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Efficacy, persistence and removal of chlorpyrifos-methyl after application against cotton leaf worm in soybean

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Abstract
Soybean crop (*Glycine max* L) is a very important economic crop belongs to leguminosae, considered one of the high potentially protein source and attacked by Cotton leaf worm *Spodoptera littoralis* (Boisd), the major pest thought-out the growing season. Therefore, this study aimed to evaluate the insecticidal activity of chlorpyrifos-methyl against the cotton leaf worm *Spodoptera littoralis* on soybean (Giza 21). Furthermore, to investigate the persistence of this tested insecticide after application under some of the environmental factors (direct sunlight, ultraviolet rays and temperature). Moreover, to evaluate the effect of some commercial processes on the safe removal of chlorpyrifos-methyl residues from soybean seeds. The results showed that chlorpyrifos-methyl was effective against cotton leaf worm. The exposure of chlorpyrifos-methyl to the direct sunlight, ultraviolet rays and different temperature degrees significantly reduced its level. Cooking procedure showed higher effect than dry heating at (90-95ºC) on removal of tested insecticide from soybean seeds. The results showed that the food processing is an important way for minimizing chlorpyrifos-methyl in the final products lower than the maximum residue limits.

Keywords: insecticide; soybean; temperature; residues; seeds

1. Introduction
In Egypt soybean considered very important crop and cultivated in more than 90000 hectare. Soybean used in wide range of foodstuffs derived for soybean. Moreover, there are an increasing consumption of soybean products as a result of the interesting properties associated with the use of soybeans which promoted the appear of a wide range of foodstuffs derived from this soybean: soybean flour, textured soybean, soybean milks, soybean infant formulas, etc [1]. Soybean oil is a concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from soybean (Glycine max).Soybean oil is popularly used in food processing and cooking. Soybean oil accounts for over 75% of the total vegetable oil in human foods [2].

Soybean crop attacked by many insects such as spider mites, aphids, cotton leaf worm, and many other pests [3]. The pests infest all parts of the soybean plant at all growth stages and can lead to yield losses from 20 to 50% thus lead the farmers into the use of pesticides to protect their crops. Among the different control measures such as cultural, mechanical, biological and chemical methods, the farmers prefer the use of chemicals to control pests because it gives quick results. Besides the usefulness of pesticides in protecting the crop, it has some drawbacks not only to environment but also to consumers in case of residues in the edible parts [2].

Organophosphorous insecticides have been widely used in agriculture especially for controlling cotton leaf worm in soybean. Their initial success was mainly based on their high toxicity, high biological specificity and rapid environmental degradation [4]. Furthermore, most organophosphorus insecticides are also toxic to non-target organisms and some appear to have relatively high environmental persistence [5]. The increasing amounts of organophosphorus pesticides released into the environment represent a major ecotoxicological hazard especially for humans and non-target animals [5-6].

Food is the basic necessity of life and food contaminated with toxic pesticides is associated with severe effects on the human health. Hence it is pertinent to explore strategies that address this situation of food safety especially for the developing countries where pesticide contamination is widespread due to indiscriminate usage and a major part of population lives below poverty line. It is therefore of significance to evaluate simple, cost

effective strategies to enhance food safety from harmful pesticides for poor populace. Food processing at domestic and industrial level would offer a suitable means to tackle the current scenario of unsafe food [7].

Food processing techniques implies the set of methods and techniques used to transform raw ingredients into food or to transform food into other forms for consumption by humans or animals either in the home or by the food processing industry. This food processing techniques aid in pesticide dissipation and reduced its residues [7].

Therefore, the present study was carried out to evaluate the bio-residual efficacy of chlorpyrifos-methyl insecticide against cotton leaf worm *Spodoptera littoralis* (Boisd.), to determine the residues of chlorpyrifos-methyl insecticide on and in whole soybean plant leaves, pods and seeds under normal field conditions, to justify the effect of different environmental factors (light and temperature) on the persistence of chlorpyrifos-methyl after application and finally to investigate the role of industrial processes on the safe removal of chlorpyrifos-methyl in and on soybean products.

2. Materials and methods

2.1. Insecticidal activity of chlorpyrifos-methyl against cotton leaf worm S. littoralis on soybean

Soybean *Glycine max* "Giza- 21" was planted at the farm of Zarzora Research Station, Etay El-Baroud, at Beheira Governorate, Egypt. A complete randomized design was used, two treatments including the control were prepared and each treatment was divided into three replicates. The area of each treatment was about 3/100 hectare. A knapsack sprayer was used for applying the insecticide at the recommended field rate. Leaves were collected randomly from each treated and untreated plots at zero time (one hour after treatment), 14 hrs (as the initial deposit),1,3,5,10 and 15 days after treatment in August. The 4th instars leaf worm larvae of the field strain which were obtained from the farm of Zarzora Research Station were exposed to the collected soybean leaves. Fifty larvae's were used for each treatment and divided into 5 replicates (10 larvae in each glass jar). Mortality counts were recorded 24 hours, after feeding of the larvae on the treated soybean leaves. The mortality percentage was corrected according to [8].

2.2. Residues of chlorpyrifos-methyl in soybean leaves, pods and seeds

Samples were collected randomly from treated and untreated plots at zero time (one hour after treatment) as the initial deposits for all parts (leaves, pods and seeds), then 1, 3, 5, 10, 20 and 49 days after treatment (harvest day). Three replicates of plant samples, 500 g each were collected. All undesired parts involved in samples were discarded and then seeds were removed out from pods. The samples (leaves) were cut into small pieces; each sub sample was taken at rate of 10 g, transferred to laboratory and kept under deep freezing until analysis of chlorpyrifos-methyl residues. Calculation of residual half-life value (RL_{50}) and decomposition rate (K) mathematically according to [9]. as shown equations 1 and 2

$$RL_{50} (t_{1/2}) = Ln_2 / K` = 0.6932 / K` \quad (1)$$

$$K = 1/t_x * Ln\ a / b_x \quad (2)$$

Where is the K refer to rate of decomposition, tx is the time in days, a is the initial residue of the compound and bx_{is} the residue of the compound at x time.

2.3. Residues analysis

2.3.1. Extraction and clean up

Ten grams of soybean leaves, pods and seeds were macerated with 25 g activated anhydrous sodium sulphate till complete mixing achieved, then 200 ml chloroform were added and blended for two minutes on high speed using warring blender. The macerate was filtered through a glass wool using amount of activated anhydrous sodium sulphate (30 g) and finally washed with 25 ml of chloroform. The filtrate was evaporated to dryness with a rotary evaporator at 35 – 40 ºC.

The clean up for chlorpyrifos-methyl was achieved according to the method of [10]. A 20 mm (i.d.) glass column was prepared by adding successively, a plug of glass wool, 5 g of activated florisil (60-100 mesh) and compact thoroughly. The column was pre-washed using 40 ml n-hexane and drained to the level of the solvent down to the top of florisil. Residue extract was transferred to the florisil column, already saturated with hexane. The column was eluted with 100 ml of the eluant (50 % methylene chloride: 48.5 % hexane: 1.5 % acetonitrile) at a rate of 5 ml/min. The collected elute was concentrated in rotary evaporator and dissolved in 2 ml of ethyl acetate for residue analysis employing gas liquid chromatography (GLC) equipped with flame photometric detector.

2.3.2. Determination procedure

Agilent Gas Liquid Chromatograph model 7890, equipped with flame photometric detector (FPD), programmed for external standardization depending on peak area that used to determine chlorpyrifos-methyl residues. Capillary column HP-5, 5% phenyl methyl siloxan 30 m × 320 µm × 0.25 µm film thicknesses was employed. Nitrogen carrier gas at flow rate 3 ml/min and septum purge flow at 3 ml/min. Flow rate of helium, air and makeup were 100, 70, and 60 ml/min, respectively. Injector and detector temperatures were 240 and 250°C, respectively. The column oven temperature was initially maintained 170°C for 1 min and increased at 25°C /min to 250°C for 5 min. Retention time at these conditions was 3.5 min.

2.3.3-Oil removal from pods and seeds extracts

Dried extracts were dissolved in 15 ml light petroleum ether (b.p., 40-60°C) and transferred quantitatively to a 125 ml separating funnel. After that extracts were partitioned using 3×30 ml acetonitrile completely saturated with light petroleum ether, and then fractions were combined together and evaporated under vacuum till dryness.

2.3.4. Recovery evaluation

The efficacy of the analytical steps used was evaluated through recovery by fortifying untreated samples of leaves, pods and seeds with known amounts of chlorpyrifos-methyl at level 10 mg Kg^{-1}. Then extraction, cleaning up and determination procedures that mentioned before were performed.

2.4. Effect of direct sunlight, UV-rays and temperature on the persistence of the tested insecticide

The effect of different environmental factors (direct sunlight, UV-rays and two temperature degrees) on the degradation rate of chlorpyrifos-methyl (active ingredient) was investigated. Stock solution of tested insecticide contains 500 µg/ml of the active ingredient (dissolved in ethyl acetate), had prepared. One millilitre aliquots of prepared stock solution, were spread uniformly as possible on uncovered Petri dishes surface (5 cm i.d.) and were left to dry at room temperature.

2.4.1. Effect of direct sunlight exposure

A group of dried Petri dishes prepared as mentioned above was exposed to the direct sunlight, then dishes were withdrawn after 0, 1, 3, 6, 12, 24, 48 and 96 hours of exposure. Residues remained in Petri dishes were quantitatively transferred to volumetric flasks using suitable solvents and then were determined by GLC as mentioned previously.

2.4.2. Effect of UV-ray exposure

A second group of dried Petri dishes prepared as mentioned before was exposed to short UV rays at 254 nm with a distance of 12 cm for 0, 1, 3, 6, 12, 24 and 48 hours of exposure in a dark isolated box. Residues remained in Petri dishes were quantitatively transferred to volumetric flasks using suitable solvents and then were determined by GLC as mentioned previously.

2.4.3. Effect of exposure to different temperature degrees

A third group of dried Petri dishes prepared as mentioned before were exposed to two temperature degrees (35 and 45 ºC) for 0, 1, 3, 6, 12, 24, 48, 96 and 144 hours in a dark electric oven provided with temperature regulating system. Residues remained in Petri dishes were quantitatively transferred to volumetric flasks using suitable solvents and then were determined by GLC as mentioned previously.

2.5. Effect of industrial commercial processes on the safe removal of chloropyrifos-methyl residues

Stock solution of used insecticide contains 299.25 µg/ml of chlorpyrifos-methyl (0.315 ml dissolved in 1000 ml tap water). A sample (10 g) seeds was soaked in 7 ml (0.5 ml stock solution + 6.5 ml tap water) in 100 ml glass beaker and stirred slowly for three hours to ensure good mixing and even distribution of chlorpyrifos-methyl according to the method described by [11]. After that the seeds were allowed to dry at room temperature.

2.5.1. Effect of dry heating at (90ºC)

The spiked seeds were ground with a mixer for 2 min; the mixture was heated at 90-95 ºC for 10 minutes with careful stirring and cooled in a refrigerator. The cooled samples were analyzed as mentioned before.

2.5.2. Effect of cooking procedure

The spiked seeds (10 g) were soaked in 50 ml of tap water and were heated at 90-95 ºC for 10 minutes with careful stirring. After that seeds were ground in a warring blender with 50 ml hot water followed by filtration through gauze. The filtrate (soybean milk) was heated at the same temperature for 10 min and the soybean milk was subjected to analysis after cooling.

2.5.3. Analytical Procedure for soybean milk

Each sample was extracted by partitioning twice with 100 ml of ethyl acetate using separating funnel 125 ml. The layer of ethyl acetate was evaporated under vacuum till dryness with a rotary evaporator (35–40ºC) according to [12].Removal of oil and the clean up for tested insecticide were done according to the method mentioned previously. The recovery for chlorpyrifos-methyl in soybean milk was 91.9%. Data were statistically analyzed according to the method described by [13].

Table 1: Percentage recovery rates of chlorpyrifos-m ethyl on soybean leaves, pods and seeds at level of 10 ppm

Insecticides used	Recovery rate (%)		
	Leaves	Pods	Seeds
Chlorpyrifos-methyl	87.2±0.56	90.51±0.43	85.61±0.61

*Values are means ± standard deviation (n = 3).
*LOD: Limit of detection. *LOD: 0.01 ppm

3. Results and discussion

2.1. Insecticidal activity of chlorpyrifos-methyl against cotton leaf worm S. littoralis on soybean

Data in Table (2) clearly showed that the initial residue of chlorpyrifos-methyl was effective against the 4th instars larvae of cotton leaf worm with mortality percentage of 99 % after application of tested insecticide. Chlorpyrifos-methyl residual toxicity decreased gradually with increasing the time after treatment. The prolongation of time to 1 day revealed less mortality, i.e. 95% followed by 58, 37, 29 and 12 % after 3, 5, 10 and 15 days of application. This is may be due to the further exposure of chlorpyrifos-methyl residues to environmental conditions that highly decreased its amounts and bio-residual effectiveness. The LT_{50} value for residual toxicity of chlorpyrifos-methyl was 80.64 hours (3.36 days) as shown in Table (2). The results obtained were agreed with those obtained by [14].who showed that chlorpyrifos-methyl was the most effective insecticide towards cotton leaf worm larvae. Also, the results in this study was in agreement with those obtained by [15-16].who concluded that chlorpyrifos-methyl gave average initial mortality 100 % for both the 2nd and 4th instars larvae of cotton leaf worm.

Table 2: Insecticidal activity of chlorpyrifos-methyl against the larvae of cotton leaf worm *S. littoralis* on soybean leaves.

Time of application (days)	Mean
Initial	99±1.9 [a]
1	95± 1.5[a]
3	58±0.35 [b]
5	37± 0.79[c]
10	29±1.1 [d]
15	12± 0.42 [e]
LT_{50} (days)	3.36

*Values are means ± standard deviation (n = 3).
*Means followed by a common letter are not significantly different at the 5% level

2.2. Residues of chlorpyrifos-methyl in soybean leaves, pods and seeds

Residues and loss rates of chlorpyrifos-methyl on and in soybean leaves, pods and seeds of the tested genotype Giza-21 were illustrated in Table (3). Data in Table (3) clearly showed that the values of chlorpyrifos-methyl residues on and in leaves, pods and seeds decreased with the time. Also there were significantly differences in chloropyrifos-methyl residues among intervals after application; continuous loss was significant

for chlorpyrifos-methyl to 15 days. The initial deposit which remained on leave one hour after treatment was 27.53 ppm. Chlorpyrifos-methyl residues gradually decreased to 12.94, 11.73, 10.78, 4.87 and 1.83 ppm indicated loss rates of 52.98, 57.41, 60.82, 82.31 and 93.34%, respectively after 1, 3, 5, 10 and 15 days of treatment. Chlorpyrifos-methyl residues at harvest day were below the detection limit (0.01 ppm) and the RL_{50} value was 2.22 days from application with degradation rate of 0.31.

The initial deposit which remained on pods after treatment was 12.41 ppm. Chlorpyrifos-methyl residues gradually decreased to 2.25, 1.68 and 0.53 ppm indicated loss rates of 73.79, 86.44 and 95.7, respectively after 1, 3, and 5 days of treatment. Chlorpyrifos-methyl residues at harvest day were below the detection limit (0.01 ppm) and the RL_{50} value was 2.52 days from application with degradation rate of 0.27.

There was no initial deposit of chlorpryifos-methyl remained on seeds after treatment. Chlorpyrifos-methyl residues gradually increased to 0.22, 0.44 and 0.83 ppm after 1, 3 and 5 days of treatment, respectively. Chlorpyrifos-methyl residues gradually decreased to 0.63 and 0.10 ppm indicating loss rats of 25 and 88% after 10 and 15 days of treatment, respectively. Chlorpyrifos-methyl residue at harvest day was below the detection limit (0.01ppm) and the RL_{50} value was 2.52 days from application with degradation rate of 0.27.

Residues and loss rates of chlorpyrifos-methyl on and in soybean leaves, pods and seeds of the tested genotype Giza-21 were decreased with the time. Since the level of residues in and on leaves and seeds were affected by many factors i.e. applied dosage, properties of insecticide, meteorological and biological factors depending on the kind as well as properties of the plant surface. The obtained data were in agreement with those reported by [17].

Soybean seeds were free from any detectable residues of chlorpyrifos-methyl at the mentioned detection limit; the maximum residue limit (MRL) of chlorpyrifos-methyl residues on soybean crop found in Codex (2009) is 0.1 ppm. The estimated pre-harvest interval (PHI) for chlorpyrifos-methyl residues on soybean crop was 15 days from application. These findings indicated that soybean plants treated with chlorpyrifos-methyl during growing and ripening stages should stay in the field about 15 days before harvesting to be consumed and marketed safely for human and animal consumption. Since the rates of soybean seeds contamination with chlorpyrifos-methyl will be below the estimated MRL´s. The obtained data were in agreement with those reported by [17].

Table 3: Residues and loss rates of chlorpyrifos-methyl on and in soybean leaves, pods and seeds.

Time of application (days)	Residues in leaves		Residues in pods		Residues in seeds	
	Mean µg /gm	% Loss	Mean µg /gm	% Loss	Mean µg /gm	% Loss
Initial	27.53±0.25 a	00.00	12.41 ±0.53a	0.00	N.D.	0.00
1	12.94±0.23 b	52.98	3.25± 0.1b	73.79	0.22 ±0.01a	0.00
3	11.73± 0.10c	57.41	1.68±0.01 c	86.44	0.44 ±0.03b	0.00
5	10.78± 0.21d	60.82	0.53±0.04 d	95.7	0.83± 0.06c	0.00
10	4.87± 0.4e	82.31	N.D.	100	0.63±0.04 d	25
15	1.83±0.01 f	93.34	N.D.	100	0.10±0.01 f	88
49 (Harvest day)	N.D.	≈100.00	N.D.	100	N.D.	100
K`	0.312	--	0.87	--	0.083	--
RL_{50} (days)	2.22	--	0.79	--	8.34	--

*N.D.: not detected. * Limit of detection (LOD) for chlorpyrifos-methyl 0.01 ppm
*Means followed by a common letter are not significantly different

3.3. Effect of direct sunlight and UV radiation on the persistence of chlorpyrifos-methyl

Data in Table (4) showed that there was a significant influence of direct sunlight and UV radiation on the dissipation rate of chlorpyrifos-methyl. Also there were significant differences in insecticide residues among intervals after exposure either to sunlight or UV radiation. A result revealed that residue of tested insecticide was decreased after one hour of exposure to direct sunlight and UV radiation by 66.15 and 69.49%, respectively. Continuous degradation was positively correlated with the exposure period that the long exposure period gave a high loss rate for the tested insecticide. Loss rate values for chlorpyrifos-methyl residues were 99.72 and 100% at

3 and 6 hours after exposure, respectively. While loss rate values for chlorpyrifos-methyl residues after exposure to UV radiation were 81.03, 82.86, 92 and 100 % at 3, 6, 12 and 24 hours after exposure, respectively.
The data showed that there was a significant influence of direct sunlight and UV radiation on the dissipation rate of chlorpyrifos-ethyl. The significant influence of sunlight or UV radiation on chlorpyrifos-ethyl has been reported [18-19]. Photodegradation by sunlight is one of the promising methods for pesticides degradation after their release to the environment. Factors that influence pesticides photodegradation are the intensity of light and properties of pesticide itself (Fred, 1997) [20]. Chlorpyrifos degradation by sunlight was faster than ultraviolet radiation and this is may be due to that chlorpyrifos has ultraviolet absorbance over 290 nm which indicate its susceptibility to photodegradation by sunlight [19]. Also may due to the thermal evaporation and light intensity consideration of sunlight [20].

Table 4: Influence of direct sunlight and UV radiation on dissipation rate of chlorpyrifos-methyl residues.

Time (hr)	Sunlight		UV radiation	
	Mean µg found	% Loss	Mean µg found	% Loss
initial	500.00 a	00.00	500.0 a	0.00
1	169± 2.4b	66.15	152.55±0.54 b	69.49
3	1.36±0.01 c	99.72	94.85±0.9 c	81.03
6	0.00 d	100	85.67 ±0.7d	82.86
12	0.00 d	100	40.22± 1.1e	92
24	00.00 d	100.00	0.16 ±0.01f	100
48	0.00 d	100	0.00 g	100
96	0.00 d	100	0.00 g	100
144	0.00 d	100	0.00 g	100
K`	1.51	---	0.311	--
$T_{1/2}$ (hr.)	0.458	--	2.22	--

* Limit of detection (LOD) for chlorpyrifos-methyl 0.01 ppm
* Values are means ± standard deviation (n = 3).
*Means followed by a common letter are not significantly different at the 5% level

3.4. Effect of different temperature degrees on chlorpyrifos-methyl persistence

The results in Table (5) revealed that the percentage loss values of chlorpyrifos-methyl residues were 10 and 17.77 % after exposure to temperature for one hour at 35 and 45°C, respectively. The degradation rate values were 2.7, 16.66, 28.99, 34.26, 45.95, 69.34, 71.82 and 99.76 % after 1, 3, 6, 12, 24, 48, 96 and 144 hours, respectively after exposure to 35°C. The degradation rate values were 39.56, 51.92, 58.36, 82.97, 99.63 and 100 % after 1,3,6,12,24 and 48 hours of exposure at 45°C, respectively. The results revealed that the calculated RL_{50} values of chloropyrifos-methyl were 16.05 and 14.5 hours, indicating degradation rates of 1.036 and 2.62 at 35 and 45°C, respectively. The results revealed that the loss rate values increased significantly by increasing the degree of temperature. There were significant differences in the insecticide levels among intervals after application either under 35 or 45°C degrees. The obtained results in this study agree with findings of [18]. who reported that the temperature significantly reduce chlorpyrifos-methyl residues. The results showed that the temperature play role in dissipation of chlorpyrifos-ethyl and dissipation rate increase with increasing temperature and prolongation of exposure [20]. found that the increasing of temperature degrees and prolongation of exposure increase the percentage of insecticide loss.

3.5. Effect of commercial processes of soybean on the removal of chlorpyrifos-methyl

Data in Table (6) showed that the residues of chlorpyrifos-methyl in soybean seeds were significantly decreased by application of the two tested commercial processes. Whereas, cooking procedure was higher than dry heating at (90-95ºC) on removal of the tested insecticide from soybean seeds. The percentage removal value of chlorpyrifos-methyl residues from soybean seeds by dry heating was 35.44% while the percentage removal value of chlorpyrifos-methyl residues from soybean seeds by cooking at (90-95 ºC) for 20 min was 70.3%.

Table 5: Influence of temperature (35 and 45°C) on the dissipation rate of chlorpyrifos-methyl residues.

Time (hr)	35 °C		45°C	
	µg found	% Loss	µg found	% Loss
Initial	500 a	00.00	500a	00.00
1	486.45±1.3 a	2.7	302.19±1.2 b	39.56
3	416.69±0.56 b	16.66	240.37±1.4c	51.92
6	356.05±0.48 c	28.99	206.87±2.1d	58.36
12	328.96±0.75 c	34.26	85.13±0.71e	82.97
24	270.25±0.67 d	45.95	1.84±0.1f	99.63
48	153.03±0.93e	69.39	0.00	100
96	140.85±1.2e	71.82	0.00	100
144	1.2±0.1 f	99.76	0.00	100
K`	0.035	--	0.260	--
$T_{1/2}$ (hr)	19.8	--	2.66	--

* Values are means ± standard deviation (n = 3).
*Means followed by a common letter are not significantly different at the 5% level

The residues of chlorpyrifos-methyl in soybean seeds were significantly decreased by application of the two tested commercial processes Thus all stages of the processing play a significant role in removing the tested insecticide from soybean seeds. The results of this study agree with those obtained by [12, 22-25].

Table 6: Influence of commercial processes on removal of chlorpyrifos-methyl on and in soybean seeds.

Commercial processes	Mean Residue(µg /gm)	% Removal
Before treatment	14.06±1.94 [a]	00.00
Dry heating	4.68±0.72 [b]	33.28
Cooking procedure	00.00[c]	70.3

*Limit of detection (LOD) for chlorpyrifos-methyl 0.01 ppm
*Values are means ± standard deviation (n = 3).
*Means followed by a common letter are not significantly different

Conclusion

From the practical point of view in the insect control program, it could be recommended to use the tested insecticide (chlorpyrifos-methyl) in area of dominant temperature not more than 40°C, during the controlling time. The exposure to the main environmental factors sunlight and temperature affected the degradation rates of tested insecticide. From all the previous data it could be concluded that the residue levels of the tested insecticide chlorpyrifos-methyl on the tested soybean genotype Giza-21 were in the safe limits for human consumption, when applied with the recommended rates. Also it could be said that, the stages of the food processing of soybean play a significant role in removing the insecticide and reduced significantly its hazards.

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