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

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Received (Geliş tarihi): 18.06.2020  
Accepted (Kabul tarihi): 10.09.2020

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) 1ara Tepkisi Üzerine Bir Ön Çalışma

ÖZ: Bu ön çalışma, çerezlik açıcığinde (Helianthus annuus L.) arbüsküler mikorizal fungus (AMF) 1un verim, dane özellikleri ve klorofil içeriği üzerine etkisini belirlemek amacıyla yapılmıştır. Deneme 2018 yılında Tavas/Denizli bölgesinde yürütülmüş ve 4 tekrarlı Tesadüf Blokları Deneme Desenine göre plantanmıştır. 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 yönden uygulamalararası farklılıkların önemli olduğu saplanmıştır. Tohum kaplama uygulamasının bitki boyu (cm), tabla çapi (cm), dane eni (mm), tek bitki verimi (g), verim (kg ha⁻¹), klorofil içeriği ve SPAD değerleri yönden önemli düzeyde daha yüksek değerlerle 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 olabilecek karnıza varılmıştır. Bir ön çalışma niteliğinde olan bu araştırma sonuçlarının birden fazla çevrede ve yılda yürütülen çalışma bulguları ile desteklenmesinde yarar vardır.

Anahtar Kelimeler: Çerezlık açıcıği, 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 cm³ 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 P₂O₅ and 35 kg ha⁻¹ K₂O 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<sup>§</sup> |
|-----------------------------------------------|
| **Soil structure** | **pH** | **Lime (%)** | **Kireç (%)** | **Organic matter (%)** | **Organic madde (%)** | **Total salt (%)** | **N (%)** | **P₂O₅ (kg ha⁻¹)** | **K₂O (kg ha⁻¹)** |
| Clayey/Killi | 8.0 | 28.4 | 1.28 | 0.01 | 0.09 | 42.6 | 988.0 |

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| Table 2. Monthly mean temperature, moisture and total monthly precipitation for 2018 (Anonim, 2018). |
|-----------------------------------------------|
| **Means** | **Moisture** | **Precipitation** |
| **Aylar** | **Ort. sco.** | **Nem** | **Yağış** |
| **(°C)** | **(%)** | **(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 |
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 coating.

| Live organisms name | Number of live organisms | pH  |
|---------------------|--------------------------|-----|
| Glomus mosseae     | 1 x 10^5                 | 7.9 |
| Glomus etunicatum  |                          |     |
| Glomus intraradices|                          |     |
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). |
| Applications | PH* (BB)(cm) | HD* (TÇ)(cm) | SPY* (TBV)(g) | Yield* (Verim)(kg ha⁻¹) |
|--------------|--------------|---------------|----------------|------------------------|
| Seed coating | 245.75 a     | 29.48 a       | 98.00 a        | 3430.0 a               |
| Soil spraying| 202.50 b     | 26.98 b       | 89.50 b        | 3132.5 b               |
| Control      | 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.

| Table 5. Mean values of seed length (SL), seed width (SW), thousand kernel weight (TKW) and hull ratio (HR). |
| Applications | SL (TB)(mm) | SW* (TE)(mm) | TKW* (BTA)(g) | HR (%) |
|--------------|-------------|--------------|---------------|--------|
| Seed coating | 19.27       | 8.60 a       | 146.81 a      | 47.59  |
| Soil spraying| 17.60       | 8.53 a       | 123.92 b      | 46.85  |
| Control      | 17.36       | 6.40 b       | 105.97 c      | 42.38  |
| LSD (α: 0.05)| 1.33        | 10.97        | 14.60         | 10.20  |
| CV (%)       | 5.52        | 10.97        | 14.60         | 10.20  |

*Same letters in a column are not significantly different at the 0.05 probability level.

| 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). |
| Applications | SPAD 1* | SPAD 2* | CCI 1* | CCI 2* |
|--------------|---------|---------|--------|--------|
| Seed coating | 72.37 a | 70.92 a | 33.23 a | 31.81 a |
| Soil spraying| 53.12 b | 53.87 b | 26.00 b | 25.94 b |
| Control      | 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 arasındaki 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.

ACKNOWLEDGEMENTS

This research article is part of the master thesis conducted by Filiz PARÇA at Aydın Adnan Menderes University, Institute of Natural and Applied Science, Field Crops Department.
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