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Harvest Time Residue of Atonik (Nitro Phenols) in Tomato and Cotton

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Abstract: A field investigation was conducted on tomato and cotton during 2002-2003 at Tamil Nadu Agricultural University, to study the harvest time residue of Atonik (nitro phenolic compound) in soil, leaf and fruit/boll. The mean recovery percent of various constituents of Atonik ranged between 85-97%. The harvest time residual level was found to be below detectable limit and it is safe for consumption. The applied nitrophenols may be degraded by various microorganisms by providing carbon and nitrogen sources or by photo oxidation.

Key words: Atonik, nitro phenolic compound, residue, below detectable limit.

INTRODUCTION

Atonik is an aromatic nitro phenolic compound, which consists of sodium ortho-nitrophenol, sodium para-nitrophenol and sodium nitro-guaiacol as active ingredients. Atonik stimulates plant activity without causing malformation or toxicity to the plants and accelerates the plasma streaming of the cells. Atonik increased the yield, which may be due to increase in the endogenous auxin level by external application[1].

Low molecular weight phenolics are usually not end products, but they undergo high rates of turnover in soil resulting from several reactions of interconversion, conjugation, polymerization and degradation. The decomposition of the phenolic fractions of soil organic matter may also occur[2], thus releasing phenolics into solution. The degradation products are either converted into phenolic radicals and hydroxy-benzoquinones by polyphenol oxidase enzymes and auto oxidation, or oxidised and decomposed to simple molecules such as phenolic acid[3]. The low molecular weight phenolics have been isolated from a variety of soils and their concentrations depend on the extraction procedures, soil types, sampling time, vegetation type, management history and many other factors[4].

Free phenolics in soil are subjected to microbial mediated processes, which accounts for their disappearance[5]. Microorganisms are capable of polymerizing phenolic acids through the activity of the enzyme polyphenol oxidases and peroxidases[3]. When these compounds were added at concentrations $\leq 0.5\ \mu g\ g^{-1}$ of soil, the growth of bacterial and fungal populations was stimulated as well as total soil respiration[4]. Since no information about the harvest time residues of Atonik on tomato and cotton is available, the study was undertaken to assess the residual level after foliar spray of Atonik at various stages of cotton and tomato.

MATERIALS AND METHODS

Field experiments were conducted under irrigated conditions to assess the effect of Atonik in cotton and tomato. The experimental design followed was factorial randomized block design in tomato and cotton with three replications by maintaining a plot size of 4x5 and 4x3 m, respectively.

Treatment details for cotton: Treatment consisted of two factors, stages of application and concentration. Stage was represented as S and concentration as T.

T_1 - Control
T_2 - Seed treatment of Atonik 3 ppm
T_3 - Foliar spray of Atonik 0.1%
T_4 - Foliar spray of Atonik 0.25%
T_5 - Foliar spray of Atonik 0.5%
S_1 - Foliar spray of Atonik during square formation stage
S_2 - Foliar spray of Atonik during square formation and flowering stages
S_3 - Foliar spray of Atonik during square formation, flowering and boll set stages

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Treatment details for tomato: The treatment consists of two factors. Factor 1 – stage of application (S) and Factor 2–concentration of Atonik (T):

T_1 - Control
T_2 - Seed treatment of Atonik 3 ppm
T_3 - Foliar spray of Atonik 0.1%
T_4 - Foliar spray of Atonik 0.2%
T_5 - Foliar spray of Atonik 0.4%
T_6 - Foliar spray of Atonik 0.8%
S_1 - Foliar spray of Atonik during flower bud initiation stage
S_2 - Foliar spray of Atonik during flower bud initiation and fruit set stages

Persistence residues of Atonik in cotton and tomato produces and soil: Leaf, fruit/boll and soil samples were collected from all the concentrations of Atonik sprayed plots and control plot of cotton and tomato, at harvest. The sample was homogenized with a small amount of distilled water. Then it was first extracted with acetone under acid phase by the existence of ortho-phosphoric acid. Acetone was removed and the residue is then extracted with ether. Subsequently, the ether layer containing ortho-nitrophenol (ONP), para-nitrophenol (PNP) and nitro-guaiacol (5NG) was extracted with 0.2N–NaOH–20% NaCl solution. Once again, ONP, PNP and 5NG were extracted with chloroform under the acidic condition by adding ortho-phosphoric acid. The chloroform extract was dried through the column packed with sodium sulfate anhydrous. Chloroform was evaporated until the residue was reduced to 2 mL. The residue was cleaned up by Thin Layer Chromatography (TLC). Then ONP, PNP and 5NG were determined by Gas Chromatography (GC). The heights of the peaks of ONP, PNP and 5NG were measured from the gas chromatogram. The quantities of ONP, PNP and 5NG referring to the calibration curves were determined. In order to modify the determined quantities to those of the sodium salts, multiplication factors 1.16 to PNP and ONP and 1.13 to 5NG were assigned. ONP was quantified using apiezon grease L column at 8.7 min. PNP and 5 NG were quantified using diethylene glycol adipate column at 10.1 min. Chromosorb W, nitrogen and ECD – ^{63}Ni were used as support column, carrier gas and detector, respectively, for the residue analysis. Recovery studies were conducted in leaf, fruit/boll and soil samples by fortifying the samples at 1 μg g^{-1} and quantified as mentioned above.

RESULTS

Fortification study and residue of Atonik in tomato and cotton: The mean recovery was 85.6, 95.61 and 89.82% from leaf, fruit and soil for tomato, respectively, at 2–5 μg level of ortho-nitrophenol (Table 1). It was 89.03, 95.13 and 89.46% for leaf, boll and soil, respectively, for cotton. Similarly a mean recovery of 89.42, 90.51 and 92.63% from leaf, fruit and soil for tomato, respectively, at 2–5 μg level

Table 1: Fortification studies in tomato and cotton with ortho-nitrophenol, para-nitrophenol and 5-nitroguaiacol

		Ortho-nitrophenol (ONP) recovery (%) level of fortification			Para-nitrophenol (PNP) recovery (%) level of fortification			5-nitroguaiacol (5NG) recovery (%) level of fortification		
Crop	Plant Parts/Soil	2 ppm	5 ppm	Mean recovery%	2 ppm	5 ppm	Mean recovery%	2 ppm	5 ppm	Mean recovery%
Tomato	Leaf	85.01	86.19	85.60	87.64	91.20	89.42	92.65	96.43	94.54
	Fruit	94.70	96.53	95.61	92.32	88.70	90.51	98.09	96.15	97.12
	Soil	89.64	90.00	89.82	93.18	92.08	92.63	91.70	95.46	93.58
Cotton	Leaf	87.19	90.88	89.03	93.21	95.87	94.54	97.19	96.41	96.80
	Boll	93.74	96.53	95.13	91.32	89.32	90.32	96.76	98.70	97.73
	Soil	90.40	88.53	89.46	93.49	91.99	92.74	93.82	94.78	94.30

Determinability of the instrument for leaves and bolls of cotton (20 g) for ONP = 0.02 μg g^{-1}, PNP = 0.02 μg g^{-1} and 5NG = 0.010 μg g^{-1}
Determinability of the instrument for leaves and fruits of tomato (20 g) for ONP = 0.02, μg g^{-1}, PNP = 0.02 μg g^{-1} and 5NG = 0.010 μg g^{-1}
Determinability of the instrument for soil of cotton and tomato (20 g) for ONP = 0.02, μg g^{-1}, PNP = 0.05 μg g^{-1} and 5NG = 0.005 μg g^{-1}

Table 2: Effect of Atonik on residue of ortho-nitrophenol (μg g^{-1}) in tomato PKM 1 and cotton

	Tomato				Cotton		
Treatments	Soil	Leaf	Fruit	Treatments	Soil	Leaf	Lint
T_1	BDL*	BDL	BDL	T_1	BDL	BDL	BDL
T_2	BDL	BDL	BDL	T_2	BDL	BDL	BDL
T_3	BDL	BDL	BDL	T_3	BDL	BDL	BDL
T_4	BDL	BDL	BDL	T_4	BDL	BDL	BDL
T_5	BDL	BDL	BDL	T_5	BDL	BDL	BDL
T_6	BDL	BDL	BDL	S_1	BDL	BDL	BDL
S_1	BDL	BDL	BDL	S_2	BDL	BDL	BDL
S_2	BDL	BDL	BDL	S_3	BDL	BDL	BDL

Table 3: Effect of Atonik on residue of para-nitrophenol (μg g^{-1}) in tomato and cotton

	Tomato				Cotton		
Treatments	Soil	Leaf	Fruit	Treatments	Soil	Leaf	Lint
T_1	BDL*	BDL	BDL	T_1	BDL	BDL	BDL
T_2	BDL	BDL	BDL	T_2	BDL	BDL	BDL
T_3	BDL	BDL	BDL	T_3	BDL	BDL	BDL
T_4	BDL	BDL	BDL	T_4	BDL	BDL	BDL
T_5	BDL	BDL	BDL	T_5	BDL	BDL	BDL
T_6	BDL	BDL	BDL	S_1	BDL	BDL	BDL
S_1	BDL	BDL	BDL	S_2	BDL	BDL	BDL
S_2	BDL	BDL	BDL	S_3	BDL	BDL	BDL

Table 4: Effect of Atonik on residue of 5 - nitroguaiacol (μg g^{-1}) in tomato and cotton

	Tomato				Cotton		
Treatment	Soil	Leaf	Fruit	Treatments	Soil	Leaf	Lint
T_1	BDL*	BDL	BDL	T_1	BDL	BDL	BDL
T_2	BDL	BDL	BDL	T_2	BDL	BDL	BDL
T_3	BDL	BDL	BDL	T_3	BDL	BDL	BDL
T_4	BDL	BDL	BDL	T_4	BDL	BDL	BDL
T_5	BDL	BDL	BDL	T_5	BDL	BDL	BDL
T_6	BDL	BDL	BDL	S_1	BDL	BDL	BDL
S_1	BDL	BDL	BDL	S_2	BDL	BDL	BDL
S_2	BDL	BDL	BDL	S_3	BDL	BDL	BDL

* - Below detectable limit.

of para-nitrophenol, whereas in cotton leaf, boll and soil had 94.54, 90.32 and 92.74%, respectively (Table 1). In tomato, the mean recovery of 5-nitroguaiacol from leaf, fruit and soil was 94.54, 97.12 and 93.58%, respectively (Table 1). Mean recovery of 5-nitroguaiacol from leaf, boll and soil of cotton was 96.80, 97.73 and 94.30%, respectively.

The minimum detectable level was 0.02, 0.02 and 0.01 μg g^{-1} for ONP, PNP and 5-NG, respectively, for cotton leaves and bolls as the weight of sample was 20 g (Table 1). The minimum detectable level for tomato leaves and fruits was 0.02, 0.02 and 0.01 μg g^{-1} for ONP, PNP and 5-NG, respectively, for tomato leaves and fruits as the weight of sample were 20 g. The minimum detectable limit for cotton and tomato soil was 0.02, 0.015 and 0.005 μg g^{-1} for ONP, PNP and 5-NG, respectively. The residues of Atonik were at below detectable limit in all the harvested produces including soil of cotton and tomato (Table 2-4).

DISCUSSION

Atonik has ortho-nitrophenol, para-nitrophenol, 5-nitroguaiacol as active ingredients in its composition. Para-nitrophenol (PNP) is a chemical commonly used in the manufacture of pesticides and pharmaceuticals. In addition, PNP is the major metabolite resulting from the microbial degradation of parathion[6] and the lethal dose for humans is reported as 5–10 mg kg^{-1} body weight[7]. Microbial degradation is primarily responsible for the removal of PNP from the environment. Several studies have reported that natural bacteria readily degrade PNP in soil[8], sediment[9], activated sludge[10] and water[9]. In most of these studies, lag phases ranging from 2 to 42 days were followed by periods of faster PNP degradation. Several studies have also examined the degradation of PNP at low concentrations[11], in the presence of inorganic nutrients and by bacteria in granular activated carbon columns[12]. With this knowledge, the tomato and cotton plants were sprayed with different concentrations of Atonik and evaluated for its residual quantity.

Both cotton and tomato had a residue level below the detectable limit for ortho-nitrophenol, para-nitrophenol and 5-nitroguaiacol in soil, leaf and fruit of tomato and soil, leaf and lint of cotton. The Biopesticide and Pollution Prevention Division of USDA had assessed the potential hazards and exposures that might result from the proposed use of Atonik in or on all food commodities and no toxicity end points for dietary, occupational or non-occupational risk characterizations were indicated[13]. The results of the present investigation are in line with the above finding.

The aerobic fission of aromatic nucleus was carried out by various mono and dioxygenase enzymes. Before ring cleavage, the aromatic ring is being hydroxylated, followed by ortho or meta cleavage[7]. Compared to pure phenol, substituted phenolic compound like Atonik had faster degradation. It may be due to the presence of relatively inert group in ortho or meta position (methyl or nitro group).

Pseudomonas partially degraded PNP or had a higher rate of incorporation of PNP into cellular biomass. Catechol is believed to be an oxidation product occurring

in the chemical pathway for the microbial degradation of PNP. Catechol is known to be common substrate for ring opening enzymes and subsequent degradation to carbon dioxide by microbial catabolic enzymes[12]. The carbon skeleton of phenol ring may be used as carbon source for the microorganism[7]. Atonik, being a nitrophenolic compound, has both C and N in the structure, therefore favour the easy degradation by microorganisms.

Coriolus versicolor produces laccase, which is a multicopper blue oxidase capable of oxidising ortho and para diphenols and anilines by oxidation and cross-linked in the presence of guaiacol[14]. Since Atonik has guaiacol as one of its constituents, it may facilitate the degradation of PNP and ONP by that enzyme. Hence, it is clear that the applied nitrophenols are degraded by various organisms and by various metabolites, but the degradation requires an initial lag period as reported by Wiggins and Alexander[11] but this, apparently did not affect the active period of degradation[15].

From the result of the experiment, it is evident that the residue is below the threshold level of contamination and it is safe for consumption. The chemical may also enhance the soil microorganism population by providing carbon and nitrogen sources.

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