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A Preliminary Study on the Response of Confectionery Sunflower (Helianthus annuus L.) to Arbuscular Mycorrhizal Fungi (AMF)

Filiz PARÇA [1] ***Aydın UNAY*** [2*]

[1]Aydın Adnan Menderes Uni. Institute of Natural and Applied Sci. Aydın/TURKEY
[2]Aydın Adnan Menderes Uni. Agricultural Fac., Field Crop Dep., Aydın/TURKEY

[1]https://orcid.org/0000-0003-2396-9327 *[2]https://orcid.org/0000-0002-7278-4428*

[*]Corresponding author's (Sorumlu yazar) e-mail: aunay@adu.edu.tr

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ABSTRACT: *This preliminary study was carried out to investigate the effect of arbuscular mycorrhizal fungi (AMF) on yield, seed characters and chlorophyll content of confectionery sunflower (**Helianthus annuus** L.). The experiment was conducted at Tavas/Denizli in the 2018 growing season. The experimental design was "Randomized Complete Block Design (RCBD)" with four replications. Seed coating and soil spraying of AMF applications were compared with control (non-treatment AMF). The differences among treatments were significant for plant height (cm), head diameter (cm), seed width (mm), single plant yield (g), thousand kernel weight (g), yield (kg ha^{-1}), chlorophyll content index (CCI) and soil plant analysis development (SPAD) values. The seed coating had significant and higher values for plant height (cm), head diameter (cm), seed width (mm), single plant yield (g), thousand kernel weight (g), yield (kg ha^{-1}), chlorophyll content index (CCI) and SPAD values, whereas hull ratio was found to be similar between AMF applications and control. It was concluded that seed coating with AMF can be profitable for confectionery sunflower producers due to increased yield and low cost. The results of this preliminary study should be supported by findings from multiple locations and years.*

Keywords: *Confectionary sunflower,* ***Helianthus annuus*** *L., arbuscular mycorrhiza, yield, hull ratio, chlorophyll content.*

Çerezlik Ayçiçeğinin (Helianthus annuus L.) Arbusküler Mikorhizal Fungus (AMF)'lara Tepkisi Üzerine Bir Ön Çalışma

ÖZ: *Bu ön çalışma, çerezlik ayçiçeğinde (**Helianthus annuus** L.) arbüsküler mikorizal fungus (AMF)'un verim, dane özellikleri ve klorofil içeriği üzerine etkisini belirlemek amacıyla yapılmıştır. Deneme 2018 yılında Tavas/Denizli bölgesinde yürütülmüş ve 4 tekerrürlü Tesadüf Blokları Deneme Desenine göre planlanmıştır. AMF'nin tohum kaplama ve üst uygulamaları kontrol ile karşılaştırılmıştır. Bitki boyu (cm), tabla çapı (cm), dane eni (mm), tek bitki verimi (g), verim (kg ha^{-1}), klorofil içeriği ve SPAD değerleri yönünden uygulamalar arası farklılıkların önemli olduğu saptanmıştır. Tohum kaplama uygulamasının bitki boyu (cm), tabla çapı (cm), dane eni (mm), tek bitki verimi (g), verim (kg ha^{-1}), klorofil içeriği ve SPAD değerleri yönünden önemli düzeyde daha yüksek değerlere sahip olduğu buna karşın kabuk oranının uygulamalar arasında benzer olduğu belirlenmiştir. Sonuçta, tohum kaplaması şeklinde AMF uygulamasının verim artışı ve düşük maliyeti nedeniyle üreticiler için karlı olabileceği kanısına varılmıştır. Bir ön çalışma niteliğinde olan bu araştırma sonuçlarının birden fazla çevrede ve yılda yürütülen çalışma bulguları ile desteklenmesinde yarar vardır.*

Anahtar Kelimeler: *Çerezlik ayçiçeği,* ***Helianthus annuus*** *L. arbüsküler mikoriza, verim, kabuk oranı, klorofil içeriği.*

INTRODUCTION

The interest in confectionery sunflower is increasing worldwide. The proportion of confectionery sunflower in the world and Turkey are 2.6% and 8.37% of total sunflower production, respectively (Anonymous, 2018). In our country, the planting area of confectionery sunflower reached 105 thousand hectares. Central Anatolia (Ankara, Kayseri and Kırıkkale) and Aegean Regions (Denizli) are the main production areas of confectionary sunflower.

Confectionery sunflower was defined as black white, black with stripes, high hull percentage and larger than the oil type seeds (Hladni *et al*., 2012). It was emphasized that there is no certified seed and not many registered hybrids in confectionery sunflower production (Pekcan *et al*., 2015). Similarly, many landraces with different grain colors and characteristics are grown for confectionery sunflower production (Tan *et al*., 2017; Tan and Kaya, 2019). Also, confectionery sunflower cultivation as monoculture negatively affected crop productivity and quality depending on plant nutrient problems.

Arbuscular mycorrhizal fungi (AMF) are cited as a promising option for sustainable agriculture (Rillig *et al*., 2016; Thirkell *et al*., 2017). With the symbiotic relationship between AMF and the crop, the plant supplies a source of carbon to fungi, while the fungi is responsible for the acquisition of immobile nutrients including macro and micronutrients beyond the range of plant's roots via their hyphae. (Bago *et al*., 2000; Govindarajulu *et al*., 2005; Jiang *et al*., 2017; Smith and Read, 2008). It was revealed that AMF is the most common type of useful microbial community in the soil (Heidari and Karami, 2014; Ibrahim, 2018). Mycorrhizal fungi induce plant growth through increasing the availability of mineral nutrients such as P, Zn and Cu (Phiri *et al*., 2003). Inoculation of roots with AMF can also reduce the harmful effects of chemical fertilizer used in conventional farming (Silva *et al*., 2015), increase plant defense mechanisms to alleviate different stresses (Mayer *et al*., 2017) and aid in weed control (Veiga *et al*., 2011). In addition, colonization of arbuscular mycorrhiza (AM) in the rhizosphere enhances growth (Jalaluddin and Hamid, 2011; Silva *et al*., 2015) and morphological parameters (Kavitha and Nelson, 2014) in sunflower. AMF applications increased head diameter, seed number in head, seed and oil yield and oil percentage when compared with non-treatment (control) (Soleimanzadeh, 2010).

In addition to increases in yield, yield components and seed quality, the percentage of N in leaves and seeds is enhanced by mycorrhizal fungi in sunflower (Gholamhoseini *et al*., 2013). It was emphasized that the SPAD (soil plant analysis development) value of plant leaves reflected tissue nitrogen levels, and that the higher SPAD indices in plants with AMF were positively correlated with a higher photosynthetic potential due to better nutrient status (Chang and Robison, 2003; Campanelli *et al*., 2012). Seed coating is shown to be the most effective method for the application of exogenous AMF for many crops such as wheat, maize and cowpea (Oliveira *et al*., 2016; Ma *et al*., 2019) although the lack of cost-effective methods restricts the application of AMF (Vosátka *et al.,* 2012; O'Callaghan, 2016) in field conditions.

Many previous studies were pot studies under greenhouse conditions. Our study is the first conducted under actual field conditions and aimed at producer practices in confectionery sunflower. Therefore, we focused on evaluating the effectiveness of AMF on yield, yield components and chlorophyll content of confectionery sunflower under field conditions.

MATERIALS and METHODS

This study was carried out in a farmer's field (Tavas/Denizli; 37° 49' N 28° 95' E) that was used to grow monocrop confectionary sunflower in recent years. Previous seasons were most damaged by mildew disease. The experiment was arranged in a Randomized Complete Block Design with four replications in 2018. The soil of the experimental area was defined as clayey, slightly alkaline, poor organic matter, non-saline, high lime and insufficient nitrogen and phosphorus (Table 1). The province of Tavas/Denizli shows a transition climate between the Mediterranean and Aegean regions with hot, dry summers and cold/mild, rainy winters. When the climate data of the 2018 sunflower growing season were evaluated, the mean temperatures of July and August and precipitation of May had the highest values (Table 2).

Mycorrhiza applications

The local cultivar of confectionery sunflower (*Helianthus annuus* L.) İnegöl Alası was used as material. To inoculate seeds with mycorrhiza, 2.0 kg of seeds was treated with 25 g mycorrhiza mixture and 0.5 lt distilled water, then the seeds were aerated to reduce moisture to 10%. The dosage and method of application were as recommended in the company's license (Anonymous, 2020). The list of fungi contents in the product are given in Table 3.

As a second application method, mycorrhiza was applied by soil spraying before first irrigation at the 6-8 leaf stage. To water one plant, 260 cm^3 of the suspension was used, which corresponds to a dose of 8.75 mg of the preparation per plant (Mikiciuk *et al*., 2019).

Cultural management

Two hours after inoculation, seeds were sown by pneumatic drill machine. Sowing norm was 0.7 m x 0.25 m in plots of ten rows measuring 7 m x 7 m. Plots were fertilized with 40 kg ha^{-1} N, 70 kg ha^{-1} P_2O_5 and 35 kg ha^{-1} K_2O before planting, and 120 kg ha^{-1} N was applied before first irrigation. Drip irrigation was applied three times. The experimental area was hoed twice for thinning at the early seedling stage and weeding at the 3-4 leaf stage. No plants showing symptoms of disease were encountered during the experimental period although downy mildew is one of the most devastating diseases for confectionery sunflower in this region. Also, there was no need to control broomrape as the critical level was not exceeded.

Measurements

Chlorophyll content and SPAD

The relative chlorophyll content (CCI; chlorophyll content index) was measured twice by two leaf-clip chlorophyll meters, the CCM-200 Plus (Apogee) and SPAD-502 meter (Konica Minolta) at the stages of flowering (DAS 74 days) and seed development (DAS 90 days) according to the method suggested by Gornik (2011).

Table 1. Soil analysis of experimental area§.
Çizelge 1. Deneme alanının toprak analiz sonuçları§.

Soil structure Toprak yapısı	pH	Lime (%) Kireç (%)	Organic matter (%) Organik madde (%)	Total salt (%) Toplam tuz (%)	N (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
Clayey/Killi	8.0	28.4	1.28	0.01	0.09	42.6	988.0

§ Soil Analysis Laboratory of Aydın Adnan Menderes University, Faculty of Agriculture.
§ Aydın Adnan Menderes Üniversitesi, Ziraat Fakültesi Toprak Analiz Laboratuvarı.

Table 2. Monthly mean temperature, moisture and total monthly precipitation for 2018 (Anonim, 2018).
Çizelge 2. 2018 yılına ait ortalama sıcaklıklar, nem ve aylık yağış miktarı (Anonim, 2018).

Months Aylar	Mean temp. Ort. sıc. (°C)	Moisture Nem (%)	Precipitation Yağış (mm)
April/Nisan	12.5	55.1	17.8
May/Mayıs	14.9	65.6	90.5
June/Haziran	18.3	61.9	39.1
July/Temmuz	22.3	49.7	29.5
August/Ağustos	22.6	51.8	20.1
September/Eylül	19.3	47.7	4.1

Table 3. Contents of biological (microbial) material used in the study.
Çizelge 3. Çalışmada kullanılan biyolojik (mikrobiyal) materyalin içeriği.

Live organisms name Canlı organizma adı			Number of live organisms Canlı organizma sayısı (propagule g^{-1})	pH
Glomus mosseae	*Glomus etunicatum*	*Glomus intraradices*	1 x 10^5	7-9

Yield and yield components

In the harvest stage, the five middle rows were used for yield estimation (kg ha^{-1}) at 10% humidity. Plant height (cm), head diameter (cm) and single plant yield (g) were measured in 10 randomly selected plants at the R9 stage (bracts yellow and brown, plant at physiological maturity) as defined by Schneiter and Miller (1981).

Kernel characteristics

Seed length (mm) and seed width (mm) was measured using a vernier caliper (Hladni *et al*., 2016). Hull ratio (%) was calculated according to the formula suggested by Baldini and Vannozzi (1996) mass of hulls removed during dehulling/mass of seed sample before dehulling at 10% moisture in laboratory. Four replicated 100-seed lots from the dried and cleaned seed samples from each parcel after harvest were weighed and averaged to determine thousand kernel weight (g) at 10% moisture.

Statistical analysis

Experimental data from seed coating, soil spraying and control were subjected to variance analysis with TARIST Statistical Package Program (Acikgoz *et al*., 1994) in accordance with the randomized block experimental design. The differences between means were compared using LSD (Steel and Torrie, 1980).

RESULTS and DISCUSSION

Grain yield

The grain yield of confectionery sunflower was significantly affected by AMF inoculation (Table 4). In addition, the differences among treatments were significant for single plant yield (g) and yield (kg ha^{-1}). Single plant yield values were 98.0 g for seed coating and 89.50 g for soil spraying compared with control (82.50 g). Similarly, yield per hectare was significantly higher in the seed coating of AMF (3430.0 kg) followed by soil spraying (3132.5 kg). Compared to the control, the yield increases with seed coating and soil spraying of AMF were 18.78% and 8.48% (542 kg ha^{-1} and 245 kg ha^{-1}), respectively. Similar findings were earlier reported by Chandrashekara *et al*. (1995), Soleimanzadeh (2010), Heidari and Karami (2014), Chaghamarani *et al*. (2019) and Kalaiyarasan *et al*. (2019) in sunflower.

Yield components

Plant height and head diameter are two important yield components positively correlated with yield (Kholghi *et al*., 2011; Sıncık and Goksoy, 2014). Table 4 shows that the differences among treatments (two mycorrhiza applications and control) were significant for plant height and head diameter. Seed coating with AMF produced significantly taller plants (245.75 cm) and increased head diameter (29.48 cm) than those of the soil spraying (202.50; 26.98 cm) and non-inoculated treatments (189.75 cm; 23.70 cm). Similarly, other studies revealed positive effects of AMF on plant height and head diameter (Soleimanzadeh, 2010; Kavitha and Nelson, 2014; Ibrahim, 2018; Kalaiyerasan *et al*., 2019).

Kernel characteristics

The characteristics of confectionery sunflower for market value are: thousand kernel weight, hull/kernel ratio and seed size (Hladni *et al*., 2016). The treatments with AMF produced a significant increase in thousand kernel weight and seed width in comparison with control (Table 5). Seed weight values of seed coating and soil spraying were 8.60 and 8.53 mm, respectively, compared with control (6.40 mm). Similarly, the effects of AMF on seed length and hull ratio were positive. It can be said that increases in seed length, width and hull ratio were reflected in thousand kernel weight. Compared to control, the increase in thousand kernel weight due to AMF varied between 16.94% and 38.54%. Soleimanzadeh (2010) and Kalaiyerasan *et al*. (2019) found that the effect of AMF on thousand kernel weight was positive. Also, it was clearly seen that both AMF applications increased hull ratio.

Chlorophyll content

Significant differences were determined among mycorrhizal treatments and control for SPAD and CCI at the flowering and seed development stages (DAS: 74 and 90). Three LSD groups were formed for all four parameters (Table 6). The effect of seed coating was better than soil spraying and control for SPAD and CCI values. Also, SPAD and CCI values were in parallel with each other. When the results were compared with the findings of Chang and Robison (2003) for hardwood, Campanelli *et al*. (2012) for globe artichoke, Glolamhoseini *et al*. (2013) for sunflower, Makarian *et al*. (2016) for maize and Fileccia *et al*. (2017) for durum wheat, they confirmed an increase in chlorophyll content by AMF application due to the increase in plant nitrogen status.

Table 4. Mean values of plant height (PH), head diameter (HD), single plant yield (SPY) and yield (kg ha^{-1}).
Çizelge 4. Bitki boyu (BB), tabla çapı (TÇ), tek bitki verimi (TBV) ve verim (kg ha^{-1})'e ilişkin ortalama değerler.

Applications Uygulamalar	PH* BB(cm)	HD* TÇ (cm)	SPY* TBV (g)	Yield* Verim (kg ha^{-1})
Seed coating / Tohum kaplama	245.75 a	29.48 a	98.00 a	3430.0 a
Soil spraying/ Toprağa püskürtme	202.50 b	26.98 b	89.50 b	3132.5 b
Control/ Kontrol	189.75 c	23.70 c	82.50 c	2887.5 c
LSD (α: 0.05)	11.49	2.47	3.21	112.3
CV (%)	3.13	5.4	14.24	2.16

*Same letters in a column are not significantly different at the 0.05 probability level.
*Aynı harfle gösterilen ortalamalar arasında önemli fark (P ≥ 0,05) yoktur.

Table 5. Mean values of seed length (SL), seed width (SW), thousand kernel weight (TKW) and hull ratio (HR).
Çizelge 5. Tane boyu (DB), tane eni (DE), bin tane ağırlığı (BDA) ve kabuk oranı (KO) özelliklerine ilişkin ortalama değerler.

Applications Uygulamalar	SL TB (mm)	SW* TE (mm)	TKW* BTA (g)	HR KO (%)
Seed coating / Tohum kaplama	19.27	8.60 a	146.81 a	47.59
Soil spraying/ Toprağa püskürtme	17.60	8.53 a	123.92 b	46.85
Control / Kontrol	17.36	6.40 b	105.97 c	42.38
LSD (α: 0.05)		1.33	9.99	
CV (%)	5.52	10.97	14.60	10.20

*Same letters in a column are not significantly different at the 0.05 probability level.
*Aynı harfle gösterilen ortalamalar arasında önemli fark (P ≥ 0,05) yoktur.

Table 6. Mean values of SPAD (soil plant analysis development) and CCI (chlorophyll content index) at two different growing stages (1; flowering stage and 2; seed development).
Çizelge 6. İki farklı büyüme döneminde (1; çiçeklenme dönemi ve 2; tane gelişimi) SPAD ve klorofil içeriğine (CCI) ilişkin ortalama değerler.

Applications Uygulamalar	SPAD 1*	SPAD 2*	CCI 1*	CCI 2*
Seed coating / Tohum kaplama	72.37 a	70.92 a	33.23 a	31.81 a
Soil spraying / Toprağa püskürtme	53.12 b	53.87 b	26.00 b	25.94 b
Control / Kontrol	40.99 c	42.18 c	20.43 c	20.80 c
LSD (α: 0.05)	0.83	1.35	0.62	0.81
CV (%)	0.95	1.47	1.38	1.87

*Same letters in a column are not significantly different at the 0.05 probability level.
*Aynı harfle gösterilen ortalamalar arasında önemli fark (P ≥ 0,05) yoktur.

CONCLUSIONS

Summarizing the main results, seed coating treatment may have important potential to promote confectionery sunflower production with increases in seed yield under field condition. Also, seed coating should be considered an efficient and easy method of AMF inoculation for crops. In addition, AMF application increased yield by 550 kg ha^{-1} with a cost of 500 TL compared to the control. The sales price of confectionery sunflower for 2018 was 5000 TL ton^{-1}, therefore, the net return can be estimated as 2250 TL ha^{-1}. This finding indicated that seed coating with AMF can result in sustainable confectionery sunflower cultivation for the farmer despite the inoculation costs. It is recommended that further research is needed to support the results of this preliminary study.

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