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Dynamics of phoxim residues in green onion and soil as influenced by arbuscular mycorrhizal fungi

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ABSTRACT

Organophosphorus pesticides in crops and soil pose a serious threat to public health and environment. Arbuscular mycorrhizal (AM) fungi may make a contribution to organophosphate degradation in soil and consequently decrease chemical residues in crops. A pot culture experiment was conducted to investigate the influences of *Glomus caledonium* 90036 and *Acaulospora mellea* ZZ on the dynamics of phoxim residues in green onion (*Allium fistulosum* L.) and soil at different harvest dates after phoxim application. Results show that mycorrhizal colonization rates of inoculated plants were higher than 70%. Shoot and root fresh weights did not vary with harvest dates but increased significantly in AM treatments. Phoxim residues in plants and soil decreased gradually with harvest dates, and markedly reduced in AM treatments. Kinetic analysis indicated that phoxim degradation in soil followed a first-order kinetic model. AM inoculation accelerated the degradation process and reduced the half-life. *G. caledonium* 90036 generally produced more pronounced effects than *A. mellea* ZZ on both the plant growth and phoxim residues in plants and soil. Our results indicate a promising potential of AM fungi for the control of organophosphate residues in vegetables, as well as for the phytoremediation of organophosphorus pesticide-contaminated soil.

1. Introduction

Organophosphorus pesticides are used worldwide as an ideal alternative to organochlorine pesticides for disease control in agriculture. In China, many investigations have reported the organophosphorus pesticide residues in vegetables exceeding food safety standards [1–3]. For example, the mean phoxim and methamidophos residues in vegetables in two Chinese villages reached 89.9 and 36.5 $\mu g\ kg^{-1}$, which surpassed the national tolerance [4]. Organophosphorus pesticides in crops and soil have posed a serious threat to public health and environment and received more attention.

Many studies have reported that arbuscular mycorrhizal (AM) fungi have positive effects on the behaviors of organic contaminants such as atrazine [5,6], PAHs [7–10], DDT [11] and *p,p*-DDE in soils [12]. Mycorrhizal effects on organic pollutants may include direct effects of enzymes secreted by hyphae, and indirect effects of enhanced root-derived enzymes, enhanced microbial activity and modified microbial composition, and unspecific effects of changes in pH, osmotic potential, redox potential, etc [6,10,11,13,14]. Thus, it is expectable that AM fungi make a direct and/or indirect contribution to enhance the degradation of organophosphorus pesticides and consequently decreasing pesticide residues in soils and crops grown on polluted sites.

Phoxim is one of the most widely used organophosphate pesticides in vegetable production in China. It has been reported that this pesticide can be degraded by bacteria [15,16] or fungi [17]. We have found that inoculation with AM fungi decrease phoxim residues in vegetables and enhance soil phosphatase activity, and may contribute to degradation of pesticides in soil [unpublished data]. However, whether AM can accelerate the degradation process of organophosphorus pesticides in soil still remains unclear. Here, we hypothesize that AM inoculation might make a contribution to phoxim degradation in phoxim-contaminated soil.

In the present study, a greenhouse pot culture experiment was carried out using green onion, one most common vegetable in China. The aims were: (1) to assess the effect of AM inoculation on the growth of green onion grown in soil applied with phoxim; (2) to identify whether AM inoculation can decrease the phoxim residues in vegetable; and (3) to assess the influence of AM inoculation on the degradation dynamics of phoxim in soil.

2. Materials and methods

2.1. AM inocula and host plants

Two AM fungal inocula, *Glomus caledonium* 90036 and *Acaulospora mellea* ZZ, were propagated on alfalfa (*Medicago sativa*

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L.) grown in a soil–sand mixture in greenhouse for 3 successive propagation cycles, each 4 months long. The inocula were air-dried and sieved (2 mm), and each consisted of a mixture of rhizospheric soil from pure pot culture containing spores, hyphae and mycorrhizal root fragments. At the same time, the control nonmycorrhizal inoculum was also prepared following the same procedure.

The seeds of green onion (*Allium fistulosum* L. cv. Fengwang) were surface sterilized in a 10% (v/v) H_2O_2 solution for 10 min, and subsequently washed several times with distilled water. Seeds of uniform size were selected for sowing.

2.2. Soil

The soil was collected from the surface horizon (0–15 cm depth) of an experimental field at Henan University of Science and Technology. After sifting through a 2 mm sieve, the soil was sterilized by autoclaving at 121 °C for 2 h and then air-dried and thoroughly mixed. The soil type is fluvo-aquic soil and soil texture is loamy soil, with the following properties: soil pH (soil/water, 1:2.5) 7.9, 1.62% organic matter, 65.3 mg kg^{-1} alkali-hydrolyzable N, 21.4 mg kg^{-1} Olsen P, 120.0 mg kg^{-1} 1 M NH_4OAc extractable K.

2.3. Pesticide, reagents and standards

The phoxim applied to soils was a commercial 40% phoxim emulsifying concentrate marketed in China. Standard substance of phoxim (0.1 mg mL^{-1}) purchased from Beijing Beihua Hengxin Biotechnology Co. Ltd. was used for phoxim analysis. All other reagents were HPLC-grade or analytical reagents.

2.4. Experimental procedure

This study was designed as a two-factor experiment: (a) inoculation treatments and (b) harvest date. Phoxim was diluted in deionized water to prepare the solution and the target concentration was 800 mg L^{-1}, twice the recommended dose. Mycorrhizal treatments received 20 g of mycorrhizal inocula, while the control treatment received an equivalent nonmycorrhizal inoculum to provide a similar microflora. The inocula were placed in the middle layer of the air-dried soil (1200 g) in pottery pots. Thus, there were 3 treatments in total with 18 replicates, giving a total of 54 pots.

The seeds were sown on 31 July 2008. Six uniform seedlings were left after emergence. Plants were grown in a greenhouse with natural light in a randomized block design. Plants were watered with tap water to maintain soil moisture at about 70% of water holding capacity during the experimental period. No fertilizers were applied in the experimental procedure.

Phoxim solutions were applied on 25 November 2008. Prior to application pots were left without watering for 2 d. The 0.5 cm depth surface soil was removed and 100 mL of phoxim solution was poured slowly into the soil around the roots. Then the surface soil was placed back.

Three randomly chosen pots in each treatment were harvested at 0, 1, 3, 5, 7, 14 d, respectively, after phoxim addition, and the soil were sampled immediately after phoxim addition to determine the initial phoxim concentrations (at 0 d). Shoots and roots were sampled separately, rinsed with distilled water, wiped with tissue paper, and weighed immediately. The sub-samples of roots were left to assess mycorrhizal colonization and other samples were stored at −20 °C for pesticide analysis. The whole pot of soil was thoroughly mixed after root harvest and 100 g fresh soil was taken up from each pot and stored at −20 °C for pesticide analysis.

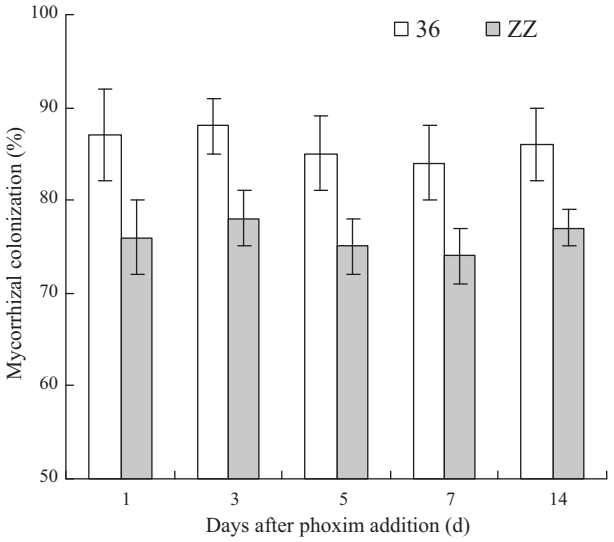

Fig. 1. Mycorrhizal colonization rates (mean ± S.E.) of green onion under different inoculation treatments and harvest dates. 36 and ZZ represent inoculation with *Glomus caledonium* 90036 and *Acaulospora mellea* ZZ, respectively.

2.5. Plant and soil analysis

Root mycorrhizal colonization was estimated after clearing and staining, using the acid fuchsin staining-grid intersect method [18].

Phoxim in vegetables was extracted following the standard method established by the Ministry of Agriculture of China [19] and phoxim in soil following Chinese National Standards [20]. Phoxim residues were analyzed using an Agilent 1100 HPLC system equipped with an auto-injector and a DAD detector. An Agilent ZORBAX SB-C18 column (150 mm × 2.1 mm i.d., 5 μm) from Agilent Technologies was used for separations. The mobile phase was a mixture of ACN–water (65:35, V/V), and the flow rate was 0.45 mL min^{-1}. The column temperature was set at 30 °C, and the DAD detector was set at a wavelength of 280 nm. The injection volume was 4 μL. The recoveries were between 95.0% and 100.4%.

2.6. Data analysis

The degradation dynamics of phoxim in soil was tested using the first-order kinetic equation, which may be expressed as

$$C_t = C_0\, e^{-kt} \quad (1)$$

where C_0 is the initial phoxim concentration, C_t is the concentration at time t, k is the first-order rate constant, and t is time.

Data were subjected to two-way ANOVA using the SPSS version 13.0 software package to determine the significance of AM inoculation, harvest date, and AM inoculation/harvest date interactions as sources of variation. Means and standard errors were calculated for three replicate values. Comparisons between means were carried out using Duncan's multiple range test at a significance level of $P < 0.05$, 0.01 or 0.001.

3. Results

3.1. Mycorrhizal colonization

Plants in the control treatment were not colonized. Mycorrhizal colonization rates of the inoculated plants were all higher than 70%, but much lower in ZZ treatment than in 36 treatment (Fig. 1). The mycorrhizal colonization rates did not vary with harvest dates.

Two-way ANOVA results show that AM inoculation had a significant effect on mycorrhizal colonization, but harvest date and

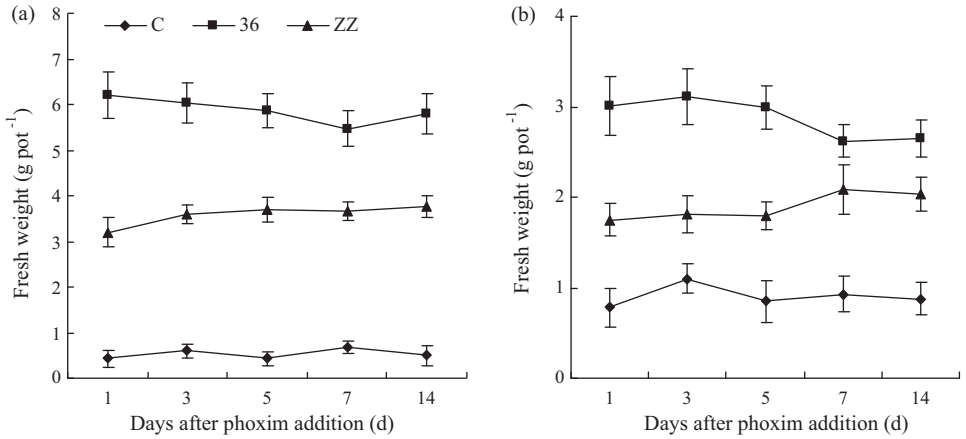

Fig. 2. Fresh weights (mean ± S.E.) of shoots (a) and roots yields (b) of green onion under different inoculation treatments and harvest dates. C, 36 and ZZ represent noninoculation, inoculation with *G. caledonium* 90036 and *A. mellea* ZZ, respectively.

Table 1
Significance level (*F*-values) of effects of different factors and factor interactions on variables based on a two-way ANOVA analysis.

Variable	Inoculation treatment	Harvest date	Inoculation treatment × harvest date
Mycorrhizal colonization	328.5***	ns	ns
Shoot fresh weight	296.7***	ns	ns
Root fresh weight	288.5***	ns	ns
Phoxim residue in shoot	203.4***	198.6***	26.4***
Phoxim residue in root	303.2***	211.0***	38.9***
Phoxim residue in soil	245.6***	372.1***	48.6***

ns: non-significant effect.
*** Significant levels: $P < 0.001$.

AM inoculation/harvest date interactions had no significant effects (Table 1).

3.2. Plant biomass

Fig. 2 shows that phoxim harvest date had no significant effects on shoot or root fresh weights in all three treatments. Both inocula markedly enhanced shoot and root fresh weights, and *G. caledonium* 90036 was much more effective than *A. mellea* ZZ. The average yield of green onion (edible part) in 36 and ZZ treatments was 11.2 and 6.8 times that of the nonmycorrhizal control, respectively.

Two-way ANOVA results show that AM inoculation had significant effects on shoot and root biomass, but harvest date and AM inoculation/harvest date interactions had no significant effects (Table 1).

3.3. Phoxim residues in vegetables

Phoxim residues in shoots of the control plants showed a sharp decrease between the 1 d and 7 d after phoxim addition, and thereafter, decreased slowly (Fig. 3a). Both AM inocula significantly decreased phoxim residues in shoots at all harvest dates. There were no significant differences between the two AM inocula except for at 3 d after phoxim addition.

Phoxim residues in roots of control plants firstly increased at 3 d after phoxim addition, and then decreased gradually (Fig. 3b). Both AM inocula significantly decreased phoxim residues in roots at all harvest dates. On the whole, the plants inoculated with *G. caledonium* 90036 had lower root phoxim residues than those inoculated with *A. mellea* ZZ.

Two-way ANOVA results show that AM inoculation, harvest date and the interactions between them all had significant effects on phoxim residues in shoots and roots (Table 1).

3.4. Phoxim residues in soil

Fig. 4 shows that phoxim residues in soil after harvest gradually decreased with harvest dates. Both AM inocula significantly

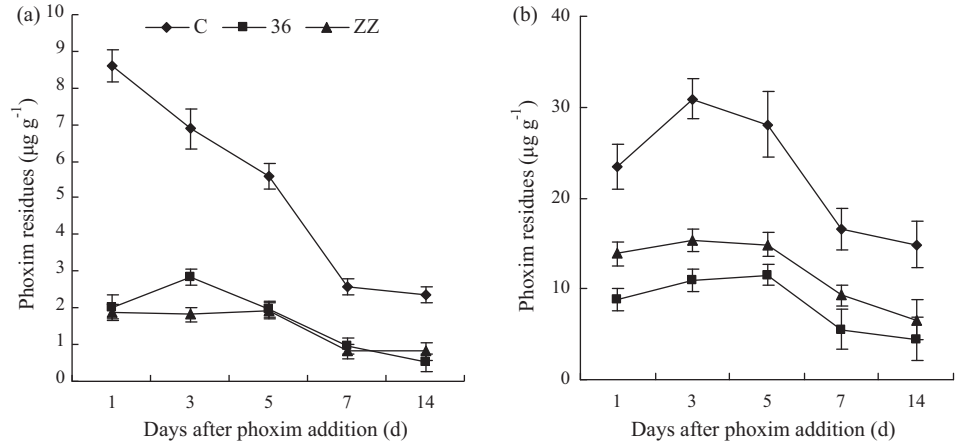

Fig. 3. Phoxim residues (mean ± S.E.) in green onion shoots (a) and roots (b) under different inoculation treatments and harvest dates. C, 36 and ZZ represent noninoculation, inoculation with *G. caledonium* 90036 and *A. mellea* ZZ, respectively.

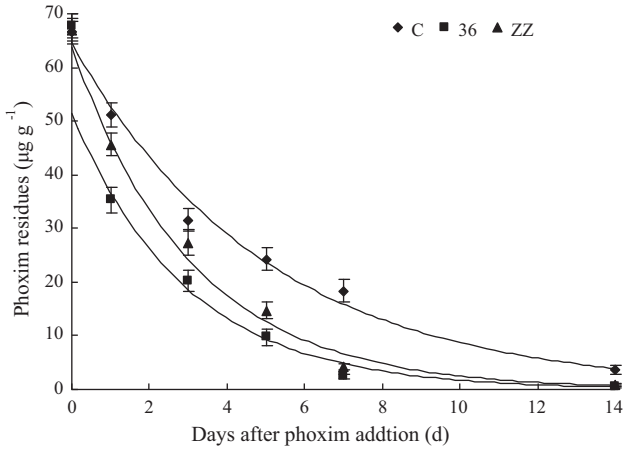

Fig. 4. Phoxim residues (mean ± S.E.) in soil grown with green onion plants under different inoculation treatments and harvest dates. C, 36 and ZZ represent noninoculation, inoculation with *G. caledonium* 90036 and *A. mellea* ZZ, respectively.

Table 2
First-order kinetic equation and half-life of phoxim in soil under different inoculation treatments and harvest dates.

Treatment	Regression equation	R^2	*P* value	Half-life (d)
C	$C_t = C_0\ e^{-0.2003t}$	0.9924	<0.001	3.5
36	$C_t = C_0\ e^{-0.3395t}$	0.9617	<0.001	2.0
ZZ	$C_t = C_0\ e^{-0.3236t}$	0.9805	<0.001	2.1

C, 36 and ZZ represent noninoculation, inoculation with *G. caledonium* 90036 and *A. mellea* ZZ, respectively.

decreased phoxim residues in soil, and *G. caledonium* 90036 showed a more effective effect than *A. mellea* ZZ (except at 14 d after phoxim addition).

Two-way ANOVA results show that AM inoculation, harvest date and the interactions between them all had significant effects on phoxim residues in soil (Table 1).

3.5. Degradation kinetics of phoxim in soil

Linear regression analysis was performed using the data in Fig. 4 and it was found that the degradation process of phoxim in soil was well fitted to the first-order kinetic equation. The rate constant *k* was obtained and half-life of phoxim was calculated as 0.693/*k*, respectively (Table 2). The high R^2 values indicated that the degradation progress of phoxim in soil could be adequately described by the first-order model.

Table 2 shows that both AM inocula accelerated the degradation of phoxim in soil. The half-life was found to be shorter in mycorrhizal treatments than in the control, with an order of C > ZZ > 36.

4. Discussion

Organophosphorus pesticides generally have little or no adverse effects on AM colonization at recommended dosages, but show deleterious effects at higher application levels [21–23]. At the recommended dose, phoxim had no significantly adverse effects on AM colonization and cardoon growth [24]. Our results show that mycorrhizal colonization rates of all inoculated plants were higher than 70%, and plant growth did not vary with harvest dates. One of the reasons may be due to that the plants have reached maturity, and the mycorrhizae have been formed when phoxim was applied. Additionally, these results also indicate low toxicity of phoxim to AM colonization.

The half-life of phoxim in soil varied from 1 d to 8 weeks in various laboratory and field experiments [25]. In our results, the half-lives were between 2.0 d and 3.5 d (Table 2), indicating that, in general, phoxim appeared to be easily degradable and less persistent in soil environments. Besides biodegradation, abiotic degradation including photodegradation and hydrolysis may also make a contribution [26], which needs a comparative study to determine which process dominates phoxim degradation in soil.

The mechanisms involved in the mycorrhizal effects on PAH degradation have been summarized as two main indirect effects including the enhanced degradation activity of other rhizosphere microorganisms and the enhanced activity of enzymes in roots and rhizosphere soil [14], which have been proved for other organic pollutants such as atrazine [6], phenanthrene [10], and DDT [11]. Our results confirm that AM inoculation accelerated the degradation process of phoxim in soil. Mycorrhizal effects on phoxim degradation may include the indirect effects such as enhanced root growth, enhanced microbial degradation activities, enhanced soil enzyme activities, and direct effects of mycorrhizal structures such as extraradical mycelium. Plant roots were greatly increased by AM fungi (Figs. 2 and 3), and they might excrete more root exudates and root-derived enzymes. Thus, microbial composition can be modified and microbial activity may be enhanced due to C input from root exudates. Soil enzyme activities can be enhanced due to higher root biomass and mycorrhizal mycelium. In another experiment, we found higher phosphatase activities in phoxim-polluted soil received AM inocula [unpublished data]. Additionally, AM fungi may also induce changes in soil pH, osmotic potential, redox potential, etc, which all need further investigations.

The direct metabolism by AM fungi may also be involved in the phoxim degradation. In a split-dish in vitro carrot mycorrhiza system free from contaminating microorganisms, extraradical hyphae of *G. intraradices* can hydrolyse organic P, and, further, that the resultant inorganic P can be taken up and transported to host roots [27]. Other studies have also found that mycorrhizal hyphae can produce phosphatase hydrolyzing organic P compounds and contribute to utilization of P by plants [28–30]. Likely, arbuscular mycorrhizae may also produce enzymes participating in phoxim degradation process. AM symbiosis can both enhance decomposition of and increase N capture from complex organic material (grass leaves) in soil [31], indicating that AM symbiosis can have saprotrophic capability. Phoxim, as an organic source of P and N, can be degraded and utilized by bacteria [15,16] and fungi [17]. Thus, it might be inferred that AM fungi participate in the catabolic process of phoxim.

We also found that AM inoculation decreased phoxim residues in green onion. In addition to the accelerated phoxim degradation included in mycorrhizal effects, there are also other explanations. One possible reason is the dilution effect caused by increased growth due to mineral nutrition especially P, which may partly explain the low phoxim residues in plants. However, dilution effect was unlikely to have been the only explanation, because colonized roots had higher biomass and larger surface area, and the phoxim residues in them should not be lower than in the nonmycorrhizal controls if they have the same or higher capacity for phoxim uptake. Phoxim is a plant-metabolized organophosphorus pesticide [32]. Thus, AM fungi may indirectly influence its metabolic process in plants through mycorrhizal effects on plant metabolic activities. On the other hand, AM fungi can colonize root cells to form arbuscules and vesicles, and thereby may directly participate in phoxim metabolism and/or cooperate with plants. Additionally, in our present experiment, only molecular form of phoxim was determined, and the metabolic intermediates of phoxim were not included but may be present in soil, plants and AM fungi. Further studies using ^{14}C and ^{32}P-labelled phoxim should be carried out to study the fate and the metabolic pathway of pesticides in mycorrhizal rhizosphere, mycorrhizal plants, and AM fungal structures such as mycelia and spores.

AM fungi react differently to pesticides [24,33,34]. In our study, *G. caledonium* 90036 showed more pronounced effects than *A. mellea* ZZ did on both the growth of green onion and phoxim residues in plants and soil, probably because of the high inoculum potential of *G. caledonium* 90036 and high compatibility between this isolate and green onion. Clearly, the interaction between AM fungi and pesticides varied with the toxicity and doses of pesticides, the tolerance of AM fungi, host plants, and the environmental factors influencing AM fungi and plants. These all need further investigations for practical application of AM fungi in future.

5. Conclusions

Here our preliminary results have provided the first evidence that AM fungi can accelerate the degradation process of organophosphorus pesticides and decrease the residues in plants and soil. Phoxim appeared low toxic to AM fungi. AM inoculation increased plant growth and decreased phoxim residues in plants and soil. Phoxim degradation in soil followed a first-order kinetic model, and AM inoculation accelerated the degradation process and reduced the half-life. Our results indicate a promising potential of AM fungi for vegetable production and the control of organophosphorus pesticide residues, as well as the phytoremediation of organophosphorus pesticide-contaminated soil.

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