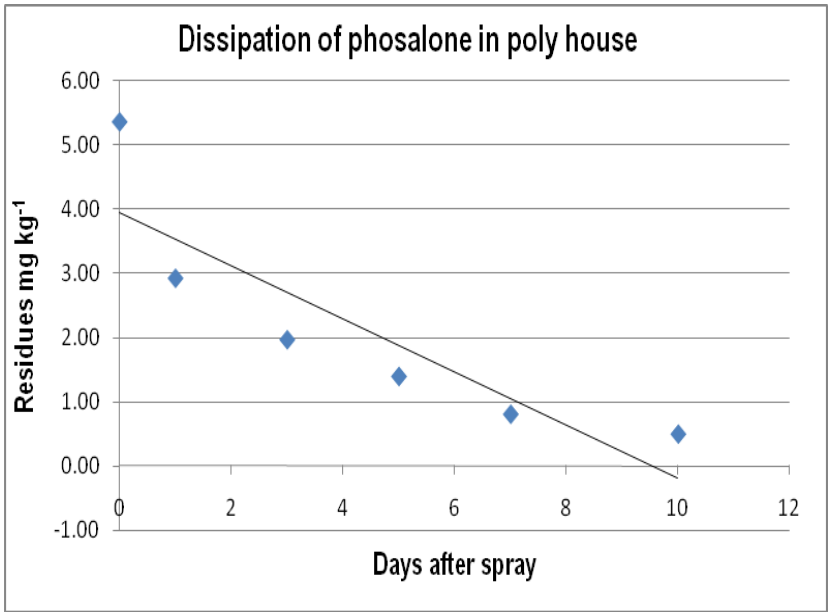

Fig..2.. Dissipation of phosalone in chilli in poly house

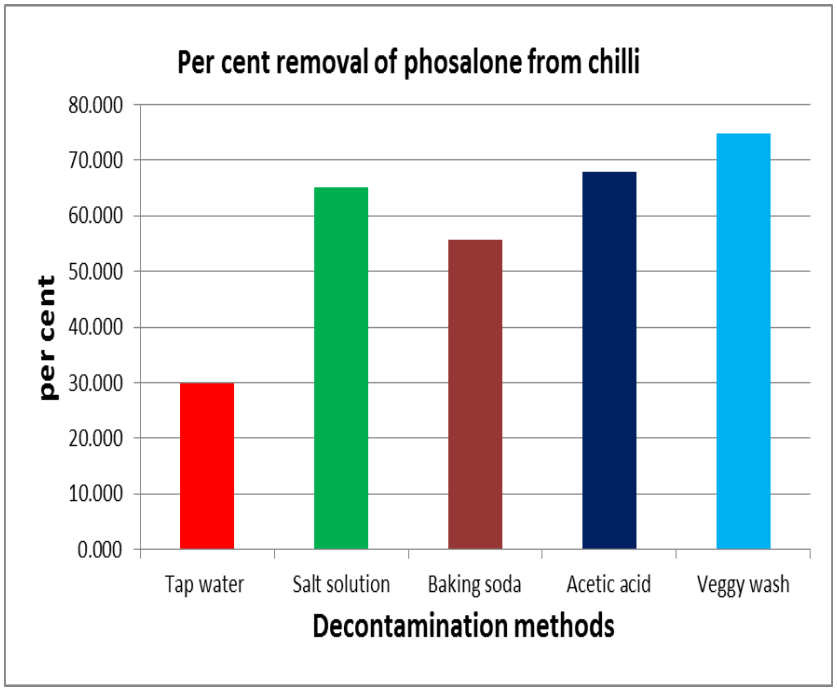

Fig. 3 Per cent removal of phosalone residues from chilli by various decontamination methods