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Effect of arbuscular mycorrhizal fungi on the accumulation of secondary metabolites in roots and reproductive organs of *Solanum nigrum*, *Digitaria sanguinalis* and *Ipomoea purpurea*

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Abstract

Background: The application of arbuscular mycorrhizal fungi (AMF) can induce the synthesis and accumulation of secondary metabolites in the tissues of host plants, thus impacting their allelopathic potential.

Materials and methods: The objective of this study was to determine the effect of three AMF species (*Rhizoglomus intraradices, Funneliformis mosseae, Rhizoglomus fasciculatum*) on photosynthetic pigments and secondary metabolites content in roots and reproductive organs of *Ipomoea purpurea* L., *Digitaria sanguinalis* L., and *Solanum nigrum* L. as a problematic weed species.

Results: Among compared weeds, the roots of *D. sanguinalis* associated with AMF accumulated the highest level of phenols. Higher content of flavonoids was obtained in roots of *S. nigrum* (7.46 mg g^{-1} FW) following colonization with *R. intraradices*. Berries of *S. nigrum* inoculated with *R. intraradices* had a higher concentration of terpenoids (21.45 mg 100 mL^{-1} of extract) than reproductive organs of *D. sanguinalis* and *I. purpurea***.** Colonization with *R. intraradices* improved total phenolics in seeds of *D. sanguinalis* compared to the reproductive organs of other weeds. These compounds released from seeds help defend against pathogen infection, consequently increasing seed production. In addition, phenylalanine ammonia lyase enzyme activity in leaves of *D. sanguinalis* colonized by *R. fasciculatum* and *F. mosseae* was 55% and 67%, respectively, higher than *I. purpurea* plants, grown in the same condition.

Conclusions: Results suggest that AMF can play a crucial role in enhancing of secondary metabolites in these three weeds, thereby improving their allelopathic potential and competitive ability.

Keywords: Mycorrhizal symbiosis, PAL activity, Photosynthetic pigments, Secondary metabolites

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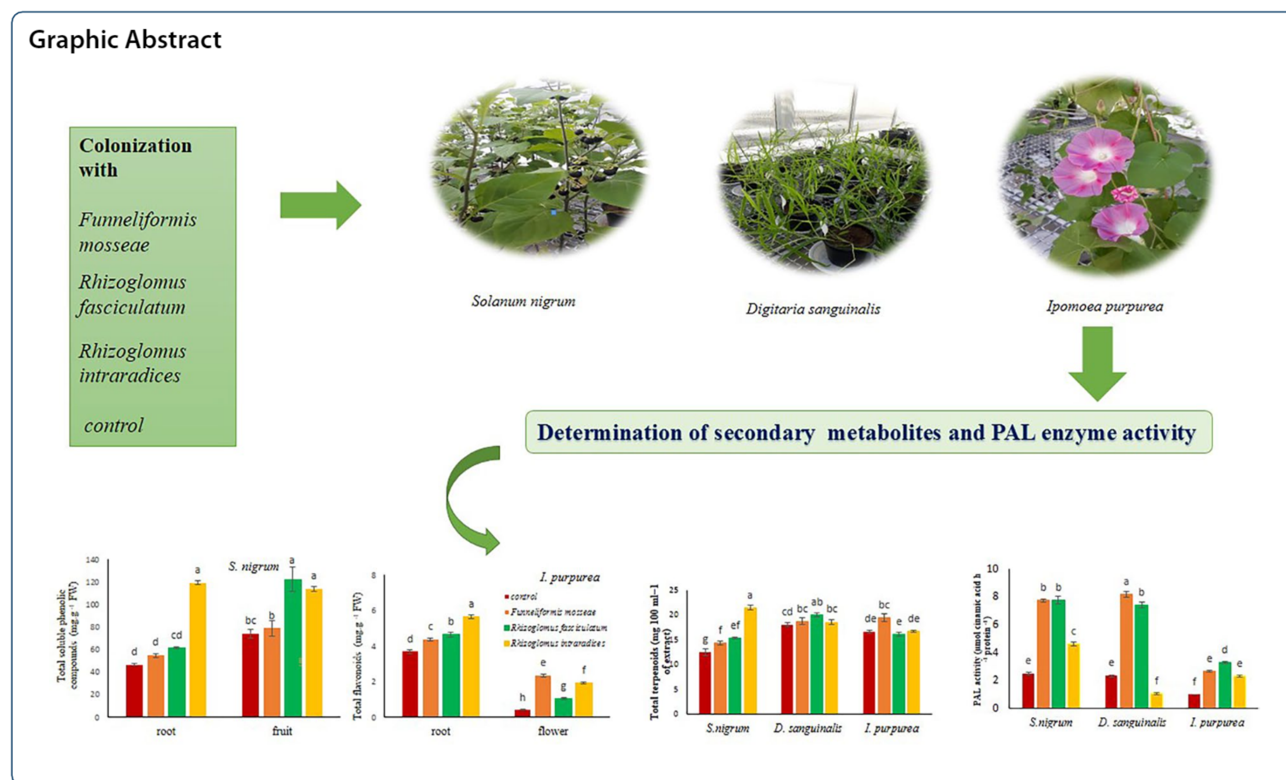

Background

Plants synthesize a broad and diverse assortment of natural products, the great majority of which do not appear to contribute directly in growth and development. These compounds have various functions such as in defence against herbivores and pathogens, attracting insects and protecting against UV light [1, 2]. In different plants, these compounds are synthesized and accumulated in roots, stems, leaves, fruits, and flowers. Most of these chemical compounds are accumulated in the vacuole, then polymerized or directly liberated, and eventually released to the environment, where they can act as allelopathic agents in the metabolism of neighbouring plants [3]. Allelochemicals are also released by weeds and inhibit growth and yield of crops [4].

Solanum nigrum L. (Solanaceae), or black nightshade, is a weed growing widely in worldwide. It mainly grows in tropical and temperate areas [5]. The berries of *S. nigrum* are revealed to have antiulcer, antioxidant, anti-inflammatory, antituberculosis, and antidiuretics effects [6]. *Ipomoea purpurea* L. Roth (Convolvulaceae), a troublesome weed of agronomic, horticultural and nursery crops, is often found in cotton, corn, and soybean fields. This weed is prolific, and can produce 8,000 seeds per season [7], which favours the infestation of fields by *I. purpurea*. *Digitaria sanguinalis* (L.) Scop. commonly known as crabgrass, is considered as an annual summer weed found in crops, turf, ruderal communities, and field margins in both tropical and temperature regions of the world [8]. Zhou et al. [9] identified three chemicals in the root exudates of *D. sanguinalis,* which may act as allelochemicals interfering with crop growth and affecting soil microbial communities.

Roots of *D. sanguinalis, S. nigrum* and *I. purpurea* can associate with arbuscular mycorrhizal fungi (AMF) present in soils [10, 11]. Previous studies indicated that AMF establishes mutualistic symbioses with flowering plants, ferns and bryophytes [12].

AMF can regulate chloroplast enzyme activity, decrease chlorophyll decomposition rate, accelerate the synthesis of important enzymes required for the chlorophyll peptide chain, promote chlorophyll synthesis, increase chlorophyll content plants, improve nutrient uptake, increasing the intensity of photosynthesis, which in turn reflects in increased biomass production [13]. In addition, some studies have also indicated that the AMF can increase the secondary metabolites content in plant organs such as seeds of *Lallemantia iberica* [14], fruits of *Solanum lycopersicum* L. [15] and leaves of *Zea mays* L. [12]. These chemical compounds also act as bioprotectants against pathogens and toxic stresses [16, 17]. For example, sesquiterpenes released from flowers of *Arabidopsis thaliana* defend plants against pathogen infection, reduce the cell death caused by the pathogen attack, and

favour seed production [18]. The release of the flavonoids luteolin by seeds of *Sesbania vesicaria* can inhibit the growth of some edaphic fungi such as *Pythium irregulare* and *Pythium ultimum* [19].

Secondary metabolites in plants are usually accumulated in organs, tissues and structures critical for the survival of the plant itself (roots, functional leaves) and its offspring (flowers, seeds and fruits). As the allelochemicals in roots and reproductive organs of *I. purpurea, D. sanguinalis* and *S. nigrum* are unknown, the general objective of this study was to evaluate the impact of three AMF (*Rhizoglomus intraradices, Rhizoglomus fasciculatum* and *Funneliformis mosseae),* susceptible to establish a symbiotic association with those weeds, on the accumulation of secondary metabolites in roots and reproductive organs of those weed species.

Materials and methods

Study design

In this study, we used seeds of *D. sanguinalis, S. nigrum* and *I. purpurea* obtained from naturally infested fields at Zanjan University Research Farm, Zanjan, Iran (36°0.41'N, 48°23' E; altitude 1,634 m). The seeds were surface sterilized with sodium hypochlorite (10%) for 5 min and subsequently washed with distilled water and then placed in Petri dishes to germinate. After seven days, the germinated seeds were transplanted into (11 cm diameter *14 cm height) plastic pots (two seedlings per pot) containing 1.1 kg autoclaved soil (for one hour at 121 °C on three consecutive days) obtained from Research Farm of University of Zanjan, New Biotechnology Research Center, Zanjan, Iran (36°0.41′ N and 48°0.28′ E; altitude 1,620 m). Soil was sandy [20] with features as follows: pH of 7.8, EC (electrical conductivity) of 0.9–1 ds/m, 0.5% organic matter [21], 0.03% nitrogen [22], 1.56 mg/kg available P [23], and 33.25 mg/kg potassium [24]. Soil pH and EC were determined using pH and EC meters (Jenway 4310, Lancashire, UK**).** Within each weed species, there were four treatments: (a) non-inoculated control plants (without AMF); (b) plants inoculated with *Rhizoglomus intraradices* (N.C. Schenck & G. S. Sm.) Sieverd, G.A. Silva& Oehl comb. nov.), (c) plants inoculated with *Rhizoglomus fasciculatum* (Thaxt.) Sieverd, G.A. Silva & Oehl comb. nov., and (d) plants inoculated with *Funneliformis mosseae* (Nicol. and Gerd.) Walker & Schüβler comb. nov. For each treatment four replicates were considered. The experiment was carried out twice, and data were obtained from both experimental rounds. AMF inoculum added to each plant consisted of 20 g of soil containing colonized root fragments from *Zea mays* and 40 spores per gram. A filtrate containing the microorganisms accompanying AMF was added to non-AMF plants. The filtrate was prepared by passing diluted mycorrhizal inoculum through a layer of 15–20 μm filter paper with particle retention of 2.5 μm (Whatman 42; GE Healthcare, Little Chalfont, UK). The plants were placed in a greenhouse at temperature of 26–29 °C (day/night) with photosynthetic photon flux density (PPFD) of 500–600 μmol m^{-2} s^{-1} and 45% relative humidity. Plants were irrigated every day with 200–250 mL of distilled water to keep the soil moisture at 75% FC and received once a week 80–100 mL of complete Hoagland solution [25].

Colonization rate (%)

Root samples of weeds were cleared and stained [26] and mycorrhizal colonization was determined by examining 1-cm root segments (50 fragments from each plant) under the microscope.

Secondary metabolites

Total phenolic content in plant organs was determined using 1 mL of each sample, mixed with 1 ml of 95% ethanol, 4 mL of deionized water, 0.5 mL of Folin–Ciocalteu reagent, and 1 mL of 0.5% sodium carbonate. Mixtures were then placed in the dark for 60 min and afterward, the absorbance rate was measured at 725 nm. Gallic acid was used as the standard solution, where the concentrations of soluble phenolic compounds were expressed as mg g^{-1} FW [27].

Total flavonoid contents of weeds were determined by the aluminium chloride colorimetric method [28]. Briefly, 0.5 mL of extract was mixed with 0.3 mL of 5% $NaNO_2$, 4.5 mL of deionized water and 600 μL of 10% AlCl3. After 6 min, the reaction was stopped by adding 2 mL of 1 M NaOH and 2 mL of deionized water. The absorbance of the samples was read at 510 nm. Flavonoids concentrations were expressed as mg g^{-1} FW, where quercetin was used as the standard.

Total terpenoid concentration was performed according to Ghorai et al. [29].

Phenylalanine ammonia lyase (PAL)

PAL activity was measured in fresh leaves (0.3 g). Enzyme was extracted with 2 mL of 50 mM boracic acid buffer (pH 8.8), containing 8 mM mercaptoethanol and 2% (w/v) PVPP. The homogenate was centrifuged at 14,000×*g* for 20 min at 4 °C. PAL assay was carried out according to the procedure of Zucker [30].

DPPH radical scavenging

DPPH reagent prepared in methanol (5 mg/100 mL, 2.0 mL) was added to each test sample (1.5 mL) and mixed with 0.5 mL of methanol. The mixture was allowed to stand for 30 min in the dark and absorbance was measured at 517 nm.

Scavenging activity was performed according to Barros et al. [31]:

$$\text{Scavenging \%} = 100 \times \left[(\text{A0}-\text{A1})/\text{A0}\right],$$

where A0 and A1 are the absorbance rate of the control and test sample, respectively.

Photosynthetic pigments and chlorophyll content index

Chlorophylls (chl a and b) and carotenoids were extracted according to the method of Arnon [32] from 0.1 g of fresh leaves in 80% acetone. Absorbance at 470, 645, 663 nm was determined using a PerkinElmer-Lambda 25 USA Spectrophotometer. Chl a, b and carotenoids concentrations were calculated by applying the equations of Lichtenthaler [33]. Chlorophyll meter (CL-O1, Hansatech instruments) was used to estimate the chlorophyll content index (CCI) in the middle part of the leaf at the beginning of the reproductive stage in each plant.

Statistical analysis

Data were subjected to an analysis of variance (ANOVA) by using PROC GLM in SAS Software (Version 9.1, SAS Institute Inc., Cary, NC). The assumption of homogeneity of variance was tested before analysing the data. The significant differences were compared by Duncan's multiple-range tests ($P \leq 0.05$). The correlations between mycorrhizal colonization and secondary metabolites were tested with Pearson's correlation coefficients.

Results

Mycorrhizal colonization

No mycorrhizal structures were found in roots of non-inoculated controls of any of the species (Fig. 1). Mycorrhizal colonization of weeds was significantly affected by the AMF species. The roots of *S. nigrum* and *I. purpurea* had higher colonization than *D. sanguinalis* plants. In *D. sanguinalis*, percentages of mycorrhizal colonization varied from 16 to 35% among different AMF species. Inoculation of *I. purpurea* with *R. fasciculatum* and *R. intraradices* increased colonization rate by 18.14 and 14.92%, respectively, as compared to *F. mosseae* (Fig. 1). In contrast, in *S. nigrum F. mosseae* and *R. fasciculatum* appeared as the most effective fungus for increasing the colonization rate.

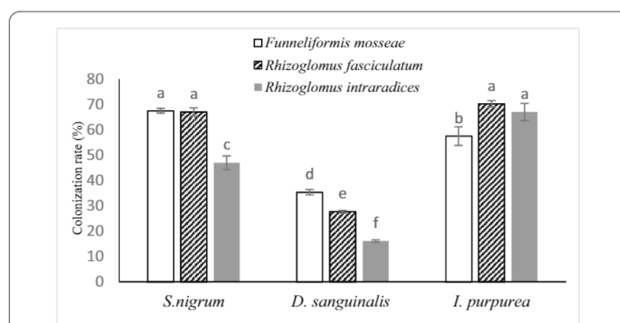

Fig. 1 AMF colonization rate (%) in roots of *Solanum nigrum*, *Digitaria sanguinalis* and *Ipomoea purpurea* at final harvest. Pots did not receive any mycorrhizal inoculum (control) or were inoculated with *F. mosseae*, *R. fasciculatum* or *R. intraradices*. Bars represent means ($n = 4$ plants) ± SE. Bars topped by the same letter indicate no significant differences between treatments at the 5% level using Duncan's multiple-range test

Secondary metabolites

There was a significant effect of AMF on the levels of total soluble phenolic compounds and total flavonoids in the roots, and reproductive organs of the studied weeds (Fig. 2). *R. intraradices* induced the accumulation of total soluble phenolic compounds in both roots and fruits of *S. nigrum* and so did *R. fasciculatum* in fruits. The concentration of phenolic compounds in roots of *R. intraradices*-inoculated *S. nigrum* plants was 60.98% higher than that of non-AMF controls (Fig. 2). In *D. sanguinalis* roots were more influenced than seeds by AMF and all the tested AMF increased the concentration of total phenolic substances. *I. purpurea* also accumulated higher amount of phenolics after its inoculation with AMF, especially in their flowers. In the *R. intraradices*-inoculated plants, reproductive organs showed higher levels of phenolics than vegetative ones (roots). Colonization with *F. mosseae, R. fasciculatum* and *R. intraradices* improved phenolic compounds in flowers of *I. purpurea* (by 50%, 55.8% and 71%, respectively) compared to the respective non-AMF plants. However, this pattern changed after AMF inoculation, so that roots and seeds of *D. sanguinalis* had quite similar concentrations of these secondary metabolites and so did roots and fruits of *S. nigrum* associated with *R. intraradices* (Table 1).

Roots of the three weed species had higher concentrations of flavonoids than reproductive organs (fruits, seeds or flowers) (Fig. 2). Colonization with *F. mosseae* and *R. intraradices* sharply promoted flavonoids in roots of *S. nigrum* (by 34% and 41%, respectively) compared to the respective control plants. Total flavonoids in roots of *D. sanguinalis* improved considerably when plants were associated with *F. mosseae*, so that *D. sanguinalis* plants colonized by *R. intraradices* species showed 25.43% more flavonoid content as compared to control plants. On the contrary, no difference in flavonoids was found in seeds of *D. sanguinalis* colonized by AMF and those collected from non-mycorrhizal plants. Colonization of *I. purpurea* with any of the three species of AMF used in this study promoted the accumulation of total flavonoids in roots and flowers. This

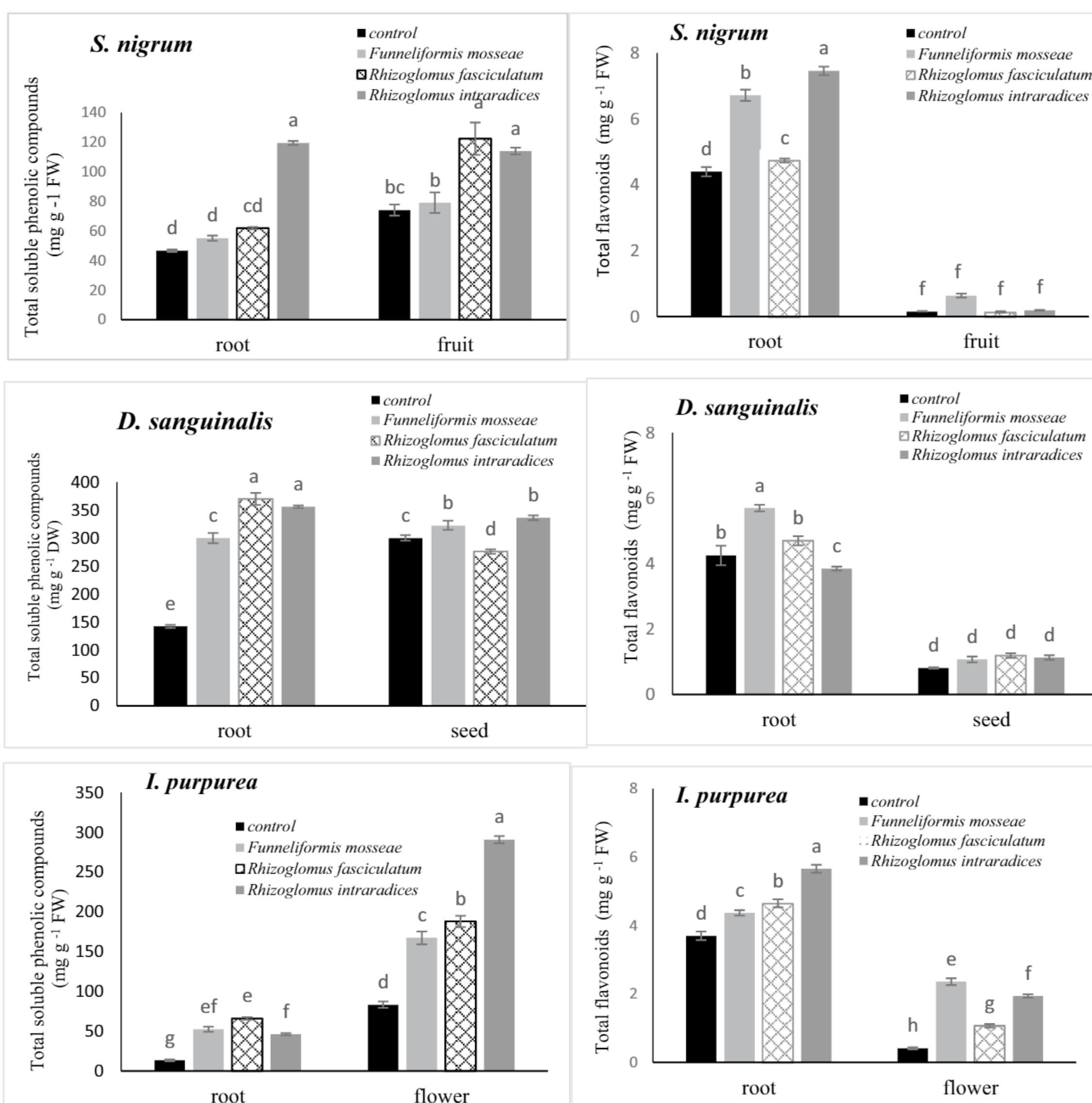

Fig. 2 Total soluble phenolic compounds (mg g^{-1} FW) and total flavonoids (mg g^{-1} FW) in different organs of *Solanum nigrum*, *Digitaria sanguinalis* and *Ipomoea purpurea* at final harvest. Pots did not receive any mycorrhizal inoculum (control) or were inoculated with *F. mosseae, R. fasciculatum or R. intraradices.* Bars represent means (n = 4 plants) ± SE. Bars topped by the same letter indicate no significant differences between treatments at the 5% level using Duncan's multiple-range test

Table 1 Analyses of variance (ANOVA) for secondary metabolites in different parts of weeds grown with three AMF

S.O.V	Df	Total phenolic compounds (mg g^{-1} FW)						Total flavonoids content (mg g^{-1} FW)					
		S. n		*D. s*		*I. p*		*S. n*		*D. s*		*I. p*	
		F	p	F	p	F	p	F	p	F	p	F	p
AMF	3	54.55	**< 0.0001**	141.98	**< 0.0001**	236.98	**< 0.0001**	129.59	**< 0.0001**	15.19	**< 0.0001**	132.55	**< 0.0001**
Plant organ	1	58.55	**< 0.0001**	13.52	**0.0012**	1810.7	**< 0.0001**	6097.78	**< 0.0001**	1394.39	**< 0.0001**	2372.8	**< 0.0001**
AMF*plant organ	3	15.05	**< 0.0001**	132.96	**< 0.0001**	133.37	**< 0.0001**	97.20	**< 0.0001**	16.80	**< 0.0001**	36.14	**< 0.0001**
Error	24	–	–	–	–	–	–	–	–	–	–	–	–
Cv %		11.70		4.32		8.07		6.56		9.65		6.04	

increase is especially evident in the flowers of plants associated with *R. intraradices* (Fig. 2).

The levels of total terpenoids in the reproductive organs of weeds (fruits of *S. nigrum*, seeds of *D. sanguinalis* and flowers of *I. purpurea)* are shown in Fig. 3. The association of weeds with AMF significantly affected the levels of total terpenoids in *S. nigrum* and *I. purpurea* (Table 2). The highest amount of total terpenoids (21.45 mg 100 ml^{-1} of extract) was found in fruits of *S. nigrum* inoculated with *R. intraradices.* In *I. purpurea*, the greatest amount of terpenoids was measured in plants colonized by *F. mosseae;* the accumulation of these compounds in the flowers was 18% higher than in those of non-mycorrhized controls. In contrast, total terpenoids in seeds of *D. sanguinalis* were similar in plants colonized by *F. mosseae* or *R. intraradices*

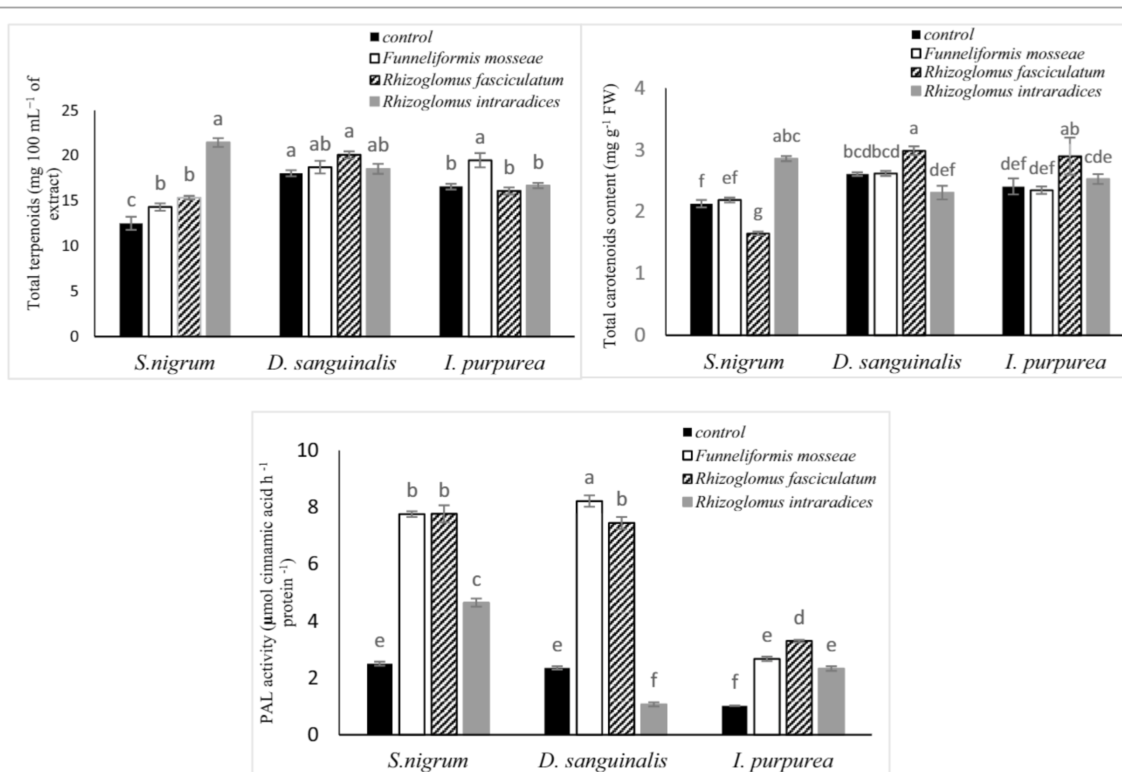

Fig. 3 Total terpenoids (mg 100 mL^{-1} of extract) in fruit of *Solanum nigrum*, seeds of *Digitaria sanguinalis* and flowers of *Ipomoea purpurea,* total carotenoids (mg g^{-1} FW) and phenylalanine ammonia lyase (PAL) (μmol cinnamic acid h $^{-1}$ protein $^{-1)}$ in leaves of weeds at final harvest. Pots did not receive any mycorrhizal inoculum (control) or were inoculated with *F. mosseae, R. fasciculatum or R. intraradices.* Bars represent means (n = 4 plants) ± SE. Bars topped by the same letter indicate no significant differences between treatments at the 5% level using Duncan's multiple-range test

Table 2 Analyses of variance (ANOVA) for secondary metabolites and PAL activity in leaves of weeds grown with three AMF

S.O.V	Df	Total terpenoids (mg 100 mL^{-1} of extract)		Total carotenoids (mg g^{-1} FW)		PAL (μmol cinnamic acid h $^{-1}$ protein $^{-1}$)	
		F	*p*	*F*	*F*	*F*	*p*
AMF	3	20.24	**< 0.0001**	1.97	0.1356	22.21	**< 0.0001**
Weeds	2	33.79	**< 0.0001**	15.64	**< 0.0001**	2.29	**< 0.0001**
AMF*weeds	6	25.61	**< 0.0001**	13.91	**< 0.0001**	5.36	**< 0.0001**
Error	36	–	–	–			
Cv%	–	5.80		9.23		6.63	

Significant differences are in bold at $p < 0.05$

and in their respective non-mycorrhized control plants (Fig. 3).

Our results showed that the mycorrhizal association can significantly affect the concentrations of total carotenoids in leaves of the three studied weeds (Table 2). While *R. intraradices* induced the accumulation of carotenoids in *S. nigrum*, *R. fasciculatum* increased the concentrations of these pigments in leaves of *D. sanguinalis* and *I. purpurea,* suggesting that *R. fasciculatum* and *R. intraradices* are very efficient in improving total carotenoids in weeds. The content of total carotenoids in leaves of *I. purpurea,* which was unaffected by *F. mosseae* and *R. intraradices*, was significantly higher when associated with *R. fasciculatum,* so that *I. purpurea* inoculated with *R. fasciculatum* showed 16.81% more total carotenoids than its respective non-AMF control (Fig. 3).

The effect of AMF and weed species on PAL activity was significant (Table 2). In leaves of both *D. sanguinalis* and *S. nigrum*, the activity of the enzyme PAL clearly enhanced when plants were inoculated with either *R. fasciculatum* or *F. mosseae*. Likewise, the PAL activity in leaves of *I. purpurea* colonized by *F. mosseae, R. fasciculatum* or *R. intraradices* was, respectively, 61%, 69%, and 56% higher than that measured in non-AMF control plants. Contrariwise, PAL activity in leaves of *D. sanguinalis* inoculated with *R. intraradices* was 54% lower as compared to non-mycorrhizal control plants (Fig. 3).

Total antioxidant capacity differed between vegetative and reproductive organs in the studied weeds. In *S. nigrum* and *D. sanguinalis* DPPH activity was higher in the reproductive (fruits and seeds) than in the vegetative (root) organs. In contrast, roots of *I. purpurea* exhibited higher DPPH activity than flowers. Moreover, the DPPH radical scavenging activity in the organs of the three weed species was significantly affected by AMF. The three tested AMF increased the antioxidant capacity in the reproductive organs of *S. nigrum* and *D. sanguinalis*. DPPH activity in fruits of *S. nigrum* colonized by with *F. mosseae, R. fasciculatum* and *R. intraradices* AMF species was 6.89, 8.98 and 2.46%, respectively, higher as compared to non-AMF control plants. Similarly, *R. intraradices* and *R. fasciculatum* also improved the antioxidant activity in the flowers of *I. purpurea,* and so did *R. fasciculatum* and *F. mosseae* in roots of this weed (Fig. 4, Table 3).

The effect of AMF on the concentrations of chlorophylls (a, b) in leaves depended on the weed species. While the highest concentrations of chlorophylls a and b

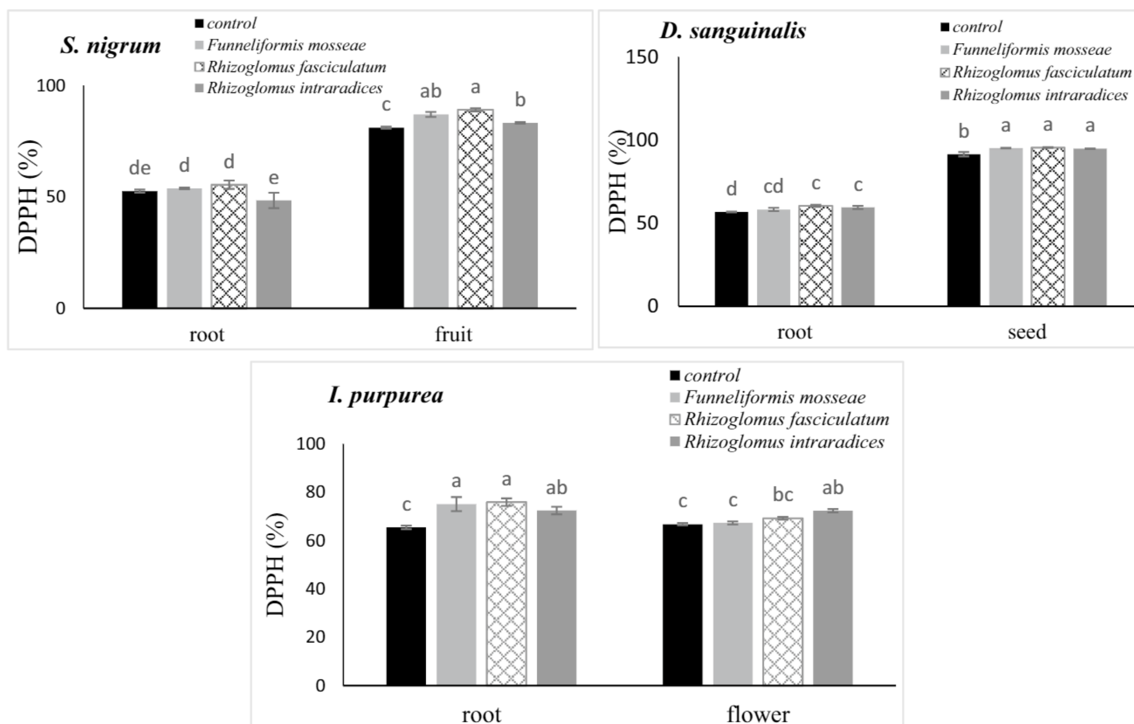

Fig. 4 DPPH radical scavenging activity (%) in different parts of *Solanum nigrum*, *Digitaria sanguinalis* and *Ipomoea purpurea* at final harvest. Pots did not receive any mycorrhizal inoculum (control) or were inoculated with *F. mosseae, R. fasciculatum or R. intraradices.* Bars represent means (n = 4 plants) ± SE. Bars topped by the same letter indicate no significant differences between treatments at the 5% level using Duncan's multiple-range test

Table 3 Analyses of variance (ANOVA) for DPPH radical scavenging activity (%) in different parts of weeds grown with three AMF

S.O.V	Df	*S. nigrum*		*D. sanguinalis*		*I. purpurea*	
		F	*p*	*F*	*p*	*F*	*p*
AMF	3	81.73	**0.0008**	33.36	**< 0.0001**	9.45	**0.0003**
Plant organ	1	21.82	**< 0.0001**	15,604.9	**< 0.0001**	11.40	**0.0025**
AMF*plant organ	3	0.25	0.227	5.72	**0.0042**	5.49	**0.0051**
Error	18	–	-	–	–	–	–
Cv%	–	4.40		1.06		3.92	

Significant differences are in bold at $p < 0.05$

were observed in *S. nigrum* associated with either *R. intraradices* or *F. mosseae*, AMF did not significantly affect the concentrations of chlorophyll a in *D. sanguinalis* and *I. purpurea* (Table 4). The chlorophyll index (SPAD) was increased by AMF colonization in the three species of weeds (Table 4).

Discussion

Unlike *D. sanguinalis*, mycorrhizal colonization of *S. nigrum* and *I. purpurea* plants with AMF species reached a high percentage (47–70%), making these weeds plants relatively stronger AMF hosts compared to *D. sanguinalis.* Since weeds are one of the major threats to the natural environment, the widespread occurrence of AMF and their important role in communities and ecosystems makes the interaction between weed and AMF key for the ecosystem functioning [34, 35].

Our results showed that roots and reproductive organs of the three investigated weeds inoculated with AMF were rich in total soluble phenolic compounds and flavonoids, substances with high allelopathic capacity, whose regulation and composition often differ below and above ground plant organs [36, 37]. Results of this study allow us to hypothesize that the secondary metabolites accumulated in the mycorrhized roots of weeds may be released into the soil through the external fungal mycelium and impact the roots of the surrounding plants [38]. Increased amounts of secondary metabolites in roots following mycorrhizal colonization may reinforce the allelopathic potential of weeds thus negatively affecting crops [39]. In forest ecosystems, chemical compounds released by invasive species can limit the growth of competing vegetation providing the invader competitive advantage [40, 41].

Some secondary metabolites belonging to the phenolics increase cell membrane permeability and induce lipid peroxidation, which finally results in plant death [42]. Earlier studies demonstrated that the increase of electrolyte leakage represents membrane integrity damage [43]. Among these phenolic compounds with allelopathic potential, catechin has been found in roots of the weed *Centaurea stoebe* and it has shown strong phytotoxicity against *Festuca idahoensis* and *Arabidopsis thaliana* [44]. Similarly, catechin has found in *Melia azedarach* fruit play an important role in its allelopathic potential [45]. In addition, other phenolic compounds including gallic

Table 4 Photosynthetic pigments and chlorophyll index (SPAD) in leaves of weeds

		Ch a	Ch b	Ch total	Chlorophyll index (SPAD)
	Control	1.05 ± 0.07 bc	0.50 ± 0.06 ab	1.55 ± 0.12 bc	12.85 ± 0.11 c
	F. mosseae	1.42 ± 0.04 a	0.58 ± 0.01 a	2.01 ± 0.0.5 a	15.45 ± 0.3 b
S. nigrum	*R. fasciculatum*	1.17 ± 0.08 b	0.57 ± 0.06 a	1.74 ± 0.09 b	16.37 ± 0.51 b
	R. intraradices	1.46 ± 0.15 a	0.59 ± 0.01 a	2.05 ± 0.15 a	23.5 ± 0.64 a
	Control	0.77 ± 0.006 d	0.35 ± 0.01 def	1.12 ± 0.011 de	3.98 ± 0.3 f
	F. mosseae	0.87 ± 0.04 cd	0.36 ± 0.01 cdef	1.23 ± 0.06 de	12.27 ± 0.47 c
D. sanguinalis	*R. fasciculatum*	0.74 ± 0.07 d	0.46 ± 0.016 bc	1.21 ± 0.08 de	5.95 ± 0.45 e
	R. intraradices	0.93 ± 0.05 cd	0.41 ± 0.01 bcde	1.35 ± 0.05 cd	6.36 ± 0.29 e
	Control	0.82 ± 0.03 d	0.39 ± 0.007 cde	1.21 ± 0.03de	7.96 ± 0.26 d
	F. mosseae	0.87 ± 0.06 cd	0.31 ± 0.02 ef	1.18 ± 0.08 de	12.85 ± 0.87 c
I. purpurea	*R. fasciculatum*	0.84 ± 0.02 cd	0.42 ± 0.05 bcd	1.27 ± 0.07 de	12.25 ± 0.32 c
	R. intraradices	0.75 ± 0.02 d	0.28 ± 0.004 f	1.03 ± 0.02e	12.75 ± 0.56 c

Data are means ($n = 4$) + SE. Within each column, means followed by the same letter are not significantly different at 5% level

acid, *p*-coumaric acid, *p*-hydroxybenzoic acid and ferulic acid, which cause reduced growth of rice, have been detected in the rhizosphere of *Ageratum conyzoides* L. [46]. Gmerek and Politycka [47] reported that the lipid peroxidation in roots of *Z. mays* L., *Raphanus sativum* L. and *Pisum sativum* L. was attributed to *p*-coumaric and ferulic acids.

Flavonoids are compounds with high antioxidant activity [48], whose biosynthesis can be inhibited by gibberellic acid [49]. They are known to inhibit the electron transport chain in the mitochondrial membrane [50]. These compounds suppress root growth, may be due to the breakage of cell homeostasis leading to allelopathic stress. Endogenous and exogenous flavonoids in various doses can affect auxin transport in roots and induce lateral root growth under stress conditions [51]. The allelopathic potential of *Dittrichia viscosa* L. may be attributed to flavonoids accumulated in its tissues [52]. Allelochemicals isolated from extracts of *Xanthium strumarium* L. exert inhibitory effects on growth of Gram-positive and Gram-negative bacterial strains and had antioxidant activity [53]. According to our results, the fruits of *S. nigrum* plants inoculated with AMF have high concentrations of phenolics and terpenoids and *R. fasciculatum* and *R. intraradices* are the most effective fungal species to improve phenolic compounds, terpenoids and DPPH. Consequently, we can infer that they can also exhibit increased antioxidant activity acting as free radical scavengers [56]. This higher accumulation of secondary metabolites in the fruits of *S. nigrum* can have practical applications for the phytotherapeutic industry.

A high concentration of secondary metabolites, such as phenols, flavonoids, anthocyanin and terpenoids, was also reported from *S. nigrum* and *D. sanguinalis* leaves [10], in addition, inoculation with *F. mosseae* species increased phenol, anthocyanin, and total terpenoid content in *S. nigrum* plants much more than *D. sanguinalis.*

The enhanced production of secondary metabolite concentrations in AM plants may be (1) due to improved mineral nutrition, and/or (2) a result of plant reaction to fungal colonization [54, 55]. Both of these mechanisms are possible explanations for the effect of AMF on the production of phenols, flavonoids and terpenoids in weeds in our study.

Carotenoids concentrations in leaves of *D. sanguinalis* and *I. purpurea* were improved by the association of these plants with *R. fasciculatum*. Carotenoids are known to be non-enzymatic antioxidant molecules that prevent the photo-oxidative damage of chlorophylls. In our study, the amount of carotenoids was enhanced in most part of the mycorrhizal plants in comparison with their respective non-mycorrhizal controls, which agrees with findings of Kumar et al. [57] working with *Vigna radiata* L.

PAL is a key enzyme in the biosynthesis of phenols, flavonoids and isoflavonoids in plants. Increased PAL activity in weeds associated with AMF may probably induce the production of flavonoids and other phenolic compounds production thus increasing their allelopathic potential. Altered gene expressions in hosts as a result of AMF colonization influence their metabolism and lead to the induction of chemical defence [58]. It was found that roots colonized by AMF had increased levels of transcripts encoding phenylalanine ammonia lyase (PAL). PAL is the first enzyme of the phenolics/phenylpropanoid pathway [59]. Since phenolic compounds are produced in weed as, *D. sanguinalis, S. nigrum* and *I. purpurea*, defence metabolites, the improved concentrations of these chemicals in weeds in our experiment might be explained by this mechanism.

Arbuscular mycorrhiza association promoted changes in chlorophyll concentration of the leaves of weeds. This result is likely due to improved nutrient uptake, resulting in overall higher photosynthetic capability [60]. Our results showed an increase in chlorophyll contents in *S. nigrum* associated with *R. intraradices*. Increased concentration of chlorophylls in *Calendula officinalis* associated with AMF was founded by Kheyri et al. [54]. A large amount of chlorophyll content in the leaves of AMF weeds, which allows plants to achieve more energy from light, could be related to enhanced uptake of phosphorus and magnesium increased transpiration, stomatal conductance, and carbon assimilation [61].

A significant positive relationship between mycorrhizal colonization and DPPH (0.832**) and terpenoids (0.853**) in fruits of *S. nigrum* was observed (Additional file 1: Table S2). In roots of *D. sanguinalis*, the root colonization is significantly correlated with total phenolics compounds (0.727**) and flavonoids (0.550*) and a positive correlation was observed between colonization rate and flavonoids and DPPH in seeds of this weed (Additional file 1: Tables S3, S4). Highly significant positive correlations were found between the mycorrhizal colonization and total phenolics, flavonoids and DPPH in roots and flowers of *I. purpurea* (Additional file 1: Tables S5, S6).

Conclusion

In conclusion, the application of AMF is a way of improving the contents of secondary metabolites, thereby increasing the allelopathic potential of these weeds. In between three AMF of species, *R. intraradices* had the highest effect in improving secondary metabolites in roots of *S. nigrum*. The higher production of secondary metabolites in the fruit of R. intraradices-inoculated S. nigrum can have practical applications in the phytotherapeutic industry. These results indicate that

the establishment of AM symbiosis induces secondary metabolite accumulation, increases PAL enzyme activity, which may be of biological significance in the interactions of colonized plants with their environments.

Abbreviations
AMF: Arbuscular mycorrhizal fungi; PAL: Phenylalanine ammonia lyase.

Supplementary Information

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Additional file 1. Additional tables.

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ARY: methodology, software, conceptualization, supervision, writing and editing. MP, NG: software, writing and editing. SR: investigation, methodology, software, writing—original draft. ARY, MP: funding acquisition. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
All authors contributed in design and preparation of the research, and they have read the final version of the manuscript.

Consent for publication
We declare our agreement.

Competing interests
The authors declare that they have no competing interests.

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