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Combining Effect of Lower Rate of Trifluralin Herbicide and Sunflower residues on mycorrhizal association with cowpea and soil nitrification

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Abstract

Field and laboratory experiments were conducted during the course of study to test if the sunflower residues along or with 50% full rate of trifluralin herbicide has any impact on mycorrhizal association and soil nitrification which are important processes for improving growth and productivity of crops. Results revealed that incorporation of sunflower residues significantly increased spores number at 2, 4 and 6 weeks of residue decomposition compared to control treatment. However, when the residues applied in combination with reduced rate of trifluralin herbicide, sporulation was appreciably decreased by sunflower residues at 3 t ha^{-1} but it remains almost the same at 6 t ha^{-1} rate of residues. Field soil amended with label rate of trifluralin showed lower sporulation during the first six weeks from beginning of the experiment. Colonization rate was appreciably increased by application of sunflower residues at 6 t ha^{-1} and decreased by label rate of herbicide and weed free treatments. The highest colonization intensity (83.4) was recorded by application of sunflower residues at 6 t ha^{-1}, followed by treatment of sunflower residues at 3 t ha^{-1} (72.2). Label rate of herbicide recorded the least colonization intensity (54.4). Incorporation of sunflower residues at 3 and 6 t ha^{-1} significantly reduced nitrification rate at all incubation periods in comparison to control. However, when the residues was applied along with the reduced rate of herbicide, nitrification rate was significantly increased over the sole application of sunflower residues at all incubation periods except 4 and 8 days incubation periods but it remains below control. Soil sterilization treatment showed the least nitrification rate at all incubation periods. In all bioassay experiments, the amounts of NH_4^+ converted in the incubated soil was significantly correlated with the amount of NO_3^- produced over the periods of incubation suggesting that soil incubation is efficient method that can be used for measuring nitrification process.It can conclude Sunflower residues amended in field soil was found to provide a good medium for growing *Glomus mosseae* fungus and best way to inhibited nitrification rate and thus may contribute in alleviating losses of NO3 used by plants and reducing the environmental pollution.

Keywords: sunflower residues, lower rate herbicide, mycorrhizal association , soil nitrification , cowpea.

تأثير جرعة منخفضه من مبيد الترفلان مع مخلفات زهرة الشمس على الفطريات الجذرية المتعايشة وعملية النترجة

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الخلاصة

نفذت تجربة حقلية ومختبرية خلال موسم الدراسة لتقييم الجهد الاليلوباثي لمخلفات زهرة الشمس بمفردها او مع نصف الكمية الموصى بها من مبيد الترفلان على الفطريات الجذرية الشجيرية Arbuscular mycorrhizaالمتعايشة مع جذور نبات اللوبياء وفي عملية النترجة في التربة والتي تعد من العمليات المهمة لتحسين النمو وزيادة الإنتاج النباتي، وبينت النتائج إن عدد السبورات في تربة الحقل المضاف إليه مخلفات زهرة الشمس ازداد معنويا عند ٢ و ٤ و ٦ أسابيع من تحلل مخلفات زهرة الشمس مقارنة بمعاملة المقارنة (بدون مخلفات). في حين ان إضافة المخلفات بمعدل ٣ طن هـ$^{-1}$ مع نصف الجرعة من مبيد الترفلان انخفض عدد السبورات بصورة ملحوظة ، الا انه لم يتغير عند إضافة المبيد مع المخلفات بمعدل ٦ طن هـ$^{-1}$. ولوحظ اقصى انخفاض لعدد السبورات في معاملة الجرعة الموصى بها من المبيد خلال الست اسابيع الاولى من تنفيذ التجربة . ازداد معدل الإصابة بالميكورايزا في الألواح التي أضيفت إليها المخلفات بمعدل ٦ طن هـ$^{-1}$، وانخفاضها في الجرعة الموصى بها من مبيد الترفلان. اما شدة الإصابة بالميكورايزا فقد سجلت أعلى درجة لها (٨٣,٤) في ألمعاملة التي أضيفت اليها المخلفات بمعدل ٦ طن هـ$^{-1}$، تليها المعاملة التي أضيفت اليها المخلفات بمعدل ٣ طن هـ$^{-1}$ (٧٢,٢). وقد انخفضت شدة الإصابة في الجرعة الموصى بها من المبيد الى (54.4). ادت أضافه مخلفات زهرة الشمس بمعدل ٣ و ٦ طن هـ$^{-1}$ الى تثبيط معنوي في معدل النترجة خلال كافة مدة التحضين المدروسة مقارنة بالمقارنة .إلا أن إضافة مخلفات زهرة الشمس مع نصف الجرعة الموصى بها زادت من معدل عملية النترجة في جميع الفترات عدا الاسبوع ٤ و٨ ألا انها ظلت بمستوى اقل من معاملة المقارنة فيما اظهرت معاملة التربة المعقمة انخفاض في معدل النترجة خلال جميع مراحل التحلل (التحضين) . ولوحظ ان كمية NH_4^+ المتحولة في التربة المحضنة قد ارتبطت ترتبط معنويا مع كمية NO_3^- المتكونة، مما يشير إلى ان استخدام طريقة تحضين التربة هي الطريقة المثلى والكفوءة في حساب عملية النترجة . ويمكن أن نستنتج ان معدلات من مخلفات زهرة الشمس االمضافة في تربة الحقل توفر وسيلة جيدة لنمو الفطريات الجذرية المتعايشة *Glomus mosseae*وتعد أفضل طريقة لتقليل معدل النترجة، وبالتالي يمكن أن تسهم في التخفيف من وطأة خسائر NO3 التي تستخدمها النباتات والحد من التلوث البيئي.

Introduction

Soil an important component of the ecosystem, serves as a medium for plant growth through the activity of microbial communities. These soil microbial communities (like bacteria, fungi and actinomycetes) play a critical role in litter decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth [1,2].

In an earlier work [3], it was found that combination of reduced rate of trifluralin herbicide with sunflower residues significantly suppressed weeds population and dry weight biomass in cowpea field and increased cowpea seeds yield similar as or even better than the full label rate of trifluralin. However, information concerning the effect of combination of sunflower residues and lower rate of trifluralin herbicide has not been explored for mycorrhizal association with cowpea roots and soil nitrification. Mycorrhiza play a significant role in providing the host plant with nutrients and water, protecting against pathogen, buffering against toxic metals and increasing tolerance to environmental stress [4]), Nitrification is a vital biological process which provides plant with available nitrate in soil [5,6] and regulation of this process by nitrification inhibitors [7].

With this in minds, the present study was conducted to test if the residues of sunflower, trifluralin herbicide alone or in combination with each other affect sporulation and colonization of arbuscular mycorrhiza associated with at roots of cowpea and soil nitrification process.

MATERIALS AND METHODS

Implementation of field experiment

The proposed study was conducted at Research Station of State Board of Agricultural Extension, Ministry of Agriculture, Waset Province, Iraq. Plots of 2.5×5 m were randomly selected and fertilized with nitrogen as urea (46% N) at 80 kg ha^{-1} and triple phosphate (46% P_2O_5) at 240 kg ha^{-1}. All phosphorus and half of nitrogen were applied with irrigation at sowing time, while the remaining amount of nitrogen was applied 4 weeks after sowing. Each field plot was treated on lines with 250 g

of arbuscular mycorrhizal (*Glomus mosseae*) inoculum (spores, hyphae and roots of the sorghum and millet host plants) before sown cowpea seeds. Seeds of cowpea were manually sown at the August 15 in all plots in 40 cm spaced crop rows keeping plant to plant distance of 20 cm. All plots received recommended irrigation water during the entire course of study.

Treatments were comprised of sunflower residue rates at 3 and 6 t ha^{-1} incorporated into field plots with and without half rate of trifluralin herbicide (1.2 L ha^{-1}). Treatments of weedy check (without sunflower residues), trifluralin at label rate (2.4 L ha^{-1}) and a weed free were also included for comparison. Weeds from weed free plots were manually removed every week by hand pulling throughout the crop's life span. Trifluralin was applied as pre-plant soil incorporation. Volume of spray (300 L ha^{-l}) was calibrated using water. Trifluralin was applied using a Knapsack hand sprayer fitted with T-Jet nozzle at a pressure of 207 k Pa.

The experiment was laid out in randomized complete block design (RCBD) with four replications. Data collected was analyzed by Fisher's analysis of variance technique. Least significance difference (LSD) test was applied at 0.05 probability level to compare treatment means [8].

Mycorrhizal studies

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 [9]. The supernatant was decanted through a nest of sieves with reducing mesh sizes from 500 µm, 250 µm, and 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 [9].

Isolation and identification of Mycorrhiza spores

Some spores were mounted in small glass capsules containing water and a drop of chloroform for identification. The identification was made according to Schenck and Smith [10].

Preparation of Mycorrhiza inoculums

Loamy soil was brought from field, autoclaved for 0.5 h and packed in 10 plastic pots of 1 kg capacity. Spores of mycorrhiza *Glomus mosseae*, identified according to Schenck and Smith [10], were extracted by procedure below and mixed with the sterilized soil. Pots of 10 kg capacity were 50% filled by field soil while the other half was filled by spores inoculated soil. Ten seeds of sorghum and 10 of millet were sown separately in 5 pots and irrigated with appropriate amount of water. Three months after planting, the above grounds of plants were cut. The soil plus roots of both plant species were taken, air dried under laboratory conditions, mixed thoroughly and used for inoculation process [11].

Spore counting

Spore counting was conducted by taking soil samples from rhizosphere of cowpea plants growing in all plots and in plots without sunflower residues (Control) at 2, 4, 6 and 8 weeks after sowing (DAS). Spore counting was also made at the end of cowpea crop maturity to determine the possible effect of test treatments on mycorrhizal population and growth. Soil samples from rhizosphere of cowpea plants growing in plots of all treatments were taken and used for counting number of spores using microscopic slide.

Mycorrhizal colonization rate (%)

Cowpea 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 minutes 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 minutes. 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 distains. Roots were allowed to distains overnight before microscopic examination (9). 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 [12].

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

Mycorrhizal intensity was calculated based on the following rate index.

0 = the root fragments was not mycorrhizaled
١-25 = one of the root fragments were mycorrhizaled
26-50 = two of the root fragments were mycorrhizaled
51-75 = three of the root fragments were mycorrhizaled
76-100 = four of the root fragments were mycorrhizaled

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

MI = Total (number of fragments × their rate index) / number of observed fragments × highest rate [12].

Effect of sunflower residues in combination with 50% full rate of herbicide on soil nitrification.

Mature plants of sunflower *cv.* Asgria were collected from the Research Farm of State Board of Agricultural Extension and Cooperation, Ministry of Agriculture at Waset Province in July 1, 2013. The plants were oven-dried at room temperature for several days, grounded by electrical grinder to pass 6 mm openings and kept under laboratory condition until use.

For nitrification bioassay, soil with characteristics listed in Table-1 was taken from field, mixed thoroughly, air-dried under shade, and passed through a 2-mm sieve. The soil was divided in to six parts of 3 kg each to conduct the treatments. The treatments were soil amended with sunflower residues at 3 and 6 g per kg soil alone and in combination with the reduced rate of trifluralin (50% of label rate). Control treatments were made by mixing 3 and 6 g of peat moss with soil to keep the organic matter the same [13]. Autoclaved sterilized soil was also included for comparison .Hundred g each soil treatments were placed in 100-ml plastic beakers. Each beaker received 20 ml (90% of soil field capacity) of aqueous solution containing enough $(NH_4)_2SO_4$ to make the NH4 nitrogen added 800 ppm. All beakers were covered with perforated polyethylene sheets to allow aeration and incubated at 28 °C under darkness. The experiment was arranged in a randomized complete block design with four replications. The amounts of NH_4 nitrogen and NO_3 nitrogen were measured at 0, 4, 8 and 12, 16, 20, 24 and 28 days after incubation using the Mgo - Devarda alloy method [14]. Nitrification rate was measured according to the following equation:

Percent nitrification rate = $\frac{NO3-N}{NH4-N+NO3-N} \times 100$.

Table 1- Some Physical and chemical properties of soil used for nitrification experiments.

*Soil property	Value
pH	7.5
EC (dS m^{-1})	1.8
NH4-N (ppm)	29.4
NO3-N (ppm)	39.3
Organic matter (%)	1.8
Sand %	18.7
Silt %	36.0
Clay %	45.3

*Each value is an average of three replications.

All beakers were covered with perforated polyethylene sheets to allow aeration and incubated at 28 °C under darkness. The experiment was arranged in a randomized complete block design with four replications. The amounts of NH_4 nitrogen and NO_3 nitrogen were measured at 0, 4, 8,and 12 days after incubation using the MgO-Devarda alloy method [15].

RESULTS and DISCUSSION

Microscopic examination of the collected samples from cowpea field revealed that the dominant (90% of the population) species of mycorrhiza is *Glomus mosseae*. The genus includes both sporocarpic and non-sporocarpic species. Those with chlamydo spores develop the spores terminally, on a single undifferentiated hypha. The spores are formed at the end of a hypha which may be

constricted at the point of attachment to the spore, have parallel side walls, or become markedly occluded at the point of attachment to the spores. The spore wall can have one to many layers, without ornamentation. Germination is either into old subtending hypha or more rarely through the spore wall.

Vesicles and arbuscular have been found in *Glomus mosseae.*

Results in table ٢ revealed that incorporation of sunflower residues significantly increased spores number at 2, 4 and 6 weeks of residue decomposition compared to control treatment. Increase the rate of residues to 6 t ha^{-1} considerably increased the number of spores. However, when the residues applied in combination with reduced rate of trifluralin herbicide, sporulation was appreciably decreased by the low rate of sunflower residues while it remains almost the same at the higher rate of the residues. Field soil amended with label rate of trifluralin showed lower sporulation during the first six weeks from beginning of the experiment compared with control. At the 8^{th} week, sporulation was almost the same among all treatments Table-2.

Table 2-Effects of 50% full rate of trifluralin herbicide and residues of sunflower *cv.* Asgria on sporulation of *Glomus mosseae* associated with *Vigna sinensis.*

Treatments*	Number of spores per 100 g air dried soil			
	Decomposition periods (week)			
	2	4	6	8
Weedy check (Control)	260	340	360	460
Residues at 3 t ha^{-1}	420	480	500	440
Residues at 3 t ha^{-1}+50% rate of trifluralin	300	320	420	440
Residues at 6 t ha^{-1}	580	500	500	540
Residues at 6 t ha^{-1}+50% rate of trifluralin	440	420	460	480
Label rate of trifluralin (2.4 L ha^{-1})	200	320	340	380
weed free	360	400	500	480
LSD ≤ 0.05	79.3	87.8	93.4	N.S

*Each number is an average of 5 replicates.

Table-3 indicted all treatments did not significantly affected colonization rate of *G. mosseae*. However, colonization rate was appreciably increased application of sunflower residues at 6 t ha^{-1} compared with control and decreased by label rate of herbicide and weed free treatments (Table 3).Sunflower residues incorporated into field soil at rate of 6 t ha^{-1} increased appreciably colonization rate compared with Label rate of trifluralin. However, combination of sunflower residues and reduced herbicide reduced colonization rate compared to sole application of respective residue rate alone but the reduced was not significantly.

Table 3-Effects of different 50% full rate of trifluralin herbicide and residues of sunflower *cv.* Asgria on colonization rate and colonization intensity of *Glomus mosseae* associated with *Vigna sinensis.*

Treatments*	colonization rate**	Colonization Intensity**
Weedy check (Control)	77.8	62.2
Residues at 3 t ha^{-1}	77.8	72.2
Residues at 3 t ha^{-1}+50% rate of trifluralin	66.7	61.1
Residues at 6 t ha^{-1}	83.3	83.4
Residues at 6 t ha^{-1}+50% rate of trifluralin	77.8	57.8
Label rate of trifluralin (2.4 L ha^{-1})	61.1	54.4
weed free	61.1	66.7
LSD ≤ 0.05	17.0	11.93

*Each number is an average of 10 replicates.** See text for explanation

Colonization intensity was significantly averted by Residues at 6 t ha^{-1}treatments. The highest colonization intensity (83.4) was recorded by application of sunflower residues at 6 t ha^{-1}, followed by treatment of sunflower residues at 3 t ha^{-1} (72.2). Label rate of herbicide recorded the least colonization intensity (54.4).

In all bioassay experiments, the amounts of NH^{+}_{4} converted in the incubated soil was significantly correlated with the amount of NO^{-}_{3} produced over the periods of incubation Table-4. Incorporation of sunflower residues at 3 and 6 t ha^{-1} significantly reduced nitrification rate at all incubation periods in comparison to control Table-5. However, when the residues were applied along with the reduced rate of herbicide, nitrification rate was significantly higher than that achieved by sunflower residues application alone at all incubation periods except 4 and 8 days incubation periods. The Soil sterilization treatment showed the least nitrification rate at all incubation periods.

Table 4-Regression and correlation coefficients equation between ammonium converted and nitrate produced in nitrification bioassays.

Treatments	Correlation coefficient Between NH_4 and NO3 *	Regression coefficient
Weedy check (Control)	0.9303*	-0.9961*
Residues at 3 t ha^{-1}	0.9847*	-1.0293*
Residues at 3 t ha^{-1}+50% rate of trifluralin	0.9888**	-1.1018*
Residues at 6 t ha^{-1}	0.9831**	-1.1197**
Residues at 6 t ha^{-1}+50% rate of trifluralin	0.9728**	-1.0057**

*, ** Correlation is significant at the 0.05 and 0.01 levels, respectively.

Table 5-Effects of different rate of sunflower residues *cv.* Asgria in combination with 50% full rate of trifluralin herbicide on nitrification.

Treatments*	Nitrification rate (%) after Incubation periods (day) **						
	4	8	12	16	20	24	28
Weedy check (Control)	22.5	41.1	49.6	70.9	71.2	78.0	87.5
Soil sterilization	2.6	5.9	3.7	2.2	3.5	1.9	10.0
Residues at 3 t ha^{-1}	11.1	26.6	33.1	49.8	57.8	74.1	85.2
Residues at 3 t ha^{-1}+50% rate of trifluralin	7.3	27.7	38.8	56.0	64.7	78.5	89.4
Residues at 6 t ha^{-1}	5.4	20.3	32.6	42.7	58.6	67.3	79.2
Residues at 6 t ha^{-} +50% rate of trifluralin	9.2	31.2	41.7	46.0	56.5	73.6	84.2
LSD ≤ 0.05	2.3	2.3	2.1	2.9	1.7	1.8	2.7

*Each number is an average of 4 replicates. ** See text for explanation

The present study revealed the sunflower residues incorporation into the field soil was not directly affected Sunflower crop but also indirectly through their positive impact on sporulation, colonization and hyphal growth of *Glomus mosseae* fungus. In our study, higher sporulation, colonization rate and colonization intensity over control was observed during the release of allelochemicals from sunflower residues into the soil, particularly at the higher rate (6 t ha^{-1}). This suggests 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 arbuscular mycorrhiza fungi are controversial. Siqueira, *et al.*[15] Indicated that allelochemicals specially phenolics stimulate mycorrhizal colonization, while others found that mycorrhizal colonization is suppressed by phenolic acids released from allelopathic plants [16-17-18].

Interestingly, application of trifluralin herbicide considerably inhibited sporulation, colonization rate and colonization intensity. However, reduced dose of trifluralin in combination with higher rate of sunflower residues scored higher sporulation and colonization rate and intensity similar to that of control treatment. It is possible that the residues may mitigate the inhibitory effect of trifluralin herbicide when applied in combination.

With respect to nitrification, the high correlation between ammonium converted and nitrate produced in all bioassay treatments suggests that the soil incubation method is very efficient in studying nitrification under laboratory conditions. The high correlation also indicates that almost all the ammonium converted is biologically oxidized to NO_3 via bacterial nitrification. This is more confirmed by the results of soil sterilization treatment which shows no conversion of NH_4 to NO_3. Similar observation was reported by Alsaadawi *et al*. (16-19), who studied the allelopathic potential of sorghum cultivars against nitrification by the soil incubation method. In all bioassay experiments, incorporation of sunflower residues significantly inhibited nitrification rate at all decomposition periods except 24 and 28 days. However, the inhibition decreased when the residues was applied with reduced rate of herbicide; nevertheless, nitrification rate remain below the control treatments.

This result is significantly important from agricultural side of view since nitrification is a key soil process that provides plant roots with the nitrogen (N) form. However, when nitrification occurs rapidly, NO^-_3 supply may exceed plant demand and lost because NO^-_3 is less tightly bound to the soil compared with NH_4^+. Such a reduction in N use efficiency represents a large economic cost, estimated to be around US$ 15 billion annually [20], In addition to environmental problems such as ground water contamination, eutrophication of surface water and climate change phenomenon. The regulation of nitrification by nitrification inhibitors to a level in which nitrification rates are in synchrony with NO^-_3 uptake by plants may alleviate these problems and help achieve a more sustainable modern agriculture. No attempts were made to isolate, identify, and quantify the nitrification inhibitors from sunflower residues. However, the phenolics identified in *Helianthus annuus* residues are reported to inhibit nitrifying bacteria and thus the nitrification process [21]. Thus it appears from this study that combination of reduced (50%) trifluralin herbicide with sunflower residues at 6 t ha^{-1} improving mycorrhizal association and inhibiting nitrification process.

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