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Original Research Article

Microbial Degradation and Residue Analysis of Atrazine in Open Field and Indoor Cultures

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Abstract

The present study aimed to investigate Atrazine residues and biodegradation by naturally occurring bacteria in open field and indoor cultures of corn and cucumber amended with Atrazine. Three soil types were employed to evaluate the effect of soil characteristics including the indigenous degraders on the fate of Atrazine different ecosystems. In the field experiment, corn grew well to its full cycle (120 days) in the presence of the Atrazine at its recommended dose (RD). Cucumber plant grew only to its full cycle in the presence of 0.5 XRD of the herbicide in soil^{E} while it did not grow in soil^{H} at any concentration of the tested herbicide with no Atrazine residues were detected in any of cucumber fruit. Higher averages of Atrazine residues (0.232 and 0.140 ppm after 25 and 50 days respectively) were detected in soil^{M} during outdoor corn cultivation compared to indoor cultivation. However, Atrazine was not detected outdoor after 75 days of its application. During indoor cucumber cultivation much lower Atrazine residue averages were detected compared to the outdoor corn cultivation. Results clearly confirmed that soil characteristics, plant type as well as environmental conditions controlled biodegradation and residue levels of Atrazine in the soil.

Keywords: Atrazine, Bacteria, Biodegradation, Growth, Open field, Residue levels

INTRODUCTION

Pesticides and their metabolites considered among the top twenty priority pollutants due to their potential impacts on the environment, wildlife, and human health. Pesticides applied directly to the aboveground parts of the plants or the pests end up in the soil in contact with soil organisms. The impacts of a wide range of pesticides on soil organisms, soil food webs, and biological processes in soil are highly variable, dependent on the type/ amount of the pesticide, soil environment and the biotic groups examined. The impact extended to non-target organisms and has disruptive effects on the biological regulatory capacity of the soil community, with damaging consequences for all soil functions (Kibblewhite *et al.,* 2007). Constant crop yields despite high use of pesticides and synthetic fertilizers recorded worldwide in the past 40 years is a result of pesticide residues in soil which is not only reducing harvest yields, but also increasing the need for synthetic fertilizers, thereby raising costs for farmers and contributing to environmental pollution (European Parliament-EU, 2010). Synthetic fertilizers especially nitrogenous compounds affect soil ecosystem functions through the impact on primary productivity and reduction in the quantity of soil organic matter input (Potera, 2007; Moeckel, 2008). Similarly, the widespread use of synthetic pesticides has several limitations and serious harmful effects on the environment and human health (Alert, 2009). This promotes a serious need for a more environmentally safe practice particularly for plant protection known as environment-friendly approach (Hobbs *et al*., 2008). Using a bioremediation technique should lead to an almost complete disappearance of pesticide pollution (Gregoire *et al*., 2009).

Although prohibited in the European Union in 2004 (Ackerman, 2007), Atrazine, {2-chloro-4-(ethylamine)-6-(isopropyl amine)-s-triazine} is a widely used herbicide

worldwide to stop pre- and post-emergence broadleaf and grassy weeds in major crops such as corn, sorghum, sugarcane and pineapples as well as chemical fallows, grassland, macadamia nuts, conifers and industrial weed control (Briggs, 2002). Atrazine has been reported to have long-term reproductive and endocrine-disrupting effects as well as being a probable human carcinogen and has epidemiological connection to low sperm levels in men (Ackerman, 2007). It may be dangerous, with implications for human birth defects, low birth weights and menstrual problems even at concentrations meeting U.S. federal standards (Duhigg, 2009). It was confirmed that even at low doses Atrazine can increase health risks (Randall-Amster, 2010).

Although toxicological effects of Atrazine on humans are weaker than other chlorinated herbicides, severe environmental problems emerged due to their persistence in soils (Dossantes *et al*., 2004). Also being a non-polar compound and moderately retained by the polar soil colloids, it can be washed out from the root zone into ground water resources, especially if applied prior to heavy rainfall or irrigation event (Randall-Amster, 2010).

Fate of Atrazine and other pesticides in soil is determined by a combination of many different factors including sorption/adsorption to soil organic matter and clays, photo-degradation, volatilization to the atmosphere, uptake by plants, transport via erosion runoff to surface water or leaching to the groundwater, chemical and/or microbiological degradation as well as environmental conditions (Swann and Eschenroeder, 1983; Aislabie and Lloyd-Jones, 1995; Wang and Liu, 2007; Shiferaw *et al.,* 2009). Biodegradation of pesticides/herbicides is greatly influenced by soil factors like moisture, temperature, pH, and organic matter content, in addition to microbial population and pesticide solubility which provide congenial environment for the break down or retention of any pesticide added in the soil. Most of the organic pesticides degrade within a short period (3-6 months) under tropical conditions (www.AgriInfo.in 2009).

Atrazine degrades in soil primarily by the action of microbes (bacteria, fungi, and actinomycetes). The best-characterized organisms that degrade Atrazine are of *Pseudomonas spp* and *Bacillus spp* as well as a number of other bacteria (Zeng *et al*., 2004; Chelinho *et al*., 2010). *Pseudomonas spp.* Are well known for their biodegradative capabilities against a wide range of recalcitrant environmental pollutants (Chelinho *et al*., 2010). Enhanced degradation due to microbial adaptation to Atrazine, occurred in some fields when applied repeatedly and used for a long time leading to a reduction in herbicidal effectiveness (Krutz *et al*., 2008).

Atrazine showed high persistence compared to other pesticides leading to high residual levels in contaminated soils. For example, Atrazine has higher stability and affinity for binding to soil particles than Malathion. They showed average degradation rates of 0.0102 and 0.1053/day corresponding to 68.1-day and 6.6-day half-life respectively where 78% of Atrazine was remained in soil after 25 days of application compared to 8% only of Malathion at the same period. These results emphasized on care needed when using persistent pesticides to control weeds in soil (Chirnside *et al*., 2009; Al-Wabel *et al*., 2010). In another study, about 25% of the total applied Atrazine remained in the soil at the end of the simulation period (120 days), almost in the solid phase (Ouyang *et al*., 2010). Moreover, it was reported that co-application of glufosinate with nitrogen may increase Atrazine persistence under field conditions thereby extending Atrazine residual weed control in adapted soils (Zablotowicz *et al*., 2008).

The present study aimed to investigate Atrazine residues and biodegradation by naturally occurring bacteria in open field and indoor cultures of corn and cucumber amended with Atrazine.

MATERIALS AND METHODS

Residual concentrations and bioremediation of the herbicide Atrazine were investigated in sandy-loam soils planted with corn from two completely different environments (Egypt and Saudi Arabia). Cucumber was planted under the same conditions in a comparative study.

Pesticide: Atrazine (80% Active Ingredient)

Atrazine was selected based on its common use for the protection of corn crop from weeds and herbs, which is recommended by Agricultural Ministries of the two countries. Technical Atrazine is colorless crystalline powder with density of 1.187 g/cm^3, low vapor pressure (40 n Pa at 20°C) and melting point (175-177°C). It is readily soluble in dimethyl sulfoxide (183 g/liter), moderately soluble in methanol (18 g/liter), diethyl ether (12g/liter), chloroform (52 g/liter), and ethyl acetate (28 g/liter) while slightly soluble in water (30 mg/liter). It is stable in the dry state, but hydrolyzed to the inactive herbicidal2-hydroxy analogue in acid or in alkaline solutions and more slowly in neutral aqueous solutions, even at elevated temperatures (Tomlin, 2006). (Figure 1)

Soil

Loam Sandy soil samples with different physical, chemical, mechanical and biological characteristics were collected from three spatially and environmentally different ecosystems (Hada Al-Shame area, Saudi Arabia ($Soil^H$), Abu El-Matameer area, El-Behaira Governorate ($Soil^M$) and El-Sharqia Governorate, Egypt ($Soil^E$)) and

Figure 1. Atrazine Chemical Structure

investigated in a comparative study for the fate of Atrazine which was expected to be different. Soil samples were collected from the top layer of the soil profiles (0-20 cm), passed through a 2-mm sieve, and stored in polyethylene bags until used. Abu El-Matameer soil was used in the outdoor (open environment) field culturing of corn while Hada Al-Shame and El-Sharqia soils were used in indoor (green house) culturing of corn and cucumber all of which were subjected to Atrazine as an effective herbicide.

Plants

Corn is grown throughout Egypt and in some areas of Saudi Arabia along all springtime in the open field. Maize is most sensitive to drought at the time of silk emergence, when the flowers are ready for pollination. Weeds compete with the crop for moisture and nutrients, making them undesirable. Cucumbers are very versatile and can been grown easily both indoors and outdoors. Greenhouse cucumber production is very popular in many areas of the world. The cucumber is a warm season crop (80 to 85^{o}F), very sensitive to cold and should not be planted until all danger of frost has passed and the soil has warmed sufficiently. It requires plenty of sunlight (full direct sun, at least 6 hours) as well as rich, well-drained soil with pH between 5.5-7.0 and high organic matter content. This crop requires a continuous supply of moisture during the growing season, with the critical time occurring at fruiting.

Application Experiments

In a long term comparative study, Atrazine was applied during cultivation of corn (indoor and outdoor) and cucumber (indoor) using different soil composition and environmental conditions. The main aim was to investigate the effect of variables such as soil characteristics, crop type, indigenous soil microorganisms and environmental conditions (temperature and water availability) on the fate of Atrazine in the soil.

Open Field (Outdoor) Corn Cultivation

Fate of Atrazine, the widely used herbicide in corn production, was traced in agricultural corn field with a long history of Atrazine application. This field lies at Abu El Matameer area, El-Behaira Governorate, Egypt (soil^{M}). The investigated field was cultivated under a crop rotation (corn-wheat) during the past 10 years. Atrazine was applied as pre-emerge dose at the rate of 750g/200-600L water/Fadden or 750g/3-4K water/Fadden both of which are recommended by the Egyptian Ministry of Agriculture for controlling weeds and pests in corn plant. Subsurface (0-20 cm depth) soil samples (500 g each) were collected at different time intervals (25, 50 and 75 day) along cultivation period (≈120 day).Samples were transported to the laboratory and prepared for pesticide residue analysis and microbiological analysis (Dehghani *et al.*,2009).

Indoor Corn and Cucumber Cultivation

This experiment was conducted using Saudi (Hada El-Sham) and Egyptian (El-Sharqia) (soils^{H+E}) with two vegetable crops, corn and cucumber. Aims were to investigate 1) effect of the crop type on the occurrence and distribution of Atrazine residues in the cultivated soils, 2) effects of Atrazine (toxicity) and soil microorganisms (biodegradation) on each other and 3) effect of the environmental conditions (temperate and arid) on the fate of Atrazine. Cultivation was carried out at King Abdul-Aziz University planetary, Jeddah during summer season simulating the natural environmental cultivation conditions. Cultivation took place in plastic pots (25 cm in diameter and 30 cm deep) with hole in the bottom for drainage. Four sets (16pot×4) were prepared, two sets (one for the corn and the other for the cucumber) for the Egyptian soil and the other two for the Saudi soil. In each set, lower dose (equivalent to half of the recommended dose) of Atrazine was applied in the first 4 pots followed by the recommended dose in the second 4 pots and finally the higher dose (equivalent to 2 folds the recommended dose concentration) in the third 4 pots. The remaining 4 pots were left free of Atrazinein and considered as controls. Four replicates of each

treatment were carried out. Each pot was packed with 5 kg air-dried soil leaving the upper 5 cm free for irrigation practices. Plant seeds were inserted; the pots were irrigated to a level equal to the field capacity for each soil. Mineral fertilizer mixture, Kemira (19% nitrogen, 19% phosphorus, 19% potassium and the trace elements iron, zinc manganese, calcium magnesium, copper and boron) was added to all pots at a rate of 0.7-1.50kg/1000L water. One week after planting, soils were sprayed once with Atrazine at the rate of 750g/200 to 600L water/Fadden, which is the dose for controlling weeds and pests in corn plant recommended by ministry of agriculture. After treatment, corn soil samples were collected at0, 25,50 and 75 days and 0, 22, 32 and 42 days for the cucumber soil samples. Samples (50g each) were taken from the upper surface zone down to roots area (0-10cm) and placed in clean plastic bags using septic tools. The collected samples were transported to the laboratory where they were immediately subjected to microbiological analysis and prepared for pesticides residue analysis.

Atrazine Residues Analysis

Extraction of Atrazine from Soil Samples

Extraction of Atrazine from soil was carried out using the method described by Polese *et al.* (2002). Extraction took place by adding 150 ml ethyl acetate that gave the high percentage recovery, to50 g soil sample in a conical flask with 20 g sodium sulfate anhydrous. The flask was covered and agitated for 3 hours in a mechanical shaker. The extract was carefully decanted and filtrated through a clean pad of cotton. Eighty ml from the filtrate was concentrated using a rotary evaporator at 40°C to remove ethyl acetate, the final reconstituted to extract was an adequate volume (1.0 ml) ethyl acetate to injection in GC without clean up.

Extraction of Atrazine from Vegetables (Corn root and Cucumber)

The method of Molloff (1975) was adopted for the extraction of Atrazine from cucumber fruits and corn plant. Fifty g of the sample was placed in the blender cup to which a constant amount (150 ml) of methanol instead of acetone was added, then blended for three minutes and filtered. Extract was shaken successfully with 100, 50 and 50 ml of methylene chloride in separatory funnel after adding 40 ml of sodium chloride solution (20%) then the water phase was discarded. The combined methylene chloride phase was dried by filtration through anhydrous sodium sulphate, and then it was evaporated just to dryness using a rotary evaporator at 40°C.

Extract Clean Up

The cleanup procedure was done according to the method of Mills *et al*. (1972). Cleanup of the extracted residue was carried out using a 25 X 2 cm chromatographic column packed with Florisil (PRg RAED, mesh size 6–100) pre-heated at 70ºC for 24 h to remove moisture, and pre-washed with 50 ml of 1 M methylene chloride. Residues from the column were eluted with 200 ml of elution solvent mixture {50% methylene chloride, 1.5% acetonitrile, 48.5% hexane (v/v/v)}. The affluent was evaporated just to dryness as previously described and the residues were ready for chromatographic determination after being re-dissolved in an appropriate volume of ethyl acetate.

Gas liquid Chromatography Determination

Hewlett Packard GC model 6890 equipped with nitrogen phosphorus Ni63 electron capture detector was used for analysis. GC conditions were as follows:PAS-5capillary column (30 m length × 0.32 mm internal diameter (id) × 0.52µm film thickness), 5% phenyl methyl polysiloxane.N_2 at flow rate of 3 ml/min was used as carrier gas. Injector and detector temperature were 230°C and 280°C respectively. The initial column temperature was 180°C for 2 min, raised at 3°C/min, and then held at 200°C for 15 min.

Calibration Curve

Stock solution (100 ppm) of Atrazine was prepared in ethyl acetate. Matrix matched calibration standard at the concentration of 2.5, 5, 10 and 40 mg/kg were prepared. Each concentration was injected under the mention chromatographic conditions. The peak area was plotted against each concentration values of r^2 was 0.9994.

Atrazine Recovery Efficiency Study

Atrazine Recovery

Recovery study was carried out to define the efficiency of the determination method. Untreated samples of soil were fortified with known amount of Atrazine active ingredient solutions. Spiked samples were then subjected to Atrazine extraction, clean-up and determination. Average recovery from spiked samples recorded 89%.

Preparation of Blank Solution

Solvent and the anhydrous sodium sulphate used in the extraction and clean up were subjected also to recovery test to detect any possible traces of the Atrazine in the solvents.

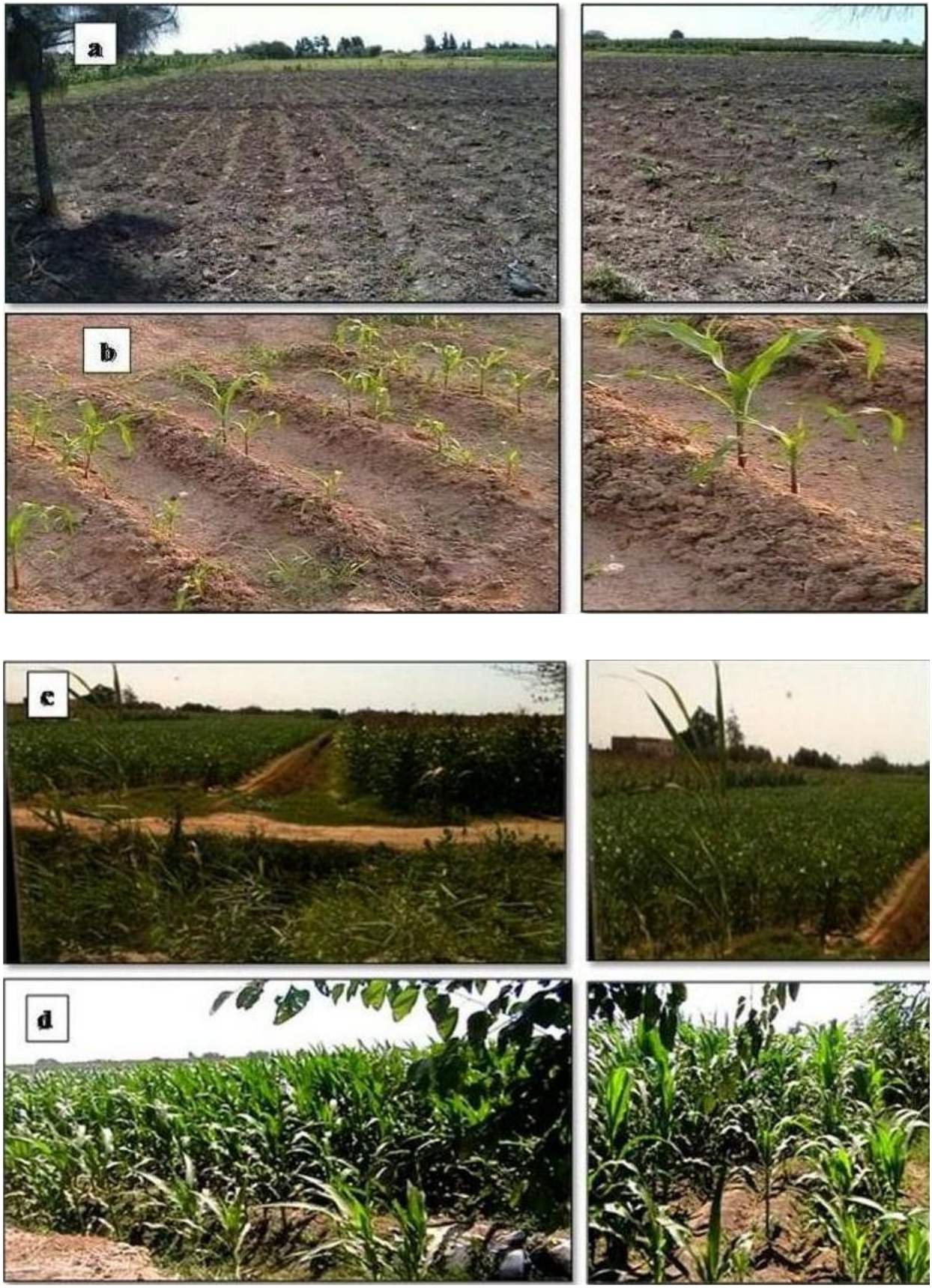


Figure 2A. Corn Plant Emerged in the Field ($Soil^{M}$) (a), Corn in the Field 10 Days (b), 25 Days (c) and 50 Days (d) After Atrazine Application

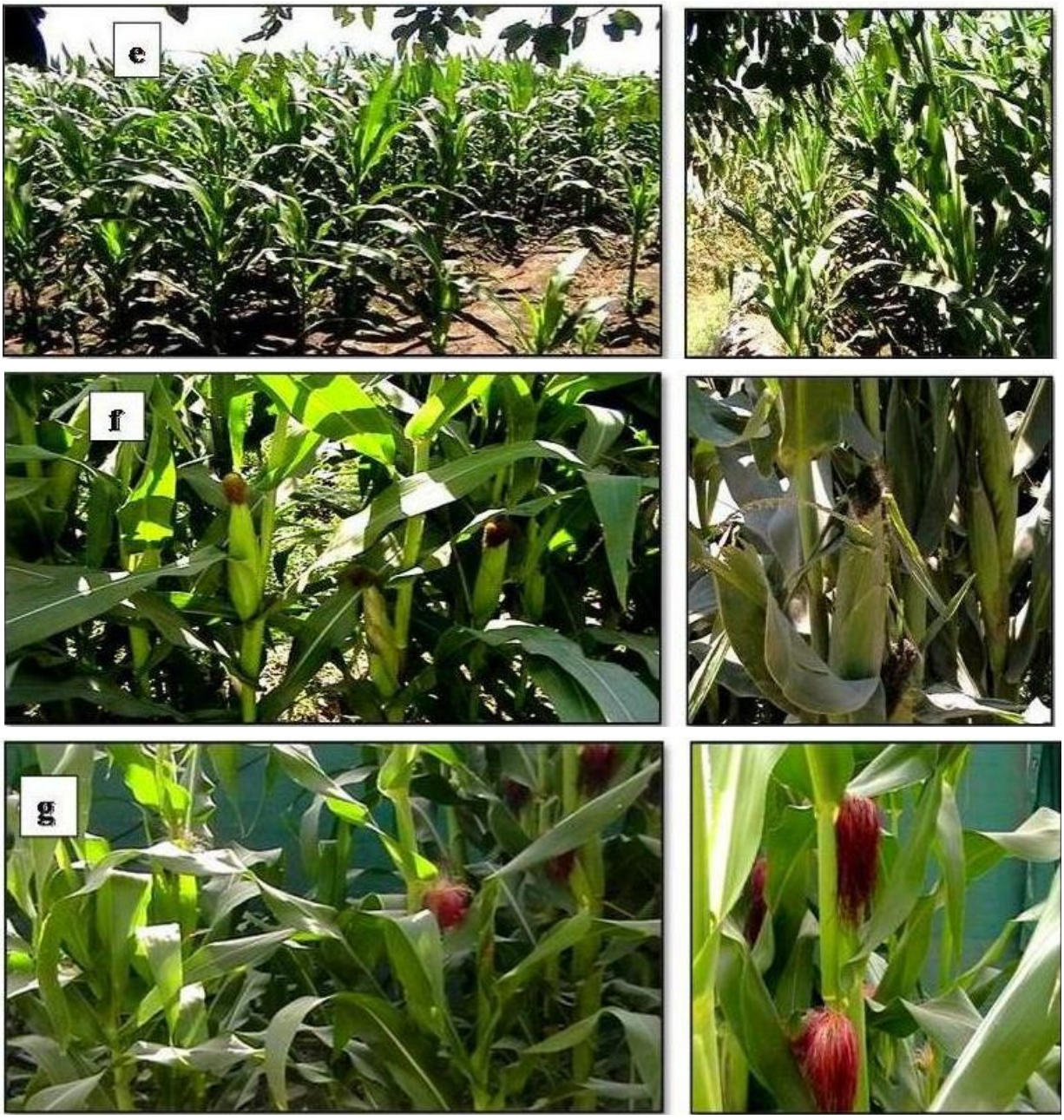
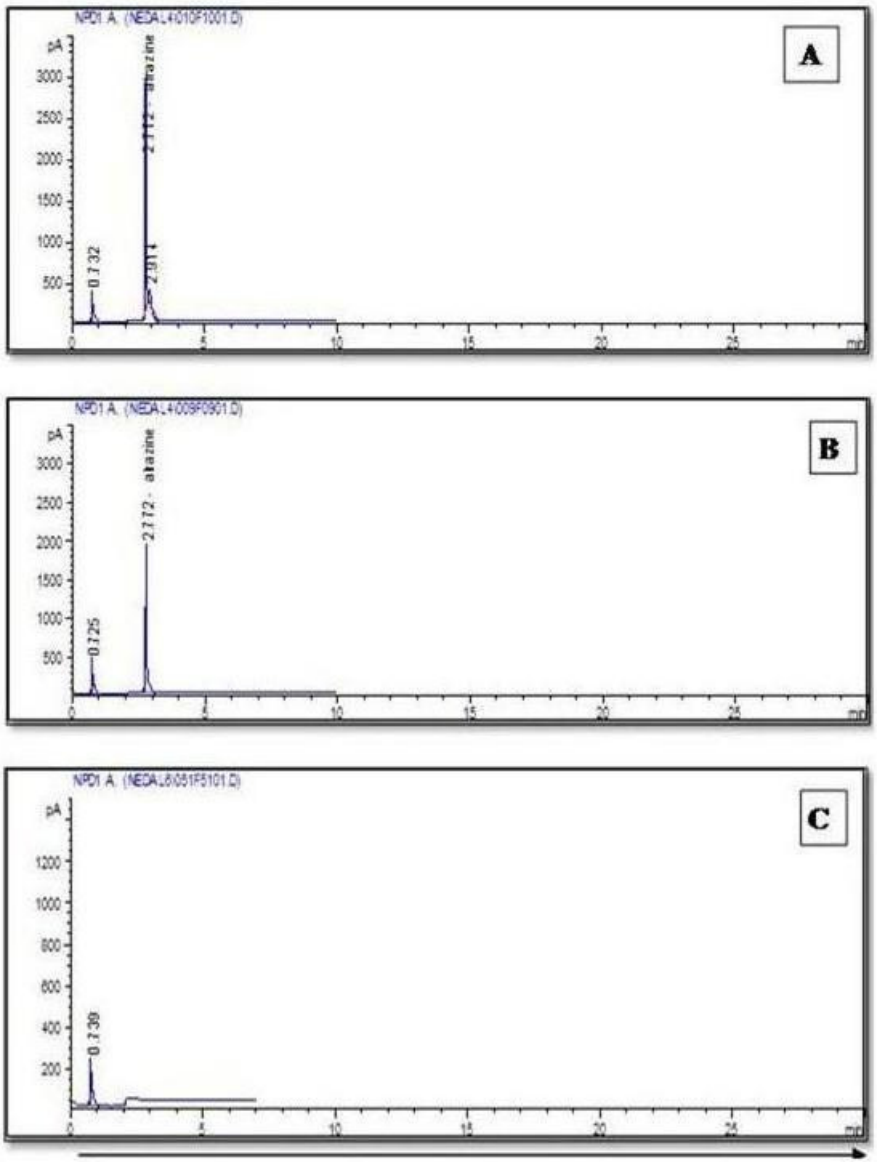


Figure 2B. Corn in the Field (Soil^{M}) 75 Day (e), 110 Days (f) and 120 Days (g) After Atrazine Application

Table 1. Atrazine Residue Averages in Soil^{M} during Corn Cultivationin the Open Field

Time (Days)	***Atrazine Residue (ppm)***			***Average***
	1	***2***	***3***	
Control (zero)	ND*	ND	ND	-
25	0.247	0.249	0.200	0.232
50	0.172	0.120	ND	0.140
75	ND	ND	ND	-



Time (min)
Figure 3. Atrazine Residue in Soil^{M} during Corn Cultivation in the Open Field after A) 25 Day, B) 50 Day and C) 75 Day of Atrazine Application

RESULTS

Open Field (Outdoor) Corn Cultivation

Corn grew well in an open agricultural field located in *Abu El-Matameer area, El-Behaira Governorate, Egypt* to its full cycle in the presence of the Atrazine at its recommended dose (Figure 2A and B). Atrazine residues in soil^{M} during corn cultivation (Table1) recorded average Atrazine residue of0.232 ppm after 25 days of Atrazine application that was decreased to 0.140 ppm after 50 days of the herbicide application (Figure 2). However, Atrazine was not detected after 75 days of its application.

Indoor Corn and Cucumber Cultivation

Indoor Corn Cultivation

Comparative indoor cultivation of corn in soils $^{H\&E}$ (Table 2 and Figure 4 A and B) revealed the following:
1. There was a general trend where soil ^{E} recorded lower Atrazine residues than soils^{H} at all the tested concentrations and exposure times attributed to their physicochemical and biological features. Also, Atrazine residues decreased regularly with time while increased with increasing the tested dose.
2. The highest residue levels were detected in both soils

Table 2. Atrazine Residue Averages in Soil^{H} and Soil^{E} during the Indoor Corn Cultivation

Time (Days)	***Soil^{H}***			***Soil^{E}***		
	Atrazine Residue (ppm)			***Atrazine Residue (ppm)***		
	1\2fold	***1fold***	***2fold***	***1\2fold***	***1fold***	***2fold***
Control (zero)	ND*	ND	ND	ND	ND	ND
25	0.92	1.26	1.58	0.011	0.02	0.05
50	0.006	0.10	0.085	0.01	0.016	0.026
75	ND	ND	0.009	ND	ND	0.002
120	0.011	0.046	0.065	0.006	0.021	0.018

ND*: *Not detected below detection limit (0.01 ppm)*

Figure 4A. Corn Plant Emerged Indoor in Soils$^{H\&E}$ a) Corn at the Zero Time, b) 25 Day, c) 50 Day and d) 75 Day after Atrazine Application

Figure 4B. Corn in Soils$^{H\&E}$ 110 Day (e) and 120 Days after Atrazine Application

after 25 day of Atrazine application. Soil^{H} recorded 0.92, 1.26 and 1.58 ppm at the three tested doses (0.5, 1.0 and 2.0 X the RD concentration) respectively compared to 0.011, 0.02 and 0.05 ppm detected in soil^{E} respectively. These values are 83.6, 63.0 and 31.6 fold higher in soil^{H} compared to soil^{E}.

3. The same trend was shown after 50 days except at the 0.5 RD applied Atrazine. Soil^{H} recorded 0.006, 0.10 and 0.085 ppm at the three tested doses respectively compared to 0.01, 0.016 and 0.026 ppm detected in soil^{E} respectively. These values are 6.3 and 3.3 fold higher in soil^{H} at the RD and 2X RD Atrazine doses respectively compared to those recorded in soil^{E} while at 0.5 RD of Atrazine, residue in soil^{E} was 1.7 fold higher than that recorded in soil^{H}.

4. After 75 days, Atrazine residues disappeared completely at 0.5 and 1.0 X the RD from both soils and were detected only at the 2.0 X RD of Atrazine with 0.009 and 0.002 ppm in soil^{H} and soil^{E} respectively. This means 4.5 fold higher herbicide residue in soil^{H} compared to

Table 3. Atrazine Residue Averages in Soil^{H} and Soil^{E} during the Indoor Cucumber Cultivation

Time (Days)	***Soil^{H}***			***Soil^{E}***		
	Atrazine Residue (ppm)			***Atrazine Residue (ppm)***		
	1\2fold	***1fold***	***2fold***	***1\2fold***	***1fold***	***2fold***
Control (zero)	ND*	ND	ND	ND	ND	ND
22	0.11	7.08	161.08	0.92	1.26	1.58
32	0.20	0.32	4.78	ND	ND	ND
42	ND	ND	ND	ND	ND	ND

ND*: *Not detected below detection limit (0.01 ppm)*

Figure 5A. Application Atrazine on Cucumber in Soils$^{H\&E}$

Figure 5B. Cucumber in a) Soil^{E} and b) Soil^{H} 22 day After Atrazine Application

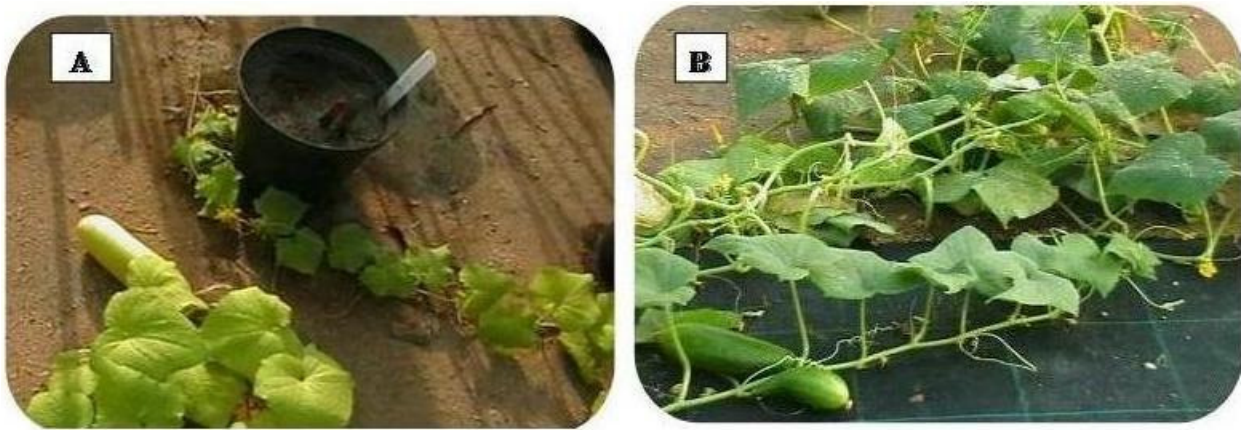


Figure 5C. Yellowish Cucumber in a)Atrazine-Treated Soil^{E} and Healthy Cucumber in b) Control Soil^{E} 32 Day after Atrazine Application

Figure 5D. Yellowish Cucumber Leaves in Atrazine-Treated Soil^{E} 42 Day after Atrazine Application

soil^{E} at this dose.

5. Surprisingly Atrazine residues were detected in soil^{H} and soil^{E} samples after 120 day of the herbicide application process. Soil^{H} recorded 0.011, 0.046 and 0.065 ppm Atrazine at 0.5, 1.0 and 2X of the RD respectively compared to 0.006, 0.021 and 0.018recorded in soil^{E} which are equivalent to 1.8, 2.2 and 3.6 fold increases in Atrazine residues in soil^{H} compared to soil^{E} at the tested doses respectively.

6. These results confirmed the effect of soil characteristics as well as herbicide concentration applied on its environmental fate and residue in the tested soils.

7. Comparing these results with the residues obtained in the outdoor cultivation in soil^{M} indicated higher averages of Atrazine residues (0.232 and 0.140 ppm after 25 and 50 days respectively) compared to the residues in the indoor cultivation. However, in the indoor cultivation Atrazine was not detected after 75 days of its application. Again, this is a clear confirmation that soil characteristics as well as environmental conditions controlled biodegradation of Atrazine which is reflected by its residues.

Indoor Cucumber Cultivation

Type of the plant is another important factor affecting the fate and residues of the applied pesticide. Therefore, a comparative indoor cultivation of cucumber in soils$^{H\&E}$ was performed for 45 days with the application of Atrazine in similar pattern as for corn. Table 3 and Figure 5 A and B concluded the following points:

1. Control samples recorded zero Atrazine residues at the zero time of the application process. As with corn, there was a general trend where soil^{E} recorded lower Atrazine residues than soil^{H} at all the tested concentrations and exposure times except at the 0.5 X of the herbicide RD after 22 days. Also Atrazine residues decreased regularly with time while increased with increasing the tested dose.

2. Cucumber plant only grows to its full cycle in the presence of 0.5 XRD of the herbicide in soil^{E} while it did not grow in soil^{H} at any concentration of the tested herbicide (Figure 5 A and B).

3. After 22 day of Atrazine application in cucumber cultivation, the highest residue levels were detected in

both soils. Soil^{H} recorded 0.11, 7.08 and 161.08 ppm at the three tested doses (0.5, 1.0 and 2.0 X the RD concentration) respectively compared to 0.92, 1.26 and 1.58 ppm detected in soil^{E} respectively. These values are 5.6, and 101.9 fold higher in soil^{H} at 1.0 and 2.0 X the herbicide RD compared to soil^{E} while at 0.5 X the RD, soil^{E} recorded 8.4 fold higher Atrazine residue compared to soil^{H}.

4. After 32 and 42 days of Atrazine application, no Atrazine was detected in soil^{E}. However, and due to weak biodegradation, Atrazine was detected in soil^{H}at elevated levels (0.20, 0.32, 4.78 ppm at the 3 tested doses respectively) after 32 days post application while it disappeared completely after 42 as in soil^{E}.

5. Comparing these results with the residues obtained in the outdoor cultivation in soil^{M} indicated remarkably much lower averages of Atrazine residues in corn (0.232 and 0.140 ppm after 25 and 50 days respectively) as well as indoor corn cultivation compared to the residues in the indoor cucumber cultivation.

6. Also no Atrazine residue was detected in any of cucumber fruit.

DISCUSSION

Outdoor cultivation in soil^{M} indicated higher averages of Atrazine residues (0.232 and 0.140 ppm after 25 and 50 days respectively) compared to the residues in the indoor cultivation attributed to soil characteristics as well as environmental conditions that control biodegradation of Atrazine. Moreover, Atrazine residues were 83.6, 63.0 and 31.6 fold higher in soil^{H} compared to soil^{E} in the indoor cultivation after 25 day of herbicide application. These values decreased to 6.3 and 3.3 fold higher in soil^{H} at theRD and 2RD Atrazine doses respectively after 50 day compared to soil^{E} while at 0.5 RD of Atrazine, residue in soil^{E} was 1.7 fold higher than that recorded in soil^{H}. These results are consistent with and supported by Umar *et al*. (2012) where competition, survival of inocula, bioavailability of organic amendments and nature of tested chemical are important factors affecting bioremediation.

Greenhouse (indoor) bioassay is a simple and sensitive tool in detecting small amounts of herbicides present in the soil (Riddle, 2012). Atrazine residues were detected in soil^{H} and soil^{E} samples after 120 day of the herbicide application process being higher in soil^{H} compared to soil^{E}. In addition, adsorption and desorption behaviour of selected pesticides is influenced by decomposition of surface crop residues called mulch that define the fate of pesticides (Aslama *et al*., 2013). The decomposition degree of mulch residues should be taken into account while predicting the fate of. Type of the plant, corn in our study, is another important factor affecting the fate and residues of the applied pesticide in greenhouse culturing (Yuexuan *et al.,* 2010; Xiuying *et al*., 2012; Ma *et al*., 2012; Ibrahim *et al.,* 2013; Ivanov *et al.,* 2013).

Herbicide degradation and efficiency of remediation technologies vary according soil type, type and concentration of the pesticide, type of the cultivated plant and the selected bioremediation technique used (Ma *et al*., 2012). Accumulation of Atrazine in the cultivated plant represents another removal route of the pesticide with the subsequent effects on the plant (Ivanov *et al*., 2013). It was also true that faster reduction rate of Atrazine residues from contaminated soil planted indoor with *Zeamaize* compared to the unplanted soil confirming that maize is useful for phytoremediation of soils contaminated with Atrazine (Ibrahim *et al*., 2013).

In the present study the height of the corn shots was shorter and fresh weight was lower in green house experiment (soil^{E+H}) compared to those recorded in the open filed cultivation (soil^{M}). This may be attributed to the sowing depth of the applied herbicide as also sown by Pacanoski *et al*., (2007). Cucumber only grows to its full cycle in the presence of 0.5 XRD of the herbicide in soil^{E} while it did not grow in soil^{H} at any concentration of the tested herbicide. There was a general trend where Atrazine residues decreased regularly with time while increased with increasing the tested dose. Also, soil^{H} recorded higher Atrazine residues than soil^{E} at all the tested concentrations (5.6 and 101.9 fold higher at 1.0 and 2.0 X the herbicide RD respectively) and exposure times except at the 0.5 X of the herbicide RD after 22 days (8.4 fold higher Atrazine residue in soil^{E} compared to soil^{H}). After 32 and 42 days of Atrazine application, no Atrazine was detected in soil^{E}. However, due to weak biodegradation, Atrazine was detected at elevated levels (0.20, 0.32, 4.78 ppm at the 3 tested doses respectively) after 32 days post application while it disappeared completely after 42 as in soil^{E}. Comparing these results with the residues obtained in the outdoor cultivation in soil^{M} indicated remarkably much lower averages of Atrazine residues in corn (0.232 and 0.140 ppm after 25 and 50 days respectively) as well as indoor corn cultivation compared to the residues in the indoor cucumber cultivation. Again, this is a clear confirmation that type of the plant is an important factor affecting the fate and residues of the applied pesticide as also reported by other workers (Robinson, 2008; Riddle, 2012; Robinson and McNaughton, 2012 and others).In that respect, it was reported that effects of mesotrione residues on visual injury, plant dry weight, and yields varied among various vegetable crops planted one year after herbicide application in field. The order of tolerance was carrot < onion < cabbage = cucumber. As found cucumber in the present study, significant yield reductions were observed in cabbage and cucumber, but not in onion leading to recommendation that these plants should not be grown the year following application of mesotrione (Riddle, 2012). Similarly, Robinson and

McNaughton (2012) recommended that cabbage, carrot, cucumber, onion, pepper, and sugar beet should not be planted the year after saflufenacil application at rates up to 200 g ha^{-1} while pea and potato can be safely planted.

In the present study Atrazine residues were not detected in cucumber fruit in contrast to the work of Yan *et al.,* (2012) on two kinds of triazine herbicides (cyanazine and Atrazine) on cucumber.

CONCLUSION

Corn grew well in the field to its full cycle in the presence of the Atrazine at its recommended dose. Greenhouse bioassay used in the indoor experiments proved to be a simple and sensitive tool in detecting small amounts of herbicides present in the soil. Cucumber plant grows to its full cycle only in the presence of 0.5 XRD of the herbicide in $soil^{E}$ while it did not grow in $soil^{H}$ at any concentration of the tested herbicide. Also no Atrazine residue was detected in any of cucumber fruit. Higher averages of Atrazine residues (0.232 and 0.140 ppm after 25 and 50 days respectively) were detected in $soil^{M}$ during outdoor corn cultivation compared to the residues in the indoor cultivation. However, in the outdoor cultivation Atrazine was not detected after 75 days of its application. Results confirmed the effect of soil characteristics, herbicide concentration applied, crop type as well as environmental conditions controlled biodegradation and fate of Atrazine in the tested environments which is reflected by its residues.

Conflict of Interest

The authors declare that they have no conflict of interest.

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