COMBINING EFFECT OF CHEVALIER HERBICIDE AND SUNFLOWER RESIDUE ON ARBUSCULAR MYCORRHIZA

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ABSTRACT

The effect of different rates of sunflower residues alone or in combination with lower dose of chevalier herbicide on arbuscular mycorrhiza associated with wheat roots was investigated during the growing season of 2012-2013. The quantitative level of total phenolics released from sunflower residues in soil was also determent by using ferulic acid as a standard phenolic acid. Results revealed that total phenolics in field soil significantly increased after incorporation of sunflower residues and reached the maximum concentration at 4 weeks of residues decomposition, then decreased significantly at 6 weeks and vanished at the end of the second month. However, the number of spores in field soil amended with sunflower residues was significantly increased at 2, 4 and 6 weeks of residue decomposition compared to control treatment (without sunflower residue). At flowering stage, it was found that sunflower residues incorporated in field soil at 3 and 6 t ha^{-1} increased number of spore by 15.68 and 23.46 % over control, while it was decreased significantly when chevalier was applied at the label dose. Chevalier at reduced (50% of the label rate) rate applied to plots amended with sunflower residue at 3 t ha^{-1} scored spore number significantly lower than that of the control treatment, but when the reduced dose was applied to plots amended with higher residues rate, the number of spores was significantly increased over the control. Sunflower residues incorporated in to field soil at rates of 3 and 6 t ha^{-1} increased rate and intensity of colonization by 48, 53% and 50, 52% of control respectively. Application of reduced dose of herbicide on plants grown in plots amended with sunflower residues significantly increased rate and intensity of colonization compared to the control.

INTRODUCTION

Soil an important component of the ecosystem, serves as a medium for plant growth through the activity of microbial communities. This soil microbial communities (like bacteria, fungi and actinomycetes) play critical role in litter decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth (8,18,21,26).However, soil microorganisms are greatly influenced by factors including the application of herbicides (17),which are applied in modern agricultural practices to attain optimum crop yields (28).

Arbuscular mycorrhiza (AM) are beneficial fungi found in soil existing symbiotically with plant roots. They enhance the growth of many plant species by increasing the efficiency of nutrient and water uptake. Since AM fungi grow and develop in soil ecosystem, they are likely to be exposed directly to toxicants or chemicals such as pesticides use in agricultural practices, which may be inhibitory to their

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development. Some pesticides have been reported to inhibit spore germination and/or hyphal growth (7,9,24). The effect of herbicides on development of AM fungi had also been reported (6). The interaction between AM systems and agrochemicals in soil is an important consideration in the development of appropriate management technologies of AM fungal populations (15).

In an earlier work (2), it has been shown that foliar application of chevalier herbicide on wheat plants grown in soil incorporated with sunflower residues at 3 and 6 ton per hectare suppressed weed emergence and growth and enhance wheat production similar to that achieved by recommended dose of herbicide. However, the effects of this combination on Mycorrhiza population and growth were not investigated; therefore, the present study was conducted to test if the residues of sunflower, chevalier herbicide alone or in combination with each other affect number of spores, colonization rate and intensity of arbuscular mycorrhiza associated with at roots of wheat at different stages of growth and development under field conditions.

MATERIALS AND METHODS

Preparation of sunflower residues

To prepare residues of sunflower plants, field plot (24×2 m) were tilled twice at the beginning of July 2012 in the Research Field of Biology Department, College of Science, and Baghdad University. Seeds of sunflower was sown in 75 cm spaced crop rows with distances of 25 cm between seeds. Plot (12×2) without crop was used as a control. Fertilizers (nitrogen as urea 46% N) and phosphorus (triple super phosphate,46% P_2O_5) (11) and irrigation were applied as recommended for this crop. At physiological maturity, the heads were removed then 50% of the sunflower plant parts of half of the plot area and whole plants of sunflower of the other half were left for drying for several days on their respective plots. These plots were then tilled twice at mid November 2012 by using a disc plough to incorporate sunflower residues in to the soil. Residue free treatment was maintained as control on an intentionally un-cropped area of the same field. Field measurements revealed that the residues of sunflower incorporated in field soil before and after removal of half of sunflower plant parts correspond to sunflower residue rate of about 6 and 3 t ha^{-1}.

Field trial

The plots that received sunflower residues at 0, 3 and 6 t ha^{-1} of previous experiment were subdivided into plots measuring 2×1.5 m. Fertilizers Nitrogen as urea (46% N) and phosphorus as triple super phosphate (46% P_2O_5) were applied to these plots as recommended for wheat crop (11). Each field plot was treated with 250 g of AM fungal inoculums. Grains of wheat cv. Abu-Grab were manually sown in all plots in 20cm apart crop rows at planting density of 120 kg ha^{-1}. All plots received equal irrigation water during the entire course of study. The experiment consists of the following treatments:

a. Control (un cultivated sunflower field)
b. Residues at 3 t ha^{-1}

c. Residues at 3 t ha^{-1}+50% of label rate of chevalier 15WG (200ml/ha)
d. Residues at 6 t ha^{-1}
e. Residues at 6 t ha^{-1}+50% of label rate of chevalier 15WG (200ml/ha)
f. Chevalier WG (Label rate)

Chevalier 15 WG herbicide (400 ml/ha Mesosulfuron + Iodosulfuron) was sprayed on weeds of their respective plots after 45 days from sowing of wheat, using hand sprayer. The experiment was conducted in split plot design with four replications for each treatment. The herbicide rates were kept in the subplot while sunflower residue rates were assigned as main plot. All plots received equal irrigated water during the growing season of the crop.

Mycorrhizal studies

Preparation of Mycorrhiza inoculums

Loamy soil was brought from field, sterilized in autoclave for 0.5 hr period for two days and packed in 10 plastic pots of 1 Kg capacity. Spores of mycorrhiza *Glomus mosseae* {identified according to Schenck and Smith, (20)} were extracted by procedure below and mixed with the upper part of pot soil. 10 seeds of sorghum and 10 of millet were sown separately in 5 pots containing kg by sorghum and 5 pots by millet) and irrigated with appropriate amount of water. Three months after planting, the above ground of plants were cut. The soil plus roots of both plant species were taken, mixed thoroughly and used for inoculation process (13).

Spore extraction:

Soil samples were collected from each plot, air dried and sieved through a 2 mm openings sieve to remove large debris. A sub sample (100 g) was taken from each sample and placed in a 500 ml beaker containing 200 ml 0.08 M sodium hexametaphosphate solution to break up clay clumps. The suspension was agitated for 5 minutes and left to settle for 15 seconds (23). The supernatant was decanted through a nest of sieves with reducing mesh sizes from 500 µm, 250 µm, 125 µm to 45 µm. This step was repeated with water twice and the debris from the 45 µm was discarded. The debris on the remaining sieves, containing the AM spores was washed and placed in 40 ml centrifuge tubes for purification. The spore suspension was centrifuged at 3000 rpm for 5 minutes, after which the supernatant was discarded. The pellet was re-suspended in 60% sucrose solution and centrifuged for another 5 minutes. The supernatant containing AM fungal spores was decanted into a 45 µm sieve and washed with water to remove sucrose on the spores (23).

Spore counting:

Based on previous study, total phenolics was increased after two weeks of residue decomposition and reach maximum accumulation in December 15, 2012 of residues decomposition and declined drastically thereafter. To test if the mycorrhizal population is correlated with the

phenolics profile during this period, spore counting was conducted by taking soil samples from rhizosphere of wheat plants growing in plots containing 6 t ha^{-1} and in plots without sunflower residues (Control) at 1, 14, 28 and 42 days after sowing (DAS).

Spore counting was also made at the end of wheat crop maturity to determine the possible effect of test treatments on mycorrhizal population and growth. Soil samples from rhizosphere of wheat plants growing in plots of all treatments were taken and used for counting number of spores using microscopic slide.

Mycorrhizal colonization rate (%):

Wheat plant roots were taken from the field at the end of crop maturity for determining the colonization rate and colonization intensity. Fresh roots were carefully washed and cut into 1-3 cm pieces. The pieces were immersed with 1% KOH solution and incubated at 70°C for 20 minters to remove the cytoplasm. The KOH solution was discarded and the roots were rinsed well with distilled water. Roots were covered with a freshly prepared alkaline H_2O_2 solution for 10 minters. The bleaching solution was discarded and the roots were rinsed with water. Roots were acidified in a 0.1 M HCl solution overnight to ensure adequate binding of stain to fungal structures. The HCl solution was discarded and roots were covered with Lacto glycerol Trypan Blue (0.05%) stain and incubated for 45 minutes at 90°C. The stain was poured off and roots were covered with lacto glycerol destain. Roots were allowed to destain overnight before microscopic examination (23). Finally, roots were mounted on microscopic slides and using a compound microscope for examined. The percentage root colonization was calculated by the following equation using a modified Line Intersect Method (14).

% Colonization =Total number of AM positive segments/Total number of segment studied × 100

Mycorrhizal intensity was calculated based on the following rate index;

0= the root fragment not mycorrhizaled

1= 1-25 of the root fragment was mycorrhizaled

2= 26-50 of the root fragment was mycorrhizaled

3= 51-75 of the root fragment was mycorrhizaled

4= 76-100 of the root fragment was mycorrhizaled

Mycorrhizal intensity (MI) was calculated according the following equation:

MI=Total(number of fragments × their rate index)/number of observed fragment × highest rate

4. Determination of total phenolics in the field

Soil samples were taken from soil of plots amended with 6 t h^{-1} and 0 t h^{-1} (control) at a depth of 30 cm at 1, 14, 28 and 42 days after sowing (DAS). The soils were mixed thoroughly and allowed to dry at room temperature for 3 days. Samples of 250 g dry soil were extracted separately in 250 ml of distilled water by shaking for 24 hrs at 200 rpm (4). Soil suspensions were filtered through Whatman No.2 filter paper under vacuum. Folin-Denis (0.5 ml) and Na_2CO_3 (one ml) were added to one ml of soil water extract and left to stand for 30 minutes. Absorbance was determined at 750 nm on aspectrophotometer (5). The total phenolic content was obtained by standard curve using different concentrations of ferulic acid.

RESULTS AND DISCUSSION

Results presented in table 1 exhibited that sunflower residues incorporated in field soil had a significant effect on number of spores. Field soil amended with sunflower residues at 3 and 6 t ha^{-1} increased number of spore by 13.44 and 19.01 % over control. However, the number of spores was decreased significantly when chevalier was applied at the label dose. Chevalier application at reduced (50% of the label rate) rate applied to plots amended with sunflower residue at 3 t ha^{-1} scored spore number significantly lower than control treatment, but when the reduced dose was applied to plots amended with higher residues rate, the number of spores was significantly increased over the control.

Result showed that sunflower residues incorporated in to field soil at rates of 3 and 6 t ha^{-1} increased colonization rate and intensity of colonization by 48, 53% and 50, 52% of control respectively (Table 1). Application of chevalier did not affect rate and intensity of colonization significantly. When reduced dose of herbicide applied on plants grown in plots amended with sunflower residues, rate and intensity of colonization increased significantly compared to the control.

Total phenolics in field soil significantly increased after incorporation of sunflower residues and reached the maximum concentration at 4 weeks of residues decomposition, then decreased significantly at 6 weeks and vanished at the end of the second month (Figure 1).

Number of spores in field soil amended with sunflower residues was significantly increased at 2, 4 and 6 weeks of residue decomposition compared to control treatment (without sunflower residue) (figure 2).

Table 1: Effects of different rates of chevalier 15 WG herbicide and sunflower residues cv. Asgrow on number of spores, colonization rate and intensity of colonization of *Glomus mosseae* associated with wheat roots

Treatments	Number of spores per 100 g dry soil	Colonization rate (%)	Intensity of colonization (%)
Weedy check (Control)	363.5	40.00	40.00
Residues at 3 t ha^{-1}	420.5	77.50	79.50
Residues at 3 t ha^{-1} + 50% of label rate of chevalier15 WG	299.0	45.00	37.50
Residues at 6 t ha^{-1}	448.8	85.00	82.75
Residues at 6 t ha^{-1} + 50% of label rate of chevalier15 WG	341.2	50.00	37.25
Chevalier15WG (Label rate)	241.5	40.00	33.75
LSD ≤ 0.05	27.9	7.41	6.97

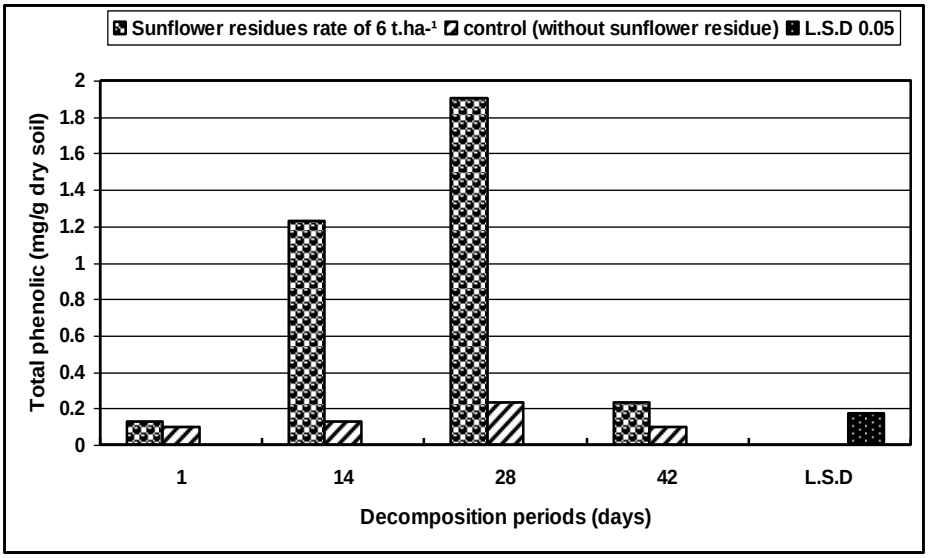

Figure 1: Total phenolics released in field soil amended with sunflower residues at 6 t ha^{-1} during different decomposition periods.

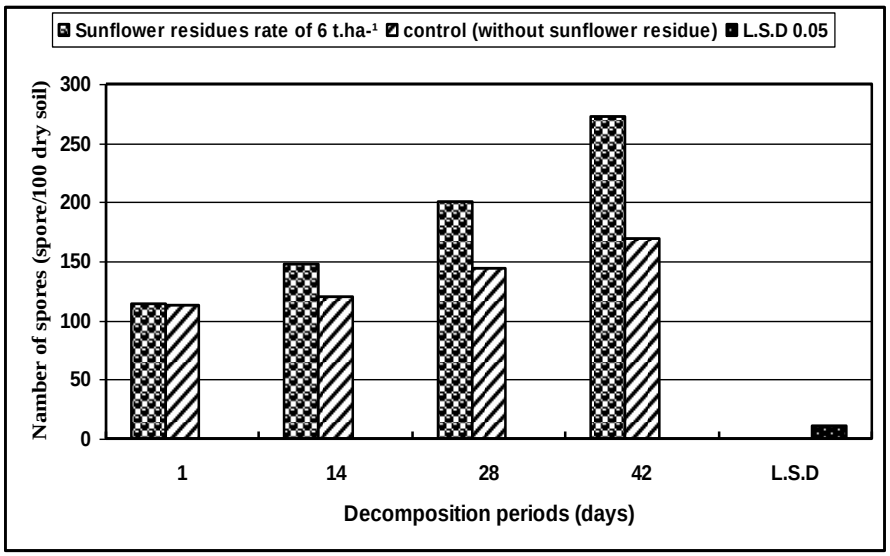

Figure 2: Sporulation of *Glomus mosseae* in field soil amended with sunflower residues at 6 t ha^{-1} during different decomposition periods.

Mycorrhizal fungi play an important role in natural and agricultural systems by absorbing N, P, K, Ca, S, Cu, and Zn from the soil and translocate them to associated plants (25). However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn by increasing the absorptive surfaces of the root (12,16). The growth of mycorrhiza is greatly influenced by the several factors such as soil organic matters, pesticides, temperature, water, structure and macro and micronutrients (6).

In an earlier work (2), it was found that application of chevalier herbicide on plots amended with allelopathic sunflower residues significantly suppressed weed population and biomass and increase yield of wheat crop. The present study revealed that the combined effect of crop residues and reduced chevalier herbicide was not directly affected wheat crop but also indirectly through their positive impact on sporulation, colonization and hyphal growth of AM. In our

study, The better sporulation, colonization and intensity was observed during the release of sunflower residues in soil (during the first two months from sowing). This suggest that the phenolics pose stimulatory or at least are not interfere with the growth of AM fungi. Reports on the effect of phenolic acids on AM fungi is controversial. Siqueira *et al.* (22) indicated that allelochemicals specially phenolics stimulate mycorrhizal colonization, while others found that mycorrhizal colonization is suppressed by phenolic acids released from allelopathic plants (10,19,27). The inhibition or stimulation of AM spore germination and hyphal growth and branching is mainly depended on concentration and type of allelochemicals present in soil rhizosphere (3).

The inhibition of AM sporulation and colonization intensity by the label dose of chevalier is coincided with general trends of the effect of most herbicides on mycorrhiza (1). However, reduced dose of chevalier in combination with higher rate of sunflower residues scored sporulation and colonization intensity similar to that of control treatment. It is possible that the residues may mitigate the effect of chevalier herbicide when applied in combination.

The results of this study lead to the conclusion that the sunflower residues amended in field soil provide a good medium for growing AM fungi and the allelochemicals released from the residues are not interfere with the test growth parameters of AM.

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تأثير مبيد الشيفالير مع مخلفات زهرة الشمس في الفطريات الجذرية الشجيرية

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الملخص

اختبر تأثير مخلفات زهرة الشمس المضافة بنسبتين مختلفة 3 و6طن هـ$^{-1}$ بمفردها أو بالتكامل مع نصف الكمية الموصى بها من مبيد الشيفالير (150 غم.هـ$^{-1}$) في نمو المايكورايزا المتعايشة مع جذور الحنطة اثناء موسم النمو 2012– 2013 وتقدير المستوى الكمي للفينولات المتحررة من تحلل مخلفات زهرة الشمس في التربة باستخدام حامض الفيروليك كمادة قياسية.أشارت النتائج بأن المركبات الفينولية في تربة الحقل تزداد معنوياً عند إضافة مخلفات زهرة الشمس ووصلت إلى أقصى تركيزاً بعد 4أسابيع من تحلل المخلفات ثم انخفضت معنوياً عند 6 أسابيع، وتتلاشى بعد شهرين.وبالمقابل فان عدد السبورات في تربة الحقل المضاف إليه مخلفات زهرة الشمس ازداد معنوياً عند 2، 4 و6 أسابيع من تحلل المخلفات مقارنة مع السيطرة (بدون مخلفات زهرة الشمس).أما عند مرحلة التزهير، فقد وجد بأن إضافة مخلفات زهرة الشمس بالتركيزين 3و6طن هـ$^{-1}$ قد أزاد من عدد السبورات بالنسبتين 15.68 و23.46 عن المقارنة على التوالي. بينما قلت معنوياً عند إضافة مبيد الشيفالير بكامل الجرعة. أما عند إضافة نصف الجرعة من المبيد مع مخلفات زهرة الشمس بتركيز 3 طن هـ$^{-1}$ فقد حققت انخفاضاً معنوياً في عدد السبورات أكثر من معاملة المقارنة، إلا إنه عند إضافة نصف الجرعة من المبيد مع مخلفات زهرة الشمس بتركيز 6طن هـ$^{-1}$، فقد ازداد عدد السبورات معنوياً مقارنة مع السيطرة. إما عند إضافة مخلفات زهرة الشمس إلى تربة بتركيز 3 طن هـ$^{-1}$ قد زادت نسبة وشدة الإصابة المايكورايزا بالنسبتين 48.53 و50.52% عن المقارنة. وقد سجلت ألواح المعاملة التي أضيف فيها المخلفات إلى تربة الحقل بالنسبتين 3 و6 طن هـ$^{-1}$ مع نصف الجرعة من المبيد زيادة معنوية في نسبة وشدة الإصابة مقارنة بالسيطرة.

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