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Residual Behavior of Chlorpyriphos on Cultivated Gherkin and Its Removal Using Various Household Risk Mitigation Methods.

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ABSTRACT

Two sprays of chlorpyriphos 20% EC were applied on cultivated gherkin (*Cucumis melo* L.) at the rate of 200 g a.i. ha^{-1}. The first spray at fruit formation stage followed by second spray at seven days interval, and the samples of gherkins were collected at regular intervals of 0 (2 h after application) 1, 3, 5, 7, 10 and 15 days after second spray. Residues were quantified by using gas chromatography. The initial disappearance of chlorpyriphos appeared to follow first order kinetics with different rates of reactions of 0.42 day^{-1} for gherkin fruits. The corresponding half-lives ($t_{1/2}$) were 1.65 days. To reduce the safe waiting period, efforts were made to decontaminate the residues from gherkin fruits by various household processing methods (viz. Washing, washing plus cooking, 2% salt water washing and peeling). Various household processing substantially reduced the residues from gherkin fruits in the range of 47.49- 73.29%. The maximum reduction (73.29%) was observed by 2% salt water washing, so consumers are suggested to follow dipping of gherkin in 2% salt water, as best risk mitigation method.

Keywords: Chlorpyriphos, Residues, Gherkin, Decontamination, Risk mitigation methods.

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INTRODUCTION

Vegetables are considered the important ingredient of the human diet for the maintenance of the health and prevention of disease in the Indian sub-continent. They are good source of vitamins that are essential for human health [1]. The total Indian meal constitutes about 150- 250g of vegetables per day [2]. A wide range of pesticides are globally used for crop protection during the cultivation of vegetables due to heavy pest infection throughout the season crop and food [3]. Among the vegetables gherkin (*Cucumis melo* L.) is a Cucurbitaceous vegetable crop cultivated in India exclusively for exports [4]. India is a traditional producer of cucumber, its export potential was discovered during the late 1980s, and since then their exports have been increasing progressively. The production of gherkin in India is concentrated in the three south states, viz. Karnataka, Tamil Nadu and Andhra Pradesh. Karnataka accounts for 60 percent of the total gherkin production and Tamil Nadu and Andhra Pradesh account for 20 percent each [5]. Gherkin is a major dietary constituent to many European countries and the USA. Hence, all the volume of gherkin produced in India is exporting [6]. Basing on the importance of the production of gherkin, farmer resort to insecticides application once in a week. Chlorpyriphos (*O, O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphosrus broad spectrum insecticidal active ingredient registered for application on more than 40 different food commodities. It is widely using on cucurbits in Andhra Pradesh. It is a stable compound in neutral and acidic conditions. It is a affective against both sucking and chewing insects. Cholinesterase inhibition is the mode of action of chlorpyriphos and is the cause of potential toxicity in human [7]. Use of pesticides by farmers is the only way to sort out the problem of insects/pests and many a time they harvest the crop without observing any waiting period. As a result, considerable quantities of these pesticides that are absorbed by vegetables, sometimes above the legal limits i.e. maximum residue limit (MRL). This is clear, as the National Monitoring data shows that the presence of residues in vegetables frequently, and on some occasions above MRL and finally they are reaching in to the human body and results in many health hazards. There are numerous studies in the literature that have examined chlorpyriphos behaviour in field vegetables [8,9]. As well as to find more efficient washing reagents for removing its residues from gherkins to reduce the health hazards [10]. However, there is a lack of published data in India for the fate of these insecticides on field grown gherkin fruits and in the processed products. Therefore the present investigation was carried out with the objectives to examine the persistence behaviour of chlorpyriphos and effect of different household mitigations on reduction of its residues in gherkin fruits.

MATERIALS AND METHODS

Insecticide and chemical reagents

Chlorpyriphos; (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), 250 mg, (Batch / lot number; SZBA 141 XV) 99.9%, purity technical grade standard were procured from sigma Aldrich, Germany, which was used for GC standardization and fortification and recovery studies in the present study. Formulated products were purchased from local vender and were employed in the field experiment. Chemicals and sorbents (Acetonitrile, hexane, primary secondary amine (PSA), sodium sulphate, magnesium sulphate, sodium chloride) used were obtained from E. Merck and Agilent companies.

Field experiment and sampling

A field experiment was conducted on gherkin crop (AJAX-F1 Hybrid) at the student's farm college of Agriculture, Acharya N.G. Ranga Agricultural University, Rajendranagar Hyderabad. During the period December, 2012 to March, 2013. Field trial was laid out in the randomized block design (RBD) and replicated three times. Plot size 3.35 x 3.35 with net size 3.00x3.00 spacing between different plots was 30 x 60 cm. Number of plants per row was 9 and rows /bed were 5.So, the total plants per bed were 9x5=45. Chlorpyriphos (Dursban 20% EC) was applied on gherkin crop at 200 g a i/ha along with untreated control at fruiting stage. Each treatment including control was replicated thrice. The formulations was diluted with water and sprayed on gherkin crop whereas control plots were sprayed with water only. Gherkin fruit samples were collected at regular intervals, 0 (2 h after spray), 1, 3, 5, 7, 10, and 15 days after second treatment in three replicates. Two Kg's of samples from each plot were collected in polythene bags and brought immediately to the laboratory then samples were made in to two portions, one portion was processed for dissipation studies and another one is processed with different house hold processing methods for the removal of chlorpyriphos residues.

Climatic conditions

The weather parameters for the season (December, 2012- March, 2013) were temperature, minimum 24.07 ^{0}C, maximum 42.47 ^{0}C; relative humidity 75%; rain fall 21.72 mm; at the experimental site.

Household risk mitigation methods

The gherkin samples collected from pesticide sprayed plots were divided in to five portions, one portion was processed as such second after plain water washing, third portion with 2% salt water washing, fourth after peeling and fifth one after washing followed by boiling/ cooking. In treatment one, each replicated sample (100g) was washed under running tap water for 2 min. In the second treatment, the gherkins (100g) were washed thoroughly under tap water for 2 min followed by boiling in 500 ml water for 5 min, and the water was discarded. The next treatment, gherkins were dipped in 500 ml 2% salt solution at room temperature, (28 ± 1^{0}C) for 5 min and washed under tap water for 2 min. In the fourth treatment, the gherkins outer layer was peeled with peeler. The field samples analyzed without any house hold techniques are designed as unprocessed control.

Extraction and clean-up

Extraction and clean- up procedure was performed as per AOAC Official Method 2007.01 QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) with little modifications. Representative 2 kgs of gherkin fruits were collected randomly from chlorpyriphos sprayed plots in polythene bags. The samples were homogenized with robot coupe blixer. Homogenized 15±0.1g sample was taken in 50ml centrifuge tube, and 30±0.1 ml acetonitrile was added. The sample was homogenized at 14000-15000 rpm for 2-3 min using heidolph silent crusher. The samples was then added with 3±0.1g sodium chloride and mixed by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube and added with 9±0.1g anhydrous sodium sulphate 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±0.01g anhydrous magnesium sulphate. The sample tube was vortexed for 30sec then followed by centrifugation for 5min at 2500-3000 rpm. The extract of about 2 ml was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1ml n-Hexane: Acetone (9:1) for GC analysis with ECD detector under standard operational conditions.

Residue determination

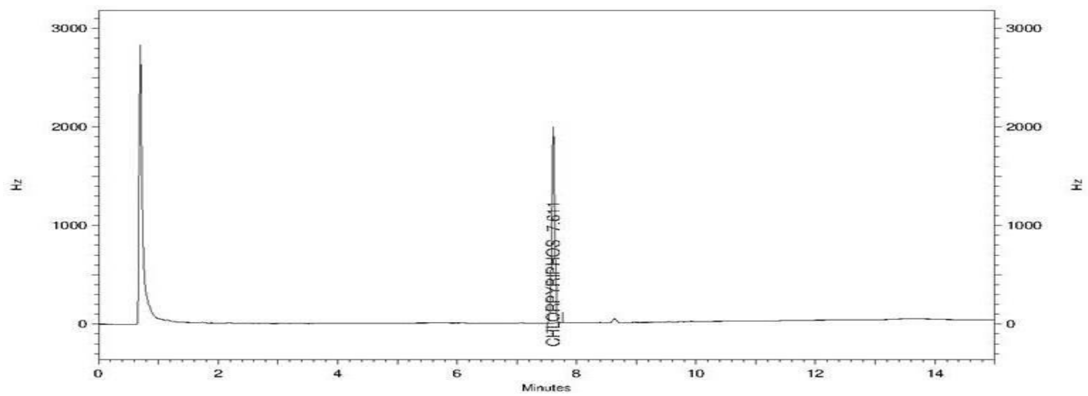

Figure 1: Chromatogram of chlorpyriphos 0.5ppm standard

Determination of chlorpyriphos residues in gherkin fruits was analyzed on Shimadzu – 2010 GC (gas chromatography) (Serial Number: C11324405589 SA) equipped with capillary column, MR-1 (30mts, 0.25 mm ID, 0.25 µm Film thickness of diphenyl/ 95% dimethyl polysiloxane) and electron capture detector (ECD). The operating parameters of the instrument were: column oven temperatures 150 ^{o}C-2 min hold-250 ^{o}C-@10 ^{o}C/min-23 min; TOTAL 35 min, injection port temperature 280 ^{0}C and detector temperature was 300^{0}C. Flow rate of nitrogen (carrier gas) was 1ml/min through column; makeup gas flow is 35ml/min and split ratio is 1: 10. Under these operating conditions the retention times of chlorpyriphos were found to be 7.61 minutes (Fig 1).

Recovery studies

In order to estimate the efficiency of the method, a recovery experiment was conducted by fortifying the untreated gherkin samples with analytical grade chlorpyriphos at the rate of 0.05, 0.25 and 0.5 mg kg^{-1}, and processed by following the methodology as described above. The result revealed that percent mean ± SD recoveries for gherkin samples at these three levels were 83.50±3.04, 89.16±0.76 and 91.00±4.76 for chlorpyriphos (Table 1). The results have been reported as such without applying any correction factor. The minimum limit of detection (LOD) was 0.01mg kg^{-1} and limit of quantification (LOQ) for chlorpyriphos in gherkin fruits were found to be 0.05 mg kg^{-1}.

Table 1: Recoveries of chlorpyriphos at various fortification levels in gherkin fruits

Pesticide	0.05mg/kg	0.25mg/kg	0.5mg/kg
	*Recovery%	*Recovery%	*Recovery%
Chlorpyriphos	83.50	89.16	91.00
Standard deviation	±3.04	±0.76	± 4.76

*Average of three replicates

RESULT AND DISCUSSION

The residual data in Table 2 showed that the initial deposits of chlorpyriphos in/ on gherkin fruits were 4.38 ppm. A rapid degradation was noticed chlorpyriphos after one day of application with values of 22.83% reduction was shown in chlorpyriphos. The progression of time after the application resulted more dissipation of residues. The first five days was critical, showing the highest rate of dissipation of chlorpyriphos from gherkin fruits, being 62.78 % respectively. On the seventh day of experiment gherkin fruits collected from where as chlorpyriphos from gherkin fruits were shown the reduction of 97.71% on 10th day, it was shown the 100% dissipation on 15th day after spraying (Fig 2). The initial disappearance of chlorpyriphos appeared to follow first order kinetics with different rates of reactions of 0.42 day^{-1} for gherkin fruits, respectively. The corresponding half- lives ($t_{1/2}$) were 1.65 chlorpyriphos in on gherkin fruits (Table 3).

Table 2: Residue data of chlorpyriphos in gherkin at regular intervals

Days after Treatment	Chlorpyriphos (200 g a.i. ha^{-1})	
	*Mean±SD	Reduction %
0 (2.h)**	4.38±0.20	-
1	3.35±0.46	22.83
3	2.40±0.34	44.06
5	1.63±0.23	62.78
7	0.26±0.11	94.06
10	0.10±0.01	97.71
15	BDL	~100
Regression equation	Y= 3.765 – 0.17 x	

BDL= below detectable level.
* Values given are mean of three replications.
** Initial deposits of the insecticide.

Table 3: Calculated half-life values of chlorpyriphos on gherkin fruits

Pesticide	Apparent rate constant (k)	Half- life time ($t_{1/2}$) in days
Chlorpyriphos	0.42	1.65

k = ½ ln (a/y) where
k = dissipation rate constant, $days^{-1}$
a= initial amount of pesticide
y= amount of pesticide at time t, and t= time in days
$t_{1/2}$ = ln (2)/ k = 0.6931/k

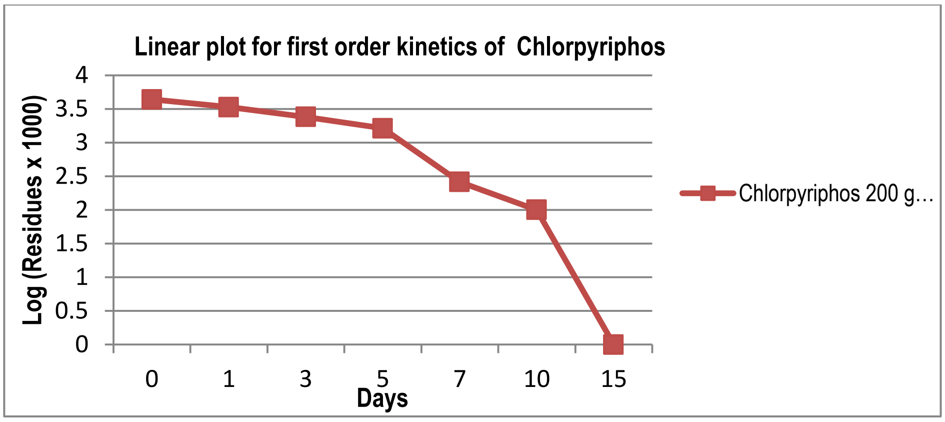

Figure 2: Dissipation of chlorpyriphos in gherkin fruits

The residual data could be concluded while in the case of chlorpyriphos 0.10 ppm residues were detected on gherkin fruits, 10 days after application. This indicated that only 10 and 15 days were long enough to reduce the residues of chlorpyriphos below the determine levels (0.05 ppm) on gherkin fruits. The codex maximum residue limits (MRLs) whereas chlorpyriphos the maximum residue limits are ranged from 0.01 to 2 mg/ kg on different vegetable commodities. However, the list lacks the MRLs for chlorpyriphos on gherkin fruits. Therefore, gherkin fruits could be marketed with apparent safety for human consumption.

When the pre-harvest intervals between treatments and harvest are not respected by the farmers, the risk of having higher pesticide levels is not negligible. In this case, the higher levels of pesticides can involve considerable economic losses if the maximum residue limits established by FAO/WHO are surpassed. So, the effect of washing by different solutions or using some household processing in removing the pesticide residues from plants may be a solution to overcome this problem.

Effect of various household risk mitigation methods

Chlorpyriphos initial residues and its removal percent as affected with different washing solutions and processing treatments on gherkin fruits were shown in Table 4. The residues of chlorpyriphos on raw unprocessed gherkin fruits after application were 4.38 mg/ kg. The washing of treated fruits under running tap water reduced these residue levels to 2.30 mg/ kg with corresponding percent removal of 47.49. The result obtained by different others [2,11,12] indicated that washing chlorpyriphos treated vegetables fruits with tap water removed a considerable amounts of residues. Washing plus cooking, it further increased to a 1.34 ppm (69.40% reduction) and 0.71 ppm (83.05% reduction) of chlorpyriphos. Washing plus cooking is seems to be effective when compare with tap water washing. While gherkin fruits were washed with 2% salt water the initial residues found on unwashed fruit to 1.17ppm (73.29%) for chlorpyriphos. When the outer layer of gherkin was peeled with peeler the reduction levels are 2.10 ppm (52.05% reduction) chlorpyriphos in/on gherkin fruit. This was followed by tap water washing. These data indicated that the tested different washing solutions or home processing procedures had varied effects on reducing or removing of chlorpyriphos residues from the tested gherkin fruits depending upon the type of mitigation methods (Fig 3). The removal studies are reported in the literature by different authors [8,11,13,14]. They found that washing with water and/ or other solutions as well as the cooking process resulted in a great reduction of pesticide residues from treated vegetable fruits and lead to the residue level lower than the maximum residue limits (MRLs).

Table 4: Effect of house hold risk mitigation methods on reduction of chlorpyriphos residues in gherkin fruits

Treatment	Chlorpyriphos (200 g a.i. ha^{-1})	
	*Mean±SD (mg kg^{-1})	Reduction %
Control	4.38±0.20	-
Tap water washing	2.30±0.01	47.49
Washing + cooking	1.34±0.01	69.40
2% salt water	1.17±0.00	73.29
Peeling	2.10±0.02	52.05

*Values given are mean of three replications.

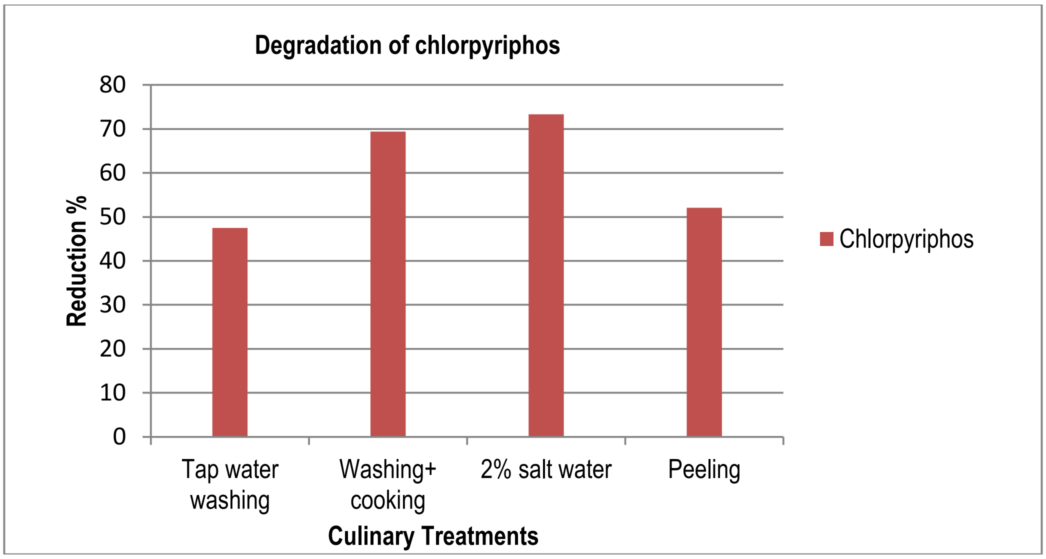

Figure 3: Degradation of chlorpyriphos in gherkin fruits under different household risk mitigation methods.

CONCLUSION

The present study provides residue data which may be useful for establishing the MRL and pre harvesting intervals for chlorpyriphos residues in/on gherkin fruits under Indian field conditions and suggests the need of implementation of these safety intervals before harvesting and marketing such vegetable fruits. Moreover, a comparison of the overall effect of different household mitigation methods indicated that levels of chlorpyriphos residues can be reduced significantly by washing plus cooking and 2% salt water washing. The reduction in residue levels makes these procedures worthwhile for adopting by the consumer. The effectiveness of different risk mitigation methods was observed in the order of tap water washing is less effective than peeling whereas washing plus cooking and 2% salt water washing have shown the equal effect in the removal of chlorpyriphos residues from gherkin fruits. Hence, to reduce the risk associated with these residues while consuming the raw gherkin, washing with 2% salt water is the best risk mitigation method before consumption.

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