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ORIGINAL PAPER

ALLELOPATHIC EFFECTS OF OLIVE OIL MILL WASTEWATER (OMW) ON SAINFOIN (*Onobrychis viciifolia* Scop.) GERMINATION

Demet ALTINDAL[1], Nüket ALTINDAL[2*]

[1] Muğla Sıtkı Koçman University, Fethiye Ali Sıtkı Mefharet Kocman Vocational School, Department of Crop and Animal Production, Organic Farming Programme, Muğla, TURKEY
[2] Uşak University, Sivaslı Vocational School, Department of Crop and Animal Production, Medical and Aromatic Plants Programme, Uşak, TURKEY

*Corresponding author mail: nuketaltindal@gmail.com

Abstract

In this research, the effect of olive mill wastewater at different concentrations (control, 1/1, 1/2, 1/3, 1/4 [OMW/distilled water] and undiluted OMW) on the seed germination of sainfoin (Onobrychis vicifolia Scop.) was studied. According to the results, the germination ratio and mean germination time showed significant differences among the treatments; sainfoin populations and sainfoin populations x treatments interaction ($p<0.01$). Germination ratio varied at 5-70 % and the highest value (70 %) was obtained from 1/2 (OMW/distilled water) application in true sainfoin seeds. In general, the OMW (added distilled water) treatments were found to increase germination ratio. The lowest germination ratio (11.67 %) was determined in sainfoin seeds collected from Eğirdir location. The highest germination ratio was obtained from 1/1 and 1/2 (OMW/distilled water) applications. Mean germination time varied in 5.0-11.0 days. Generally, high OMW concentrations (except undiluted OMW) extended mean germination time in sainfoin seeds. In all OMW applications, it was shortened in wild sainfoid seeds (ES), but not in true sainfoid (TS) seeds. Additionally, germination time weren't affected as positive from OMW applications (except undiluted OMW) in TS seeds. The longest mean germination time (9.0 day) was in ES seeds. According to treatments, germination in the shortest time occurred in 1/4 (OWM/distilled water) treatment. As a result, olive oil wastewater (OMW) mixed to irrigation water in certain doses may increase seed germination and can be used as fertilizer in agriculture.

Key words: Allelopathic, germination, olive mill wastewater, sainfoin

INTRODUCTION

Sainfoin (*Onobrychis viciifolia* Scop.) is belonging to the family of Leguminosae and is as an important plant in Mediterranean, Black and Caspian Seas, Europe and Northern Russia. Sainfoin reported to be originated from Turkey, Iran and Europe. Sainfoin was introduced to the northern Great Plains of the U.S. in Montana and North Dakota in the 1960s from Turkey (Gray, 2006). Ditterline (1973) reported that sainfoin was first cultivated in southern France in 1582 (Anonymous, 2018a) and sainfoin culture first decribed in 1629.

Sainfoin is rich in protein and minerals (P, Mg and K etc.) but its calcium and sodium concentrations are lower than in other legumes (Spedding and Diekmahns, 1972). It is preferred as the forage in the feeding of animals such as cattle, sheep, deer, elk and some wildlife. In addition, pink flowers are used by bees to produce honey. Sainfoin seeds are consumed by birds and rodents in the early autumn. Sainfoin fruit (pod) each contains a single seed. Sainfoin seed is dark olive green, brown or black and 4-6 mm in size (Anonymous, 2018b). Most of the legumes such as sainfoin have hard case (testa) to protect the seed. There is fruit crust (pericarp) on this case. Hard fruit crust may prevent the uptake of water necessary for germination. The hard-shelled seed sainfoin, when the seeds are ripe, fruit crust does not open and therefore are harvested together (Akgun et al., 2007). Fruit crust of sainfoin slows the absorption of water in sainfoin (Carleton et al., 1968). Frame (2007) declared that hard seeds occurred at 15-20 % in true sainfoin. Additionally, seeds of wild sainfoin is harder than true sainfoin. It is important to improve the germination charatceristics of these plants to be able to use them in breeding. There are some methods, used to increase the seed coat permeability and break seed dormancy, which are moisturizing, growth regulators and pre-germination.

Akgun et al. (2007) reported that seeds of true sainfoin grown in Isparta-Turkey germinated but did not observed germination in wild sainfoin populations and it is utmost important to improve germination rates of these plants for using in breeding.

Altındal and Altındal (2013) studied allelopathic effects of sage and thyme oil *in vitro* conditions in sainfoin. Authors reported that they have positive effect on the seed germination, shoot and root growth, thus it was declared that these oils can be used as growth stimulus in *in vitro* studies. Hydro-priming probably enhances water uptake and exits inhibitors from pod in soaking period and reduces mechanical restriction of pods in sainfoin (Noorbakhshian et al., 2011).

Different applications affect germination in plants (Okatan, 2017).To enhance productivity in agriculture many methods are being applied. One of these methods is to eliminate the effects of the toxic substances. For this purpose, a lot of researchs on irrigation water of agricultural lands as well as natural water resources maintain. Today, olive mill wastewater is used as irrigation water by giving directly to agricultural fields. In large of olive mill wastewater (OMW) are produced in Turkey, Tunisia, Israel, Italy and Spain during the olive oil production process. In the Mediterranean region, the annual production of OMW exceeds 30 million m^3 (Gebreyohannes at al., 2016). Generally, the amount of wastewater during the production of olive oil is 0.5-1.5 m3 / tons of olives. As a result, 60 kg blackwater (OMW) is obtained from 100 kg of olives (Çetin, 2009).

Olive oil wastewater (OMW) contains sugar, pectin, tannin, nitrogen compounds, volatile oil acids, organic acids, phenols, lipids and mineral substances (Buchmann et al., 2015; Mseddi et al., 2015; Rahmanian et al., 2014).

Main organic and mineral substances in wastewater are shown in Table 1. The main products of this wastewater irrigated farmlands are wheat and soybean (Aybeke et al., 2000).

Table 1. The characteristic values of the OMW (Naija et al., 2014).

Mean composition of OMW organic substances	**Classic oil mill (Kg/m^3)**	**Continued oil mill (Kg/m^3)**
Total sugars	20-80	5-26
Nitrogenous substances	5-20	4-17
Organic acids	5-10	2-4
Alcohols	10-15	3-5
Pectins, mucilages, tannis	10-15	2-5
Polyphenols	10-24	3-8
Fat	0.3-10	5-23
Mineral composition of OMW	**Classic oil mill (Kg/m^3)**	**Continued oil mill (Kg/m^3)**
P	1.1	0.3
K	7.2	2.7
Ca	0.7	0.2
Na	0.9	0.3
Fe	0.07	0.02
CO_3	3.7	1.0
SO_3	0.4	0.15
Cl_2	0.3	0.1
SiO_2	0.05	0.02

A lot of research was conducted on the effects of olive oil wastewater on the seed germination. Perez et al. (1986) reported that undiluted wastewater had the greatest depressive effect, and followed by deionized wastewater and organic matter.

OMW has a phytotoxic properties while seed germination, germination capacity and plant growth might negatively inhibited (Ouzounidou and Asfi, 2012). Cavallaro et al. (2014) reported that the agronomic use of OMW is limited by reasons such as high salinity, acidity, phytotoxic and antimicrobial effects. In another research, high concentrations of OMW reported to increase stem length in peas, tomatoes and stimulated germination in some plants (Mseddi et al., 2015).

Rusan et al. (2015) determined effects of OMW at concentrations of 25-50-75 and 100 % on germination in barley. They reported that OMW at concentrations of 50-75 and 100 % was the phytotoxic and completely inhibited germination.

The mean objective of this work was to investigate the effects of olive oil wastewater (OMW) treatment on the germination of sainfoin seeds, which is grown naturally around the world and grown in turkey for animal feeding.

MATERIALS AND METHODS

In this study, true sainfoin (TS) and wild sainfoin seeds (ES) were used which were collected from Eğirdir region (in Turkey) . The olive mill wastewater (OMW) was obtained from olive oil mills located in Muğla-Dalaman (Mediterranean region in Turkey). The olive mill wastewater at different concentrations (control, 1/1, 1/2, 1/3, 1/4 [OMW/distilled water], OMW [undiluted]) were used to determine the impact on the seed germination of sainfoin.

Experimental Studies

This research was designed according to the complately randomized block design and each treatment was replicated three times. Each replication consisted of 20 sainfoin seeds which were placed between 2 filter paper (Whatman No 1) in a petri dish (9 cm). Untreated (control) seeds were moised in tap water and the other seeds were moised in OMW/distilled water and undiluted OMW solutions prepared at concentrations mentioned and then incubated at room temperature (20-22 °C) for 14 days. During this period, germination ratio (%) and mean germination time (day) of sainfoin seeds were determined. Seeds were scored as germinated when a radicle protrusion of approximately 2 mm was observed (Mackay et al., 1995). After sowing seeds, germination ratio were recorded 14^{th} day and germination ratio also was calculated (Ruan et al., 2002).

Statistical analysis

SAS (Statistical Analysis System) program was used to evaluate the data obtained from the experiments and Duncan's multiple range test was used to compare means after ANOVA.

RESULTS AND DISCUSSION

According to the results obtained, the germination ratio (%) and the mean germination time (day) are significantly different in terms of levels according to population sainfoin and applications ($p<0.01$).

Germination ratio (%)

Sainfoin seeds showed different react against treatments and thus the significant interaction ($p<0.01$) was obtained for populations and applications. Germination ratio in populations ranged from between 5.00 to 70.00 %. While germination ratio was 17.50 % in untreated ture sainfoin seeds, it was 70.00 % (the highest) in the 1/2 (OMW/distilled water) application and the lowest value (7.50 %) was obtained from OMW application (Figure 1).

Germination ratio (5.00-25.00 %) varied according to different applications in sainfoin seeds (ES) collected from natural areas of Eğirdir and was 10.00 % in untreated (control) seeds. In treatments, germination ratio increased by 25.00 % when seeds moised in 1:1 concentration. However, the lowest value (5.00 %) was counted in 1:3 OMW/distilled water and OMW applications. In both populations, undiluted OMW treatment was reduced germination ratio (Figure 1).

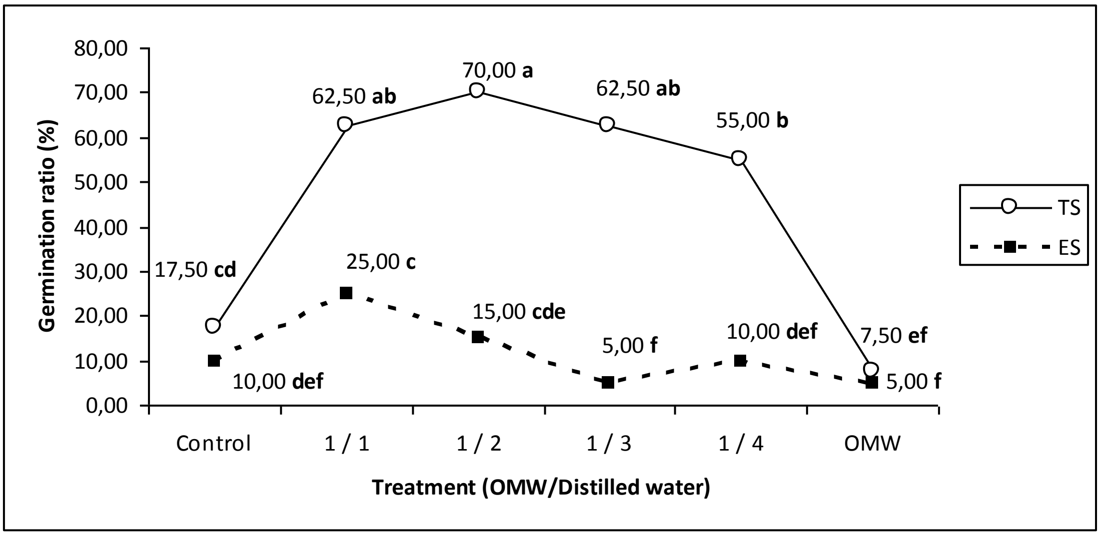

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Figure 1. Effect of populations x treatments on germination ratio (%)

Results indicated that all treatments significantly affected germination ratio in sainfoin seeds. In increased OMW concentration (except undiluted OMW), germination ratio always decreased. The highest germination (43.75) % recorded in 1/1 (OMW/distilled water) application. Germination ratio of true sainfoin seeds was higher than ES seeds (Table 2).

Table 2. Effect of treatments and sainfoin populations on germination ratio and mean germination time.

	Populations		Treatments					
Characters	**CS**	**ES**	**Control**	**1/1 OMW/Distilled water**	**1/2 OMW/Distilled water**	**1/3 OMW/Distilled water**	**1/4 OMW/Distilled water**	**OMW**
Germination ratio (%)	45.83**a**	11.67**b**	13.75**c**	43.75**a**	42.50**a**	33.75**b**	32.50**b**	6.25**d**
Mean germination time (days)	7.00**b**	9.00**a**	8.00**c**	9.50**a**	8.50**b**	7.17**d**	6.83 **d**	9.50**a**

Values followed by the same letter or letters in same row are not significantly different at 1% according to Duncan's multiple range test.

Mean Germination Time (day)

Sainfoin seeds showed different reactions against treatments in terms of mean germination time and thus the significant interaction ($p<0.01$) was determine between populations and applications. Mean germination time ranged from 5.0 to 11.0 days. The longest mean germination time (11.0 day) was obtained from untreated (control) and 1:1 OMW/distilled water in ES. 1:4 (OMW/distilled water) treatment in both populations showed a similar result (7.0 day) (Figure 2.). Additionally, mean germination day shortened at decreasing OMW / distilled doses (1/1, 1/2, 1/3). Compared with control groups, undiluted OMW prolonged mean germination time in true sainfoin (TS), but not in ES. In general, the shortest mean germination time occurred in true sainfoin seeds (Figure 2 and Table 2).

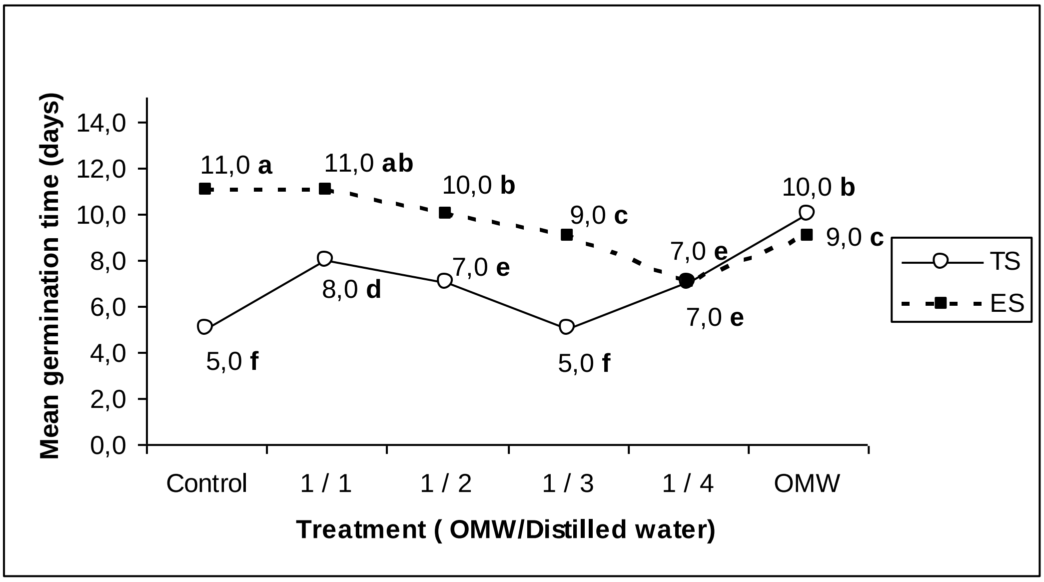

Figure 2. Effect of population x treatments on mean germination time (days)

In present study, mean germination time was significant according to populations and the shortest germination time measured from true sainfoin (TS). Germination time showed variation among the treatments. Nevertheless, mean germination time at 1/4 (OMW/distilled water) concentration was shorter than the control (untreated OMW) (Table 2).

Results of our experiment indicated that germination ability of both sainfoin seeds were affected by OMW treatments. In a previous study, Aybeke et al. (2000) reported that they applied OMW olive oil to the wheat seeds and high rate of germination was observed in undiluted OMW and 1/10.000 concentration treatments, and lowest (or no) germination was determined at different concentrations (1/10, 1/100 and 1/1000).

In present study, undiluted OMW found to significantly reduce germination ratio of TS and ES seeds and it was 7.50 and 5.00 %, respectively (Figure 1). These results are in accordance with the results of some previous researchs. Similarly, Krogmeier and Bremner (1989) declared that germinability of *T. durum* Desf. seeds are affected by the application of OMW and could reduce in treatment up to 1:8 dilution and that undiluted OMW treatment to be inhibited of germination completely induced.

To induce the germination of seeds, enzymes such as α and β-amylases are needed. When seeds are soaked in water, these enzymes become active and α-amylase serves to mobilise the starch reserves in the endosperm during germination (Helland et al., 2002) and both enzymes degrades starch faster (Das and Sen-Mandi, 1992), while β-amylase is an essential enzyme for germination (Nandi et al., 1995). In an earlier study, high concentrations of OMW were negatively effected by α and β-amylases activites used plant seeds (Muscolo et al., 2010). Mentioned researches explained the reason of raw OMW and its high concentrations negatively effected the germination ability of sainfoin seeds in present study.

In general, the effect on germination characters of OMW solutions including distilled water was positive. In such case, it can be suggested that, water should be added to OMW solutions to increase germination ratio of seeds subjected to hydropriming for 24 h observed as described in an earlier study on safflower (Ashrafi and Razmjoo, 2010), wheat (Basra et al., 2002) and forage crops (Artola et al., 2003).

The results obtained in the our study showed that the seed germination reduced by high doses of OMW. This negative effect resulted from that OMW includes the phenolic and organic acid content (Isidori et al., 2005) and occurs during germination and seedling development in plant (Krogmeier et al., 1989).

Seed size effects germination characters. Wild sainfoin seeds belonging Eğirdir location used in our research are smaller than true sainfoins (TS). On this account, in our experiment, germination ratio of wild sainfoin (ES) were found to be lower than true sainfoin. In an earlier study, the effects on characters such as germination of sainfoin hydroprimed seed was evaluated and seed size and seed priming on gramination character were completely significant. In untreated (control) seeds, large seeds germinated more and faster than small seeds. Additionally, beneficial reserve components for germination were greater in the large seeds in compared to small size. Noorbakhshian (Noorbakhshian et al., 2011).

Sainfoin has hard case (testa) which protect the seed and its fruit crust was hard. Thus, it may prevent the uptake of solutions, and water is necessary for germination(Peel et al., 2004). Our results confirm these informations because seed testa of wild sainfoin (ES) are harder than true sainfoin (TS).

As a conclusion, germination ratios of wild sainfoin seeds (ES) collected from the natural flora and true sainfoin (TS) seeds increased at the applications of low concentrations of OMW, but decreased in the undiluted OMW application. To shorten, the olive mill wastewater (OMW) can be used to improve the soil by increasing the germination by giving a certain amount of irrigation water and also may be applied as a fertilizer.

REFERENCES

Akgun I., Altindal N., Karadogan T., 2007. Effect of GA_3 and KNO_3 applications on seed germination in sainfoin (*Onobrychis sativa* Lam. and *Onobrychis ssp).* Turkish 7th. Field Crops Congress, Erzurum/Turkey. 25-27 June 2007, 230-234.

Altindal N., Altindal D., 2013. Allelopathic effect of sage and Turkish oregano volatile oils on *in vitro* in sainfoin (*Onobrychis viciifolia*). Journal of Essential Oil Bearing Plants, 16(3): 328-333.

Anonymous 2018a. Sainfoin haylage. Available at http://www.hydehallhaylage.co.uk/index.html.

Anonymous 2018b. Fact sheet-USDA plants database. Available at http//www. plants.usda.gov/factsheet/pdf/fs_onvi.pdf.

Artola A., Cariilo-Castaneda G., Garcia de los Santos G., 2003. Hydropriming: A strategy to increase *Lotus corniculatus* L. sees vigor. Seed Sci. and Technol., 31:455-463.

Ashrafi E., Razmjoo K., 2010. Effects of priming on seed germination and field emergence of safflower (*Carthamus tinctorius* L.). Seed Sci. Technol., 38:675-681.

Aybeke M., Olgun G., Sıdal U., Kolankaya D., 2000. Zeytinyağı fabrikası atık suyunun buğday (*Triticum aestivum* L.) kök ucu hücrelerindeki mitoz bölünme ve total protein miktarı üzerine etkisi. Turkish Journal Biology, 24:127-140.

Basra S.M.A., Zia M.N., Metmood T., Afzal I., Khaliq A., 2002. Comparison of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. Pakistan Journal Arid Agric., 5:11-16.

Buchmann C., Felten A., Peikert B., Munoz K., Bandow N., Dag A., Schaumann G.E., 2015. Development of phytotoxicity and composition of a soil treated with olive mill wastewater (OMW): an incubation study. Plant Soil, 386:99-112.

Carleton A.E., Cooper C.S., Wiesner L.E., 1968. Effect of seed pod and temperature on speed of germination and seedling elongation of sainfoin (*Onobrychis viciaefolia* Scop.). Agronomy Journal, 60(1):81-84.

Cavallaro V., Maucineri C., Barbera A.C., 2014. *Lolium multiflorum* Lam. cvs germination under simulated olive mill wastewater salinity and pH stress. Ecological Engineering, 71:113-117.

Çetin M., 2009. Zeytin karasuyunun aerobik stabilizasyonunda biyo-ayrışma kinetiğinin belirlenmesi. Yüksek Lisans Tezi. Balıkesir Üniversitesi, Fen Bilimleri Enstitüsü, Çevre Mühendisliği Anabilim Dalı, 127 sayfa.

Das G., Sen-Mandi S., 1992. Scutellar amylase activity in naturally aged and accelerated aged wheat seeds. Ann. Bot., 69:497-501.

Ditterline R.L., 1973. Yield and yield components of sainfoin (*Onobrychis viciaefolia* Scop.) seed and an evaluation of its use as a protein supplement. Montana States University, Crop and Soil Science, Doctor Philosopy, Bozeman, Montana. 96 pp.

Frame J., 2007. *Onobrychis viciifolia* Scop. Available at http://www.fao.org/AG/agpc/doc/Gbase/DATA/Pf000347.html.

Gebreyohannes A.Y., Mazzei R., Giorno L., 2016. Trends and current practices of olive mill wastewater treatment: Application of integrated membrane process and its future perspective, Separation and Purification Technology, 163:45-60.

Gray F.A., 2006. Shoshone' sainfoin, plant sciences timely information series. Department of Plant Sciences, College of Agriculture, University of Wyonming, No:2, July 2006, Available at http://cordis.europa.eu/result/rcn/161034_en.html.

Helland M.H., Wicklund T., Narvhus J.A., 2002. Effect of germination time on alpha-amylase production and viscosity of maize porridge. Food Res. Int., 35:315-321.

Isidori M., Lavorgna M., Nardelli A., Parrella A., 2005. Model study on the effect of 15 phenolic olive mill wastewater constituents on seed germination and *Vibrio fischeri* metabolism. J. Agric. Food Chem., 53 (21):8414-8417.

Krogmeier M.G., Bremner J.M., 1989. Effects of phenolic acids on seed germination and seedling growth in soil. Biology and Fertility of Soils, 8, 116-122.

Mackay W.A., Davis T.D., Sankhla D., 1995. Influence of scarification and temperature treatments on seed germination of Lupinus havardii. Seed Science & Technology, 23:815-821.

Mseddi S., Chaari L., Belaid C., Chakchouk I., Kallel M., 2015. Volarization of treated olive mill wastewater in fertigation practice. Environ Sci Pollut Research, DOI 10.1007/s11356-015-4353-6.

Muscolo A., Sidari M., Mallamaci C., Attina E., 2010. Effect of olive mill wastewater on seeed germination and seedling growth. Terr Aquat Environ Toxicol., 4 (1):75-83.

Naija D.S., Boussaadia O., Dhiab A.B., Mariem F.B., Braham M., 2014. Valorization of the olive sector effluents as potential fertilizers and their ımpact on biological, physical and chemical properties of the soil. Research Journal of Agriculture and Environmental Management, 3(9):450-459.

Nandi S., Das G., Sen-Mandi S., 1995. β-amylase activity as an index for germination potential in rice. Ann Bot., 75:463-467.

Noorbakhshian S.J., Nabipour M., Meskarbashee M., Amooaghaie R., 2011. Evaluation of germination and biochemical indexes of sainfoin hydroprimed seed. American-Eurasian J. Agric.&Environ. Sci., 11 (5):671-678.

Okatan, V. (2017). GA3 Uygulamalarının Malta Eriği (Eriobotrya japonica) Tohumlarının Çimlenmesi ve Çöğür Gelişimi Üzerine Etkileri. GÜFBED/GUSTIJ (2017), 7(2), 309-313.

Ouzounidou G., Asfi M., 2012. Determination of olive mill wastewater toxic effects on three mint species grown in hydroponic culture. Journal of Plant Nutrition, 35:726-738.

Peel M.D., Asay K.H., Johnson D.A., Waldron B.L., 2004. Forage production of sainfoin across an irrigation gradient. Crop Science, 44:614-619.

Pérez J.D., Esteban E., Gomez M., Gallardo L.F., 1986. Effects of waste water from olive processing on seed germination and early plant growth of different vegetable species. Journal of Environmental Science and Health, Part B 21, 349-357.

Rahmanian N., Jafari S.M., Galanakis C.M., 2014. Recovery and removal of phenolic compounds from olive mill wastewater. Journal of the American Oil Chemists' Society, 91:1-18.

Ruan S., Xue Q., Tylkowska K., 2002. The influence of priming on germination of rice (*Oryzo sativa* L.) seeds and seedling emergence and performance in flooded soil. Seed Science & Technology, 30:61-67.

Rusan M.J., Albalasmeh A.A., Zuraiqi S., Bashabsheh M., 2015. Evaluation of phytotoxicity effect olive mill wastewater treated by different technologies on seed germination of barley (*Hordeum vulgare* L.). Environ Sci Pollut Res., 22(12):9127-35.

Spedding C.R.W., Diekmahns E.C., 1972. Grasses and legumes in British agriculture. Bulletin No. 49, Commonwealth Bureau of Pastures and Field Crops, Farnham Royal, 511 pp.