Allelopathic Impact of *Rhazya stricta* Dence and *Artemisia monosperma* Delile on Plant Growth and the Structural Colonization of AM Fungi

[1]**Abdulaziz Abdullah Al-Qarawi;** [1]**Elsied Fathi Abdullah;** [1]**Thbit Safer Al-Shahrani;** and [2]**Mashail N Alzain**

[1]Dept. of Plant Production, College of Food & Agriculture Sciences, King Saud University, Riyadh, KSA

[2]Dept. of Biology, College of Science, Princess Nora Bent Abdulrahman University Riyadh, KSA

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Correspondent Researcher:
Abdulaziz Abdullah Al-Qarawi
E-mail: alqarawi@ksu.edu.sa

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ABSTRACT

The present study was conducted to evaluate the allelopathic potential of two invasive plants namely Rhazya stricta and Artemisia monosperma on the growth of Zea mays (agricultural plant) and Lasiurus scindicus (range plant) inoculated with arbuscular mycorrhizal fungi. The results indicated that the leaf aqueous extract (10%; 30%; 50%, w/v) of both R. stricta and A. monosperma caused gradual allelopathic effects on plant height, shoot dry weight, root dry weight and structural colonization of AM fungi of both the agricultural and range plants. However, the higher concentration of plant extract (50%, w/v) caused stimulatory effect in plant growth parameters and structural colonization of AM fungi. Also, the root exudates of the invasive plants have reported similar allelopathic impact against the morphological characters of both R. stricta and A. monosperma and their structural colonization of AM fungi. The mycorrhizal spore count, most probable number of propagules and inoculums potential in the soil of the invasive plants were less as compared with control

التأثير الأليلوباثي لنباتي الحرمل *Rhazya stricta* والعاذر *Artemisia monosperma* على نموالنباتات واستعمارها بفطريات الجذور التكافلية

[1]**عبد العزيز عبد الله القرعاوي،** [1]**السيد فتحي عبد الله،** [1]**ثبيت سفر الشهراني،** و [2]**مشاعل ناصر الزين**

[1]قسم الإنتاج النباتي، كلية علوم الأغذية والزراعة، جامعة الملك سعود
الرياض، المملكة العربية السعودية

[2] قسم الأحياء، كلية العلوم، جامعة الأميرة نورة بنت عبد الرحمن
الرياض ، المملكة العربية السعودية

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بريد الكتروني:
alqarawi@ksu.edu.sa

الكلمات الدالة

الاليلو باثي، نبات الحرمل، نبات العاذر، فطريات الجذور التكافلية (الميكروهيزا).

المُستلخص

لقد أجريت هذه الدراسة لتقييم الأثار الاليلوباثية (التثبيطي) لنباتي الحرمل *Rhazya stricta* والعاذر *Artemisia monosperma* على نمو نبات الذرة الشامية *Zay mays* كنبات منزرع ونبات الضعة *(scindicus Lasiurus)* كنبات رعوي، الملقحة بفطريات الجذور التكافلية (الميكروهيزا). فتشير النتائج إلى أن التراكيز المختلفة لمستخلص أوراق نباتي الحرمل والعاذر (10 % و30 % و50 % وزن/ حجم) لها تأثير تثبيطي تدريجي طردي على كل من ارتفاع النباتات والوزن الجاف للمجموع الخضري والوزن الجاف للمجموع الجذري وعلى نسبة استعمار فطريات الجذور التكافلية (الميكروهيزا) لجذور النباتات المستقبلة سواء المنزرع (الذرة) أوالرعوي (الضعة). مع ذلك، المستخلصات ذات التركيز العالي من أوراق كل من النباتين (50 % وزن / حجم) له تأثير تنشيطي على صفات نمو النباتات المستقبلة وعلى نسبة استعمار جذورها بفطريات الميكروهيزا. أيضاً للمستخلص الجذري من النباتين المانحين (الحرمل والعاذر) نفس التأثير التثبيطي على صفات النمو وعلى استعمار فطريات الميكروهيزا لجذور النبات المستقبل. لقد تم تقدير تأثير النباتين الغازيين (الحرمل والعاذر) على كثافة فطريات الجذور التكافلية بطريقة العد المباشر للأبواغ والعدد الأكثر أحتمالاً وعدد الوحدات التكاثرية بتقدير نسبة الاستعمار وكانت أقل تحت النباتين مقارنة بالشاهدة.

Introduction

The range plants (RP) are considered the most important natural sources in many arid and semiarid countries including Saudi Arabia (SA). Many biotic and abiotic problems have been faced the growth and reproduction of RP which decreases the efforts for rehabilitation of degraded rangelands in SA (Alqarawi and Abd_Allah, 2010); (Alqarawi *et al.,* 2014). The impact of invasive plants is one of the most important biotic factor establish virtual monoculture hence decrease the diversity in range plant communities (Hierro and Callaway 2003). The allelopathy has been suggested as a mechanism for the impressive success of invasive plants by establishing virtual monoculture and may contribute to the ability of particular exotic species to become dominants in invaded plant communities (Hierro and Callaway 2003); (Gilani *et al.,* 2007).

Arbuscular mycorrhizal fungi (AMF) are soil myobiota belong to Phylum Glomeromycota, are the most important members of soil fungi which form symbiotic relationships with the majority of higher plants (Smith and Read 2008), hence play an important role in the uptake of plant nutrients such as P, N, Cu, as well as Zn, and water (Van der Heijden *et al*. 2006) which stimulate the plant growth in natural ecosystems. Moreover, AMF can improve the plant tolerance against some abiotic stresses such as salinity (Estrada *et al*. 2013), heavy metals (Zaefarian *et al*., 2011) as well as drought (Saraswati *et al.,* 2012) and biotic stresses such as plant diseases control (Akhtar *et al.,* 2011).

Rhazya stricta Decne. (Apocynaceae) is an important medicinal plant used in traditional and indigenous medicinal herbal drugs to cure various diseases in many countries involve SA (Ali *et al.,* 2000); (Abbas *et al.,* 2002); (Ahmad *et al.,* 2004); (Baeshen *et al.,* 2010) due to it is rich in biochemical materials containing anticancer indole alkaloids (Gilani *et al.,* 2007). It was among top three allelopathic plants while studying allelopathic effects of 81 medicinal plants species (Gilani *et al.,* 2010). In another connection, *R. stricta* has been reported as evergreen invasive and unpalatable plant introduced newly to overlook natural plant communities and range lands of SA (Assaeed and Al-Doss, 2001 & 2002; Emad El Deen, 2005). Many researchers have reported *R. stricta* as poisonous shrub with very wide allelopathic impact against many range plants (Khan *et al.,* 2011); (Mutawakil, 2012) consequently *R. stricta* is extensively invading large areas of deteriorated rangelands in SA (Assaeed and Al Doss, 2002); (Baeshen *et al.,* 2010); (Khan *et al.,* 2011).

Artemisia monosperma Delile is one of the most dominate and widely distributed invasive plant in SA (Chaudhary, 1989); (Daur, 2012) with medicinal importance (Stavri *et al.,* 2005) and play an important role in sand stabilizer (Kutiel, 2001); (Tsoar *et al.,* 2005); (Lortie and Turkington 2008). The highly allelopathic impact of *A. monosperma* against range as well as agricultural plants has been reported (Assaeed, 2003); (Al-Watban and Salama, 2012) in SA. The allelopathic potential of *A. monosperma* mainly depends up on its highly content of bioactive materials such as sterols, terpenes, flavonoids, saponins and tannins (Kanitah, 2011).

The present paper aimed to study the allelopathic potential of *R. stricta* and *A. monosperma* on growth and the structural colonization of arbuscular mycorrhizal fungi associated with roots of both Zea **mays** and *Lasiurus scindicus* plants.

Materials and Methods

(1) Sample Collection

Fresh leaf and root samples of *R. stricta* and *Artemisia monosperma* were collected from King Khalid Centre (KKC) of Wildlife Research and Development at Thumama, Riyadh, Saudi Arabia.

(2) Preparation of Leaf Extracts

Known amount (10, 30, and 50 g) of fresh leaves of *R. stricta* and *A. monosperma*) were soaked in 1L of distilled water for 24 hours. The extracts were filtered through Whatman No.1 filter paper and used for irrigation of the test plant (*Z. maize* L. and *L. scindicus*).

(3) Effect of Root Exudates

Both *Zea maize* and *Lasiurus scindicus* were sown in plastic pots (40 Kg in capacity) in replicates and incubated at alternative temperatures 25^{o}C /35^{o}C with 12h light for four months. The soil samples were collected for investigation of structural colonization and to get root exudates. The soil was flooded with water and filtered, the soil filtrates

(4) The Experimental Design

Seeds of both *Z. maize* and *L. scindicus* were sown in cone shape plastic pots (4.0 cm in diameter, 21 cm height) as one seed/cone. The cones were incubated in growth chamber at alternative temperatures of 20/30^{o}C with 12h light for one month. The cones of each treatment were irrigated with 10 ml of each extract (leaf extract [10%; 30%; 50%, w/v] and root exudates [as described above]) weekly up to end of the experiment (one month after the germination). The fertilization was carried out using Hoagland solution (P-free) weekly (10 ml/cone). Three replicates were used from each treatment. At end of the experiment, the plants were removed, the morphological data were taken and roots were fixed for mycorrhizal investigation.

(5) Examination of Received Plants to Colonize with AM Fungi

The examined plants were *Z. maize* and *L. scindicus*. Seeds of such plants sown in the experimental soil in cone shape plastic pots (4.0 cm in diameter, 21 cm height). The plant extract of both *R. stricta* and *A. monosperma* were added in rate of 10 ml from each concentration weekly up to end of the experiment (one month after sowing). The irrigation was carried with tap water supplemented with 10 ml of 10% Hogland solution (P-free). At end of the experiment, the roots were washed and the cleaned root segments were placed in 10% KOH and heated to 90^{o}C for 30-60 mins. The roots stained according to Phillips and Hayman (1970) using trypan blue stain. Root parts (1.0 cm) were cited on glass slide (30 part/slide) and examined. The structural colonization of AM fungi associated with roots. Presence of hyphae, vesicles, and arbuscules were recorded and analyzed for determining the structural colonization according to Trouvelot *et al.,* (1986).

Mycelial colonization was regarded as total AM colonization. Percent colonization was calculated by the following formula:

$$\% \text{ Colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segment studied}} \text{ X } 100$$

Allelopathic impact of *R. stricta* and *A. monosperma* were studied on spore count, most probable number (MPN) and mycorrhizal inoculum potential (MIP) of AMF. The invasive plants (*R. stricta* and *A. monosperma*) were sown in cone shape plastic pots (4.0 cm in diameter, 21 cm height) with agricultural soil sample was collected from Derab farm for one month at 2030^{o}C /30^{o}C with 12h light. Control soil samples with Dirab farm were used as reference.

(6) Spore Counting of AMF

The spore counting of AMF carried out in the soil samples according to (Pacioni,1992).

(7) Most Probable Number (MPN) of AMF

The soil samples of *R. stricta*, *A. monosperma* and control were used for sown *Z. maize* in cone shape plastic pots (4.0 cm in diameter, 21 cm height) for one month at 20/30^{o}C with 12h light. Autoclaved soil used as reference. The roots of *Z. maize* were collected, stained and the structural colonization of AM fungi associated with roots were investigated for as described before according to (Trouvelot *et al.,* 1986). The most probable number (MPN) of AMF was calculated according to (Alexander, 1982).

(8) Statistical Analysis

In each experiment, the data were subjected to analysis of variance and the means were compared using the protected least significant difference values.

Results and Discussion

(1) Results

The results indicated that the concentrations (10% & 30%, w/v) of aqueous leaf extract of *R. stricta* caused gradual decrease in plant height, shoot dry weight, shoot dry weight and structural colonization of AM fungi associated with roots of *Zea mays* in directly proportional with concentrations plant extract (Table 1). At the further concentration of *R. stricta* (50%), it was observed an increase in shoot dry weight and structural colonization of AM fungi associated with roots compared with the lower concentrations (10 & 30 %, w/v).

Table 1: Effect of Aqueous Leaf Extract of *R. stricta* on Growth and AMF Infection in *Z. mays* Plants.

Aqueous Leaf Extract (%)	Plant Height (Cm)	Shoot Dry Weight (Gm)	Infection (F %)	Colonization (M%)	Arbuscles (A%)
Control (Non)	39.66 ± 3.7 A	0.17± 0.04 A	81.66± 10.13 A	6.70± 1.23 a	2.98 ± 1.31 A
10	34.00± 1.00 Ab	0.10± 0.005 A	64.76 ± 18.85A	8.42± 0.58 a	0.98± 0.23 A
30	33.33± 0.88 Ab	0.12± 0.005 A	57.60± 5.98 A	9.38± 5.64 a	3.34± 1.85 A
50	27.66± 0.33 B	0.14± 0.07 A	64.03± 8.59 A	10.81± 4.51 a	6.47± 2.70 A

(*Infection* [F] *and the Percentage of Colonization* [M] *and the Percentage Arbuscles* [A])

Table 2: Effect of Aqueous Leaf Extract of *A. monosperma* on Growth and AMF Infection in *Z. mays* Plants.

Aqueous Leaf Extract	Plant Height (Cm)	Shoot Dry Weight (Gm)	Infection (F %)	Colonization (M%)	Arbuscles (A%)
Control (Non)	29.00± 2.08 a	0.08± 0.005 A	86.00± 6.65 A	27.99± 10.60 a	5.96± 1.89 A
10 (%)	35.00± 2.08 a	0.11± 0.008 A	68.20± 10.32?	15.71± 3.69?	4.43± 0.68
30 (%)	28.66± 0.88 Ab	0.07± 0.008 A	53.33± 10.72 B	5.73± 0.91 b	2.22± 0.72 A
50 (%)	33.33± 3.38 a	0.13± 0.03 A	60.50± 11.25 A	5.88± 3.18 b	0.92± 0.45 B

(*Infection* [F] *and the Percentage of Colonization* [M] *and the Percentage Arbuscles* [A])

The impact of aqueous leaf extract of *A. monosperma* on growth and AMF infection in *Z. mays* plants was studied and data shown in Table 2. The results indicated that 10%, w/v of aqueous leaf extract of *A. monosperma* caused significant an increase in plant height and shoot dry weight, however all the structural colonization of AM fungi associated with roots were decreased as compared with the control treatment. At 30% of the plant extract (*A. monosperma*) caused slight decrease in plant growth (plant height and shoot dry weight) and all the structural colonization of AM fungi associated with roots were decreased as compared with the control and 10% (w/v) of plant extract (Table 2). The further concentration (50%) of the plant extract caused an increase in plant height, shoot dry weight, infection (F) and colonization (M) however the arbuscules (A) was decreased (Table 2).

The results in Table 3 indicated that the aqueous leaf extract of *R. stricta* at 10 (w/v) concentration caused decrease in plant height and infection (F) of AM fungi accompanied with an increase in the colonization (M) and arbuscules (A) of AMF in *L. scindicus* plants. Also, 30 (w/v) concentration caused similar effect compared with the control plants. At 50% 30% (w/v) concentration, the results indicated that the plant growth (plant height and shoot dry weight), and infection (F) were decreased, however, the other structural colonization of AM fungi [colonization (M) and arbuscules (A)] associated with roots were increased.

Table 3: Effect of Aqueous Leaf Extract of *R. stricta* on Growth and AMF Infection in *L. scindicus* plants.

Aqueous Leaf Extract (%)	Plant Height (Cm)	Shoot Dry Weight (Gm)	Infection (F %)	Colonization (M%)	Arbuscles (A%)
Control (Non)	29.33± 2.33 A	0.04± 0.008 A	65.83± 3.08 A	0.83± 0.1 A	0.65± 0.13 A
10	22.66± 0.66 B	0.04± 0.005 A	50.00± 11.54 A	1.30± 0.1 A	0.81± 0.05 A
30	26.66± 1.85 Ab	0.05± 0.01 A	58.66± 12.86 Ab	0.76± 0.13 A	0.51± 0.07 A
50	25.33± 1.20 Ab	0.04± 0.005 A	29.66± 3.75 B	2.93± 2.38 A	0.86± 0.53 A

(*Infection* [F] *and the Percentage of Colonization* [M] *and the Percentage Arbuscles* [A])

The effect of aqueous extract of *A. monosperma* leaves on height, dry weight and the structural colonization (infection [F], colonization [M], arbuscules [A]) of AM fungi of *L. scindicus* was studied and data are shown in Table 4. The results indicated that the plant height and arbuscules [A] of *L. scindicus* were decreased with 10% (w/v) concentration, however shoot dry weight and colonization [M] were increased as compared with control plants (Table 4). At 30% (w/v) concentration, plant height, infection [F] and arbuscules [A] were decreased however, colonization [M] was increased, no change observed in shoot dry weight at such concentration. At 50% concentration, plant height, colonization [M] and arbuscules [A] were increased however no change in shoot dry weight was observed. Also, at the same concentration, the infection [F] of AM fungi associated with roots of *L. scindicus* was decreased (Table 4).

Table 4: Effect of Aqueous Leaf Extract of *A. monosperma* on Growth and AMF Infection in *L. scindicus* Plants.

Aqueous Leaf Extract (%)	Plant Height (Cm)	Shoot Dry Weight (Gm)	Infection (F %)	Colonization (M%)	Arbuscles (A%)
Control (Non)	25.00± 3.60 A	0.05± 0.01 A	71.73± 7.17 A	1.63± 0.1 A	1.07± 0.11 A
10	23.00± 1.15 A	0.06± 0.005 A	71.33± 10.36 A	1.80± 0.66 A	0.56± 0.03 B
30	23.00± 4.35 A	0.05± 0.006 A	41.33± 12.14 A	2.36± 1.81 A	0.47± 0.47 A
50	25.66± 2.96 A	0.05± 0.011 A	49.66± 11.40 A	3.46± 2.11 A	1.37± 0.43 A

(*Infection* [F] *and the Percentage of Colonization* [M] *and the Percentage Arbuscles* [A])

The root aqueous exudates of both *R. stricta* and *A. monosperma* caused visible decrease in height and dry weight of maize plant as well as the structural colonization (infection [F], colonization [M], arbuscules [A]) of AM fungi associated with their roots in similar antagonistic levels (Table 5). On the other hand, the root exudates of both *R. stricta* was more effective in suppression the structural colonization of AM fungi associated with maize roots than *A. monosperma* as compared with control plant.

In continuous context, the results in Table 6 indicated clearly that, the root exudates of both *R. stricta* and *A. monosperma* caused significant decrease in plant height and dry weight of *L. scindicus* plant in similar antagonistic level by nearly 50 % as compared with control plant. The infection (F) of AM fungi associated with roots of *L. scindicus* was responded negatively against the root exudates of both *R. stricta* and *A. monosperma*. However, the plant extracts were activators to the percentage of both colonization and arbuscules (A) of AM fungi associate with root of *L. scindicus* plant.

Table 5: Effect of Aqueous Root Excudate of *R. stricta* and *A. monosperma* on Growth and AMF Infection in *Z.mays* Plants.

Aqueous Root Excaudate	Plant Height (Cm)	Shoot Dry Weight (Gm)	Infection (F %)	Colonization (M%)	Arbuscles (A%)
Control (Non)	45.83± 7.86 Ab	0.46± 0.19 A	89.20± 3.33 A	15.11± 7.48 A	3.25± 1.48 aA
R. stricta	27.16± 4.59 B	0.12± 0.03 A	36.46± 7.15 A	3.09± 1.52 A	2.44± 1.37 a
A. monosperma	36.66± 4.34 A	0.02± 0.04 A	48.11± 10.66 B	9.43± 4.27 A	3.09± 0.92 a

(*Infection* [F] *and the Percentage of Colonization* [M] *and the Percentage Arbuscles* [A])

Table 6: Effect of Aqueous Root Excaudate of *R. stricta* and *A. monosperma* on Growth and AMF Infection in *L. scindicus* Plants.

Aqueous Root Excaudate	Plant Height (Cm)	Shoot Dry Weight (Gm)	Infection (F %)	Colonization (M%)	Arbuscles (A%)
Control (Non)	53.66± 5.99 A	0.30± 0.07 A	77.90± 4.75 A	0.77± .015 A	0.46 ± 0.12 A
R. stricta	20.83± 0.83 B	0.20± 0.13 A	16.17± 6.53 B	3.64± 2.03 A	2.66± 1.61 A
A. monosperma	21.50± 2.04 B	0.06± 0.01 A	33.08± 8.72 B	3.54± 1.95 A	1.26± 0.56 A

(*Infection [*F] *and the Percentage of Colonization* [M] *and the Percentage Arbuscles* [A])

The mycorrhizal investigations of soil associated with growth of both *R. stricta* and *A. monosperma* were carried out in comparison with control soil to study the allelopathic potential of the invasive plants on AM fungi (Table 7). The results indicated that the invasive range plants have significant allelopathic potential against the spore count, most probable number of propagules and mycorrhizal inoculums potential in their soil as compared with control soil, *R. stricta* was more effective in suppression both propagules and mycorrhizal inoculums than *A. monosperma*. (Table 7).

Table 7: Spore Count, Most Probable Number (MPN) and Mycorrhizal Inoculum Potential (MIP) of AMF Associated with Soil of *R. stricta*, *A. monosperma* and control with Dirab Farm Soil.

Soil Type	Spore Count (Spore / 25 Gm Soil)	MPN of AMF (Propagules/ Cm^{-3} Soi)	Mycorrhizal Inoculum Potential (MIP) (F%)
Control (Non)	282 ± 7.2 a	17.0 ± 5.6 a	83.83 ± 5.51 a
R. Stricta	100.0 ± 10.40 b	1.3 ± 0.43 c	11.25 ± 8.77 b
A. monosperma	103.0 ± 4.93 b	3.1 ± 1.02 b	18.75 ± 5.19 b

(2) Discussion

Z. mays and *L. scindicus* are an important agricultural and range plants used as forage, respectively in SA, hence we selected them as model of target plants. The results in our paper suggested highly allelopathic impact of leaf extract of both *R. stricta* and *A. monosperma* on growth of both *Z. mays* and *L. scindicus* plants as well as the structural colonization of arbuscular mycorrhizal fungi associated with their roots. Our findings are in agreement with Assaeed and (Al-Doss 2001); (Khan *et al.,* 2011); (Mutawakil 2012) who reported *R. stricta* as invasive plant has very effective allelopathic impact against *L. scindicus*; Z. mays and *V. faba*, respectively. In parallel with *R. stricta* and *A. monosperma* caused significant decrease in the structural colonization of arbuscular mycorrhizal fungi associated with their roots of target plants (*Z. mays* and *L. scindicus*). In agreement with our results, (Assaeed, 2003); (Tsoar *et al*., 2005); (Lortie and Turkington, 2008) and (Al-Watban and Salama, 2012) who reported the highly allelopathic impact of *A. monosperma* against many agricultural and range plants in many ecosystems. The effective allelopathic potential of leaves and root of *R. stricta* (Ali *et al.,* 2000; (Stöckigt *et al.,* 2002); (Gilani *et al.,* 2007 & 2010); (Elkady, 2013) as well as *A. monosperma* (Assaeed, 2003); (Al-Watban and Salama, 2012) mainly depend up on the chemical composition of their leaves and root which contain high concentration of allelochemicals such as indole alkaloids, terpenoid, alkaloids and their derivatives. In this respect, many alkaloids have been reported from all aerial and underground parts *R. stricta* (Ali *et al.,* 2000); (Sultana *et al.,* 2005); (Gilani *et al.,* 2007); (Elkady 2013). Also, *A. monosperma* reported to contain several phytochemicals such as volatile oil (Saleh, 1985), flavonoids (Ismail *et al.,* 1989); (Abu-Niaaj *et al.,* 1993); (Elgamal *et al.,* 1997), alkaloids (Zaki *et al.,* 1984) and coumarins (Hammoda *et al.,* 2008) which were acts as allelochemicals against many plants. Our findings reported that the leaf and root exudates of both *R. stricta* and *A. monosperma* caused significant decrease in the infection [F] of AM fungi associated with roots of *Z.* **mays** and *L. scindicus* as compared with control plants (*Z. mays* and *L. scindicus*, in absence of exudates of both *R. stricta* and *A. monosperma*). The plant-microbe interaction is very complex biological system. The root exudates play an important and significant role as messengers that encourage the physical and biological interactions between soil microbe and roots (Bais *et al.,* 2006); (Smith and Read, 2008); (Tahat and Sijam, 2012). The allelochemicals of root and leave the invasive plants were inhibitory agents against the spore germination of AM fungi (Vyvyan, 2002); (Bainard *et al.,* 2009). Arbuscular mycorrhizal fungi and endophytes directly and indirectly have been influenced the interactions between invasive and native plants via modifications of soil microbial communities mediated by allelochemicals produced by those fungi and plants (Anaya *et al.,* 2013); (Nasim, 2013). On the other hand, the colonization [M] and the percentage arbuscules [A] of AM fungi associated with roots of crop and native plant were increased by the invasive plant extracts. Such increase in colonization and arbuscules percentage is a systemic resistance mechanism of AM fungi to alleviate the allelochemicals stress and increase plant uptake of soil nutrients (Giasson **et al.,** 2008); (Evelin *et al.,* 2009); (Tahat and Sijam, 2012); (Andrade *et al.,* 2013).

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