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Level and Fate of Etoxazole in Green Bean (*Phaseolus vulgaris*)

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Abstract The objective of this study was to estimate the total etoxazole residues balance (residue in pods, leaves and soil under the treated plant) in green bean and for identification of the degradative metabolites of etoxazole in soil under the treated plant. The results showed half life ($t_{1/2}$) values of 3.13, 2.73 and 2.11 days for etoxazole in green bean pods, leaves and soil, respectively. According to the maximum residue limits (MRL) the pre harvest intervals (PHI) of etoxazole on green bean pods was 4-days after the treatment. The results of GC–Ms analysis of soil extracts under the treated plant showed that, at zero time unchanged etoxazole was found. The proportion of etoxazole in soil extracts detected decreased with the time. GC–Ms analysis of soil extracts show the presence of compound having the formula of $C_{14}H_{23}NO_2$ which was suggested to be 2-amino-2(4-tert-butyl-2-ethoxyphenyl) ethanol. The other founded compound has the formula $C_{13}H_{18}O_3$ and suggested to be 4-tert-butyl-2-ethoxybenzoic acid.

Keywords Etoxazole · Residues · Soil · Green bean

Since most organic pesticide chemicals are by their very nature toxic, and since many of them degrade to toxic materials, it is important to know their transitory and ultimate fate, both in amount and composition, in or on plants (Neidert and Saschenbreker 1996). Although most insecticides are subject to weathering and other losses under field conditions, many of the synthetic organic insecticides persist on or in the commodity in decreasing amounts for remarkably long periods of time. Nearly all these chemicals are readily soluble in plant oils and waxes; this common property places them all under suspicion as food contaminants (Ripley and Edgington 1983). If residues of these insecticides remaining on food crops at harvest are above the safe level, they may cause persons consuming such food to be poisoned. Pesticides reach the soil by direct or indirect way then those chemicals are exposed to the degradation by either chemical/or microbial pathways involved with the soil system (Gruzdyev et al. 1983). It is well known that the persistence of a pesticide in soil depends upon physical and chemical properties of the soil as well as certain environmental conditions such as temperature and the pesticide itself (Hirahara et al. 1997). The present work was initiated for (1) estimation of the total etoxazole residue balance (residue in pods, leaves and soil under the treated plant) in green bean after plant treatment at the recommended dosage for pest control, (2) identification of the degradative metabolites of etoxazole in soil under the treated plant.

Materials and Methods

Green bean (*Phaseolus vulgaris*) was planted at El-Menofiya Governorate, Egypt on 26 February 2005 in plots 1/100 of feddan each (1 feddan = 4,200 m^2). The plots received the normal agronomic practices through the experimental period. Treatment was carried out by knapsack sprayer equipped with one nozzle. The commercial formulation Baroque SC (10% etoxazole) was used. Three randomized plots were treated on 11 May 2005 by etoxazole at the maximum dose recommended by the manufacturers (4 g a.i. per feddan) and one untreated plots was left to serve as control. The amount

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of formulated pesticide required for 1 feddan was diluted in 400 L of water. There was no rainfall at any time during the experimental period. The average daily temperature during the experiment was from 19 to 27°C. Sampling was performed by randomly collecting from various places of the experimental plots according to the FAO/WHO (1986) recommendations. Three replicate samples (about one kg each) were collected from pesticide treated green bean pods, the leaves of the treated plant and the soil under the treated plant. Samples were taken 2 and 24 h after the pesticide application. Subsequent samples were taken 3, 6, 9, 12, 15 and 21 days after treatment. During experiment, a control sample was taken in each sampling time. Immediately after collecting the samples, the samples were homogenized and subsampling was done where three representative samples of 50 g were taken. Samples was then placed into polyethylene containers and frozen at −20°C. The freezed subsamples were left to reach room temperature. Plant samples were then placed in the blender cup with 150 mL of acetone and blended for 3 min, and then the extract was filtered through dry pad cotton into a graduated cylinder. The filtrates were transferred into 250 mL round bottom flask and concentrated to remove the acetone with a rotary vacuum evaporator at 35°C. The concentrated solution was transferred into 500 mL separatory funnel and it was shaken with 100 mL of 5% aqueous sodium chloride solution and 80 mL of dichloromethane for 5 min. The dichloromethane layer was drained. The aqueous phase was then re-extracted with an additional 50 mL of dichloromethane. The combined two dichloromethane layer were dried with anhydrous sodium sulfate and concentrated to dryness with a rotary vacuum evaporator at a rate below 35°C. 50 g of the soil sample was shaken mechanically with 200 mL of acetone: water (80:20 v/v) for 1 h in 500 mL stopper conical flask. The extract was carefully decanted and filtered through a clean pad of cotton. 150 mL from the filtrate was concentrated by using a rotary vacuum evaporator on water bath set at 35°C to remove acetone and then extracted twice by 100 mL dichloromethane. The combined dichloromethane was dried through anhydrous sodium sulfate and then evaporated near dryness at 35°C using a rotary vacuum evaporator. The plant and soil extract was then cleaned up using a chromatographic column (1.5 cm i.d. × 40 cm) prepared by adding a plug of glass wool, 6 g activated florisil (60–100 mesh) and 2 g of anhydrous sodium sulfate. The column was pre washed with a bout 40 mL of n-hexane and then the extract was eluted with 170 mL of n-hexane: acetone (97:3 v/v). 150 mL of the elute was collected after discarding the initial 20 mL. The collected elute was concentrated to dryness with a rotary vacuum evaporator at a rate below 35°C. The obtained residue was dissolved in a proper volume of acetonitrile for HPLC determination. Aliquot of soil extract without clean up was subjected to GC–MS analysis for identification of degradative metabolites. The rate constant (k) and half-life ($t_{1/2}$) value was calculated from the first–order rate equation as following

$$C_t = C_0 e^{-kt}$$

Where C_t is the concentration of pesticide at any time t, C_0 is the initial concentration (both concentrations expressed in mg/kg) and k is the rat constant ($days^{-1}$). The half-life ($t_{1/2}$) was determined from the equation

$$t_{1/2} = \ln 2/k.$$

Estimation of etoxazole residues were preformed by Agilent 1100 HPLC equipped with diode array detector. The column was Zorbax SB C18 (150 mm × 4.6 mm i.d. × 5 μm film thickness) and the mobile phase was (methanol/acetonitrile/water) (45/40/15 v/v/v). The flow rate used was 0.8 mL/min., and the injection volume was 20 μL. At this condition the retention time of etoxazole was 5.8 min. GC–MS analysis were performed with an Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector with a direct capillary interface and fused silica capillary column HP-5MS (30 m × 320 μm × 0.25 μm film thickness). Helium was used as carrier gas at approximately 1.0 mL/min. The solvent delay was 3 min. A 1 μl sample was injected into the GC using splitless mode. The injector was operated at 280°C. The column temperature was maintained at 150°C for 3 min and the programmed at 30°C/min to 260°C and held to 5 min. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 320°C and the quadruple temperature 150°C. The electron multiplier voltage was maintained 1,050 v above auto tune. Untreated samples of green bean pods, the leaves of the plants and soil were spiked with known amounts of etoxazole prior to extraction and cleaning up for recovery tests. These samples were passed through the entire process of extraction then cleaned up and analyzed as previously described. Recovery assays were performed in the 0.1–1 mg kg^{-1} range. At each fortification level, three replicate were analysed. The quantification of recovery was carried out with standard dissolved into pure solvent. Results of recovery are presented in Table 1.

Data were statistically evaluated by one-way analysis of variance (ANOVA). All statistical analyses were done using the statistical package for social sciences (SPSS 16.0) program.

Results and Discussion

Data in Table 2 demonstrated the initial deposits, dissipation rate and half life time periods of etoxazole in/on green

Table 1 Recoveries and relative standard deviations for etoxazole on green bean pods, leaves and soil at various fortification level

Fortified level (mg/kg) (n* = 3)	Green bean pods		Leaves		Soil	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
0.1	87	4.9	80.2	0.8	94.6	4.8
0.5	91	3.8	85.3	2.1	96	7.2
1	85	5.3	89	5.3	89.9	3.2

n* = number of samples

bean pods, leaf and soil under the treated plant. The average initial deposits of etoxazole (2 h after application) were 2.33 ± 0.15, 18.05 ± 0.20 and 1.32 ± 0.03 mg/kg, in pods, leaf and soil, respectively. The etoxazole residues were decreased with the time. The residues amount decreased to 1.66 ± 0.07 mg/kg, in green bean pods within the first 24 h after application.

Following that period, etoxazole residues in/on green pods decreased to 1.05 ± 0.07, 0.72 ± 0.05, 0.54 ± 0.01, 0.29 ± 0.008, and 0.15 ± 0.07 kg/mg, at 3, 6, 9, 12 and 15 days after spraying, respectively. Samples taken 21 days after treatment contained no detectable amount of etoxazole (below the detection limit 0.02 mg/kg) under the analytical conditions used. The residues of etoxazole was dissipated in green bean pods and leaves to undetectable limits twenty-one days after plant treatment, while it reach to 0.03 ± 0.008 kg/mg, in soil. The dissipation of the pesticide residues in/on crops depends on environmental condition, type of application, plant species, dosage, and interval between application, the relation between the treated surface and its weight and living state of the plant surface, in addition to harvest time (Khay et al. 2008; Cabras et al.1990). The results also showed half life ($t_{1/2}$) values of 3.13, 2.73 and 2.11 days for etoxazole in green bean pods, leaves and soil, respectively. As shown in Table 2 the maximum allowable concentration of etoxazole in green bean pods was 1.00 mg/kg, as adopted by FAO/WHO Codex Alimentarious commission (2009). It can thus be concluded that the pre harvest intervals of etoxazole on green bean pods was 4-days after the last treatment. The results of GC–Ms analysis of soil extracts under the treated plant showed that, at zero time unchanged etoxazole was found. The proportion of etoxazole in soil extracts detected decreased with the time. GC–Ms analysis of soil extracts show a compound have molecular ion peak ($M^{+\cdot}$) at m/z 238 and fragment 224, 216, 207, 198, 191, 178, 166, 159, 151, 143, 135, 124, 104, 97, 85, 78, 71, 64 and 57 which corresponding to the formula $C_{14}H_{23}NO_2$ which suggested to be 2-amino-2(4-tert-butyl-2-ethoxyphenyl)ethanol, which is the major degradates of etoxazole in soil. The other founded compounds are of M-2 at m/z 220 and fragment 205, 189, 183, 177, 167, 161, 155, 145, 139, 133, 127, 119, 111, 105, 97, 91, 81, 73, 67, 57, and 51 which corresponding to formula $C_{13}H_{18}O_3$ and suggested to be 4-tert-butyl-2-ethoxybenzoic acid which is the miner intermediate degradates of etoxazole in soil.

Table 2 Dissipation of etoxazole residues (mg kg^{-1} ± SD) in/on green bean pods, leaf and soil

Time (days)	Green bean pods		Leaves		Soil	
	Residue level mean ± SD	Dissipation %	Residue level mean ± SD	Dissipation %	Residue level mean ± SD	Dissipation %
Zero	2.33 ± 0.15	0.00	18.05 ± 0.20	0.00	1.32 ± 0.03	0.00
1	1.66 ± 0.07	28.75	13.97 ± 0.21	22.60	1.14 ± 0.01	13.69
3	1.05 ± 0.07	54.93	8.63 ± 0.13	52.18	0.99 ± 0.007	25.0
6	0.72 ± 0.05	69.09	4.32 ± 0.04	76.06	0.66 ± 0.03	50.0
9	0.54 ± 0.01	76.82	2.55 ± 0.0.8	85.87	0.45 ± 0.04	65.90
12	0.29 ± 0.008	87.55	1.515 ± 0.14	91.60	0.21 ± 0.02	84.09
15	0.15 ± 0.07	93.56	0.75 ± 0.05	95.84	0.15 ± 0.01	88.63
21	ND*	–	ND	–	0.03 ± 0.008	97.72
MRL	1		–		–	
k ($days^{-1}$)	0.221		0.253		0.327	
$t_{1/2}$(days)	3.13		2.73		2.11	

* not detectable

References

Cabras P, Spanedda L, Cabitza F, Cubeddu M, Martini M, Brandolini G (1990) Pirimicarb and its metabolites residues in lettuce. Influence of cultural environment. J Agric Food Chem 38(3):879–882

FAO/WHO (1986) Recommended methods of sampling for determination of pesticide residues, vol. VIII, pt VI, 2nd edn

FAO/WHO (2009) Codex Alimentarious commission, pesticide residues in food. Maximum residues limit

Gruzdyev G, Zinchenko V, Kalinin V, Slovtsov R (1983) The chemical protection of plants. Mir Publishers Inc, New York

Hirahara Y, Naramuro K, Sayato Y (1997) Studies on behavior of decomposition of pesticides in environment. Japan J Toxicol Environ Health 43(4):221–229

Khay S, Choi J, Abd El-Aty M (2008) Dissipation behavior of lufenuron, benzoyphenylurea insecticide, in/on Chines cabbage applied by foliar spraying under greenhouse condition. Bull Environ Contam Toxicol 81:369–372

Neidert E, Saschenbreker P (1996) Occurrence of pesticide residues in selected agricultural food commodities available in Canada. J Assoc Off Anal Chem 79:549–566

Ripley B, Edgington L (1983) International and external plant residues and relationships to activity of pesticides. Plant protection for human welfare, 10th. International congress of plant protection (Croydon England British Crop Protection Council) 2: 545–553