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Dissipation of Organophosphorus Pesticides in Green Onion (*Allium fistulosum* L), Cultivated in Forced System Called "Barbacoas"

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In the Zulia State, green onion (*Allium fistulosum* L.) is the main horticultural crop, which is grown in forced production systems called "barbacoas" or growing trays. The pest that attack it reduce its foliage area, affecting its growth, appearance and overall quality. The leaf miner, *Liriomyza trifolli* (Burguess), along with thrips, constitute the primary pest which affect this crop. Pest control is effected through the use of diazinon, an organophosphorus pesticide. Another main threat to green onion is know as alternaria or purple spot, caused by the *Alternaria porri* (Ellis), Ciferri fungus. This fungus attacks the plants' foliage, especially in warm and humid environments.

For consumers' protection, many countries have restricted the use of these chemicals, establishing legal restrictions to control residue levels in food products, known as Maximum Residue Limits (MRLs). Persistence pesticides studies have been carried out in Venezuela in other crops like tomatoes (*3*) and guava (*10*). However, researches in green onion has not been carried out in our country. To prevent the presence of pesticide residue above the MRLs, the time between pesticide application and harvest must be determined, since the pesticide decay time depends on crop type, pesticide and environmental conditions. In this investigation, the persistence of organophosphorus pesticides diazinon, malathion and parathion were measured in green onion samples grown in "barbacoas" on the Bajo Sector in the municipality of San Francisco, Zulia, Venezuela.

MATERIALS AND METHODS

Standard pesticides of high purity; diazinon (98.9%), malathion (98.5%), and parathion (99.2 %) Dr. Ehrenstorfer GMBH (Germany) were used to prepare the initial solutions (1000 µg/mL) of each pesticide in HPLC grade ethyl acetate (Baker, USA). Calibration solutions and spiked solutions were derived from these initial solutions, diluted in grade HPLC ethyl acetate and methanol, respectively.

Triphenyl phosphate (99%, Riedel de Haën) was used as an internal standard. An initial solution of 1000 µg/mL was prepared in ethyl acetate. Analytical reagent grade anhydrous sodium sulfate (Riedel de Haën) was used to eliminate water residue during the extraction process.

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Sodium chloride (Fisher Scientific) was used to obtain salting out effect in the extraction process. Acetone HPLC (Fisher) was used to prepare the ethyl acetate/acetone (90:10) mix. Air, He, H_2 and N_2 of high purity were obtained from AGA of Venezuela. Activated charcoal 6-60 (Fisher Scientific, USA) and Florisil (Riedel de Haën), 60-10 mesh was used to clean up green onion extracts. Commercial formulation of diazinon: danol 60 %, malathion 57 % and parathion E-50, at 50 % were used in the persistence study.

Pesticide residues were analyzed using an Auto System Perkin-Elmer gas chromatograph, equipped with a Nitrogen-Phosphorus detector, a Perkin-Elmer automatic sampler and a capillary column of 30 m x 0.53 mm x 1.2 μm film thickness, of 5% phenyl - 95% methyl silicon AT-5 (Alltech, USA). A personal computer equipped with Turbochrom Navigator 4.1 software was used to record chromatograms and integrate peak areas.

For sampling, two grams of green onion, 2.0 g of sodium sulphate and 0.2 g of sodium chloride were used and ethyl acetate/acetone (90:10) was used as an extraction solvent. The cleansing of green onion extracts was performed through 200 mg activated charcoal cartridges and 700 mg of florisil. The cartridges were conditioned with 10 mL of methanol and 10 mL of ethyl acetate/acetone (90:10), after which colored extract was passed through the cartridge at a flow rate of 5 mL/min.

The pesticide residues were eluted by adding seven 5 mL portions of ethyl acetate/acetone (90:10). The extracts obtained were placed in test tubes and evaporated with a stream of nitrogen at 35 °C, until the final volume was 2 mL. It was then transferred to a vial and spiked with triphenyl phosphate (Internal Standard), then injected in duplicate samples of 1 μL in the gas chromatograph for quantification.

The persistence study was made on November and December 2002, on green onion cultivated in growing trays ("barbacoas") at "El Bajo" area in the municipality of San Francisco, Zulia (Venezuela). Four barbacoas of 10 m^2 each were used, for a total area of 40 m^2. An experimental parcel design was used, dividing them at random with four repetitions. In addition, a blank tray was prepared, to which no organophosphorus pesticides were applied.

Commercial formulations of organophosphorus pesticides were used, in dose recommended by the manufacturer: malathion 57 at 2.5 L/ha (1425 g of active ingredient/ha), parathion 50 ethyl at 1.5 L/ha (750 g of active ingredient/ha) and danol: diazinon 60-E at 1.5 L/ha (900 g of active ingredient/ha). Only one application of the pesticides was made, by means of sprinkling with a manual back sprinkler.

The pesticide concentration in green onion samples was monitored over time in each repetition beginning day 0 (one hour after application) and then during the following days: 2, 4, 6, 9, 11 and 13. This rendered a total of 28 samples taken on 7 sampling. At each sampling date, four plants were taken per repetition (300 g) and placed in dry, clean previously identified bags. The samples were then transported to the laboratory for extraction, cleaning and persistence analysis was carried out by duplicate. The same was done with the blank samples. Experimental split-plot design, complete randomized was used. Variance analysis was used as a statistical analysis technique, in order to determine the possible effects that the study factors (crop, pesticides and time) could have upon the concentration of pesticide (mg/g).

RESULTS AND DISCUSSION

The limits of detectability for the method used, following the Miller, (1993) methodology. The lowest concentration detected by this method was 0.0017 µg/g for malathion. Researches in guava (Sánchez J, 2005) reported 0.0165 µg/g as the detection limit for malathion. The results yielded by this investigation were higher.

The diazinon, malathion and parathion concentration in green onion obtained in day 0 was 8.88 µg/g, 16.12 µg/g and 10.50 µg/g, respectively (Table 1).

Table 1. Residues of organophosphorus (µg/g) in green onion crops (*Allium fitulosum* L.) and dissipation percentages over time

Days after application	Diazinon (µg/g) X ± s	%	Malathion (µg/g) X ± s	%	Parathion (µg/g) X ± s	%
0	8.88 ± 0.50	0.00	16.12 ± 0.83	0.00	10.50 ± 0.56	0.00
2	4.28 ± 0.27	51.82	8.60 ± 0.35	46.69	5.22 ± 0.18	50.24
4	0.09 ± 0.03	99.00	0.07 ± 0.02	99.58	0.11 ± 0.04	98.92
6	0.04 ± 0.02	99.61	0.02 ± 0.01	99.86	0.04 ± 0.02	99.59
9	0.02 ± 0.01	99.83	0.01 ± 0.003	99.94	0.01 ± 0.005	99.88
11	0.09 ± 0.001	99.91	0.004 ± 0.005	99.98	0.008 ± 0.001	99.92
13	*ND	100	**ND	100	***ND	100

X: Mean of concentration (µg/g)
%: Percentages of dissipation (days)
s: Standard deviation
*ND: Not detectable < 0.0061 µg/g
**ND: Not detectable < 0.0017 µg/g
***ND: Not detectable < 0.0020 µg/g

The second day after aplication, a drop of concentration was observed in diazinon residues, obtaining 4.23 µg/g corresponding to a 51.82 % dissipation. This behavior may be associated with the apparent volatilization of the pesticide in the crop (Tsakiris et al. 2002, Al-Samarie et al. 1988), which can be attributed to contact pesticides. The same behavior of diazinon was observed in malathion and parathion, showing a drop in residue concentrations to 8.59 µg/g corresponding to a 46.69% dissipation and 5.22 µg/g; or 50.24 % dissipation.

In day four after application, a drop of concentration was observed obtaining 99.00 % for diazinon, 99.58 % malathion and 98.92 % parathion dissipation in green onion samples. Between days six and eleven, a slow drop in residue concentration of the samples was observed. The mean concentrations obtained for diazinon were 0.035 and 0.008 µg/g, with dissipation percentages of 99.61 % and 99.91 %. Similar results in sweet pepper were obtained (Al-Samariee et al. 1988) at the sixth day after the initial application of diazinon, where its dissipation rate was slow. The concentration means of malathion obtained in both days were 0.023 and 0.004 µg/g, with dissipation percentages of 99.86 % and 99.98 %. In studies reported for other crops, malathion's behavior was different (Prieto et al. 2002, Podhorniak et al. 2001, Holland et al. 1994). This research was carried out by applying pesticides in green onion crops, with higher results in dissipation percentage. This difference can probably be accounted for due to the crop physiology (fruit and leaves).

If such is the case, the malathion could have penetrated deeper into the tissue of the green onion leaves, causing an rapid dissipation in time. The variation in residue levels between fruit and leaves after the application of pesticides could be attributed to morphological and biochemical differences in their constitution.

The parathion average concentration obtained the sixth and eleventh days was 0.043 µg/g and 0.008 µg/g respectively, corresponding to 99.59 % and 99.92 % dissipation. Different results were obtained in persistence studies done in must and grape wines 0.091 µg/mL on day 1 for must (Ettiene et al. 1997). The slow dissipation of parathion in green onion crop could be attributed to the plant's physiology (Tsakiris et al. 2002).

On day 13 after initial application, residues of diazinon, malathion and parathion were not detected in green onion samples analyzed.

Figure 1 shows an established tendency through a regression study based on the data analysis of each of pesticides, where lots of mathematicals models were evaluated, either linear or non-linear and it was obtained that the model that yielded the best adjustments of data was the Harris model (Farazdaghi, et al. 1968), which is a non-linear regression model which general formula is: $y = 1/(a+bx^c)$. The yield-density models are widely used, especially in agricultural

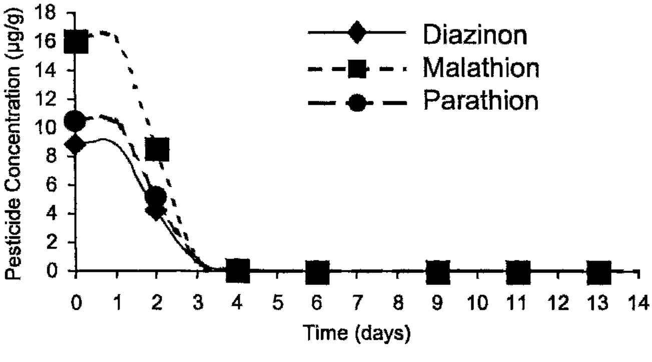

Figure 1. Dissipation of diazinon, malathion and parathion (µg/g) under time (days) in green onion (*Allium fistulosum* L.)

applications (Seber et al. 1989). These models historically have been used to model the relationship between the yield of a crop and the spacing or density or planting.

The non-linear regression equation for the diazinon dose applied to green onion was: $y = 1/(0.1126+0.0014*time_{(days)}^{6.4332})$, for malathion was: $y = 1/(0.062+0.00021*time_{(days)}^{8.018})$ and for parathion was: $y = 1/(0.095+0.001*time_{(days)}^{6.42})$. The determination coefficient was high for green onion, $R^2 = 0.9999$, demonstrating once again an appropriate adjustment of the non-linear Harris regression model to the data.

The neperian logarithm (ln) for the concentration of diazinon, malathion and parathion as a function of time was plotted. A straight line was obtained, showing a kinetic pattern of degradation of first order (Figure 2).

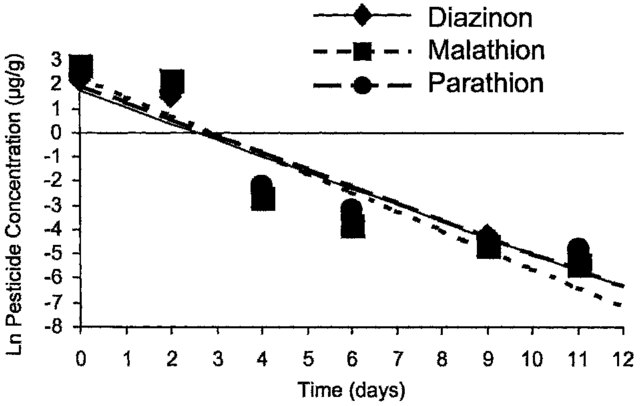

Figure 2. Logarithmic dissipation of diazinon, malathion and parathion in green onion (*Allium fistulosum* L.)

The linear regression equation for the dose applied to green onion was: $y = 1.7136-0.6715*t$ with a $R^2 = 0.8846$ ($P< 0.01$) and correlation coefficient of 0.9405 ($P< 0.01$) for diazinon, $y = 2.26668-0.79*t$, $R^2 = 0.8671$, with a correlation coefficient of 0.9312 ($P< 0.01$) for malathion and $y = 1.943-0.69*t$, $R^2 = 0.8959$ and correlation coefficient of 0.9465 ($P< 0.01$) for parathion.

The average half-lives ($t_{1/2}$) for residues of diazinon were calculated. The $t_{1/2}$ obtained for diazinon in green onion was 1.03 days. This result was similar to the reported in a persistence study of tomato crops in the Mara municipality, Zulia, Venezuela (Prieto et al. 2002), which yielded a half-life of 1.14 days for diazinon.

The malathion concentration half-life ($t_{1/2}$) was 0.87 days. Similar results were obtained for malathion in studies carried out in must and grape wine (Ettiene et al. 1997) with half-lives of 0.50. In guava crops, half-life of 0.29 days were reported (Sánchez et al. 2005).

The parathion concentration half-life ($t_{1/2}$) was 1.01 days. Different results were obtained in persistence studies done in must and grape wines: 0.50 days (Ettiene et al. 1997). This is due to the degradation of pesticide through the way of the grape in the plant to the final obtention of must. Were reported $t_{1/2}$ for parathion in tomatoes of 1.14 days (Prieto et al. 2002). This shows that this pesticide can act as a systemic and contact depending on the crop. The linear behavior of dissipation in both cases can be due to the chemical mechanism of pesticide degradation, what could be proved by tomatoes research that has been done on similar pesticides.

Table 2 shows approximate waiting times to reach Maximum Residue Limits (MRLs) (EAO/OMS 1993), after one application of the organophosphorus pesticides diazinon, malathion and parathion in green onion. The waiting times were higher for diazinon and parathion; 6 days, as opposed to 4 days for malathion, establishing a comparision with MRLs, it is possible to establish that by assuming the conditions of study in this work, six days after the application of the commercial formulations, the residual concentration of diazinon and parathion in green onion were under the MRLs, whereas in the malathion case, 4 days after the application, its concentration was lower to MRLs.

Table 2. Approximate waiting times for residues to reach Maximum Residue Limits (MRLs) after one application of organophosphorus in green onion (*Allium fistulosum* L.)

Pesticide	Application rate (g/L)	Residues (µg/g)	K (days^{-1})	$t_{1/2}$ (days)	MRLs codex tolerance (mg/Kg)	Approximate waiting time (days)
Diazinon	3.6	8.88	-0.67	1.03	0.05	6
Malathion	5.7	16.12	-0.79	0.87	0.50	4
Parathion	3.0	10.50	-0.69	1.01	0.05	6

MRLs: Maximum Residues limits recommended by FAO/WHO

The general manufacturer's recommendations for diazinon and malathion is to apply 15 days prior to harvest and 30 days for parathion. This investigation indicates that at the thirteenth day after application, diazinon and parathion residues not exist. These can not cause health damage to the consumer if the vegetables are consumed before harvest time. On the other hand, the widespread use of these pesticides can cause the residues to remain in the environment. This study shows that the diazinon and parathion concentration evaluated in green onion was below the Maximum Residue Limit at day 11.

A statistical analysis was performed to study the effect of pesticide concentrations in green onion and their dissipation over time (Table 1). This analysis establishes that there are significant differences ($P<0.05$) between pesticide concentrations in green onion crop and their dissipation time.

Generally, contact pesticides have short persistence periods in plants and in the environment (Cremlin 1995, Liapis et al. 1994). However, the results yielded in our investigation show that the organophosphorus pesticides present different behaviors and dissipation times amongst themselves.

The residue variations in green onion tissue after the initial application may be attributed to its morphological characteristics and its biochemical constitution. Similar results were reported in peaches spiked with diazinon, by the twentieth day some quantities of the pesticide were able to move systematically. The residues were higher in leaves than in the fruit (Sanghi et al. 2001). Diazinon, malathion and parathion are accounted for as non-systemic pesticides (Sanghi et al. 2001).

LITERATURE CITED

Al-Samariee A I, Shaker K A M, Al-Bassomy M A (1988) Residue levels in three organophosphorus insecticides in sweet pepper grown in commercial greenhouse. Pestic Sci 22:189-194

Cremlin R (1995) Plaguicidas modernos y su acción bioquímica. Editorial Limusa, México

EAO/OMS (1993) Codex Alimentarius. Residuos de plaguicidas en alimentos. Programa conjunto FAO/OMS, sobre normas alimentarias del Codex Alimentarius. Vol 2

Ettiene G, Prieto A, Medina D, Buscema I, Sandoval L, Unda L (1997) Residues of organophosphorus pesticide in must and grape wine. Rev Téc Ing Univ Zulia 20:223-230

Farazdaghi H and Harris P M (1968) Plant competition and crop yield. Nature 217:289-290

Holland P, Mc Naughton D, Malcom C (1994) Multiresidue analysis of pesticides in wines by solid-phase extraction. J AOAC International 17:79-86

Liapis K S, Miliadis G E, Aplada-Sarlis P (1994) Persistence of monocrothophos residuos in green house tomatoes. Bull Environ Contam Toxicol 53:303-308

Miller J C (1993) Estadística para la química analítica. Addison-Wesley Iberoamerican, Wilmington, Delaware

Podhorniak V L, Negron J F, Griffith F D Jr (2001) Gas chromatography with pulsed flame photometric detection multiresidue-method for organophosphate pesticide and metabolite residues at the parts-per-billion level in representative commodities of fruits and vegetables crop groups. J AOAC International 84:873-890

Prieto A, Molero D, González G, Buscema I, Ettiene G, Medina D (2002) Persistence of methamidophos, diazinon and malathion in tomatoes. Bull Environ Contam Toxicol 69:479-485

Sánchez Jesús, Ettiene Gretty, Buscema Ignacio y Medina Deisy (2005) Persistencia de los insecticidas organofosforados malathion y chlorpiryphos en guayaba (*Psidium guajava* L.) Rev Fac Agron (LUZ) 22:75

Sanghi R, Tewari V (2001) Monitoring of pesticides residues in summer fruits and vegetables from Kanpur, India. Bull Environ Contam Toxicol 67:587-593

Seber G A F and Wild C J (1989) Nonlinear regression. Wiley Inter Science, New York

Tsakiris I, Toutoudaki M, Nikitovic D, Danis T, Stratis I, Tsatsakis A (2002) Field study for degradation of methyl parathion in apples cultivated with integrated crop management system. Bull Environ Contam Toxicol 69:771-778