Bull Environ Contam Toxicol (2011) 87:703–707
DOI 10.1007/s00128-011-0403-z

Residue Dynamics of Tebuconazole and Quinalphos in Immature Onion Bulb with Leaves, Mature Onion Bulb and Soil

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Received: 12 May 2011 / Accepted: 2 September 2011 / Published online: 16 September 2011

Abstract Residue persistence of tebuconazole and quinalphos in immature onion bulb with leaves (spring onion), mature onion bulb and soil was studied following their spray applications 3 times. The applications were untreated control; tebuconazole @ 187.5 and 375 g a.i. ha^{-1}; quinalphos @ 300 and 600 g a.i. ha^{-1}. Initial residue deposits of tebuconazole in immature onion bulb with leaves from the two treatments were 0.628 and 1.228 mg kg^{-1}. The residues of tebuconazole dissipated with the half-life of 5 and 7.7 days. The safe pre-harvest intervals (PHI) for consumption of immature onion bulb with leaves were 16 and 35 days, respectively. Initial residue deposits of quinalphos in immature onion bulb with leaves from the two treatments were 0.864 and 2.283 mg kg^{-1}. Loss of quinalphos residues from immature onion bulb with leaves was very fast. The residues dissipated with the half-life of 1.7 and 2.6 days and the required PHI was 5 and 11 days, respectively. At harvest mature onion bulbs were free from residues of both tebuconazole and quinalphos.

Keywords Half-life · Onion · Pre-harvest interval · Quinalphos · Tebuconazole

Onion is an important vegetable consumed not only in India but all over the world. India is world's second largest producer of onion. This crop is prone to various insects and diseases. Tebuconazole ((*RS*)- 1-(4-Chlorophenyl)- 4,4-dimethyl- 3-(1,2,4-triazol-1-ylmethyl) pentan- 3-ol), a triazole broad spectrum fungicide with special strength against Alternaria spp., Blumeriella spp., Botryosphaeria spp., etc. It gives good control of purple blotch disease of onion (Wickramaarachchi et al. 2004). Purple blotch infection often follows injury caused by germinating *Botrytis* spores (small, whitish, sunken spots), thrips, wind-blown soil or pollution. Individual leaves are more susceptible to infection as they age and young emerging leaves become more susceptible as bulbs mature (Anonymous 2007). Folicur, a formulation of tebuconazole reduced the incidence of white rot by almost 50% in transplanted onions (Jaime et al. 1999). Tebuconazole and benomyl have been reported to be effective for the control of fusarium basal rot (Soares and Kurozowa 1998) and rust (Koike et al. 2001) of garlic.

Thrips are one of the major pests of onion, can ruin the crop completely. They cut the epidermis of leaves or stems and suck the plant sap. This results in white silvery blotches on deformed leaves. Quinalphos (O,O-diethyl Oquinoxalin- 2-yl-phosphorothioate) is used as a selective insecticide to control thrips with high efficiency. Quinalphos is a non-systemic, synthetic organophosphate, acaricide, acting as a cholinesterase inhibitor. The major use of quinalphos in farming is to protect corn, cotton, and fruit trees against insects (Sameer and Muniswamy 2010). Quinalphos is a broad spectrum insecticide with translaminar activity. After spraying on leaf surface, one-third is absorbed by leaf and penetrates into the plant due to translaminar action. Quinalphos persists on the plant surface for about 2 weeks and hence give better control of sucking and chewing insect pest. Both quinalphos and tebuconazole are important pesticides in onion cultivation. Onion is used world over as spring onion (immature onion with leaves) and as onion bulbs. This experiment was therefore carried out to evaluate the residue persistence of

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quinalphos and tebuconazole individually in immature onion with leaves, mature onion bulb and in soil after harvest.

Materials and Methods

Tebuconazole (99.5% purity) and its formulation, Folicur 250 EC were obtained from M/S Bayer Crop Science Limited (Mumbai, India). Reference standards of quinalphos, (99.3%, purity) were obtained from Sigma-Aldrich, Germany. Quinalphos formulation, Vazra 25 EC was obtained from Cheminova India Ltd. All reagents and solvents used were of analytical grade. The stock solution of quinalphos and tebuconazole was prepared at 1,000 mg mL^{-1} in analytical grade acetone. Working standards were prepared with suitable dilutions. All other chemicals used were of analytical grade.

The residue study of tebuconazole and quinalphos in onion was carried out in randomized block designs (RBD) at the experimental farm of Indian Institute of Horticultural Research, Bangalore. Tebuconazole (Folicur 250 EC) spray application was given 3 times and at 2 concentrations, i.e. @ 187.5 g a.i ha^{-1} and 375 g a.i ha^{-1} of the formulated product. The first spray application was given 30 days after transplanting, further 2 sprays were given at 10 days intervals. Quinalphos (Vazra 25 EC) spray application was given to onion crop at 2 concentrations, i.e. standard dose 300 g a.i ha^{-1} and double dose 600 g a.i ha^{-1}. The first spray application was given 30 days after transplanting; further 2 sprays were given at 15 days intervals. Untreated control plots were kept for comparison. For every treatment 5 plots were taken. Residue analysis of both the pesticides was carried out at 0 (within 2 h of the last spray), 1, 3, 5, 7, 10, 15 and 20 days after last/3rd spray.

On every sampling day approximately 500 g immature onion bulb including leaves (spring onion) were harvested from each plot. Samples from all 5 plots were pooled together, cleaned by removing the roots and soil, cut into small pieces and mixed in a homogenizer. At harvest mature onions (2.5 kg) were cleaned up by removing the roots, soil, cut into small pieces and homogenized. Each plot soil samples were collected from 3 × 3 grid with a total of 5 sampling sites. From each treatment soil samples (5 kg) were collected, pooled together, air dried and passed through a 2 mm sieve for analysis.

Residue analysis of tebuconazole in onion was carried out in the following manner. A 25 g portion immature onion with leaves was homogenized with 80 mL acetone in a Waring blender and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed twice with 50 mL acetone and the combined extracts were collected in a 500 mL flask. The acetone was concentrated under reduced pressure in a rotary vacuum evaporator. The aqueous extracts were transferred into a 1 L separatory funnel and diluted with 80 mL of distilled water. The aqueous phase was partitioned into 150 mL dichloromethane: toluene (1:1, v v^{-1}) (50 + 50 + 50) after adding 25 mL saturated sodium chloride solution and dried over anhydrous sodium sulphate. The combined dichloromethane: toluene was concentrated to 5 mL. Clean-up of the samples were carried out with neutral alumina as the adsorbent. In a glass column neutral alumina (5 g) was loaded between two 1 cm layers of sodium sulphate and the column was pre-washed with hexane. The concentrated extracts were passed through the chromatographic column and tebuconazole was eluted with 100 mL of hexane: dichloromethane (1:1, v v^{-1}). The elute was concentrated to dryness and redissolved in 10 mL of distilled acetone for analysis by gas liquid chromatography (GLC). Harvest time mature onion bulb (50 g) was processed in a similar manner A represantative 100 g soil samples in triplicate was taken for analysis and processed in a similar manner like onion described above without any clean-up.

For residue analysis of quinalphos a 25 g portion of representative immature onion bulb with leaves (spring onion) was homogenized in a Waring blender with 80 mL acetone and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed twice with 50 mL acetone and the combined extracts were collected in a 500 mL flask. The extract was evaporated in a flash evaporator and the aqueous fraction was extracted with 50 + 50 + 50 mL dichloromethane after adding 25 mL saturated sodium chloride solution. The combined dichloromethane fraction was dried over anhydrous sodium sulphate and evaporated to 10 mL. To the flask containing dichloromethane fraction 500 mg activated charcoal was added, mixed thoroughly and kept for 30 min with intermittent shaking. Further it was filtered with Whatman filter paper no: 1, concentrated and redissolved with 10 mL acetone for analysis by GLC. Harvest time mature onion bulb (50 g) was processed in a similar manner. A representative 100 g soil samples (3 replicates) were taken for analysis and processed as per the method described above without any charcoal clean-up.

In order to estimate the efficiency of the method, a recovery experiment was conducted by spiking untreated samples (immature onion bulb with leaves, mature onion bulb and soil) with analytical grade tebuconazole @ 0.05, 0.1, 0.5 and 1 mg kg^{-1} (5 replications) and processed as per the analytical method described above. The recovery study of quinalphos was carried out at the spiked level of 0.01, 0.05, 0.1 and 0.50 mg kg^{-1} in immature onion bulb with leaves, mature onion bulb and soil. The residue data was subjected to statistical analysis according to Hoskins (1961) to compute the residual half-life ($t_{1/2}$) and pre-harvest interval. A gas chromatogram (Shimadzu GC-2010)

equipped with flame thermal detector (FTD) was used for analysis of tebuconazole and quinalphos residues in onion and soil. One μL sample was injected with an auto sampler (AOC-20i auto injector). A capillary column, Agilent DB-1 (30 m × 0.25 mm i.d.) was used and the injector was kept in split mode with split ratio of 5. Ultra pure nitrogen was used as carrier gas at a flow rate of 1.0 mL min^{-1}. Hydrogen and air flow rate was maintained as 3 and 145 mL min^{-1}. The oven temperature was initially maintained at 140°C hold 5 min and programmed at 4°C min^{-1} to 250°C with hold time of 5 min and at 10°C min^{-1} to 280°C with hold time of 10 min. Injector and detector temperatures were maintained at 280 and 300°C. Under the above conditions retention times of tebuconazole and quinalphos was 27.54 and 20.6 min, respectively.

Results and Discussion

Recovery study carried out as per the method described above showed that recovery of tebuconazole in immature onion bulb with leaves was in the range of 92.52–93.84%, mature onion bulb, 90.16–92.36% and in soil the recovery was 93.04–94.12%. Recovery of quinalphos from immature onion bulb with leaves was in the range of 89.08–92.42%, mature onion bulb 90.14–91.76% and in soil the recovery was 95.27–98.68%. The results of the recovery study are presented in Table 1.

The residues of tebuconazole were estimated in immature onion (including leaves) over a period of 20 days. Initial residues of tebuconazole in onion from treatments at recommended and double the recommended doses were 0.628 and 1.228 mg kg^{-1}, respectively (Table 2). The residues at recommended dose persisted for 10 days and double dose for 15 days. The residues of tebuconazole dissipated with the half-life of 5 and 7.7 days. Residues of tebuconazole in mature onion and soil at harvest (40 days after the last application) from treatment at recommended and double doses were below the quantifiable limit of 0.05 mg kg^{-1}. The pre-harvest interval calculated based on the MRL/LOQ value of 0.05 mg kg^{-1} showed that for recommended dose of treatment it was 15.9 days, while for double dose it was 34.2 days.

Table 1 Recovery of tebuconazole and quinalphos residues from immature onion bulb with leaves, mature onion bulb and soil at various spiked levels

Tebuconazole				Quinalphos			
Spiked concentration (mg kg^{-1})	Recovery (%) ± SD[a]			Spiked concentration (mg kg^{-1})	Recovery (%) ± SD[a]		
	Immature onion bulb with leaves	Mature onion bulb	Soil		Immature onion bulb with leaves	Mature onion bulb	Soil
0.05	92.66 ± 3.84	91.02 ± 2.62	93.22 ± 2.83	0.01	89.08 ± 2.24	90.14 ± 5.39	95.66 ± 3.58
0.10	92.52 ± 4.32	90.16 ± 5.84	93.04 ± 3.58	0.05	90.50 ± 4.62	91.36 ± 2.15	95.27 ± 4.55
0.50	93.55 ± 2.92	91.85 ± 6.32	93.52 ± 2.84	0.10	91.04 ± 3.11	91.76 ± 6.08	96.33 ± 2.10
1.00	93.84 ± 4.56	92.36 ± 2.71	94.12 ± 3.65	0.50	92.42 ± 2.68	91.55 ± 4.18	98.68 ± 1.84

[a] Average of five replicate analyses ± standard deviation

Table 2 Residues of tebuconazole in immature onion bulb with leaves, mature onion bulb and soil

Days after application	Residues of tebuconazole on onion (mg kg^{-1})								
	Control	Application @ 187.5 g a.i ha^{-1}				Application @ 375 g a.i ha^{-1}			
		R_1	R_2	R_3	Mean	R_1	R_2	R_3	Mean
0	ND	0.648	0.655	0.582	0.628 ± 0.040	1.256	1.094	1.335	1.228 ± 0.123
1	ND	0.443	0.429	0.453	0.442 ± 0.012	0.947	0.901	0.928	0.925 ± 0.023
3	ND	0.385	0.335	0.365	0.362 ± 0.025	0.796	0.786	0.801	0.794 ± 0.008
5	ND	0.309	0.307	0.292	0.303 ± 0.009	0.704	0.659	0.686	0.683 ± 0.023
7	ND	0.258	0.249	0.228	0.245 ± 0.015	0.563	0.577	0.542	0.561 ± 0.018
10	ND	0.102	0.116	0.101	0.106 ± 0.008	0.472	0.461	0.453	0.462 ± 0.010
15	ND	BDL	BDL	BDL	BDL	0.282	0.296	0.272	0.283 ± 0.012
20	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mature onion bulb & soil at harvest	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Limit of Quantification (LOQ)–0.05 mg kg^{-1}, Below detection limit (BDL) < 0.05 mg kg^{-1}

Table 3 Residues of quinalphos in immature onion bulb with leaves, mature onion bulb and soil

Days after application	Residues of quinalphos on onion (mg kg^{-1})								
	Control	Application @ 300 g a.i. ha^{-1}				Application @ 600 g a.i. ha^{-1}			
		R_1	R_2	R_3	Mean	R_1	R_2	R_3	Mean
0	BDL	0.838	0.898	0.856	0.864 ± 0.030	2.21	2.36	2.280	2.283 ± 0.075
1	BDL	0.213	0.203	0.209	0.208 ± 0.005	0.73	0.745	0.737	0.737 ± 0.007
3	BDL	0.083	0.085	0.082	0.083 ± 0.001	0.216	0.201	0.208	0.208 ± 0.008
5	BDL	0.034	0.033	0.035	0.034 ± 0.001	0.122	0.126	0.125	0.124 ± 0.002
7	BDL	0.017	0.017	0.019	0.018 ± 0.001	0.067	0.068	0.064	0.066 ± 0.002
10	BDL	0.01	0.01	0.011	0.01 ± 0.0006	0.053	0.054	0.056	0.054 ± 0.001
15	BDL	BDL	BDL	BDL	BDL	0.028	0.029	0.027	0.028 ± 0.001
20	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mature onion bulb & soil at harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Limit of Quantification (LOQ)–0.01 mg kg^{-1}, Below detection limit (BDL) < 0.01 mg kg^{-1}

The results of the residue study of quinalphos in onion is given in Table 3. Initial deposits of 0.864 and 2.283 mg kg^{-1} of quinalphos was recovered from treatments at 300 and 600 g a.i ha^{-1}, respectively. Dissipation of quinalphos residues from immature onion bulb with leaves was very fast from both the treatments with 90% residue loss after 3 days. The residues dissipated at the half-life of 1.7 and 2.6 days. Based on the persistence study and MRL value of 0.05 (MRL fixed by European Union) the safe pre-harvest interval recommended for consumption of immature onion with leaves from treatment at recommended and double dose were 5 and 10.5 days, respectively. Residues of quinalphos in mature onion bulb at harvest and soil from treatment at both doses were below the quantifiable limit of 0.01 mg kg^{-1}.

Supervised field trials of tebuconazole in onion have been conducted in countries like Australia, Brazil, France Germany etc. In all such studies the residues were estimated in onion bulb and leaves (green material) separately. Though one study conducted in France has reported residues in the whole plant material. In India the immature onion bulb along with leaves (popularly known as spring onion) is very widely used as a vegetable. Therefore our study has been conducted to evaluate residues in immature onion along with leaves as well as in mature onion bulb. The study conducted in whole plant material in France showed that 2 applications of EW 250 formulation of tebuconazole applied @ 0.25 kg a.i. ha^{-1} resulted initial residue deposits of 1.3 mg kg^{-1}. The residues reached below the limit of quantification level of 0.05 mg kg^{-1} by 14 days (Anonymous 2008). Similar results were obtained from the present study where three applications of tebuconazole 250 EC applied @ 187.5 and 375 g a.i. ha^{-1} resulted in residue deposits of 0.628 and 1.228 mg kg^{-1} and the residues reached below the LOQ by 15 days. Studies conducted to evaluate tebuconazole residues in bulb and green material separately have reported that the residue level of tebuconazole in onion bulb was always near the LOQ level. The residue levels in the green material were reportedly very high, being 3.2–6.3 mg kg^{-1} (Anonymous 2008).

Information on residue study of quinalphos on onion is not available to our knowledge. However some information is available on residue of quinalphos on other vegetables. Aktar et al. (2008) reported that quinalphos residues in okra from treatment @ 500–1000 g a.i. ha^{-1} required a pre-harvest interval (PHI) of 7 days. Similar results were obtained from the present study where treatment @ 300 g a.i. ha^{-1} required PHI of 5 days, while treatment @ 600 g a.i. ha^{-1} required PHI of 10 days. From the results obtained from the present study it is evident that if tebuconazole is used on onion at the dosage recommended (187.5 g a.i. ha^{-1}) for consumption of spring onion a PHI of 16 days to be observed. If the dosage is doubled the required PHI is 35 days, which means consumption of spring onion is not recommended. If quinalphos alone is used a PHI of 11 days should be observed for consumption of spring onion. But mature onion bulbs were free from residues of both tebuconazole and quinalphos from all the treatments mentioned above.

Acknowledgments The authors thank Director, IIHR, Bangalore and ICAR, New Delhi for providing the facilities for carrying out this study.

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