



Journal of Biological Sciences

ISSN 1727-3048

science
alert

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Monitoring the Residue Level of Three Selected Pesticides in Red Amaranth

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Abstract: A study was undertaken in the Department of Biochemistry, Bangladesh Agricultural University, Mymensingh to monitor the level of three selected pesticides at various dose levels. In this case edible part of red amaranth was extracted and analyzed for applied pesticide residues. It was found that after one day 0.0065 μg of cypermethrin retained per g of plant sample, which was 0.23% of the applied dose. While on the 3rd day of spray the level of residue was found to decrease (0.0024 $\mu\text{g g}^{-1}$) which accounted for 0.085% of the applied dose. In case of higher dose applied, the residue level of chlorpyrifos was 0.0173 $\mu\text{g g}^{-1}$, whereas the level increased to 0.0237 $\mu\text{g g}^{-1}$ on 3rd day of application. It seemed that the uptake of chlorpyrifos by red amaranth from soil and its accumulation therein was higher on 3rd day of application. The residue level of carbofuran was very low both at 1st and 3rd day of application. However, an increasing trend of incorporation was observed on 3rd day of application. It is remarkable to note that when higher level of chlorpyrifos and carbofuran were jointly applied, the amount of chlorpyrifos as residue increased but the level of carbofuran decreased. Finally carbofuran could not be traced after 72 h of joint application. So study indicated that chlorpyrifos might exert antagonistic effect on the uptake/accumulation of carbofuran in plant system.

Key words: Red amaranth, cypermethrin, chlorpyrifos, carbofuran

INTRODUCTION

Red amaranth (*Amaranthus tricolor* L.) is one of the most important leafy vegetables in Bangladesh. It is tasty and nutritious. It can be grown throughout the year and can be harvested in a very short time. For vegetables and seed production, red amaranth is best grown in winter. It has been growing in Bangladesh both in winter and summer seasons^[1].

The chief nutritive value of red amaranth lies in their content of β -carotene (precursor of vitamin A) and vitamin C. It contains carotene (11.94 mg), vitamin C (43.0 mg), calcium (374.0 mg), carbohydrate (5.0 g), protein (5.3 g), fat (0.1 g) and calories (43.0 K Cal) 100 g^{-1} of edible portion^[2].

Amaranthus spinosus or *Amaranthus viridis* is thorny, pigweed and the leaves of which possess mucilaginous properties. The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes with good results. The leaves make a good emollient preparation available in some of the philipino villages for insect bites, sunburn and regular burns. In India the roots are used as a decoction for treating eczema and cobra bites and scorpion stings^[3,4].

Vegetables are a good source of vitamins and fibres and are beneficial for health but on the other hand, the publicity regarding the excess use of pesticides in agriculture has created a certain apprehension and fear in the public as to the presence of pesticide residues in their daily food. The public is confused and alarmed about their food safety. Moreover the production of vegetables is being crippled in Bangladesh because of pest attack.

Farmers of Bangladesh are not conscious to that extent about the essence of rules and regulations of pesticide use as outlined in the pesticide ordinance of the country. While producing vegetables, fruits and food grains they use pesticides and bring those to the market without considering duration and the effect of pesticides they used. The consumers buy those products that may contain a large amount of pesticide as residue and consume these without taking care of its removal.

Consumption of vegetables with excess in amount of pesticides beyond acceptable daily intake (ADI) limit may cause health hazard and may create problem to the environment including pollution of air, water and soil.

However there is very little information available on the monitoring of pesticide residues in vegetables and fruits that are grown by the farmers in Bangladesh and

sold in the market. Considering their potentialities, a piece of work was undertaken with the objectives to measure the residue level of the applied pesticide in the edible portion of red amaranth so as to make apprehension free consumable product.

MATERIALS AND METHODS

The experiment was conducted by growing the plants in pot in roof condition at the experimental site of Karim Bhaban 2nd floor roof, BAU, Mymensingh during the period from April to July, 2002. The analysis of pesticide residues were carried out at the Agrochemical Residue Research and Analysis Laboratory, Institute of Food and Radiation Biology (IFRB) Bangladesh Atomic Energy Research Establishment (BAERE), Savar, Dhaka.

Pesticides were collected from the local market of Mymensingh. The experiment was conducted at the site of Department of Biochemistry. Geographically the experimental area is located at 24°75' N latitude and 90°50' E longitude having an altitude of 18 m.

The soil was collected from Karim Bhaban area, BAU, Mymensingh and air dried for several days. The soil samples were collected from 15-30 cm depth of the experimental field before first opening of the soil. The soil was silty loam type.

The experimental area is situated under sub tropical climate, characterised by three distinct seasons the monsoon the rainy season (May-October), the winter or dry season (November-February) and the pre monsoon or hot season (March-April). Red Amaranth is a short growing (2 Months) vegetables crop. It can be grown in all the three seasons in Bangladesh. The winter climate condition of Bangladesh is more favourable for red amaranth cultivation.

The experiment was laid out in a Randomized Complete Block Design with four replications.

The crop used in this study was red amaranth. The seeds were collected from Mymensingh Nuton Bazar, Mymensingh. The seeds were healthy, vigorous, plummy, well matured and free from mixture of other crop seeds and extraneous materials.

Medium size pots (0.04 m³) were used in this experiment. Each pot was filled with 10 kg of the sun-dried soil. Plant propagates, inert materials, visible insects and pests were removed from the soil. The sundry soil was thoroughly mixed with well decomposed compost @ 2.79 g pot⁻¹.

There were twelve different treatments of pesticide to reduce hairy caterpillar infestation in red amaranth which were evaluated against control having no pesticide treatment. The treatment included in the study were as follows:

- T₁ = Application of cypermethrin (trade name: basathrin 10 EC) @ 1 ml L⁻¹ of water at 21 days after emergence (DAE) (2.8 µg g⁻¹ each pot)
- T₂ = Application of cypermethrin @ 1.5 ml L⁻¹ of water at 21 (4.2 µg g⁻¹ each pot)
- T₃ = Application of chlorpyrifos @ 0.5 kg a.i ha⁻¹ at 21 (2.4 µg g⁻¹ each pot)
- T₄ = Application of chlorpyrifos @ 0.66 kg a.i ha⁻¹ at 21 (3.23 µg g⁻¹ each pot)
- T₅ = Application of carbofuran @ 1.5 kg a.i ha⁻¹ at 21 (7.35 µg g⁻¹ each pot)
- T₆ = Application of carbofuran @ 2.0 kg a.i ha⁻¹ at 21 (9.8 µg g⁻¹ each pot)
- T₇ = Application of cypermethrin @ 1 ml L⁻¹ of water at 21 (2.8 µg g⁻¹ each pot) and chlorpyrifos @ 0.5 kg a.i ha⁻¹ at 21 (2.4 µg g⁻¹ each pot)
- T₈ = Application of cypermethrin @ 1.5 ml L⁻¹ of water at 21 (4.2 µg g⁻¹ each pot) and chlorpyrifos @ 0.66 kg a.i ha⁻¹ at 21 (DAE) (3.23 µg g⁻¹ each pot)
- T₉ = Application of cypermethrin @ 1 ml L⁻¹ of water at 21 (2.8 µg g⁻¹ each pot) and carbofuran @ 1.5 kg a.i ha⁻¹ at 21 (7.35 µg g⁻¹ each pot)
- T₁₀ = Application of cypermethrin @ 1.5 ml L⁻¹ of water at 21 (4.2 µg g⁻¹ each pot) and carbofuran @ 2.0 kg a.i ha⁻¹ at 21 (9.8 µg g⁻¹ each pot)
- T₁₁ = Application of chlorpyrifos @ 0.5 kg a.i ha⁻¹ at 21 (2.8 µg g⁻¹ each pot) and carbofuran @ 1.5 kg a.i ha⁻¹ at 21 (7.3 µg g⁻¹ each pot)
- T₁₂ = Application of chlorpyrifos @ 0.66 kg a.i ha⁻¹ at 21 (3.23 µg g⁻¹ each pot) and carbofuran @ 2.0 kg a.i ha⁻¹ at 21 (9.8 µg g⁻¹ each pot)
- T₁₃ = Control (without pesticide)

Application of insecticides: Cypermethrin was applied by mixing with water. The mixture within the spray tank was shaken well and sprayed covering the whole plant. Chlorpyrifos and carbofuran were directly applied by mixing with the soil surrounding the plant.

Determination of concentration of pesticides residues in red amaranth: Residues of pesticide was determined by the method of DFG Manual of Pesticide Residue Analysis^[9].

Sample extraction: Fifty grams of vegetables were chopped in small pieces and homogenized with acetonitrile in a waring blender. Hundred ml of acetonitrile was added and blended for 2-3 min at moderate to high speed. The homogenate was filtered by passing through glass wool and transferred the filtrate to a jar and extracted with an additional 100 ml acetonitrile. Filtered as before and combined the filtrates. An aliquot of one half of it was taken (equivalent to 25 g sample) into 1 L

separatory funnel. Hundred ml n-hexane was added and shaken vigorously for 1-min by venting pressure. Two percent aqueous NaCl solution (300 ml) was added and mixed thoroughly by vigorous tumbling for 15 sec and allowed the two layers to separate. Aqueous and hexane layer was collected separately. Then the aqueous layer was transferred into separatory funnel and 50 ml n-hexane was added and shaken, as previously done. Subsequently the aqueous phase was extracted with another portion of 50 ml n-hexane. Extraction was done three times with 50 ml of n-hexane each time.

Clean-up procedure: To avoid the co-extractives from the extract, the silica gel column chromatography was applied. Following procedures were taken out for silica gel column chromatography:

Silica gel preparation: Silica gel, 60-100 mesh (magnesium silicate) was heated for 6 h at 250°C, then stored in a tightly stoppered desiccator and allowed to cool. The freshly activated silica gel was then partly deactivated with drop-wise addition of distilled water (2% V/W, water/silica gel) at 40°C, with constant stirring for half an hour. Silica gel was partly deactivated to increase the polarity of the absorbent; which is very essential for perfect clean-up.

Cleaning of adsorbents: Silica gel have to be solvent extracted. Each reagent was first reflexed with methanol or dichloromethane in a Soxhlet apparatus for 8 h, then with n-hexane for the same period. The solvent was removed by a rotary evaporator operating at low speed, until the sorbent starts falling down as fine particles. Reagents were dried in a drying oven at 0.01 m bar. Silica gel was dried in a normal oven at 120°C for 4 h. This served to activate silica. The sorbent was allowed to cool in the oven (under vacuum to avoid uptake of contaminants from the atmosphere). Deactivation was done by adding water to the fully active sorbent (5% by weight to silica).

Column preparation: A fresh chromatographic glass column (accurately washed and rinsed) was taken and packed with glass wool at the end and then was set with a stand. The column was rinsed twice with acetone and once with n-hexane (double distilled). About two-third of the column was filled with double distilled hexane and then 5 g of deactivated silica gel was slowly poured down into the column with the flow of solvent and the column was packed by gently tapping the tube with a non-contaminated glass rod to avoid the formation of bubble in the column. The silica gel of the column was covered with an approximate 0.1 cm layer of anhydrous

sodium sulfate (BDH) to remove the water (if any) from the extracts.

Elution procedure: The evaporated extract was rinsed with a small portion of eluting mixture and was allowed to percolate. The rinsed content was again applied to the column and was allowed to percolate. The column was eluted with 100 ml hexane (double distilled) and diethyl ether (Hexane: Diethyl ether, 98:2) at a flow rate of 1-2 drops/sec (5 ml min⁻¹). The eluted extract was collected in a stoppered flask and evaporated by a Rotary Vacuum Evaporator under mild pressure. Finally the concentrate was completely evaporated by flow of nitrogen.

Determination of pesticide by high performance liquid chromatography (HPLC): The HPLC (Model Water 486) was used for carbamate, organophosphorus and synthetic pyrethroide detection. High Performance Liquid Chromatography (HPLC) fitted with C18 column was used for all the samples analysis. Acetonitrile: Water (65:35) was used as mobile phase. Flow rate maintained at 1 ml min⁻¹ at 3000 PSI UV-detector was used at 254 nm wave length {Chart speed (CS) 0.5; sensitivity (Si) 0.1; attenuation of the integrator (AT) 4}.

Identification and quantification procedure: Tentative identification of the suspected insecticide was carried out in relation to the retention times (RT) of the pure analytical standard of that insecticide.

Quantification of the identified pesticide was performed by the calibration technique. For this purpose, calibration curve for each pesticide was prepared by injecting equal volumes of different concentrated standard solutions into HPLC. The calibration curve was drawn by plotting the obtained peak area that was read from the calibration curve.

RESULTS AND DISCUSSION

Cypermethrin residue in red amaranth: On the 21 day of emergence cypermethrin was first applied at the rate of 2.8 µg g⁻¹ of leafy part of the plant. This was considered as recommended dose. After 1 day i.e. 24 h plant sample was collected and analyzed. It appears from the Table 1 0.0065 µg of cypermethrin retained per gram of edible part of red amaranth which accounted for only 0.232% of the applied dose. While on 3rd day of spray the plant sample was collected and analysed. The level of residue was found to decrease 0.0024 µg g⁻¹, which accounted for 0.085% of the applied dose. It is quite evident from the Table 1 that % incorporation of cypermethrin was 0.74

fold less on 3rd day of spray when compared to 1st day of spray. In the T₂ treatment 4.2 µg of cypermethrin was applied per gram of sample which was considered as higher dose. In this case residue level on the 1st day of spray was 0.0111 µg g⁻¹ of edible leaf of red amaranth. The percent incorporation in this case was 0.264. However, on the 3rd day of spray the residue level was reduced to 0.0029 µg g⁻¹ of sample and the percent incorporation was quite less (0.069%) on 3rd day of the spray. It is to be noted that the level of cypermethrin decreased with the increases of days. This might be due to volatile nature of this pesticide. It is perhaps due to rapid degradation and decomposition^[6] of pesticide.

Chlorpyrifos residue in red amaranth: In another set of experiment 2.4 µg of chlorpyrifos per gram of soil sample was applied. On 1st day the residue level was 0.0173 µg g⁻¹ of leafy sample (Table 2). Whereas the level increased to 0.0237 µg g⁻¹ of sample which further revealed that % incorporation of this pesticide was high (0.987) on 3rd day of application. On the other hand the percent incorporation was less (0.720) on 1st day of application. However, from the result it appears that uptake of chlorpyrifos by red amaranth from soil and its

accumulation in the plant was higher on 3rd day of application. It was probably due to slow hydrolysis of the compound in the soil^[7].

Carbofuran residue in red amaranth: In this experiment 2 levels of carbofuran were employed: one is recommend dose and the other one is higher dose. Both the levels were used on the 21 days of emergence. The sample were collected for both the levels after one day (24 h) and on the 23rd day i.e. 72 h of application. The recommended dose of 7.35 µg g⁻¹ and higher dose of 9.8 µg g⁻¹ of soil sample were used. The residue level of carbofuran was very low both at 1st and 3rd day of application (Table 3). However % incorporation was little higher (0.0021) on 3rd day of application compared to 1st day of application (0.0010).

An increasing trend of incorporation (0.0042%) was observed on 3rd days of higher dose application. In general carbofuran is rapidly absorbed into the roots of the plants and distributed to stems and leaves where it is slowly metabolized to non-toxic substances^[7]. However it was revealed from the results that % incorporation of chlorpyrifos residue was higher than the carbofuran in the plants of red amaranth (Table 2 and 3).

Table 1: Cypermethrin residue in edible portion of red amaranth

Time of cypermethrin application (day)	Treatments	Applied dose (µg g ⁻¹)	Residues in edible leaf µg g ⁻¹	% Incorporation
1st day	T ₁	2.8	0.0065	0.232
3rd day	T ₁	2.8	0.0024	0.085
1st day	T ₂	4.2	0.0111	0.264
3rd day	T ₂	4.2	0.0029	0.069

Treatments of cypermethrin after 21 days of emergence (DAE)

- 1st day = T₁ = Cypermethrin @ 1 ml L⁻¹ of water and samples collected after 24 h
- 3rd day = T₁ = Cypermethrin @ 1 ml L⁻¹ of water and samples collected after 72 h
- 1st day = T₂ = Cypermethrin @ 1.5 ml L⁻¹ of water and samples collected after 24 h
- 3rd day = T₂ = Cypermethrin @ 1.5 ml L⁻¹ of water and samples collected after 72 h

Table 2: Chlorpyrifos residue in edible portion of red amaranth

Time of chlorpyrifos application (day)	Treatments	Applied dose (µg g ⁻¹)	Residues in edible leaf (µg g ⁻¹)	% Incorporation
1st day	T ₃	2.40	0.0173	0.720
3rd day	T ₃	2.40	0.0237	0.987
1st day	T ₄	3.23	0.0241	0.746
3rd day	T ₄	3.23	0.0321	0.993

Treatment of chlorpyrifos after 21 days of emergence (DAE)

- 1st day = T₃ = Chlorpyrifos @ 0.5 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₃ = Chlorpyrifos @ 0.5 kg a.i ha⁻¹ and samples collected after 72 h
- 1st day = T₄ = Chlorpyrifos @ 0.66 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₄ = Chlorpyrifos @ 0.66 kg a.i ha⁻¹ and samples collected after 72 h

Table 3: Carbofuran residue in edible portion of red amaranth

Time of carbofuran application (day)	Treatments	Applied dose (µg g ⁻¹)	Residue in edible leaf (µg g ⁻¹)	% Incorporation
1st day	T ₅	7.35	0.00008	0.0010
3rd day	T ₅	7.35	0.00016	0.0021
1st day	T ₆	9.80	0.00034	0.0043
3rd day	T ₆	9.80	0.00042	0.0042

Treatment of carbofuran after 21 days of emergence (DAE)

- 1st day = T₅ = Carbofuran @ 1.5 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₅ = Carbofuran @ 1.5 kg a.i ha⁻¹ and samples collected after 72 h
- 1st day = T₆ = Carbofuran @ 2.0 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₆ = Carbofuran @ 2.0 kg a.i ha⁻¹ and samples collected after 72 h

Table 4: Joint effect of pesticides and their residue levels in edible portion of red amaranth

Time of pesticides application (day)	Treatments	Name of the pesticides	Applied dose ($\mu\text{g g}^{-1}$)	Residue in edible leaf ($\mu\text{g g}^{-1}$)	% Incorporation
1st day	T ₇	Cypermethrin and chlorpyrifos	2.8 and 2.4	0.005 (Cypermethrin) and chlorpyrifos not detected	0.1785 -
3rd day	T ₇	Cypermethrin and chlorpyrifos	2.8 and 2.4	0.0025 (Cypermethrin) and chlorpyrifos not detected	0.0892 -
1st day	T ₈	Cypermethrin and chlorpyrifos	4.2 and 3.23	0.0046 (Cypermethrin) and chlorpyrifos not detected	0.1095 -
3rd day	T ₈	Cypermethrin and chlorpyrifos	4.2 and 3.23	0.0018 (Cypermethrin) and 0.0004 (chlorpyrifos)	0.0428 and 0.0123
1st day	T ₉	Cypermethrin and carbofuran	2.8 and 7.35	0.0093 (Cypermethrin) and 0.0024 (carbofuran)	0.3321 and 0.3265
3rd day	T ₉	Cypermethrin and carbofuran	2.8 and 7.35	0.005 (Cypermethrin) and 0.0019 (carbofuran)	0.1785 and 0.0252
1st day	T ₁₀	Cypermethrin and carbofuran	4.2 and 9.8	0.0054 (Cypermethrin) and 0.0016 (carbofuran)	0.1285 and 0.0163
3rd day	T ₁₀	Cypermethrin and carbofuran	4.2 and 9.8	0.0041 (Cypermethrin) and 0.0081 (carbofuran)	0.976 and 0.0083
1st day	T ₁₁	Chlorpyrifos and carbofuran	2.8 and 7.3	Chlorpyrifos not detected and 0.0004 (carbofuran)	- 0.0054
3rd day	T ₁₁	Chlorpyrifos and carbofuran	2.8 and 7.3	0.0100 (Chlorpyrifos) and 0.0006 (carbofuran)	0.3571 and 0.0082
1st day	T ₁₂	Chlorpyrifos and carbofuran	3.23 and 9.8	0.0027 (Chlorpyrifos) and 0.0001 (carbofuran)	0.0835 and 0.0010
3rd day	T ₁₂	Chlorpyrifos and carbofuran	3.23 and 9.8	0.0057 (Chlorpyrifos) and carbofuran not detected	0.1764 -

Pesticides (cypermethrin, chlorpyrifos and carbofuran) were applied at 21 days after emergence (DAE)

- 1st day = T₇ = Cypermethrin @ 1 ml L⁻¹ of water and chlorpyrifos @ 0.5 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₇ = Cypermethrin @ 1 ml L⁻¹ of water and chlorpyrifos @ 0.5 kg a.i ha⁻¹ and samples collected after 72 h
- 1st day = T₈ = Cypermethrin @ 1.5 ml L⁻¹ of water and chlorpyrifos @ 0.66 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₈ = Cypermethrin @ 1.5 ml L⁻¹ of water and chlorpyrifos @ 0.66 kg a.i ha⁻¹ and samples collected after 72 h
- 1st day = T₉ = Cypermethrin @ 1 ml L⁻¹ of water and carbofuran @ 1.5 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₉ = Cypermethrin @ 1 ml L⁻¹ of water and carbofuran @ 1.5 kg a.i ha⁻¹ and samples collected after 72 h
- 1st day = T₁₀ = Cypermethrin @ 1.5 ml L⁻¹ of water and carbofuran @ 2.0 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₁₀ = Cypermethrin @ 1.5 ml L⁻¹ of water and carbofuran @ 2.0 kg a.i ha⁻¹ and samples collected after 72 h
- 1st day = T₁₁ = Chlorpyrifos @ 0.5 kg a.i ha⁻¹ and carbofuran @ 1.5 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₁₁ = Chlorpyrifos @ 0.5 kg a.i ha⁻¹ and carbofuran @ 1.5 kg a.i ha⁻¹ and samples collected after 72 h
- 1st day = T₁₂ = Chlorpyrifos @ 0.66 kg a.i ha⁻¹ and carbofuran @ 2.0 kg a.i ha⁻¹ and samples collected after 24 h
- 3rd day = T₁₂ = Chlorpyrifos @ 0.66 kg a.i ha⁻¹ and carbofuran @ 2.0 kg a.i ha⁻¹ and samples collected after 72 h

Joint effect of different pesticides and their residue levels in red amaranth:

Cypermethrin was applied at two different levels viz., 2.8 and 4.2 $\mu\text{g g}^{-1}$ along with two levels of chlorpyrifos (2.4 and 3.23 $\mu\text{g g}^{-1}$) and carbofuran (7.3 and 9.8 $\mu\text{g g}^{-1}$) on to the plant or soil to study their retention on the plant or uptake by the plant of red amaranth. The results presented in Table 4 revealed that 0.005 $\mu\text{g g}^{-1}$ of cypermethrin could be detected after 24 hours of application when cypermethrin at the rate of 2.8 $\mu\text{g g}^{-1}$ and chlorpyrifos 2.4 $\mu\text{g g}^{-1}$ were applied, but no trace of chlorpyrifos could be observed. The incorporation percentage was 0.1785 of the cypermethrin. After 72 h of application 0.0025 $\mu\text{g g}^{-1}$ cypermethrin was present as residue in edible leaf where chlorpyrifos showed no residual effect. Percentage of incorporation of cypermethrin was 0.0892. It is therefore apparent that quantity of cypermethrin residue reduced with laps of time and in combination with cypermethrin, chlorpyrifos left no residue.

In another treatment 0.0046 $\mu\text{g g}^{-1}$ of cypermethrin was obtained in the edible part of the plant when

cypermethrin and chlorpyrifos @ 4.2 and 3.23 $\mu\text{g g}^{-1}$ were applied in plant and soil respectively. No residue of chlorpyrifos was detected in the plant after 24 h of the treatment. However after 72 h 0.0004 $\mu\text{g g}^{-1}$ of chlorpyrifos was detected. It also appears from the table that the residue level of cypermethrin decreased with the increase of time intervals. On the other hand the residue level of chlorpyrifos was detected after 72 h, which was not detected after 24 h. It is evident that the uptake of cypermethrin is more on the 1st day of the treatment. However it disappears after a certain time intervals. This might be due to rapid degradation and decomposition of chlorpyrifos.

Table 4 also showed the effect of combined application of cypermethrin and carbofuran. The applied dose of cypermethrin and carbofuran were 2.8 and 7.35 $\mu\text{g g}^{-1}$, respectively. The residue level of cypermethrin and carbofuran was 0.0093 and 0.0024 $\mu\text{g g}^{-1}$, respectively. The % incorporation was 0.3321 and 0.3265 in case of cypermethrin and carbofuran, respectively. After 72 h 0.005 and

0.0019 $\mu\text{g g}^{-1}$ of cypermethrin and carbofuran were detected. In this case % incorporation was 0.1785 for cypermethrin and 0.0252 for carbofuran.

The joint application of higher level of both cypermethrin (4.2 $\mu\text{g g}^{-1}$) and carbofuran (9.8 $\mu\text{g g}^{-1}$) slightly increased the amount of cypermethrin residue and reduced that of carbofuran. Both the pesticide residues reduced after 72 h as compared to that of lower levels and consequently their percent incorporation in red amaranth decreased with time.

When chlorpyrifos and carbofuran were applied jointly at lower levels (2.8 and 7.3 $\mu\text{g g}^{-1}$, respectively) there was no residue of chlorpyrifos could be detected though there was a very negligible amount of carbofuran (0.0004 $\mu\text{g g}^{-1}$). But after 72 h a very little amount of chlorpyrifos (0.01 $\mu\text{g g}^{-1}$) and slightly increased the level (over 24 h) of carbofuran residues were detected. It is remarkable that when higher level of these two pesticides were jointly employed, the amount of chlorpyrifos as residue in red amaranth increased but carbofuran reduced and after 72 h presence of carbofuran in plants could not be traced. This indicates that chlorpyrifos exerted antagonistic effect on the accumulation of carbofuran in plant system of red amaranth.

It can be noted from the findings of the present study that when cypermethrin and chlorpyrifos were applied jointly, after 24 h of application a very little amount of cypermethrin retained on the plant as residue which further reduced to about half of the former residual amount: after 72 h. But, the accumulation of chlorpyrifos could not be detected neither after 24 h nor after 72 h when chlorpyrifos and cypermethrin were jointly applied. On the other hand when higher levels of these two pesticides were employed the amount of cypermethrin detected after 24 h was about the same as that of the lower level and further it reduced with higher level of chlorpyrifos after 72 h. With higher level of chlorpyrifos no residue could be detected after 24 h when applied with higher level of cypermethrin but after 72 h a very scanty amount was observed to be a up taken by red amaranth.

On the contrary when cypermethrin and carbofuran were jointly applied the amount of cypermethrin detected as residue was higher than that of the former two cases. But it was revealed that after 72 h of application the residual amount of both the pesticides decreased and reached to a half.

It appears that for early and safe harvest of the leafy vegetables of red amaranth joint application of cypermethrin and chlorpyrifos may be beneficial while for late harvest cypermethrin with carbofuran may be applied. Application of higher level of these two pesticide did not influence the uptake or retention even after 72 h harvest.

As to the effect of joint application of chlorpyrifos and carbofuran, after 24 h no residue of chlorpyrifos could be detected but a little amount of carbofuran (0.0004 $\mu\text{g g}^{-1}$) was accumulated in edible portion of red amaranth. The presence of both the insecticides could be detected after 72 h of application and with the increase in levels, the residue level of chlorpyrifos increased and that of carbofuran decreased and at higher level after 72 h of application the presence of carbofuran could not be detected. From this experiment it appears that chlorpyrifos when applied jointly the residue level of carbofuran reduced with increase in level and increase of time and vice-versa.

REFERENCES

1. Islam, M.S., J.A. Khatoon, M. Alamgir and M.A. Hossain, 2003. Nutritional status of red amaranth as influenced by selected pesticides. *Pak. J. Biol. Sci.*, 6: 2044-2049.
2. Begum, M.N., 2000. *Swastha-pusti Babosthapon* (In Bengali). 1st Edn. Bangla Academy, Dhaka-1000, pp: 138.
3. Kirtikar, K.R. and B.D. Basu, 1918. *Indian Medicinal Plants*. Parts I and II. Allabadad.
4. Colonel, R.N. and M.D. Chopra, 1958. *Indigenous Drugs of India*. U. N. Dhur and Sons Ltd. Calcutta.
5. DFG Manual of Pesticide Residue Analysis, 1987. Organochlorine and Organophosphorus pesticides, Method 5-9, Pesticide Commission-VCH, Weinheim, Germany, 1: 297-307.
6. Gpeen, M.B., G.S. Hartley and T.F. West, 1987. *Chemicals for Crop Improvement and Pest Management*. 3rd Edn. Pergamon press, Headington Hill Hall, Oxford OX3 OBW, England, pp: 115.
7. Ramulu, U.S.S, 1992. *Chemistry of Insecticides and Fungicides*. 2nd Edn., pp: 135, 193.