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# *Funneliformis mosseae* alters seed essential oil content and composition of dill in intercropping with common bean

Weria Weisany[a,b,*], Yaghoub Raei[a], Kazem Ghassemi-Golezani[a]

[a] Department of Plant Ecophysiology, Faculty of Agriculture, University of Tabriz, Iran
[b] Safe Crop Centre, Istituto Agrario di S. Michele all'Adige, Via Mach 1, S. Michele all'Adige, TN 38010, Italy

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ABSTRACT

Intercropping and arbuscular mycorrhizal (AM) fungi colonization are important strategies for improving crop yield and quality. Thus, two experiments were carried out with factorial arrangement based on randomized complete block design with three replications, to investigate the influence of AM fungi on common bean yield and essential oil (EO) quantity and quality of dill seed at different cropping systems in 2013 and 2014. The factors were cropping systems including (a) common bean (*Phaseolus vulgaris* L.) sole cropping (40 plants $m^{-2}$), (b) dill (*Anethum graveolens* L.) sole cropping at different densities (25, 50 and 75 plants $m^{-2}$) and (c) the additive intercropping of common bean + dill (25 + 40, 50 + 40 and 75 + 40 plants $m^{-2}$). All these treatments were applied with or without AM colonization. In both cropping systems, the AM colonization significantly increased the seed and EO yields as compared with non-inoculated plants. The land equivalent ratio (LER), time equivalent ratio (ATER), land utilization efficiency (LUE) and relative value total (RVT) were higher at intercropping compared with sole cropping. The aggressivity (A) and relative crowding coefficient (K) values were greater for common bean than for dill, whereas the corresponding values for dill were lower in intercropping with common bean. In both years, the *Funneliformis mosseae* colonization improved yield and intercropping indices, compared with non-inoculated plants. The content of α-phellandrene, limonene, β-phellandrene, dill-ether and carvone were enhanced in seed EO obtained from AM-inoculated and intercropped dill plants, while AM colonization resulted in a lesser content of α-phellandrene, limonene, β-phellandrene, iso-dihydrocarveol and thymol in sole cropped dill plants.

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## 1. Introduction

Intercropping may be a useful strategy to grow crops simultaneously, offering to improve resource utilization such as solar radiation, nutrients and water during growth and development. This is also an important method for sustainable crop production, particularly when inputs are limited (Agegnehu et al., 2006). Some of the potential benefits of intercropping are: enhancing the crop yield and quality (Blaser et al., 2007), reducing soil erosion (Kremer and Kussman, 2008), improving nitrogen fixation (Singer 2008) and land-use efficiency (Agegnehu et al., 2006a,b; Dhima et al., 2007a,b). There are many indices used in the literature to evaluate the potential advantages of intercrops and species interactions. Several indices such as land equivalent ratio (LER), time equivalent ratio (ATER), relative value total (RVT), land utilization efficiency (LUE), relative crowding coefficient (K), and aggressivity (A) are used to describe the competition and the economic advantage of intercropped plants (Banik et al., 2000; Ghosh, 2004; Midya et al., 2005). Intercropping of peanut (*Arachis hypogaea* L.) with corn (*Zea mays* L.) produced more yield than sole cropping of these plants (Inal et al., 2007). Sorghum was a dominant plant in intercropping of soybean and sorghum (Misra et al., 2006). In contrast, few studies were concentrated on the effects of AM colonization on annual legume intercropping with annual medicinal plant.

Dill (*Anethum graveolens* L.) is an important essential oil (EO) bearing plant that originally comes from Eastern Mediterranean. The dill is useful as aromatic, carminative, mildly diuretic, galactogogue, stimulant and stomachic. It is also used to increase the flow of milk in nursing mothers, to help prevent colic, for bad breath, cough, cold and flu, period pains (Setorki et al., 2013; Hosseinzadeh et al., 2002). The most important EO compounds in this plant are carvone and phellandrene, and the most important compounds from the fully grown seeds are carvone and limenene (Nejatzadeh

* Corresponding author at: Safe Crop Centre, Istituto Agrario di S. Michele all'Adige, Via Mach 1, S. Michele all'Adige, TN 38010, Italy. Fax: +39 041615515.
*E-mail address:* Weria.Wisany@gmail.com (W. Weisany).

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and Gholami, 2014). Some of the EO components were used in industry. For example, carvone was identified as an effective potato sprout inhibitor (Hartmans et al., 1995), it can also inhibit the growth of certain fungi (Farag et al., 1989) and microorganisms (Vokou et al., 1993) and act as an insect repellent (Su, 1985). Other EO ingredients such as myristicin and apiole were identified as natural insecticides (Duke, 2001).

Diverse factors can influence the quality and composition of EO as final product, i.e., the biotic and abiotic factors, identification of species, the amount of nutrients on the soil, planting and harvesting (Sá et al., 2015). Among the biotic factors, AM fungi colonization can influence the production of active ingredients in medicinal and aromatic plants (Karagiannidis et al., 2011). AM symbiosis is the most important type of mycorrhiza, which reported to occur in many plants (Bonfante and Genre, 2008). AM fungi can considerably influence plant growth (Sohrabi et al., 2012a,b), nutrients uptake and transport (Smith and Read, 2008; Clark and Zeto, 2000), water status (E.I-Tohamy et al., 1999) and chlorophyll content (Beltrano and Ronco, 2007). According to Gupta et al. (2002) mycorrhizal colonization can enhance oil content and yield of *Mentha arvensis* L. plants. Freitas et al. (2004) also reported an increase of 89% in the EO and menthol contents of *M. arvensis* plants due to colonization with AM. Colonization of rose-scented geranium plants with one of *Acaulospora laevis*, *Gigaspora margarita*, *Glomus fasciculatum* or *Glomus mosseae* recorded resulted in higher EO yield (21.1%), compared with non-mycorrhizal plants (Venkateshwar Rao et al., 2002). Environmental conditions may influence and change some of EO components such as α-pinene, p-cimene, α-terpinene, and linalool in coriander (Carrubba et al., 2002), or α-thujene, α-terpinene, β-phellandrene, and camphor in fennel (Carrubba et al., 2005). Maffei and Mucciarelli (2003) observed that the total EO and menthol contents of peppermint could be positively affected by intercropping with soybean (*Glycine* max M.).

Different techniques could be used to improve productivity and yield of EO bearing plants (Carrubba et al., 2006). AM symbiosis has a great potential to promote crop productivity and ecosystem sustainability in agricultural systems (Gianinazzi et al., 2010). Thus, the overall aims of the present study were to verify if AM fungi can provide an efficient and natural way of improving the seed yield of dill and common bean plants in intercropping systems, and at the same time, increase the production of seed EO. Furthermore, to determine the proper intercropping indices for these crops. More specifically, to investigate whether the composition of the dill seed EO also changes via the AM fungi and intercropping by testing two hypotheses. Firstly, AM fungi and intercropping may increase seed yield of dill and common bean and this, in turn, will increase the yield performance. Second, symbioses between plants and AM fungi may produce greater changes in seed EO yield and composition of dill under intercropping systems.

## 2. Materials and methods

### 2.1. Experimental design

Two experiments were conducted in the Agriculture and Natural Resources Research Center of Kurdistan Province in 2013 and 2014. Soil samples (from 10 to 15 cm depth) were randomly collected in 2013 from 8 points using a soil auger. All soil samples were air dried at laboratory for 3 days and then crushed and sieved through a 2 mm sieve. Soil samples were taken from plots with different treatments at harvest time. Subsequently, post-harvest physico-chemical profile of soils were determined (Table 1). Weather changes during the experiments are shown in Fig. 1. The experiments were arranged as factorial based on randomized complete block design with three replications. The factors were cropping systems including: (a) common bean (*Phaseolus vulgaris* L.) sole cropping (C40 = 40 plants $m^{-2}$), (b) dill (*Anethum graveolens* L.) sole cropping at different densities (D25, D50 and D75: 25, 50 and 75 plants $m^{-2}$, respectively) and (c) the additive intercropping of common bean + dill (25 + 40, 50 + 40 and 75 + 40 plants $m^{-2}$). All these treatments were applied with (+AM) or without (−AM) arbuscular mycorrhiza colonization.

The experimental site has been cultivated with oilseed rape (*Brassica napus* L.) in 2012. Primary tillage was carried out in October 2012 and 2013. A mounted moldboard plow and a tandem disk harrow were performed in April. The soil contained efficient populations of native *Rhizobium gallicum*.The crops were managed on the basis of organic farming practices without pesticide or fertilizer use.

Sowing dates of dill and common bean were 4 April, 2013 and 11 April, 2014. No mechanical weeding was performed after sowing. Dill was spaced 50 cm × 2.66 cm, 50 cm × 4 cm and 50 cm × 8 cm for 75, 50 and 25 plants $m^{-2}$, respectively, and common bean was spaced 50 cm × 5 cm for 40 plants $m^{-2}$ in sole croppings. Dill was spaced 25 cm × 2.66 cm, 25 cm × 4 cm and 25 cm × 8 cm for 25 + 40, 50 + 40 and 75 + 40 plants $m^{-2}$ in intercropping systems, respectively.

Dill plants were harvested at 15 weeks after sowing, while the common bean plants were harvested at 17 weeks after sowing. The experimental area was rain-fed, and supplementary irrigations were caried out during the dry weather as required.

The inoculum consisted of colonized root fragments, sand, AM hyphae, and spores. The inoculum was mixed with an inert material for dilution and homogenizing the distribution in the soil. Thirty-g inoculum was added to each plot (4m × 5m) at sowing time just below the seeds. The arbuscular mycorrhiza fungi (*Funneliformis mosseae*) was obtained from the University of Tabriz, Iran.

### 2.2. Competition indices

#### 2.2.1. The land equivalent ratio

The land equivalent ratio (LER) is defined as the land equivalent needed for growing either crops together compared with the land area needed to a pure culture of each crop. In this study we used seed weight as yield parameter. The LER was calculated as (Mead and Willey, 1980):

$$LER = \left(\frac{Y_{ab}}{Y_a} + \frac{Y_{ba}}{Y_b}\right)$$

where $Y_a$ and $Y_b$ are the yields of common bean and dill, respectively, as monocrops and $Y_{ab}$ and $Y_{ba}$ are the yields of common bean and dill, respectively, as intercrops. LER values higher than 1

**Table 1**
Post-harvest physico-chemical properties of the soil of experimental area.

| Texture | Organic carbon% | pH (1:2.5) | Electrical conductivity ($dSm^{-1}$) (1:2.5) | K | P | Ca | Na | Zn | Mn | Fe |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ($mg\ kg^{-1}$ soil) | | | | | | |
| Sandy clay loam | 1.14 | 7.12 | 0.072 | 131 | 12.2 | 1150.1 | 450.2 | 0.476 | 7.054 | 6.97 |

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