Int. J. Environ. Sci. Technol. (2015) 12:3509–3518
DOI 10.1007/s13762-015-0772-7

ORIGINAL PAPER

# Evaluation of the phytoremediation potential of three plant species for azoxystrobin-contaminated soil

**A. A. Romeh**

Received: 27 September 2014 / Revised: 3 January 2015 / Accepted: 28 January 2015 / Published online: 17 February 2015


**Abstract** Azoxystrobin is a broad-spectrum, systemic and soil-applied fungicide used for crop protection on more than 80 different crops. Azoxystrobin use has induced water pollution and ecotoxicological effects upon aquatic organisms, as well as heath issues. Such issues may be solved by phytoremediation. Here, we tested *Plantago major* L., *Helianthus annus* L. and *Glycine max* L. to clean soils under laboratory conditions. Results show that the accumulation efficiency of azoxystrobin and azoxystrobin acid in roots was higher than those of leaves. *G. max* roots were an efficient accumulator of azoxystrobin (25.32 mg/kg), followed by *P. major* roots (20.62 mg/kg) and *H. annus* roots (18.29 mg/kg), within 10 days, respectively. In the leaves, azoxystrobin significantly translocated into the *P. major* leaves and reached the maximum after 10 days of exposure (15.03 mg/kg), followed by *H. annus* leaves (9.8 mg/kg), while it reached the maximum after 3 days of exposure (3.12 mg/kg) in *G. max* leaves. Azoxystrobin acid significantly accumulated in *P. major* roots more than the *G. max* and *H. annus* roots. In the leaves, azoxystrobin acid significantly accumulated in *G. max* more than *P. major* and *H. annus*. The presence of *P. major* with Tween 80 had effects on azoxystrobin desorption from soil, plant uptake metabolism and translocation more than *P. major* alone.

**Keywords** Phytoremediation · *P. major* · *G. max* · *H. annus* · Azoxystrobin · Soil

## Introduction

Azoxystrobin [methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate] (Fig. 1), a strobilurin fungicide, is a broad-spectrum, systemic and soil-applied fungicide that has been approved for use on more than 80 different crops in 72 countries, representing over 400 crop/disease systems (Bartlett et al. 2002). It is effective against four major groups of plant pathogenic fungi including ascomcetes (e.g., powdery mildews), basidiomycetes (e.g., rusts), deutoromycetes (e.g., rice blast) and oomycetes (e.g., downy mildew) (Bartlett et al. 2002). The mode of action is by inhibition of mitochondrial respiration in fungi. It inhibits spore germination, mycelial growth and spore production of fungi (Bartlett et al. 2002). It is active at very low doses against a wide range of fungal pathogens.

Regarding their environmental fate, azoxystrobin is a moderately persistent chemical with half-life ($t_{1/2}$) values in soil ranging from 14 to over 90 days (United States Environmental Protection Agency 1997; Karanasios et al. 2010). It is more persistent in the aerobic soil than the anaerobic soil with half-life values of 107.47 and 62.69 days, respectively (Bending et al. 2007). In field trials, azoxystrobin disappeared quite slowly in grapes ($t_{1/2}$ = 15.2 days) (Lentzarizos et al. 2006) and on peppers ($t_{1/2}$ = 15.21 days) (Fenoll et al. 2008). Azoxystrobin has a high potential to leach down to groundwater in some soil

**Electronic supplementary material** The online version of this article (doi:10.1007/s13762-015-0772-7) contains supplementary material, which is available to authorized users.

A. A. Romeh (✉)
Plant Production Department, Faculty of Technology and Development, Zagazig University, Zagazig, Egypt
e-mail: ahmedromeh2006@yahoo.com

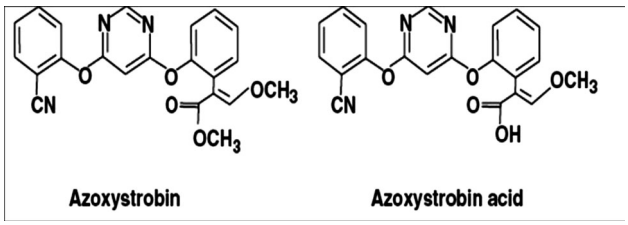


**Fig. 1** The chemical structure of azoxystrobin and azoxystrobin acid

types, as it is weakly sorbed in coarse textured soils (Cohen et al. 1984). Azoxystrobin was detected in a high proportion of the water samples (30–41 %) (Gregoire et al. 2010). Azoxystrobin acid (Fig. 1), a major metabolite of the fungicide, is highly soluble in water (860 mg/L) and exhibits low binding affinity in most soil types (US EPA 1997) and therefore has high potential to leach down in soils. The formation of azoxystrobin acid was more abundant and faster under alkaline (pH 9) condition than neutral (pH 7) or acidic (pH 4) conditions (Singh et al. 2010). Ecotoxicological testing has established that azoxystrobin is toxic to freshwater and marine invertebrates and also fish (US EPA 1997).

Phytoremediation is a strategy that uses plant to degrade, stabilize and remove contaminants from soils, water and wastes (Yu and Gu 2008; Kuo et al. 2014). Phytoremediation is an environmentally sound technology for pollution prevention, control and remediation. There have been several studies focused on the phytoremediation of pesticides (Romeh and Mohammed 2013; Romeh 2014, Zhang et al. 2014, Ibáñez et al. 2014). Sunflower (*Helianthus annuus* L.) is a relatively high-biomass and fast-growing accumulator plant which has the ability to take up and accumulate metals and radionuclides (Tassi et al. 2008; Tahmasbian and Sinegani 2014). Soybean, *Glycine max* L., produced peroxidase enzymes (McEldoon et al. 1995). Peroxidase enzymes play an important role in the oxidative metabolism of xenobiotics in plants and play a role as an oxidative stress enzyme (Roy et al. 1992). The broadleaved plantain (*Plantago major* L.) is a perennial weed and commonly found by roadsides, in meadow land, cultivated fields, waste areas and canals water. The seed and husks of this plant contain high level of fiber which expand and become highly gelatinous when soaked in water (Sharifa et al. 2008). The combined use of plants and solubility enhancement agents such as surfactants has been proposed for improving phytoremediation strategies. These techniques are based on the ability of agents to increase the water solubility of hydrophobic organic compounds (HOCs) through micellization and surface tension reduction (Saichek and Reddy 2004) and to promote desorption, bio-degradation and phytoremediation processes (Wang and Keller 2009). Different classes of surfactants are employed for soil remediation depending on the nature of removed contaminants. Synthetic surfactants such as Tween 80 are reported to remove organic pollutants from contaminated soils by a mechanism involving repartition of pollutants into the surfactant micellar phases formed in water. However, this process would take place only when the surfactant solution reaches the surfaces of soil particles, and pollutants may be desorbed into the micellar phases (Cuypers et al. 2002). Because of their cold-water solubility, low critical micelle concentration (CMC) and relatively low microbial toxicity, nonionic surfactants have attracted particular interest in promoting desorption, microbial degradation and plant uptake of the hydrophobic organic contaminants (HOCs; Zhao et al. 2005). Several studies have looked into the use of surfactant-enhanced phytoremediation (Smith et al. 2004, Wu et al. 2008). The objective of this work was to evaluate the potential of using three plants, plantain (*Plantago major* L.), sunflower (*Helianthus annus* L.) and soybean (*Glycine max* L.), for the phytoremediation of azoxystrobin-contaminated soil. The use of solubilization agent such as the surfactant, Tween 80, for enhancing the availability and uptake of azoxystrobin-contaminated soil by *P. major* was evaluated. The experiment was designed in the Department of Plant Production, Faculty of Technology and Development, Zagazig University, Egypt on June 15, 2014.

## Materials and methods

### Pesticide and plants

Azoxystrobin (Amistar 25 % SC.) [methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate]. Pesticide was obtained from a local manufacturer, Syngenta agro Egypt, 4 Yanbu St. Dokki, Giza governorate, Egypt.

The common broadleaf plantain (*Plantago major* L.) were obtained as seedlings in phytoremediation experiment from meadow land in Zagazig University, Zagazig, Sharkia governorate, Egypt. Sunflower, *Helianthus annus* L., and soybean, *Glycine max* L., seeds were germinated in vermiculite-growing medium and kept moist during the initial

growth period (1 week), which may vary according to plant type. After the germination period, only healthy seedlings with uniform size were selected. Plants were then gently removed from growing medium, and the roots were rinsed off from any adhering material before transferring to the soil.

## Experimental design

To evaluate the removal of azoxystrobin from the soil, five treatments were performed in this experiment, and each treatment consisted of five replicates: (1) azoxystrobin-contaminated soil without plants, (2) azoxystrobin-contaminated soil with *P. major* only (each pot contained one seedling of *P. major*), (3) azoxystrobin-contaminated soil with *H. annus* seedling (each pot contained twenty seedling of *H. annus*), (4) azoxystrobin-contaminated soil with soybean plants (each pot contained twenty seedling of *G. max*) and (5) azoxystrobin-contaminated soil with *P. major* and amended with polyoxyethylene sorbitan monooleate (Tween 80) at 9.2 mg/L, corresponding to 0.5 critical micelle concentration (CMC), where the CMC of Tween 80 was determined as 13–45 mg/L (Edwards et al. 1991; Mitton et al. 2012) (each pot contained one seedling of *P. major*). A whole-plant uptake experiment was performed on soil in a pot experiment for 14-day exposure. Air-dried sieved clay loam soil (organic matter, 1.79 %, pH 7.8, electric conductivity 2.36) was obtained from Aboutwala, Menia EL-Kamh province, Sharkia governorate, Egypt, and then placed in plastic pots. The pots were filled with 500 g of air-dried soil. After planting, azoxystrobin dissolved in acetone was spiked into the 150 ml of distilled water used for irrigation to obtain original concentrations of 20 mg/kg. The irrigation water containing azoxystrobin was dropped into the pots with a caution to avoid the direct contact of plant shoots. The treatment without azoxystrobin spiked into the soil acted as the control. After 1, 3, 7, 10 and 14 days, exposed and control plants were collected. Plant roots from soil were rinsed in running tap water for 2 min and were blotted dry. The plants were dissected into individual roots, stalk and leaves in the case of *H. annus* and *G. max*, while to roots and leaves in the case of *P. major;* then, 4 g of leaves, 4 g of roots, 4 g stalks and 20 g of soil were analyzed for the pesticide. All pots were watered with 50 ml tap water every 4 days or additionally watered when necessary.

## Residue analysis

### *Azoxystrobin extraction and analysis*

The root, stalk and leaf samples were chopped and blended using a food cutter. A 4.0 g portion of the chopped tissue was transferred into the glass jar of a blender and homogenized with 50 ml of acetonitrile/water (90:10 v/v) for 2 min. The homogenate was passed through a filter paper, and the filtrate was partitioned with an equivalent volume of dichloromethane plus half of equivalent volumes of 5 % NaCl solution in a separating funnel for about 1 min, filtered through anhydrous $Na_2SO_4$ and evaporated to dryness under reduced pressure. The residue was redissolved in hexane/dichloromethane (50:50) and transferred to solid-phase extraction (SPE) clean up to separate out interfering substances. The silica cartridge was conditioned with 5 ml of hexane/dichloromethane (50:50 v/v) followed by sample loading and washing with 5 ml of dichloromethane and ethyl acetate (90:10 v/v). Azoxystrobin was eluted from the SPS cartridge using 5 ml of dichloromethane and ethyl acetate (70:30 v/v). The elute was evaporated to near dryness and quantified with 1 ml of acetonitrile, and aliquot (10 µl) was injected into the HPLC (Sundravadana et al. 2008).

Soil samples (20 g) were transferred to 250-ml stoppered conical flasks, and 100 ml of ethyl acetate and 25 g of anhydrous sodium sulfate were added to each flask. The samples were equilibrated on a rotary shaker for 1 h. The ethyl acetate fraction was transferred to a 250-ml beaker, and the soil was extracted again in a similar manner. A total of three extractions were performed, the ethyl acetate fractions were pooled and evaporated to dryness at room temperature, and the residue was redissolved in 1.0 ml of petroleum ether before cleanup (Ghosh and Neera 2009). A glass column was preconditioned with 30 ml petroleum and then packed with 2.0 g florisil, 1.0 g alumina-N and 0.3 g activated carbon between two 1.5-cm layers of anhydrous sodium sulfate on top of a glass wool plug. Concentrated extracts were poured on top of the column, washed with 30 ml dichloromethane to remove any impurities and eluted with 60 ml methanol/ethyl acetate at a ratio of 5:95 (v/v). The elution was concentrated by evaporation to dryness under vacuum using a rotary evaporator at 40 °C and dried under a gentle nitrogen stream (Wang et al. 2013).

*HPLC analysis*

Samples (soil, root, stalk and leaf extracts) were analysed for azoxystrobin and metabolite azoxystrobin acid using high-performance liquid chromatography (HPLC) according to Singh and Shashi 2010). A 10 µl aliquot of samples was injected into HPLC system and analyzed using a C18 reversed-phase column [250 mm × 4 mm (i.d.)] and eluted isocratically with a mobile phase consisting of acetonitrile and acidic water (0.1 % ortho-phosphoric acid) (70:30, v/v) at the rate of 1 ml/min. The UV detection was made at 220 nm for azoxystrobin and azoxystrobin acid. Under these conditions, the retention times (RT's) of azoxystrobin and metabolite azoxystrobin acid were 4.2 and 2.8 min, respectively.

The performance of HPLC method was assessed by evaluating quality parameters, such as recovery values and limits of detection (LOD). The limit of detection (LOD) and quantification (LOQ) were evaluated using the following equations (Thomsen et al. 2003): $\text{LOD} = 3.3\ S_0/b$ and $\text{LOQ} = 10S_0/b$ (3). Where $S_0$ is the standard deviation of the calibration line and $b$ is the slope. LOD and LOQ of azoxystrobin were 0.004 and 0.019 mg/kg of samples. Recoveries of azoxystrobin at different fortification levels, i.e., 0.05, 0.1 and 0.2 mg/kg, were determined from root, stalk, leaf and soil samples. No interfering peaks were observed on the chromatogram of the samples under the selected conditions. The recoveries obtained for roots, stalks, leaves and soils were the average range of 86.5–92.3 %, 87.0–93.5 %, 85.0–90.3 % and 81.7–85.3 %, respectively.

Data analysis

The rate of degradation ($K$) and half-life ($t_{1/2}$) was obtained from the following equation by Gomaa and Belal (1975). The rate of degradation ($K$) = 2.303 × slope. Half-life ($t_{1/2}$) = 0.693/K.

Data were evaluated statistically by one-way ANOVA, and comparison of mean values (mean ± SD) was done by using Tukey's honestly significant difference test at $p \leq 0.05$. The CoStat 6.311 CoHort Statistical Software was used for the analysis.

## Results and discussion

Kinetics of pesticide removals

Data obtained during the present investigation revealed that all experimental sets containing plants removed a substantial amount of azoxystrobin. As shown in Table 1, azoxystrobin concentrations in soil with *P. major*, *G. max* and *H. annus* reduced by 40–91.6 %, 39.45–91.6 % and

**Table 1** Degradation of azoxystrobin in soil planted with three species plants

| Treatments | Days after application | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 7 | 10 | 14 | *T*1/2 (per days) | Kr (per days) | AUC (per days) |
| In soil | | | | | | | | |
| mg/kg | 16.68 a | 14.77 a | 12.32 a | 9.23 a | 7.74 fa | 11.56 | 0.06 | 168.52 |
| ±SD | ±1.0 | ±0.9 | ±0.85 | ±0.72 | ±0.62 | | | |
| % loss | 16.6 | 26.15 | 38.4 | 53.85 | 61.3 | | | |
| In soil *Plantago major* | | | | | | | | |
| mg/kg | 12.0 b | 9.29 bc | 5.34 bc | 3.71 bc | 1.68 b | 5.05 | 0.14 | 86.24 |
| ±SD | ±0.7 | ±0.5 | ±0.3 | ±0.3 | ±0.08 | | | |
| % loss | 40.0 | 53.55 | 73.3 | 81.45 | 91.6 | | | |
| In soil *Glycine max* | | | | | | | | |
| mg/kg | 12.11 b | 8.63 c | 4.5 c | 2.88 c | 1.68 b | 4.32 | 0.16 | 78.77 |
| ±SDa | ±0.76 | ±0.44 | ±0.3 | ±0.22 | ±0.15 | | | |
| % loss | 39.45 | 56.85 | 77.5 | 85.6 | 91.6 | | | |
| In soil *Helianthus annus* | | | | | | | | |
| mg/kg | 12.94 b | 10.66 b | 6.31 b | 4.58 b | 1.77 b | 5.46 | 1.13 | 98.49 |
| ±SD | ±0.81 | ±0.61 | ±0.45 | ±0.32 | ±0.14 | | | |
| % loss | 35.3 | 46.7 | 68.45 | 77.1 | 91.15 | | | |

T1/2, half-life; kr, disappearance rate constant; AUCs, areas under the curve represent compound concentration during the period of study; *SD*, standard deviation. Different letters indicate significantly different values (Tukey's honestly significant difference test at $p \leq 0.05$, $n = 3$)

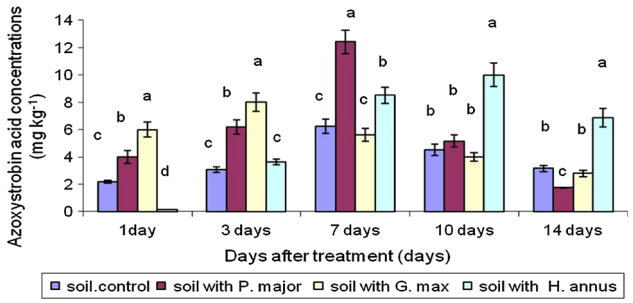


**Fig. 2** Degradation of azoxystrobin acid in soil planted with three species plants, *Plantago major*, *Glycine max* and *Helianthus annus*. *Different letters* indicate significantly different values (Tukey's honestly significant difference test at $p \leq 0.05$, $n = 3$)

35.3–91.15 % throughout 1- to 14-day exposure, compared with 16.6–61.6 % in the control. The half-life value ($t_{1/2}$) of azoxystrobin, calculated by first-order reaction, for soil with *G. max*, *P. major* and *H. annus* was found to be 4.32, 5.05 and 5.46 days, respectively, compared with 11.56 days for soil alone (Table 1). Results with the disappearance rate constant ($k_r$) values revealed that azoxystrobin had the highest $k_r$ value and shortest $t_{1/2}$ in all experimental sets containing plants, while azoxystrobin had the lowest $k_r$ and longest $t_{1/2}$ in unplanted soil.

The disappearance of azoxystrobin was coupled with the appearance of the metabolite product, azoxystrobin acid as a major degradation product in soil (Fig. 2). The degradation product azoxystrobin acid was detected in the all soil samples. The degradation product azoxystrobin acid was increased in the unplanted soil, soil planted with *P. major* and soil planted with *H. annus* for 7 days, then decreased gradually to the end of experiment, while increased in the soil planted with *G. max* for 3 days, then decreased gradually to the end of experiment (Fig. 2). Soil planted with *P. major* produced significant accumulation of azoxystrobin acid for 7 days when compared with soil planted with *G. max* or *H. annus* (Fig. 2). These data demonstrated that most of the azoxystrobin disappearance by the tested three plants may be attributed to the uptake potential and transformation or degradation by the enzyme induction capability of the plant or by microorganisms in the plant root zone. Only one of them contributes to the reduction of a contaminant or connected them (Yao et al. 2009; Deepali et al. 2009).

## The role of different plants in azoxystrobin uptake and translocation

Azoxystrobin concentrations (mg/kg) in different parts of *P. major*, *H. annus* and G. *max* are shown in Fig. 3a–c.

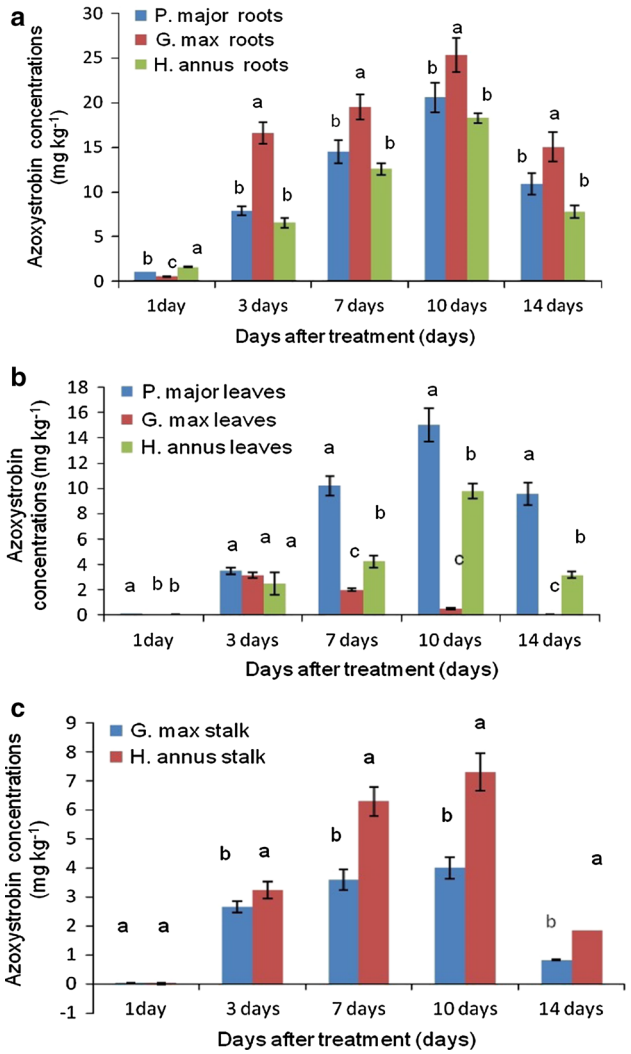


**Fig. 3** Uptake and translocation of azoxystrobin using three species plants, *Plantago major* L., *Glycine max* L. and *Helianthus annus*. **a** Azoxystrobin in roots, **b** azoxystrobin in leaves, **c** azoxystrobin in stalks. *Different letters* indicate significantly different values (Tukey's honestly significant difference test at $p \leq 0.05$, $n = 3$)

Root concentrations of the azoxystrobin were always higher than those of the leaves of all three plants. Also, azoxystrobin significantly accumulated in *G. max* roots more than that in the *P. major* and *H. annus* roots. *G. max* roots were an efficient accumulator of azoxystrobin (25.32 mg/kg), followed by *P. major* roots (20.62 mg/kg) and *H. annus* roots (18.29 mg/kg), within 10 days, respectively (Fig. 3a). In the leaves, azoxystrobin significantly translocated into the *P. major* leaves and reached the maximum after 10 days of exposure (15.03 mg/kg), followed by *H. annus* leaves (9.8 mg/kg), while it reached the maximum after 3 days of exposure (3.12 μg/g) in *G.*

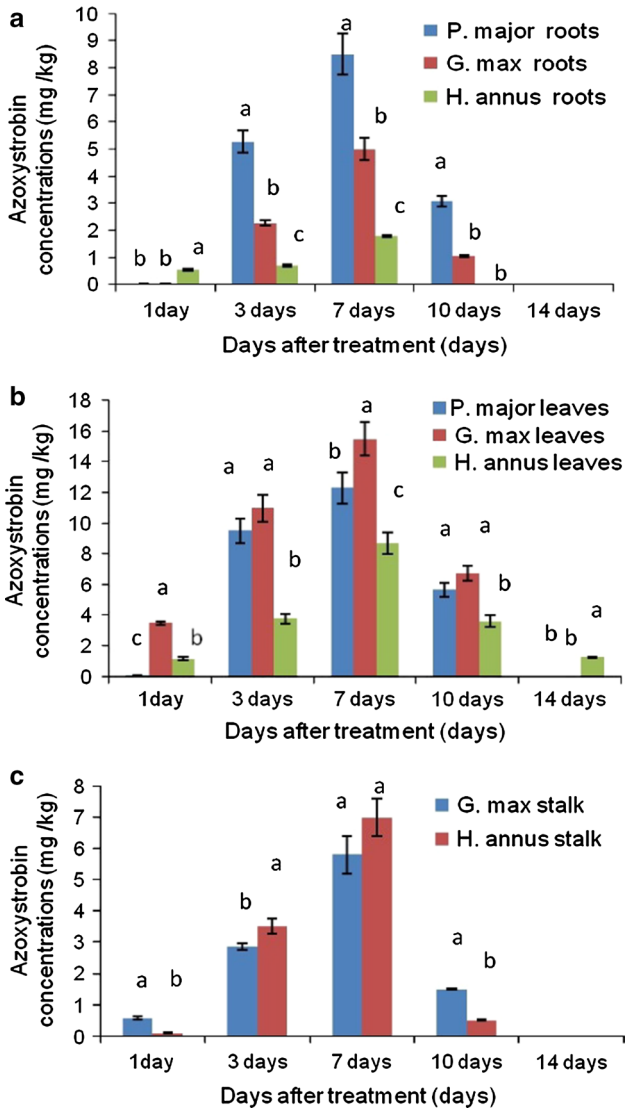


**Fig. 4** Uptake and translocation azoxystrobin acid using three species plants, *Plantago major* L., *Glycine max* L. and *Helianthus annus*. **a** Azoxystrobin acid in roots, **b** azoxystrobin acid in leaves and **c** azoxystrobin acid in stalks. *Different letters* indicate significantly different values (Tukey's honestly significant difference test at $p \leq 0.05$, $n = 3$)

*max* leaves (Fig. 3b). In the stalks, *H. annus* stalks could accumulate more azoxystrobin (7.3 mg/kg) than the *G. max* stalks (4.0 mg/kg) within 10 days of exposure (Fig. 3c). *G. max* showed significant effect on uptake of azoxystrobin into roots, and the uptake ratio was about 1.38 and 1.22 times higher when compared with *H. annus* and *P. major,* respectively, while *P. major* showed significant effect on translocation of azoxystrobin into leaves, and the uptake ratio was about 4.82 and 1.53 times higher when compared with *G. max* and *H. annus*, respectively. In the stalks, *H. annus* showed significant effect on translocation of azoxystrobin, and the uptake ratio was about 1.83 times higher when compared with *G. max* (Fig. 3a–c). It has been observed that roots were important in accumulating compounds due to their direct exposure to toxic chemicals with underground parts, and transporting the compounds to above-ground organs, shoots (Azmat et al. 2009). The uptake and translocation of organic compounds are dependent on hydrophobicity (lipophilicity), solubility, polarity, molecular weight, plant species and environmental factors (Turgut 2005). Lipophilicity is the most important property of a chemical in determining its movement into and within a plant and is related to the n-octanol/water partition coefficient ($K_{ow}$) value. For uptake, log $K_{ow}$ must be typically between 0.5 and 3.0. Compounds with larger log $K_{ow}$ are hydrophobic and may adsorb strongly onto roots, while smaller log $K_{ow}$ are too hydrophilic to pass through cell membrane (Bouldin et al. 2006). Azoxystrobin is a moderately hydrophobic compound (log $K_{ow}$ 2.64) and is likely to partially adsorb onto roots or be taken up by roots and move across cell membranes to reach the above-ground portion of plants. Also, potential uptakes of organic contamination are influenced by evapotranspiration (Chefetz 2003).

The uptake and distribution of azoxystrobin acid using *P. major*, *H. annus* and G. *max* plants is presented in Fig. 4a–c. The disappearance of azoxystrobin was coupled with the appearance of the metabolite product, azoxystrobin acid as a major degradation product in roots and leaves of experimental plants. The accumulation efficiency of azoxystrobin acid in root was higher than that of leaf. Also, azoxystrobin acid significantly accumulated in *P. major* roots more than that in the *G. max* and *H. annus* roots (Fig. 4a). In the leaves, azoxystrobin acid significantly accumulated in *G. max* more than that in *P. major* and *H. annus* (Fig. 4b). In the stalk, azoxystrobin acid significantly accumulated in *H. annus* more than that in *G. max* (Fig. 4c).

Interestingly, azoxystrobin significantly accumulated in *G. max* roots more than that in *P. major* and *H. annus*, while low concentrations were found in *G. max* leaves. The disappearance of azoxystrobin in *G. max* leaves was coupled with the appearance of the metabolite product azoxystrobin acid with the higher concentrations (Figs. 3a, b, 4b). Several studies showed that soybean, *G. max,* produced peroxidase enzymes (McEldoon et al. 1995). Peroxidase enzymes play an important role in the oxidative metabolism of xenobiotics in plants and play a role as an oxidative stress enzyme (Roy et al. 1992). Peroxidases are constitutive and inducible enzymes that are expressed as soluble, extracellular and membrane-bound proteins from some plant tissues, where oxidation of aromatic substrates occurs using $H_2O_2$ as a co-substrate (González et al. 2006). Also, azoxystrobin can be hydrolyzed to azoxystrobin acid.

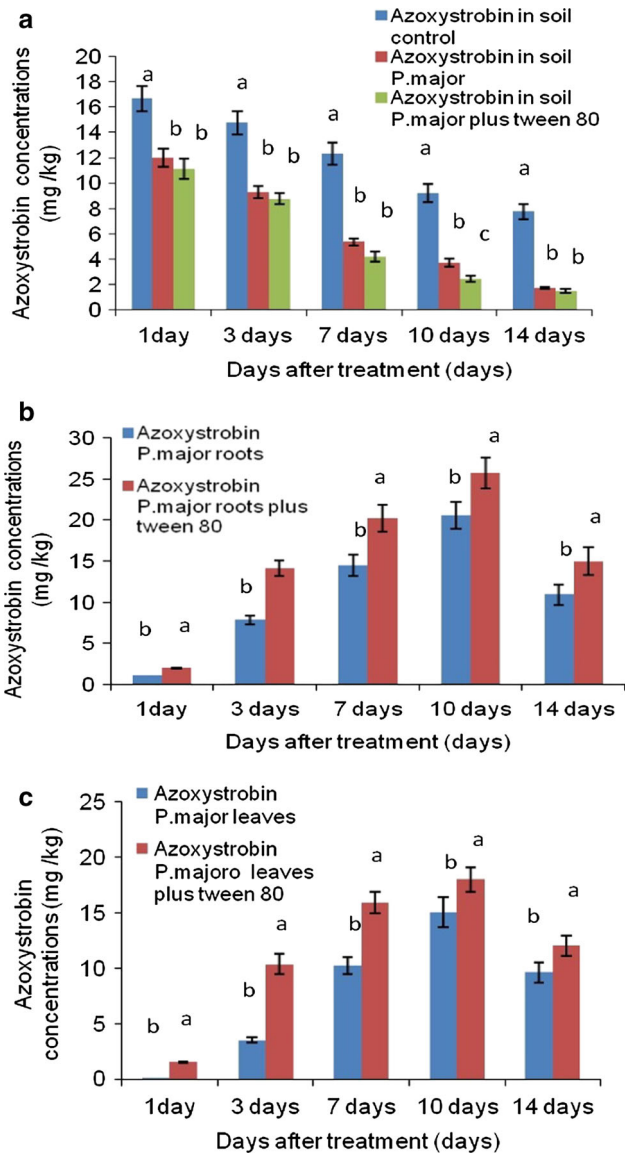


**Fig. 5** Efficiency of solubility-enhancing agent (Tween 80) in phytoremediation of azoxystrobin-contaminated soil using *Plantago major*. **a** Azoxystrobin in soil, **b** azoxystrobin in roots and **c** azoxystrobin in leaves. *Different letters* indicate significantly different values (Tukey's honestly significant difference test at $p \leq 0.05$, $n = 3$)

The hydrolysis of azoxystrobin may be another way to form azoxystrobin acid.

## Improvement in the performance of phytoremediation using Tween 80

Azoxystrobin concentrations (μg/g) in different parts of *P. major* and *P. major* with Tween 80 are shown in Fig. 4a–c.

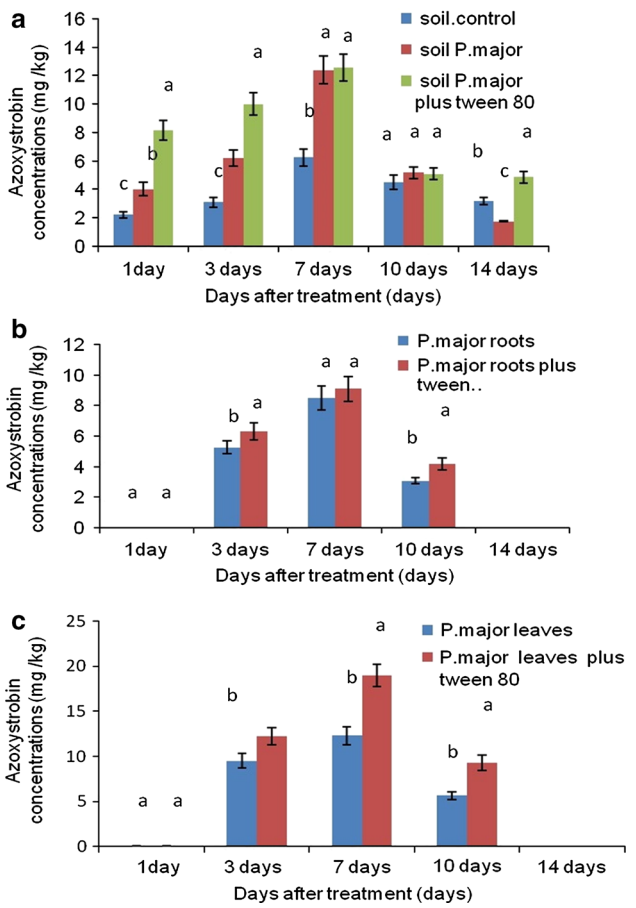


**Fig. 6** Efficiency of solubility- enhancing agent (Tween 80) in phytoremediation of azoxystrobin acid-contaminated soil using *Plantago major*. **a** Azoxystrobin in soil, **b** azoxystrobin in roots and **c** azoxystrobin in leaves. *Different letters* indicate significantly different values (Tukey's honestly significant difference test at $p \leq 0.05$, $n = 3$

The presence of nonionic surfactant Tween 80 with *P. major* enhanced azoxystrobin desorption from soil, plant uptake metabolism in roots and translocation in leaves than *P. major*. Tween 80 with *P. major* produced a synergistic effect on azoxystrobin uptake and translocation, and showed significant accumulate of azoxystrobin in root (25.72 mg/kg) and leaves (18.0 mg/kg) when compared with *P. major* roots (20.62 mg/kg) and *P. major* leaves (15.03 mg/kg) at 10 days, respectively (Figs. 4a–c, 6). The presence of Tween 80 with *P. major* showed significant effect on uptake and translocation of azoxystrobin into roots and leaves at 10 days, the uptake and translocation ratio was about 1.25 and 1.19 times higher when compared with *P. major* alone, respectively. (Fig. 4a, c). Tween 80 with *P. major* was superior to *P. major* in accumulation

and translocation of azoxystrobin into roots and leaves. The percentage removal of azoxystrobin in the soil using *P. major* plus Tween 80 was greater than that using *P. major* but statistically was not significantly different between the two treatments ($p > 0.05$) (Fig. 4a). The highest accumulation of azoxystrobin acid was found in the soil planted with *P. major* plus Tween 80 than *P. major* alone (Fig. 5a–c). The degradation product azoxystrobin acid was increased in the soil planted with *P. major* with Tween 80 for 7 days, then decreased gradually to the end of experiment. As is shown in Fig. 5a–c, azoxystrobin acid significantly accumulated in soil, *P. major* roots and leaves with Tween 80 more than that in the *P. major* alone. The increase in availability mediated by *P. major* with Tween 80 is a dynamic process; from our results, it can be seen that as the contaminant is released from soils, the residue may be taken up by plant. Surfactants may be used for remediation of contaminated soils and sediments for their ability to enhance solubility of hydrophobic compounds (Xu et al. 2006). Several studies have looked into the use of surfactant-enhanced phytoremediation (Smith et al. 2004; Wu et al. 2008). Their amphiphilic characteristics facilitate the release of organic compounds from the sorbed phase, increasing their aqueous concentrations and bioavailability (González et al. 2006; Ussawarujikulchai et al. 2008). Surfactants are known to improve the pollutant desorption/mass transfer into the water phase by decreasing the interfacial tension between the water and the pollutants and by accumulating them in the micelles (Xu et al. 2006). The presence of some nonionic surfactants including polyoxyethylene sorbitan monooleate (Tween 80) and polyoxyethylene(23) dodecanol (Brij35) at relatively low concentrations resulted in significant positive effects on phytoremediation for pyrene-contaminated soil (Yan-Zheng et al. 2007). Tween 80 amendment caused a depletion on p,p_-DDT desorption from soils in willow plants, *Salix sp*. (Mitton et al. 2012). The magnitude of pesticide desorption is dependent on surfactant and pesticide concentration (González et al. 2006). Treatment of Villa Regina soil with Tween 80 at 2 CMC leads to higher desorption than at 10 CMC (González et al. 2006). In this work, surfactant concentration was used at sub-CMC levels in order to avoid plant toxicity and high surfactant loads in soil and desorption. Thus, it could be hypothesized that azoxystrobin desorption by Tween 80 in this soil may be dependent on surfactant concentration with an optimal level at about 0.5 CMC. On the other hand, other authors have shown that Tween 80 increase diazinon availability at a wide range of concentrations (0.75 mg/L to 10 g/L) (Hernandez-Soriano et al. 2010). However, this result was obtained in a soil with lower clay content.

## Conclusion

In conclusion, our study found that *Glycine max* L. and *Plantago major* L. were the most suitable plant species for phytoremediation of azoxystrobin-contaminated soil. In addition, Tween 80 with *P. major* could act as an enhancing agent for the phytoremediation of azoxystrobin from the contaminated soil.

**Acknowledgments** The author is most grateful to the Central Laboratory for soil, food and feedstuffs, Faculty of Development and Technology, Zagazig University, Zagazig, Egypt, for their collaboration in this research.

## References

Azmat R, Haider S, Riaz M (2009) An inverse relation between Pb2+ and Ca2+ ions accumulation in *Phaseolus mungo* and *Lens culinaris* under Pb stress. Pak J Bot 41:2289–2295

Bartlett DW, Clough JM, Godwin JR, Hall AA, Hamer M, ParrDobrzanski B (2002) Review: the strobilurin fungicides. Pest Manag Sci 58:649–662

Bending GD, Rodriguez-Cruz MS, Lincoln DS (2007) Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere 69:82–88

Bouldin JL, Farris JL, Moore MT, Smith SJ, Cooper M (2006) Hydroponic uptake of atrazine and lambda-cyhalothrin in *Juncus effusus* and *Ludwigia peploides*. Chemosphere 65:1049–1057

Chefetz B (2003) Sorption of phenathrene and atrazine by plant cuticular fractions. Environ Toxicol Chem 22:2492–2498

Cohen SZ, Creeger SM, Carsel RF, Enfield CG (1984) Potential pesticide contamination of groundwater from agricultural uses. In: Kruegar RF, Seiber JN (ed) Treatment and disposal of pesticide wastes. ACS symposium series no. 259. American Chemical Society, Washington, pp 297–325

Cuypers C, Pancras T, Grotenhuis T, Rulkens W (2002) The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-b-cyclodextrin and Triton X-100 extraction techniques. Chemosphere 46:1235–1245

Deepali L, Korade M, Fulekar H (2009) Rhizosphere remediation of chlorpyrifos in mycorrhizospheric soil using ryegrass. J Hazard Mater 172:1344–1350

Edwards DA, Luthy RG, Liu Z (1991) Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions. Environ Sci Technol 25:127–133

Fenoll J, Encarnaci O, Pilar H, Alfredo L, Pilar F (2008) Strobilurin residue levels in greenhouse-grown pepper and under cold-storage conditions. J Sci Food Agric 89:299–303

Ghosh RK, Neera S (2009) Leaching behaviour of azoxystrobin and metabolites in soil columns. Manag Sci 65:1009–1014

Gomaa EA, Belal MH (1975) Determination of dimethoate residues in some vegetable and cotton plant. Zagazig J Agric Res 2:215–221

González PS, Capozucca CE, Tigier HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from wastewater, by peroxidases of tomato hairy root cultures. Enzyme Microb Technol 39:647–653

Gregoire C, Payraudeau S, Domange N (2010) Use and fate of 17 pesticides applied on a vineyard catchment. Int J Environ Anal Chem 90:406–420

Hernandez-Soriano MC, Mingorance MD, Peña A (2010) Desorption of two organophosphorous pesticides from soil with wastewater and surfactant solutions. J Environ Manag 95:S223–S227

Ibáñez SG, Merini LJ, Barros GG (2014) *Vicia sativa*–rhizospheric bacteria interactions to improve phenol remediation. Int J Environ Sci Technol 11(1679):1690

Karanasios E, Tsiropoulos NG, Karpouzas DG, Menkissoglu-Spiroudi U (2010) Novel biomixtures based on local Mediterranean lignocellulosic materials: evaluation for use in biobed systems. Chemosphere 80:914–921

Kuo HC, Juang DF, Yang L, Kuo WC, Wu YM (2014) Phytoremediation of soil contaminated by heavy oil with plants colonized by mycorrhizal fungi Int. J Environ Sci Technol 11:1661–1668

Lentzarizos C, Avramides EJ, Kokkinaki K (2006) Residues of azoxystrobin from grapes to raisins. J Agric Food Chem 54:138–141

McEldoon JP, Pokora AR, Dordick JS (1995) Lignin peroxidase type activity of soybean peroxidase. Enzyme Microb Technol 17:359–365

Mitton FM, Mariana G, Aranzazu P, Karina S (2012) Effects of amendments on soil availability and phytoremediation potential of aged p, p_-DDT, p, p_-DDE and p, p_-DDD residues by willow plants (Salix sp.). J Hazard Mater 203–204:62–68

Romeh AA (2014) Phytoremediation of cyanophos insecticide by *Plantago major* L. in water. J Environ Health Sci Eng 12:2–8

Romeh AA, Mohammed YH (2013) Chlorpyrifos insecticide uptake by plantain from polluted water and soil. Environ Chem Lett 11:163–170

Roy S, Ihantola R, Hanninen O (1992) Peroxidase activity in lake macrophytes and its relation to pollution tolerance. Environ Exp Bot 32:457–464

Saichek RE, Reddy KR (2004) Evaluation of surfactants/cosolvents for desorption/solubilization of phenanthrene in clay soils. Int J Environ Stud 61:587–604

Sharifa AA, Neoh YL, Iswadi MI, Khairul O, Abdul Halim MM, Jamaludin Mohamed A, Hing HL (2008) Effects of methanol, ethanol and aqueous extract of *Plantago major* on gram positive bacteria, gram negative bacteria and yeast. Ann Microsc 8:42–44

Singh N, Shashi B (2010) Effect of moisture and compost on fate of azoxystrobin in soils. J Environ Sci Health Part B 45:676–681

Singh N, Singh SB, Mukerjee I, Gupta S, Gajbhiye VT, Sharma PK, Goel M, Dureja P (2010) Metabolism of $^{14}$C-azoxystrobin in water at different pH. J Environ Sci Health Part B 45:123–127

Smith E, Smith J, Naidu R, Juhasz A (2004) Desorption of DDT from a contaminated soil using cosolvent and surfactant washing in batch experiments. Water Air Soil Pollut 151:71–86

Sundravadana S, Alice D, Samiyappana R, Kuttalamb S (2008) Determination of azoxystrobin residue by UV detection high performance liquid chromatography in mango. J Braz Chem Soc 19:60–63

Tahmasbian A, Sinegani AS (2014) Chelate-assisted phytoextraction of cadmium from a mine soil by negatively charged sunflower. Int J Environ Sci Technol 11:695–702

Tassi E, Pouget J, Petruzzelli G, Barbafieri M (2008) The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. Chemosphere 71:66–73

Thomsen V, Schatzlein D, Mercuro D (2003) Limits of detection in spectroscopy. Spectroscopy 18:112–114

Turgut C (2005) Uptake and modeling of pesticides by roots and shoots of parrot feather (*Myriophyllum aquaticum*). Environ Sci Pollut Res 12:342–346

United States Environmental Protection Agency (1997) Pesticide fact sheet: azoxystrobin. http://www.epa.gov/opprd001/ factsheets/azoxystr.pdf

Ussawarujikulchai A, LahaS Tansel B (2008) Synergistic effects of organic contaminants and soil organic matter on the soil–water partitioning and effectiveness of a nonionic surfactant (Triton X-100). Bioremed J 12:88–97

Wang P, Keller AA (2009) Partitioning of hydrophobic pesticides within a soil–water–anionic surfactant system. Water Res 43:706–714

Wang S, Haibin S, Yanping L (2013) Dissipation and residue of azoxystrobin in banana under field Condition. Environ Monit Assess 185:7757–7761

Wu N, Zhang S, Huang H, Shan X, Christie P, Wang Y (2008) DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant. Environ Pollut 151:569–575

Xu J, Xu Y, Shugui D (2006) Effect of surfactants on desorption of aldicarb from spiked soil. Chemosphere 62:1630–1635

Yan-Zheng G, Ling W, Zhu L, Zhao B, Zheng Q (2007) Surfactant-enhanced phytoremediation of soils contaminated with hydrophobic organic contaminants: potential and assessment. Pedosphere 17:409–418

Yao B, Gu X, Jing S, He L, Xiao N (2009) Microbial activity and degradation of atrazine in poplar rhizosphere during the soil phytoremediation. Scientia Silvae Sinicae 45:149–152

Yu X, Gu JD (2008) The role of EDTA in phytoextraction of hexavalent and trivalent chromium by two willow trees. Ecotoxicology 17:143–150

Zhang SJ, Li TX, Huang HG (2014) Phytoremediation of cadmium using plant species of *Athyrium wardii* (Hook.). Int J Environ Sci Technol 11(757):764

Zhao BW, Zhu LZ, Gao YZ (2005) A novel solubilization of phenanthrene using Winsor I microemulsion-based sodium castor oil sulfonate. J Hazard Mater 119:205–211