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Effect of *Funnelliformis mosseae* Inoculation on Phytoremediation of Atrazine by the Aquatic Plant *Canna indica* L. var. *flava* Roxb.

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Abstract: Atrazine residue in water poses a serious threat to the environment and to human health. One method to reduce levels of atrazine in the environment is by phytoremediation, a potential technology for *in-situ* remediation. However, as atrazine is a herbicide, it damages the growth of plants and weakens the effect of phytoremediation. In this work, a pot culture experiment was conducted to investigate the effect of *Funnelliformis mosseae* inoculation on the phytoremediation of atrazine by *Canna indica* L. var. *flava* Roxb.. The results demonstrated that *C. indica* was found as a novel tolerant species, and that inoculation with *F. mosseae* can alleviate the physiological inhibition of atrazine in the growth of plants and promote photosynthetic. Furthermore, *C. indica* inoculated with *F. mosseae* exhibited a greater efficiency to remove atrazine and to lower atrazine residue concentrations than plants without inoculation. With inoculation of *F. mosseae*, the maximum removal rates increased from 68.064% to 95.670%, while the concentration with the highest removal rates changed from 1.489 mg Ł$^{-1}$ to 7.363 mg Ł$^{-1}$. Inoculation of *F. mosseae* contributed 2.2% to 52.0% to the removal rate. This study shows that *C. indica* inoculated with *F. mosseae* may ultimately serve as a viable phytoremediation solution for *in-situ* remediation.

Keywords: Atrazine, Arbuscular mycorrhizal fungi, Phytoremediation, Inoculation, Removal，Aquatic

1. Introduction

As a chloro-s-triazine herbicide, atrazine has been widely used in agricultural and forestry applications to inhibit photosynthesis of plants.[1,2] Due to its extensive application and moderately high solubility (ca. 34.7 mg Ł$^{-1}$at 26°C), atrazine can enter waterways by surface runoff and leaching into groundwater.[2] Since the 1980s, the presence of atrazine in the environment has raised concerns as this herbicide is suspected to be a potential human carcinogen;[3] atrazine has been identified to have endocrine disrupting effects.[1] As a consequence, atrazine has been classified as one of the major anthropogenic pollutants, which requires immediate attention and effective

development of methods for its decontamination.[4,5]

Phytoremediation, the use of plants to alleviate organic contamination, has become a potentially cost-effective alternative to traditional practices for *in-situ* remediation.[6,7] Phytoremediation processes of aquatic vegetation within aqueous systems appear to be essential components of herbicide mitigation.[8,9] However, in the peak period of herbicide application, atrazine concentrations have dramatically increased in environmental waters.[10,11] The high concentration of atrazine may cause severe damage to aquatic plants, some of which can be terminal.[12,13] These effects will seriously reduce the effectiveness of phytoremediation, and they provide a threat to the stability of the phytoremediation system.

The utilisation of artificial wetlands and macrophyte-cultured ponds for the treatment of agricultural drainage water, sewage and industrial effluents are currently being developed. Aquatic plant-based water treatment systems have proved effective and economical in improving the quality of wastewater containing excess organic pollutants.[14] Previous studies have shown that *C. indica* can be used to remediate contaminants in water. As an ornamental plant of tropical origin, *C. indica* is tolerant of soil with a high water content, such as wetlands or river banks, thus making it suitable for soil remediation as well as for wastewater treatment in wetland areas.[15] Historically, numerous investigations have focused on the adsorption and removal of heavy metals, while the potential remediation of organic pollutants has been largely overlooked. Wilson suggested that *Canna hybrida* might be a suitable species for phytoremediation of simazine,[16] and Cheng found *C. indica* Linn. exhibited a potential for the phytoremediation of the pesticide triazophos.[17] However, the influence of *C. indica* on the phytoremediation of herbicides, such as atrazine; further investigations are required.

Arbuscular mycorrhizal (AM) fungi are ubiquitous symbiotic associations found in both natural and agricultural ecosystems, including organic-contaminated sites.[18-20] AM fungi can significantly improve plant growth by increasing nutrition uptake and alleviating toxicity of pollutants.[21,22] AM fungi can also provide direct links between the growing medium and plant roots, and consequently they may significantly influence the uptake and translocation of organics by plants, as well as the dissipation and degradation of organics in soils, including atrazine.[23,24]

It has been reported that atrazine can be degraded by ectomycorrhizal and ericoid mycorrhizal fungi.[25,26] Investigations into the phytoremediation potential of atrazine usually focus on resistant plants, such as *Zea mays*[24,27] and concentrate on the remediation of contaminated soil.[12,28,29] However, few investigations have been undertaken on the phytoremediation of atrazine in water, especially by common aquatic plants. Taking into account that atrazine is prone to leaching into groundwater and is readily removed by surface runoff, immediate attention and the effective development of methods for the decontamination of polluted water is urgently required. For this investigation the aquatic plant *Canna indica* L. var. *flava* Roxb. was selected, and AM fungi inoculation was undertaken. With two treatments and eight atrazine concentrations, a pot experiment was carried out to: (1) assess the effects of *Funnelliformis mosseae* inoculation on the growth and photosynthesis of the

atrazine-treated aquatic plant *C. indica*; and (2) identify whether *F. mosseae* inoculation can remove atrazine from contaminated media and decrease the atrazine residue in *C. indica*. The remediation potential of atrazine contaminated water by *C. indica* with AM fungi inoculation was systematically evaluated.

2. Materials and Methods

2.1. Plant culture and AM fungi inoculation

The original inoculum of the AM fungi *F. mosseae* was provided by the Life Science College of Heilongjiang University (Harbin, China). The inoculum contained about 20-25 *F. mosseae* spores per g, and the spores were used as original inoculum in the propagation. The mycorrhizal inoculum was propagated in a soil-sand-vermiculite substrate (volume ratio of 2:5:3) with white clover (*Trifolium repens* L.) as host plants in pot cultures for about three months under greenhouse conditions. The soil-sand-vermiculite substrate used in the propagation was autoclaved three times for 2 h at 121 ℃; there was a one day interval between each autoclave treatment. Prior to the beginning of the experiment, the inoculated roots of the white clover were cut into <1 cm lengths, air dried, homogenized with the compounded substrate and sieved (<2 mm).[30]

Seedlings of *C. indica* L. var. *flava* Roxb. with similar shapes and sizes(about 15 cm in height) were chosen for the experiment. The substrate for the plant culture was vermiculite; this was autoclaved for 2 h at 121 ℃. Three seedlings were transplanted and cultivated in a plot with the same weight of sterile vermiculite. Two inoculation treatments (CK and FM) were set up according to non-inoculation and inoculation:

CK: Control, each plot received 36g of the sterile mycorrhizal inoculum as the non-inoculation treatment. The sterile mycorrhizal inoculum was obtained by autoclaving the inoculums three times for 2 h at 121 ℃; there was a one day interval between each autoclave treatment.

FM: Inoculated with *F. mosseae*, each plot received 36g of the mycorrhizal inoculum as the inoculation treatment.

All of the experiment pots were placed in a random pattern in a greenhouse; and their relative positions were changed weekly to minimize the effects of environmental gradients. The light/dark cycle was 14/10 h, with light time at 30 ℃ and dark time at 20 ℃.[31]

2.2. Atrazine exposure

A month after inoculation, the two inoculation treatments were exposed to eight different concentrations of atrazine. The concentrations were 0, 0.1, 0.5, 1, 3, 5, 10 and 15 mg L^{-1}. A zero concentration was used as a blank for control purposes. Each concentration for each treatment used triplicate polyvinylchloride containers with 5 L of Hoagland solution containing the corresponding concentration of atrazine. Three pots of plants were placed into each container. Hoagland solution was added to each container to the total volume of 5 L every two days. With the same culture condition, plants were exposed to atrazine for 21 days, after which the plants were sampled for the assays of shoot height, root length and biomass.

After exposure to atrazine for 14 days the leaf area of the plants was measured using a leaf area meter (Model LI-3100, Licor, Lincoln, NE), these measurements corresponded with the photosynthesis measurements. The total leaf area (TLA), blighted leaf area (BLA) and the effective leaf area (ELA) were measured, respectively.

The relative shoot height growth of the plants, inoculated with and without *F. mosseae*, was compared after exposure to atrazine for 21 days. The relative shoot height growth (RSHG) was calculated according to the equation:

$$RSHG=H-H_0 \quad (1)$$

where H and H_0 are, respectively, the final and initial shoot height of the plants(cm).

Mycorrhizal dependency was evaluated using the relative mycorrhizal dependency index (MDI)[32] as follows:

$$MDI = (X_{AMF}-X_{non\text{-}AMF})/X_{AMF} \quad (2)$$

where X_{AMF} was the value (height, length and biomass) of the plants in the mycorrhizal inoculation treatment; $X_{non\text{-}AMF}$ was the value of the control plants.

2.3. Determination of root colonization rate

To estimate the proportion of total root length infected by *F. mosseae*, a 1 g sample of fresh roots was randomly taken and cut into approximately 1 cm pieces. Root segments were immersed in 10% (v/v) KOH for 30 min at 100 ℃ in a water bath, rinsed with water, neutralized with 2% (v/v) HCl for 3 times, rinsed with water again, and then stained with 5% lactic acid-fuchsin for 30 min at 90 ℃ in a water bath. The 5% lactic acid-fuchsin contained 0.75 g acid fuchsin, 100 ml 85 % (v/v) lactic acid and 100 ml glycerinum within 200 ml staining solution. The root colonization rate was determined using the grid line intersect method.[33] In summary, this method required the stained root segments to be arranged lengthwise on a thin layer of polyvinyl acetate mountant on a microscope slide. A hairline graticule inserted into the eyepiece of a compound microscope acted as a line of intersection with the roots. Fungal structures at each intersection were calculated by observation at 100× magnification. Photographs of the roots were taken using an Olympus camera connected to the microscope.

2.4. Measurement of photosynthesis

The net photosynthetic rate (*Anet*) was measured using a CIRAS-2 portable photosynthesis system (PP systems, Hitchin, UK) on the middle region of the second youngest fully developed leaf of each plant. The following parameters were calculated: (1) the instantaneous water-use efficiency (WUE) was expressed as A_{net}/E, and the intrinsic water-use efficiency (WUE') was expressed as A_{net}/g_s;[34] (2) the carboxylation capacity (carboxylation efficiency, CUE) was expressed as A_{net}/C_i;[35] (3) the light-use efficiency (LUE) was expressed as A_{net}/PAR.[36] The measurements were recorded between 8:00-11:00 a.m. after being exposed to atrazine for 14 days; three replicates per pot were cultivated at 25℃ under a relative humidity of 75% and a CO_2 concentration of 360 μmol·mol^{-1}.[31] The level of incident Photosynthetic Photon Flux

Density (PPFD) was conducted at 2000μmol m^{-2} s^{-1}. Each measurement was taken after 3-15 min to allow the corresponding photosynthesis to stabilize following the variation in PPFD level after the incident light had changed.[37]

2.5. Atrazine analysis of plant and water samples

Plant samples (fresh plant material, divided into shoots and roots) were cut into small pieces using a mechanical slicer, then extracted using a dichloromethane-petroleum ether system, rotary evaporated and cleaned with Florisil on a solid phase extraction column.[8]

An anhydrous Na_2SO_4 column was used to remove any water from the solvents before they were evaporated and exchanged by acetonitrile until a final volume of 2 mL was achieved. The samples were then analysed using high-performance liquid chromatography (HPLC; Shimadzu, LC-10A). Recovery of atrazine from spiked plant samples averaged 93.15% (n = 5, RSD < 2.1%).

For the water samples, a water solution containing atrazine was filtered through a 0.22 μm filter unit, and analyzed using HPLC. The concentrations in the water samples were high enough to be quantified without extraction, compared to Chu's reports with 0.01 mM (about 2 mg L^{-1}) initial concentration of atrazine.[38]

A C18-BDS column (5 μm, 25 × 0.46 cm) was used with a 60% acetonitrile (ACN) in water (v/v) mixture as the mobile phase at a flow rate of 1.0 mL/min. The optimal wavelength for atrazine detection was selected as 220 nm.[39] The retention time of atrazine was 6.47 min. The limit of detection was 4.878 μg L^{-1}, and the limit of quantification was 16.260 μg L^{-1} for this atrazine analysis method. Atrazine (>99.1% purity) was purchased from Sigma-Aldrich (St Louis, USA).

2.6. Statistical analysis

All statistical analyses were performed using SPSS ver. 17.0 of Statistical Software Package (SPSS Inc., Chicago, IL). Standard error (SE) was used as a measure of variance.[40] One-way ANOVA (Duncan test) was performed to ascertain whether parameters were significantly different among treatments (α=0.05). Bivariate correlation analyses were performed among biomass and concentration of residual atrazine in the plants. Significance was accepted at $p<0.05$ in all cases (Person's correlation).

3. Results and Discussion

3.1. Mycorrhizal root colonization and plant growth

After inoculation for 30 days, *C. indica* roots were infected by arbuscular mycorrhizal fungi *F. mosseae* to form mycorrhizal symbionts. As shown in Fig. 1, the hypha, arbuscules and vesicles can be clearly observed. The hypha passed through intercellular space and developed into arbuscules in the roots. The root colonization rate of *C. indica* was measured at 19.4±3.7% after *F. mosseae* inoculation for 30 days. The root colonization rate showed low frequency of AMF colonization in *C. indica* (below 25%), this being similar to the results reported by Wang.[41] Note that the colonization rate was not significantly affected by atrazine exposure, as shown in

Fig.2. This means that the mycorrhizal root colonization in this study was steady, especially in the high atrazine concentration levels. This may be a positive basis for the phytoremediation of atrazine. By contrast, roots of the non-inoculated *C. indica* remained undetectable.

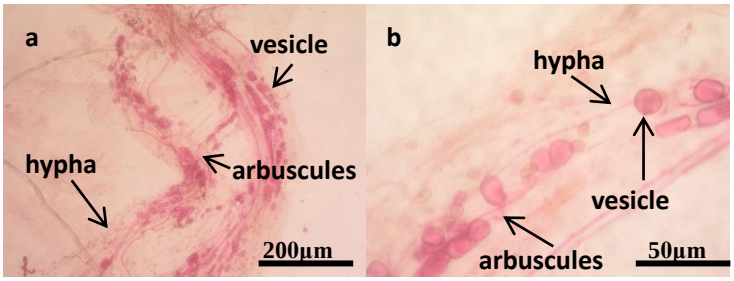

Fig. 1. The structure of arbuscular mycorrhizal fungi. Fig. a: under a 100× light microscope; Fig. b: under a 400× light microscope.

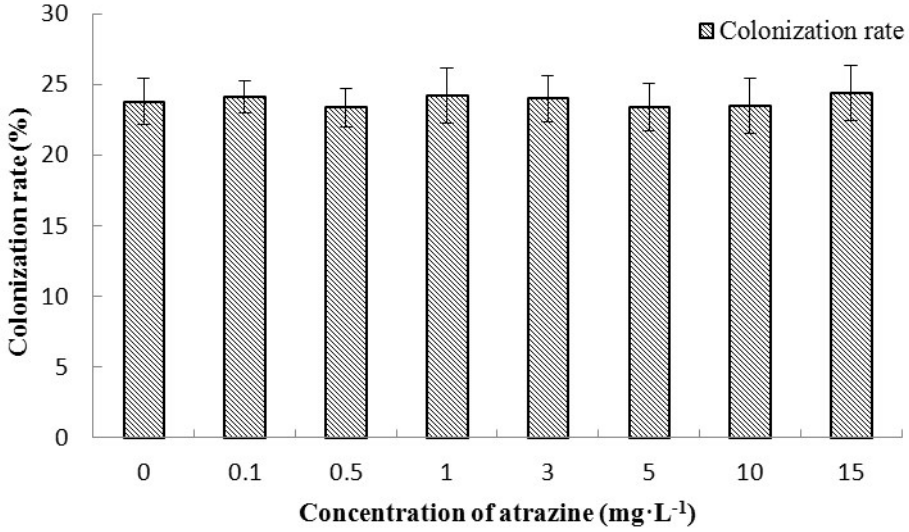

Fig. 2. The colonization rates after 21 days exposure to atrazine of FM treatment. FM: Inoculated with *F. mosseae.*

In our study, *C. indica* under CK and FM treatments were exposed to eight different concentrations of atrazine for 21 days. After exposure, parts of *C. indica* leaves were damaged by atrazine which caused the leaves to be abnormal, curled, shortened or blighted. Fig. 3 shows the leaf area of *C. indica* after exposure to atrazine in this experiment. The total leaf area (TLA) and effective leaf area (ELA) tended to decrease with increasing concentrations of atrazine for both treatments, while the blighted leaf area (BLA) showed an inverse trend (Fig. 3 a). Furthermore, in the CK treatment, the leaves appeared to be blighted when the concentration of atrazine reached 1 mg L^{-1} or more; the extent of blight on the leaves increased with increasing concentration of atrazine. The limiting concentration of atrazine resulting in leaf blight was 3 mg L^{-1} in the FM treatment. Previous research by Pritsa reported the reduction of TLA and leaf blight with atrazine pollutions. It was found that the TLA

of corn decreased in the presence of atrazine, though the effect was not linearly correlated to the concentration of atrazine.[42] This result seems to correspond to the results from our investigation.

The influence of *F. mosseae* inoculation on ELA can also be seen in Fig. 3 b. The difference between the CK and FM treatments indicates the contribution of *F. mosseae* inoculation for the ELA. It can be seen that inoculation with *F. mosseae* significantly promoted ELA development ($p<0.01$), however with increasing concentration of atrazine, the ELA of both treatments decreased sharply. Furthermore, the inoculation of AM Fungi contributed to a certain amount of ELA in the maximum concentration of atrazine (15 mg L^{-1}) rather than the total death of leaves for the CK treatment with lower concentration of atrazine (10 mg L^{-1}). Inoculation with *F. mosseae* significantly increased the leaf area of *C. indica* with exposure to atrazine. This was similar to the results reported by Todeschini which studied the response of poplar clones to copper stress with *F. mosseae* inoculation.[43] This confirms that AM fungi inoculation can promote the improved tolerance of *C. indica* in atrazine contaminated water, especially for high atrazine concentrations. These results indicate a positive signal for the phytoremediation of atrazine, a finding which will be discussed later in this section.

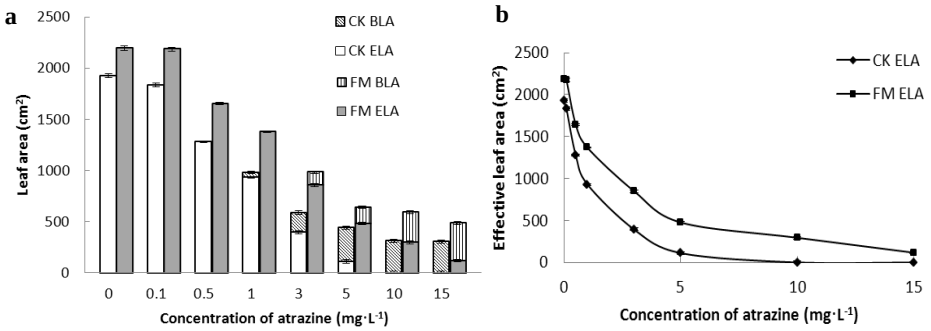

Fig. 3. The leaf area of *C. indica* after 21 days exposure to atrazine. CK: Control; FM: Inoculated with *F. mosseae*. ELA: the leaf area of effective parts; BLA: the leaf area of blighted parts.

Exposure to atrazine caused curling and blighting of the *C. indica* leaves, as well as having negative effects on the growth of the plants. Fig. 4 shows the relative shoot height growth (RSHG) of the host plants to different atrazine concentrations. It can be seen that the RSHG results of the two treatments exhibited similar trends, and that the RSHG values gradually reduced with increasing atrazine concentration. The RSHG of FM was significantly greater than that of CK at every concentration ($p<0.05$), a result which indicates that the application of mycorrhizal inoculation resulted in an increase from 29.5% to 82.2% in the plant RSHG at different atrazine concentrations. This indicated that the shoot height growth of the plants could be enhanced by inoculation with *F. mosseae*.

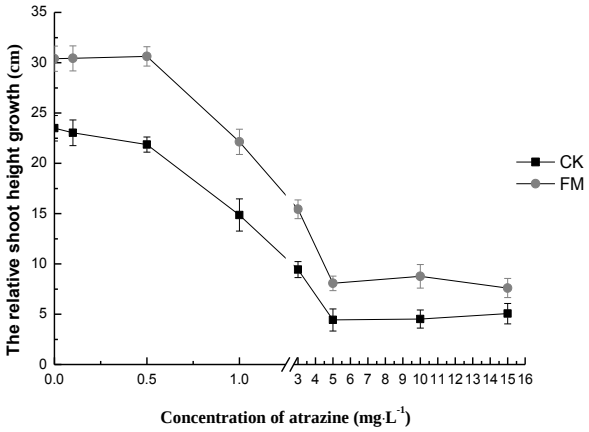

Fig. 4. The relative shoot height growth of *C. indica* after 21 days exposure to atrazine. CK: Control; FM: Inoculated with *F. mosseae*.

Fig. 5 shows the results from the biomass experiment with increasing concentration of atrazine exposure. It can be seen that the biomass of *C. indica* changed with increasing concentrations of atrazine; the biomass of the above- and under-ground parts of *C. indica* gradually decreased with increasing atrazine concentration. Compared to the CK treatment, inoculation with *F. mosseae* significantly increased the biomass of the above- and under-ground parts of *C. indica* at all atrazine concentrations ($p<0.05$). Application of mycorrhizal inoculation resulted in an increase from 23.1% to 96.6% in the biomass of the above-ground parts, and an increase from 21.0% to 67.7% in the biomass of the under-ground parts of *C. indica* at different atrazine concentrations. The effect of mycorrhizal inoculation on biomass from our experiment differed from the results reported by Huang.[24] Huang reported that mycorrhizal colonization increased root biomass at low atrazine concentrations, but it decreased shoot biomass. The reason of the difference between the results may be complicated as different arbuscular mycorrhizal fungi were used on different plants under different growing conditions. It should also be noted that *C. indica* has tuberous stems which belong to the under-ground parts. Wu reported that AM colonization significantly increased the biomass of maize leaves and stems in the soil treatments of phenanthrene (PHE) and pyrene(PYR) spiked-soils in the central compartment only, and the biomass of maize root in the soil treatments of PHE+PYR spiked-soils in all the three compartments;[44] these results were similar to our results of mycorrhizal inoculation on *C. indica* biomass.

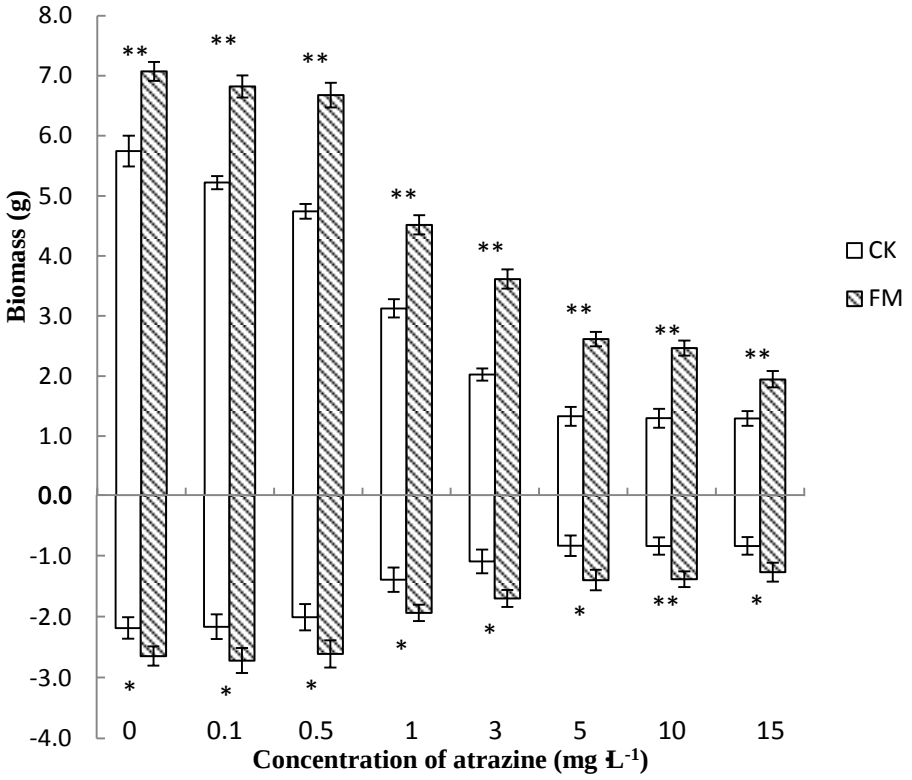

Fig. 5. The above- (above X-axis) and under- (below X-axis) ground parts biomass of *C. indica* 21 days after atrazine exposure. CK: Control; FM: Inoculated with *F. mosseae*. * Indicate significance at $p<0.05$ and **at $p<0.01$.

The influence of *F. mosseae* inoculation on RSHG and biomass of *C. indica* was further investigated by using the mycorrhizal dependency index (MDI). Fig. 6 shows the MDI profile of the corresponding indices in this study. It can be seen that the three MDI curves share a similar trend, though the values vary with differing concentrations of atrazine. Corresponding to the biomass results, the effect of mycorrhizal inoculation on the biomass of the above-ground parts was greater than the biomass of the under-ground parts. Fitting the relationship between MDI and concentration of atrazine, the equations and parameters are shown in Table 1. All of the MDI values reached their maximum levels between a concentration level of 8 to 9 mg $Ł^{-1}$ atrazine, indicating a high mycorrhizal dependency of *F. mosseae*. It can be deduced that inoculation with *F. mosseae* played the greatest role on *C. indica* growth at this concentration of atrazine, indicating that *F. mosseae* inoculation can improve the tolerance of *C. indica* in atrazine polluted water, especially at high concentration levels.

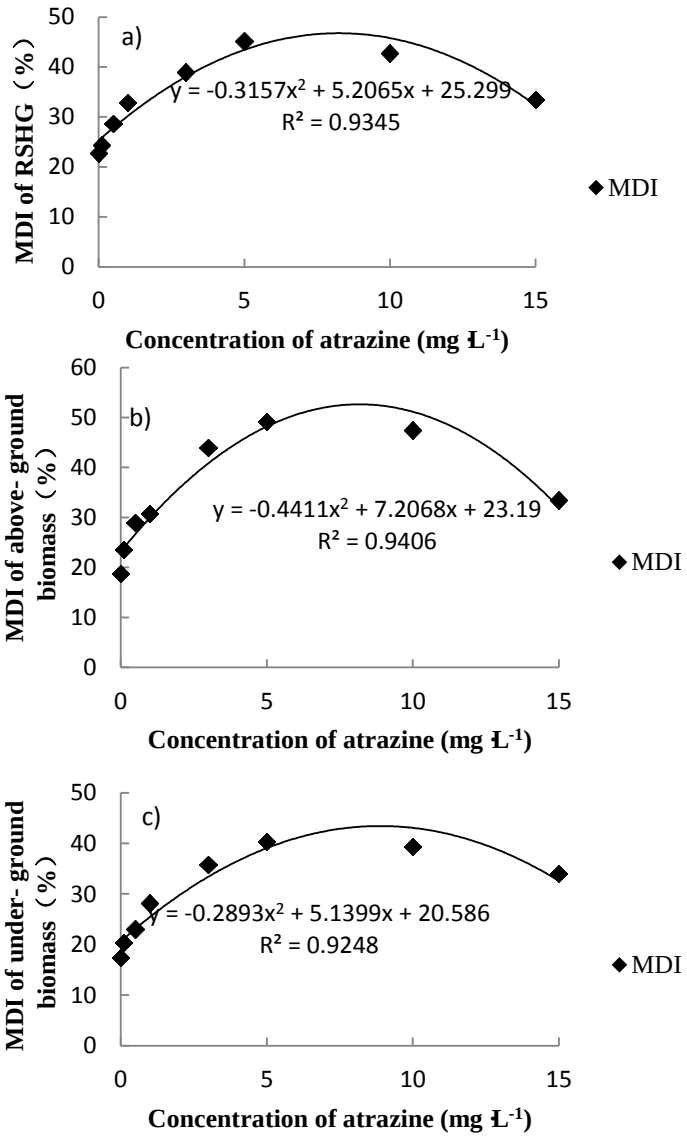

Fig. 6. Mycorrhizal dependency index (MDI) of the relative shoot height growth (RSHG) and biomass of *C. indica* 21 days after atrazine exposure. a: MDI of RSHG; b: MDI of above-ground biomass; c: MDI of under-ground biomass.

Table 1. Parameters of relationship between mycorrhizal dependency index (MDI) and the concentration of atrazine

MDI	Curve-fitted equation	R^2	The fitting maximum value	Concentration of inflection point
RSHG	$y=-0.3157x^2+5.2065x+25.299$	0.9345	46.7653	8.2460
Above-ground biomass	$y=-0.4411x^2+7.2068x+23.19$	0.9406	52.6266	8.1691
Under-ground biomass	$y=-0.2893x^2+5.1399x+20.586$	0.9248	43.4157	8.8833

x: concentration of atrazine; y: MDI.

All of the previous results indicate that *C. indica* is found as a novel tolerant species for atrazine, and that the toleration level of pollution appeared to be at 1mg L^{-1} (the concentration of a heavy field application). Previous investigations have suggested that all plant species are capable of detoxifying atrazine at different rates, but tolerant species could work more rapidly.[45] On the other hand, AM fungi exhibited positive effects on plant establishment and survival in contaminated environments by increasing nutrient uptake, improving drought tolerance, and potentially protecting roots from plant pathogens.[46-49] Taking into account that results of *C. indica* with *F. mosseae* inoculation showed higher relative shoot height growth and healthier leaves than non-inoculated plants, it seems to be true that the inoculation of *F. mosseae* can relieve the damage of atrazine on plants, and it can be beneficial as *C. indica* can tolerate higher concentrations of atrazine.

3.2. Photosynthesis of plants

Fig. 7 shows the maximum net photosynthetic rate of *C. indica* inoculated with and without *F. mosseae*. It can be seen that after exposure to atrazine for 14 days, the photosynthesis of both CK and FM treatments were inhibited. The maximum net photosynthetic rates ($A_{net\max}$) of both treatments reduced with an increase in concentration of atrazine. Atrazine is a known triazinic herbicide which kills target weeds by interfering with the normal function of photosynthesis;[50] its main phytotoxic effect is the inhibition of photosynthesis by blocking electron transport during the Hill reaction of the photosystem II.[51,52] Therefore, the enhanced photosynthetic rate of FM treatment in the present study indicates a beneficial effect on photosynthesis induced by inoculation with *F. mosseae*. With the assistance of AM fungi FM treatment showed the highest maximum net photosynthetic rates with an increase from 8.2% to 822.2% at different atrazine concentrations. Furthermore, there was a strong relationship between $A_{net\max}$ and atrazine concentration of both CK and FM treatments. The difference between the two treatments was an indication of AM fungi effects on photosynthesis of plants; the difference got bigger with increasing concentration of atrazine. This indicates that the effect of *F. mosseae* symbiosis on photosynthesis increased with the increase of atrazine stress. Combined with the previous results, the enhanced photosynthetic rate implies beneficial effects of inoculation with *F. mosseae* on *C. indica* growth and biomass.

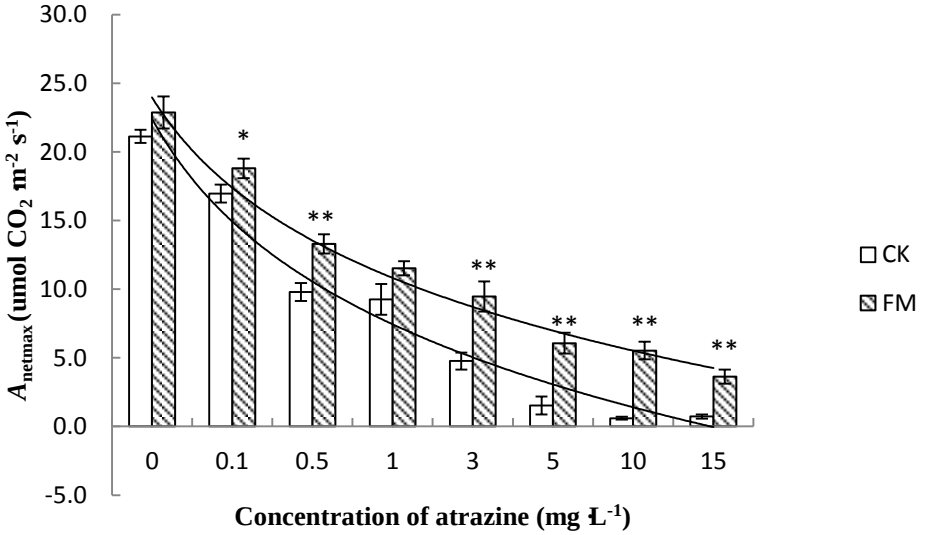

Fig. 7. Effect of *F. mosseae* on the maximum net photosynthetic rates ($A_{net\max}$) of *C. indica*. CK: Control; FM: Inoculated with *F. mosseae*. * Indicate significance at $p<0.05$ and **at $p<0.01$.

Photosynthesis was further investigated by analysing the corresponding photosynthetic parameters (Fig. 8). The transpiration fluxes (E) and stomatal conductance (g_s) exhibited a decreasing tendency with increasing concentrations of atrazine, while CO_2 concentrations (C_i) showed an inverse trend. Our results indicate that parameters such as instantaneous water-use efficiency (WUE), intrinsic water-use efficiency (WUE'), carboxylation efficiency (CUE) and light-use efficiency of plants all decreased with exposure to atrazine. However, the FM treatment exhibited beneficial changes. *F. mosseae* symbiosis increased transpiration fluxes (E) and stomatal conductance (g_s), whilst it decreased the intercellular CO_2 concentration. As a result, inoculation with *F. mosseae* increased WUE, WUE', CUE and LUE of plants. Furthermore, the differences of the parameters between plants with or without inoculation appeared to be significant above the 1 mg L^{-1} concentration of atrazine ($p<0.05$). All of the parameters which were affected by mycorrhizal colonization enabled the host plants to use water (WUE and WUE'), carboxylates (CUE) and light (LUE) more efficiently.[31]

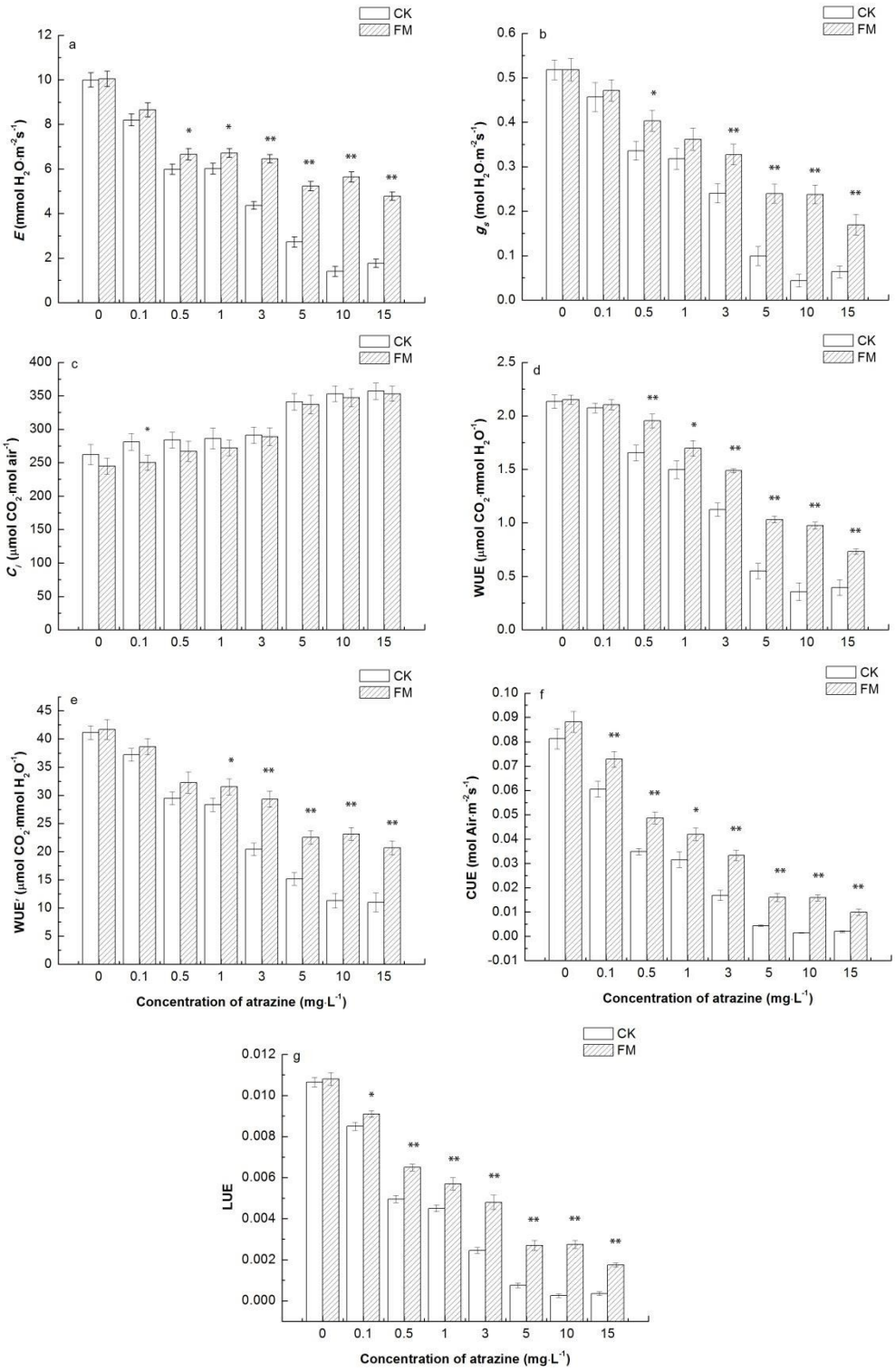

Fig. 8. Effect of *F. mosseae* on photosynthetic parameters of *C. indica*. a: transpiration rate (*E*), b: stomatal conductance (g_s), c: intercellular CO_2 concentration (C_i), d: instantaneous water-use efficiency (WUE), e: intrinsic water-use efficiency (WUE'), f: carboxylation efficiency (CUE), g: light-use efficiency (LUE). CK: Control; FM: Inoculated with *F. mosseae*. * Indicate significance at $p<0.05$ and **at $p<0.01$.

In our investigation, the usage efficiency of water, carboxylates and light was

evidently enhanced by *F. mosseae* inoculation. Previous research has also showed that plants inoculated with AM fungi maintain relatively higher water status, an occurrence which is enhanced at a low water potential by improving the extraction of water by roots and increasing the activity and hydraulic conductivity of roots.[53] With consideration of hydroponics in this investigation, this should not be the main reason for the positive effect of *F. mosseae* inoculation on photosynthesis. Another possible reason for the positive effects of AM fungi may be due to physiological changes that influenced the process.[54] However, the physiological changes were proved not to be the only reason for the effect of AM fungi on host plant photosynthesis. Taking into account the fact that *C. indica* inoculated with *F. mosseae* showed higher values of WUE, WUE' and CUE with larger TLA and ELA than non-inoculated plants, AM fungi most likely promoted leaf development with photosynthesis. However, Wu found that the relationship between photosynthetic rates and leaf area was not always positively correlated.[31] The differences between the results of our investigation and those of Wu may be due to differences in culture methods and plant species used in the experiments. The photosynthetic characteristics of mycorrhizal plants are more complex than non-mycorrhizal plants because their symbioses with AM fungi are intricate biological interactions.[55] As a result, the influence of AM fungi in this process should be assessed further.

3.3. Removal rates of atrazine by phytoremediation with F. mosseae inoculation

To determine the capacity of removing atrazine and the relative contribution of *F. mosseae* inoculation, the variation of the concentration of atrazine in the different treatments during the experiment was monitored. Removal percentages of atrazine in the different treatments at the end of the 21 day exposure period were assessed (Fig. 9). As a result, inoculation with *F. mosseae* decreased the atrazine residual concentration and promoted higher removal rates, indicating a beneficial effect on the removal of atrazine. Furthermore, the beneficial effect appeared to be significant above the concentration of 3 mg L^{-1} atrazine ($p<0.05$). There was a strong relationship between removal rates and concentration of atrazine for CK and FM treatments. The difference between the two treatments appeared to be an indication of the AM fungi effects on the removal rates of atrazine; the difference between the two treatments got bigger remarkably with an increasing concentration of atrazine. This indicated that the beneficial effect of *F. mosseae* on atrazine removal was significantly enhanced with an increase in atrazine stress.

According to the equations of the two fitting curves, removal rates of treatment CK achieved the maximum value of 68.064% at a concentration of 1.489 mg L^{-1}, while removal rates of treatment FM reached the maximum value at 95.670% at a concentration of 7.363 mg L^{-1}. The relative contribution of *F. mosseae* inoculation on atrazine removal rates changed from 2.2 to 52.0% according to the different concentrations, and it achieved the maximum value of 52.0% at a concentration of 15 mg L^{-1}. The atrazine removal rate and the optimal applicable concentration of atrazine were therefore enhanced with the inoculation of *F. mosseae* in this investigation.

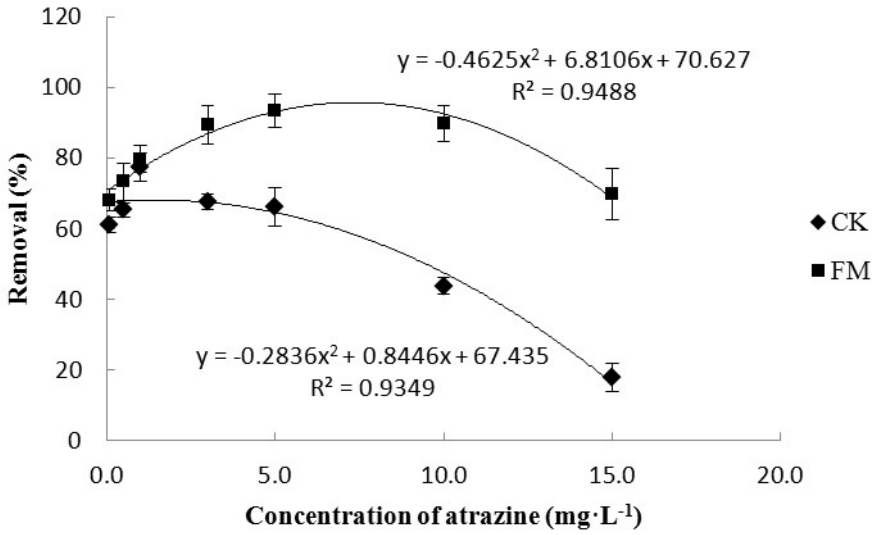

Fig. 9. Effect of *F. mosseae* on atrazine removal in water. CK: Control; FM: Inoculated with *F. mosseae*.

According to the removal rates, plants enabled the residual concentration of atrazine in water to significantly decrease, especially plants inoculated with *F. mosseae*. This indicates that AM fungi enhanced the dissipation of atrazine in water, a result similar to that of Huang in soil experiments.[24] The effect of AM fungi on the dissipation of atrazine was also similar to results reported by Kuo on heavy oil removal: systems with plants colonized by mycorrhizal fungi could speed up the phytoremediation rates and significantly increase the removal efficiencies, resulting in the removal of larger amounts of heavy oil from soil than systems without inoculation.[56] Furthermore, AM inoculation exhibited positive effects on the degradation of other organic pollutants.[57-60]

The mechanisms behind the removal of organic pollutants by AM inoculation have been unclear until now. One possible mechanism is the plant uptake of atrazine from water. With enhanced plant biomass, mycorrhizal plants can uptake more atrazine than nonmycorrhizal controls. This is true in our study since the application of mycorrhizal inoculation resulted in an increase from 23.1% to 96.6% in the biomass of above-ground *C. indica* parts, and an increase from 21.0% to 67.7% in the biomass of under-ground *C. indica* parts at different atrazine concentrations. However, the removal rates of atrazine were enhanced from 11.5% to 292.8% with the assistance of *F. mosseae* inoculation; this result appeared to be higher than expected. Other mechanisms therefore may be involved, such as enhanced microbial degradation activity and enhanced enzyme activity in the rhizosphere region; mechanisms which have been highlighted in recent investigations.[23,61,62] In addition, AM fungi directly decompose and degrade organic pollutants.[63-65]

3.4. Concentrations of atrazine in plants

To evaluate the phytoremediation effect of *C. indica* with *F. mosseae* inoculation, the concentration of atrazine in plants was examined. Results indicated that atrazine

was not detected in *C. indica* under non-atrazine application, while the atrazine concentration in both above- and under-ground *C. indica* parts increased with an increase in the concentration of atrazine application in both CK and FM treatments (Fig. 10). For both treatments, atrazine concentrations were higher in the under-ground parts than in the above-ground parts. In addition, *F. mosseae* inoculation significantly decreased atrazine concentrations in both above- and under-ground parts of *C. indica* ($p<0.05$). There was a strong relationship between the concentration of atrazine in plants and the application concentration of atrazine. The difference between CK and FM was an indication of significant effects of AM fungi on reducing atrazine residues in plants. As shown in Fig. 10, the effect of *F. mosseae* on reducing atrazine residues in *C. indica* increased remarkably with increasing concentration of atrazine. These results appear to be similar to the results from the removal rates in Fig. 9.

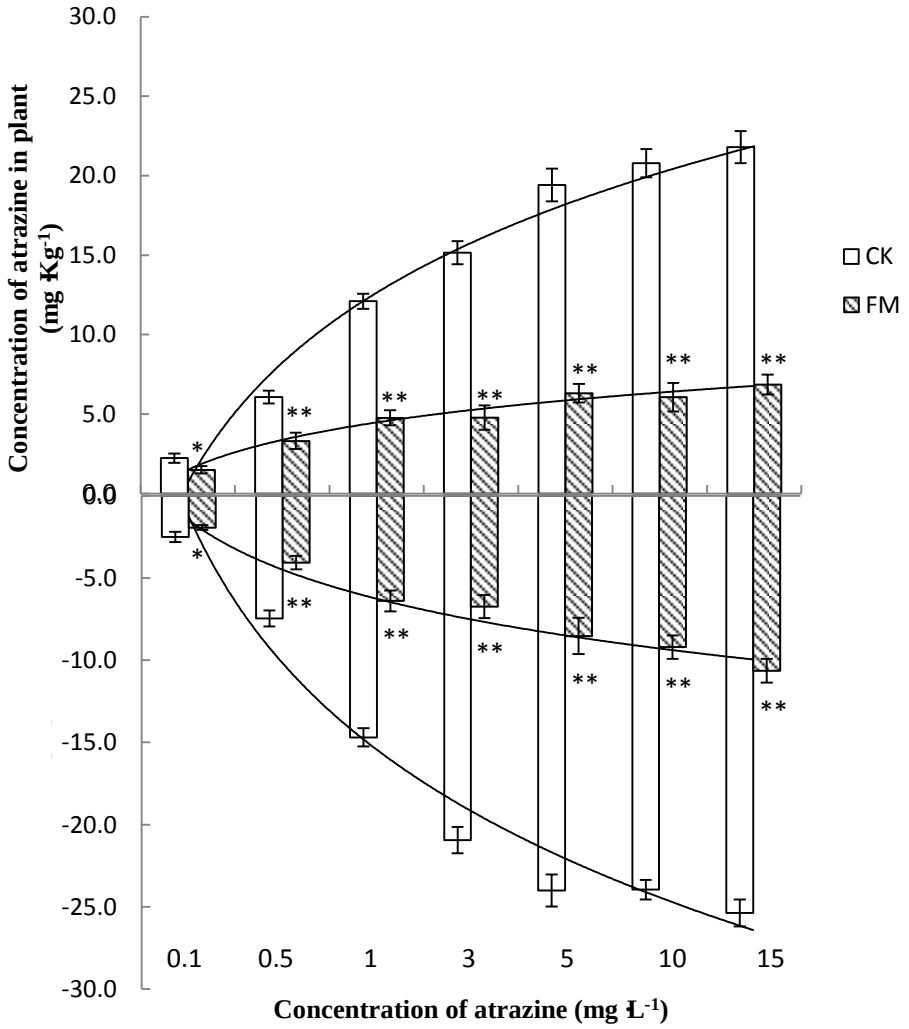

Fig. 10. The concentration of atrazine in above- (above X-axis) and under- (below X-axis) ground parts of *C. indica* 21 days after atrazine exposure. CK: Control; FM: Inoculated with *F. mosseae*. * Indicate significance at $p<0.05$ and **at $p<0.01$.

From our results, it can be seen that inoculation with *F. mosseae* exhibited beneficial effects on the removal of atrazine in water solution and on the *C. indica* plants. Due to the symbioses of AM fungi and the plants, the mechanism of decreased atrazine concentration by AM inoculation appears to be complex. As highlighted in section 3.1, the biomass of the host plants were remarkably enhanced with the assistance of AM fungi. The bivariate correlations among biomass and concentration of residual atrazine in treatments CK and FM are shown in Table 2 and Fig. 11. All bivariate correlations are negative and significant ($|r|>0.96$, $p<0.01$). This indicates that plant growth had a dilution effect on the concentration of residual atrazine in *C. indica*. Comparing the results of FM and CK treatments in Fig. 11, it can be deduced that inoculation of AM fungi can sharply increase the slopes of the negative correlations. Therefore, the dilution effect on the residual atrazine in mycorrhizal plants is highlighted, a result which was also identified by Leyval in a heavy metal remediation study.[66] The dilution effect caused by enhanced plant growth may therefore account for the lower concentrations of atrazine in mycorrhizal plants.

Table 2. Correlation coefficients among biomass and the concentration of residual atrazine in plants

Variables		CK		FM	
		Biomass of above-ground parts	Biomass of under-ground parts	Biomass of above-ground parts	Biomass of under-ground parts
Concentration of atrazine in plant	Pearson Correlation	-0.989**	-0.994**	-0.964**	-0.971**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000
	N	7	7	7	7

CK: Control; FM: Inoculated with *F. mosseae*; ** Indicate significance at $p<0.01$.

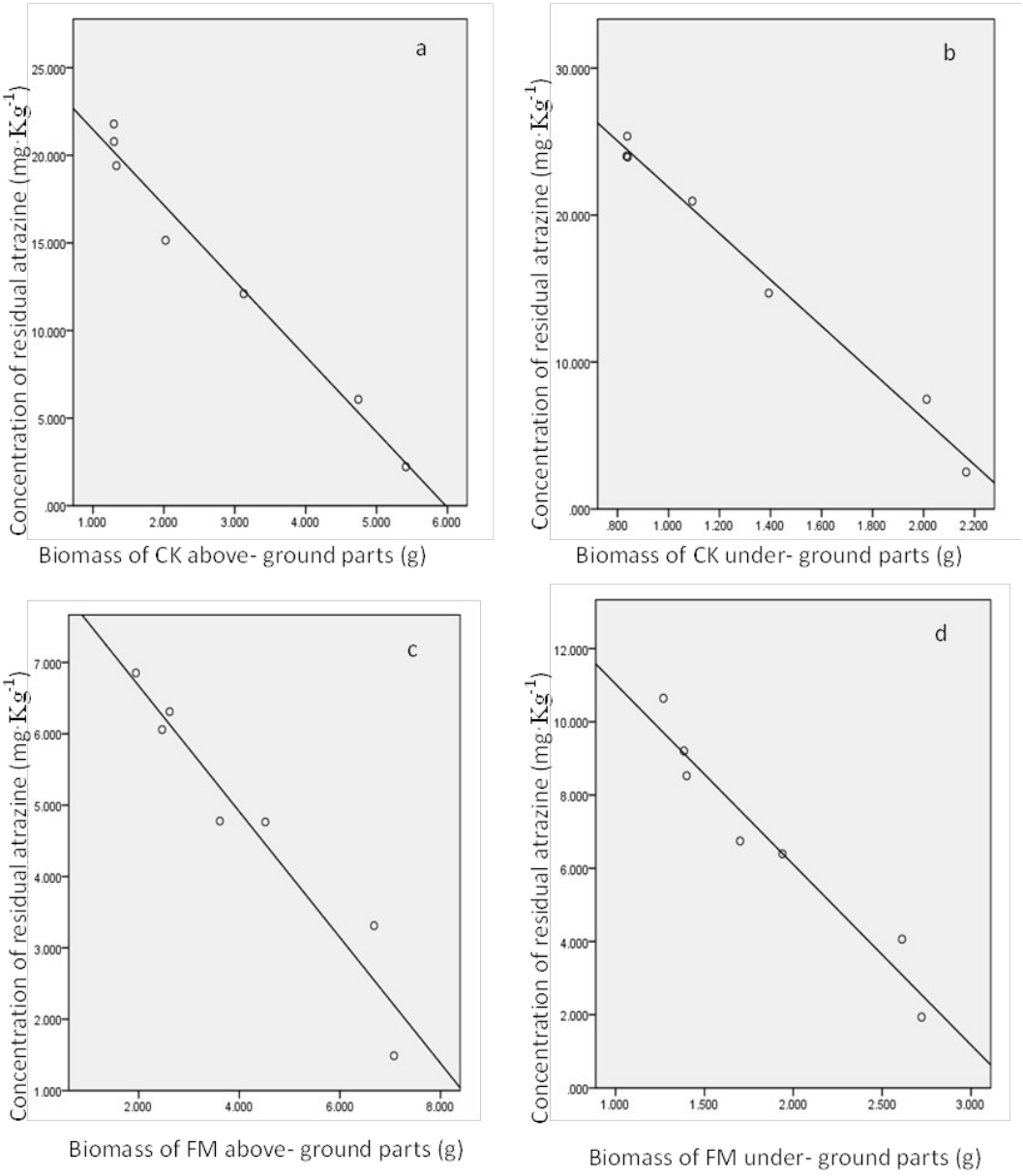

Fig. 11. Bivariate correlations among biomass and the concentration of residual atrazine in plants. a: above-ground parts of CK; b: under-ground parts of CK; c: above-ground parts of FM; d: under-ground parts of FM. CK: Control; FM: Inoculated with *F. mosseae*.

However, the dilution effect appears unlikely to be the only explanation for lower concentrations of atrazine in mycorrhizal plants, although mycorrhizal plants exhibit higher biomasses. If we suppose that mycorrhizal plants have the same or higher capacity for atrazine uptake, the residue of atrazine in their tissues should be much more than that in the nonmycorrhizal controls. However, results from our experiments show that the residue of atrazine in mycorrhizal plants was even than that in the nonmycorrhizal controls. As Fig. 12 shows, the atrazine residue in plants is a small part of the total amount of atrazine in the treatments. The main distributions of atrazine appear to exist as residue in water and in the removal parts. Furthermore, the

removal of atrazine in water and in plants has been shown to be enhanced by inoculation with AM fungi. Therefore, there should be other mechanistic explanations. AM fungi may indirectly influence the atrazine metabolic process in plants through mycorrhizal effects on plant metabolic activities. In addition, the modified rhizosphere enzymes and enhanced microbial degradation activity by mycorrhizal roots[23] may account for the mycorrhizal effects on atrazine removal under certain conditions as atrazine can be degraded and utilized by bacteria.[67-69] This can account for the fact that inoculation with AM fungi decreases residues in both water and plants. However, AM fungi are believed to have no direct catabolism or co-oxidation on pollutants such as PAH because they have very limited saprophytic capacities.[70] Some AM fungi can enhance the decomposition of complex organic material (grass leaves) in soils which therefore can increase N capture, indicating that AM symbiosis possesses saprotrophic capabilities.[71] As mycelium has been reported to exhibit a more important influence than mycorrhizal roots on atrazine degradation,[23] it might also be inferred that AM fungi play a direct role in the catabolic process of atrazine in water.

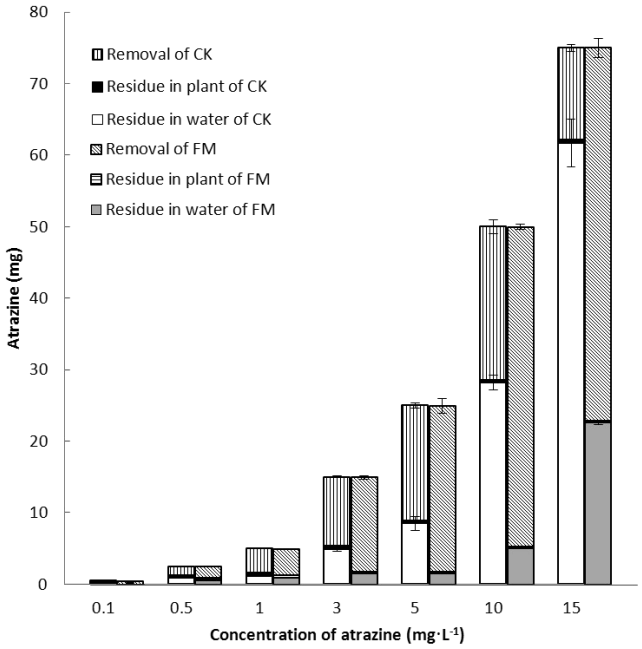

Fig. 12. Distribution of atrazine in CK and FM treatments, including residue in water, residue in plants and the removal. CK: Control; FM: Inoculated with *F. mosseae.*

Our results have provided evidence that AM fungi can simultaneously increase biomass and decrease the concentrations of herbicides in both plants and water, although the effects vary with the concentration of the applied herbicide. Our results also indicate a promising potential of AM fungi for the phytoremediation of herbicide contaminated water, and *C. indica* with *F. mosseae* colonization may ultimately serve as viable phytoremediation agents in the natural environment. Future studies should be undertaken to clarify the mechanisms of mycorrhizal effects on atrazine removal.

4. Conclusion

This investigation demonstrated that *Canna indica* L. var. *flava* Roxb. is a pollution tolerant species; the growth, biomass and photosynthesis of *C. indica* were negatively affected by atrazine exposure. The inoculation of *F. mosseae* evidently alleviated the atrazine physiological inhibition for the growth of plants. *C. indica* with *F. mosseae* colonization exhibited higher atrazine removal efficiencies and lower atrazine residue concentrations in plants than the nonmycorrhizal controls. The arbuscular mycorrhizal fungi *F. mosseae* could efficiently decrease the concentration of atrazine in water with a contribution of 2.2% to 52.0% in the removal of atrazine, and it played an important role in the remediation process. This investigation has shown that *C. indica* with *F. mosseae* colonization may ultimately serve as viable phytoremediation agents in the natural environment.

Acknowledgements

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Funnelliformis mosseae inoculation exhibited a beneficial effect on phytoremediation of atrazine in water by the aquatic plant *Canna indica* L..