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Productivity and allelopathy of nine milk thistle (*Silybum marianum*) biotypes in weedy and weed-free conditions

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ABSTRACT

Studies on productivity and allelopathic potential under reduced inputs of the milk thistle (*Silybum marianum* [L.] Gaertn.) biotypes, an alternative energy source and medicinal plant, have not been studied to support farmers' decisions. A split-plot field experiment was conducted to compare the productivity and allelopathic potential of nine milk thistle biotypes under weedy and weed-free conditions without fertilization and irrigation. The biotypes were the main plots subdivided into weedy and weed-free subplots. Averaged across years and biotypes, weed-free and weedy milk thistle produced 8.03 and 6.37 t/ha dry biomass, respectively. Weed competition slightly reduced the productivity of all biotypes. Averaged across years and biotypes, fruit yield in weed-free and weedy treatments was 1.41 and 1.02 t/ha, respectively. The two commercial biotypes originating from Bulgaria were the most productive (averaged seed yield equal to 1.61 and 1.32 t/ha/year in weed-free and weedy conditions, respectively), while most of the biotypes were allelopathic inhibitors against rigid ryegrass (*Lolium rigidum* Gaudin) and wild mustard (*Sinapis arvensis* L.). In conclusion, some milk thistle biotypes provide adequate dry biomass (for energy use) and seeds (for silymarin production) under Mediterranean-reduced input conditions. Commercial biotypes were slightly more productive than the native biotypes.

Keywords: rigid ryegrass, fruit yield, medicinal plant, weed competition, wild mustard

INTRODUCTION

The milk thistle (*Silybum marianum* [L.] Gaertn.) is an annual or occasionally biennial plant belonging to the Asteraceae family. Native to the Mediterranean region, it has spread globally owing to its medicinal and economic importance (Andrzejewska et al., 2011). Milk thistle is a promising energy crop, particularly in low-fertility regions, because it adapts to various soil types and tolerates summer drought (Andrzejewska et al., 2011; Tsiaousi et al., 2019). It is also valued for its medicinal properties, primarily because of the presence of 3-oxyflavone silymarin, a compound in its fruit cover. In particular, the four pharmacological (anti-hepatotoxic and hepatoprotective) flavonolignans silychristin, silydianin, silybin and isosilybin are included in silymarin (Ibrahim et al., 2007; Karkanis et al., 2011).

Recent studies have suggested that milk thistle possesses allelopathic properties that can affect the growth of surrounding plant species (Zrar and Ali, 2023). Sultana and Asaduzzaman (2012) and Tsiaousi et al. (2019) also demonstrated that milk thistle extracts reduced the germination rates of specific weeds, indicating their potential role in natural weed suppression. These allelopathic effects, likely driven by silymarin and other secondary metabolites such as saponins and phenolic acids (Lucini et al., 2016), could be crucial in developing sustainable farming practices, bioactive compounds and weed management strategies. Additionally, Zrar and Ali (2023) reported that benzene derivatives are the primary allelochemicals found in the root and stem extracts of milk thistle.

Given its various applications, milk thistle could offer an economically viable solution for areas suffering from land degradation (Cherlet et al., 2018). In line with the EU's strategic goals (Commission of the European Communities, 2006), it holds promise as a multipurpose crop, particularly in the Mediterranean, where farmers have shown increased interest. However, limited field data exist on its performance, particularly regarding the existing biotypes, biomass or seed yield and competitive ability under minimal inputs (e.g., irrigation, fertilization or weed control). Furthermore, silymarin content varies significantly among biotypes (Arampatzis et al., 2019; Tran et al., 2024), making this knowledge essential for farmers. Consequently, this study aimed to assess under both weedy and weed-free conditions, without the use of fertilizers or irrigation: i) the biomass and fruit productivity of nine milk thistle biotypes (seven native from Greece and two commercial from Bulgaria) and ii) their allelopathic potential against two serious winter weeds.

MATERIALS AND METHODS

Seed collection

During May and June 2017, milk thistle seeds were collected from mature weed plants (biotypes) from seven geographic regions of Greece (Table 1). Seeds of two commercial milk thistle biotypes, originating from different geographic regions of Bulgaria, were obtained (Table 1). Seeds were stored at 4 °C until used in the field experiment.

Region of the experiment

In 2017, a field experiment was established at the University Farm of Thessaly in Larissa, central Greece (longitude 22°22′48′′ E, latitude 39°37′25′′ N; elevation 81-82 m) and repeated twice in 2018 and 2019. The soil was sandy clay loam (Vertic Chromoxerent), comprising 576 g/kg sand, 173 g/kg silt and 251 g/kg clay, with an organic carbon content of 5.5 g/kg, pH of 7.9 (1:2 H_2O solution) and cation exchange capacity (CEC) of 42 meq/100 g soil. Pre-seeding soil analysis (mid-September in year 1) indicated the initial levels of the main nutrients [nitrate at 150.0 mg/kg, phosphorus (Olsen P) 19.6 mg/kg and potassium (acetate extractable K) 287.9 mg/kg]. By year 3, these values were 147.0 mg/kg, 13.1 mg/kg and 156.0 mg/kg, respectively. Before the trial, legumes were cultivated in the field for three consecutive years.

Visual assessments conducted during the previous season indicated that the experimental site was naturally infested with winter annual weeds, including the grasses wild oat (*Avena sterilis* ssp. *ludoviciana* L.) and rigid ryegrass (*Lolium rigidum* Gaudin), as well as the broadleaf species common fumitory (*Fumaria officinalis* L.), wild mustard (*Sinapis arvensis* L.), ivy-leaved speedwell

Table 1. Milk thistle biotypes used in the experiment and their origin

Biotype	Region	Longitude	Latitude
B1	University Farm, Larissa, Greece	22°22′48′′ E	39°37′25′′ N
B2	Industrial area of Karditsa, Greece	22°08′52′′ E	39°46′60′′ N
B3	Kastoria, Greece	21°24′77′′ E	40°51′04′′ N
B4	Trastenik, Bulgaria (MK FERA VITA)	24°28′12′′ E	43°31′09′′ N
B5	Industrial area of Thessaloniki, Greece	22°78′20′′ E	40°67′25′′ N
B6	Sparti, Greece	22°42′92′′ E	37°07′03′′ N
B7	Tyzha, Bulgaria (BIOFORM)	25°05′01′′ E	42°38′60′′ N
B8	Gonnoi, Larissa, Greece	22°46′54′′ E	39°90′84′′ N
B9	Myrina, Karditsa, Greece	21°96′14′′ E	39°40′59′′ N

(*Veronica hederifolia* L.), common field poppy (*Papaver rhoeas* L.), common henbit (*Lamium amplexicaule* L.), shepherd's purse (*Capsella bursa-pastoris* L.) and knotgrass (*Polygonum aviculare* L.). Monthly rainfall and temperature averages near the experimental site are shown in Figure 1, while the continental climate (cold winters, hot summers and low spring rainfall) prevailed in the region of the experiment.

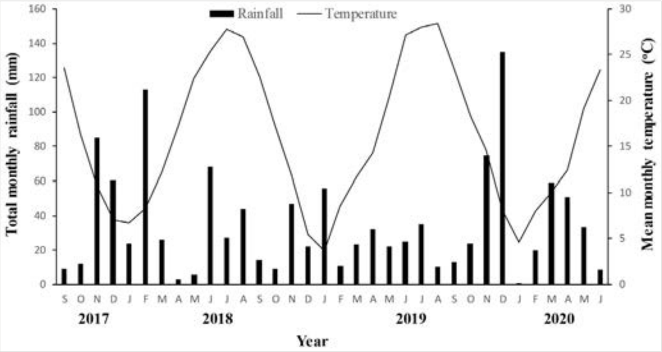


Figure 1. Total monthly rainfall and mean monthly temperature during the experiments

Design and treatments

The soil preparation involved plowing in early October, followed by disk harrowing. Simultaneously, 2 kg/ha chlorpyriphos (O-O-Diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate) was applied for insect control. Nine milk thistle biotypes, collected from different regions (Table 1), were hand-seeded with two seeds per hole in year 1 and three seeds in years 2 and 3 to account for reduced emergence in year 1. Seeds were sown on 13 October 2017, 10 October 2018, and 16 October 2019, at 20 cm intervals within rows spaced 50 cm apart, aiming for a maximum density of approximately 100,000 plants/ha. The same experimental area was used each year, with no fertilizer applied.

A split-plot design with four replications was employed, with biotypes as the main plots (11 × 4 m) and subdivided into weedy and weed-free subplots (5 × 4 m), including eight milk thistle rows. Subplots were separated by 1-m wide alleys, and manual weed removal in weed-free subplots was performed in early December and early February each year. Irrigation was not provided, and deltamethrin [(1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid (S)-cyano(3-phenoxyphenyl) methyl ester] was applied once annually to control *Rhinocyllus conicus* (Coleoptera).

Data collection

The milk thistle stand was evaluated six weeks after seeding (WAS) in the two central rows of each subplot. Weed densities in weedy subplots were measured at 22 WAS (in late March each year) in a 5 × 1 m area, which was delimited by three rows. Weed plants were harvested when milk thistle leaves reached 90% of their maximum mass (BBCH code 49), while most of which were at the growth stage of the visible flower buds (Martinelli et al., 2015). This stage marks the critical competition period between milk thistle and weeds. During sampling, the fifth row of milk thistle in each subplot, along with winter weeds from a 5 x 1 m area, was cut at the soil surface. The number of weed plants, milk thistle height and total fresh weight of each biotype were recorded.

At harvest, milk thistle yield components [dry biomass and head (capitula) number] per square meter were determined by hand-harvesting entire plants from two 5-m-long rows in each subplot on 30 May 2018, 20 May 2019 and 25 May 2020. After each harvest, the remaining biomass was removed to simulate real farm conditions, and the yields were adjusted to 15% moisture content. The fruit yield was measured after manually removing and threshing the heads.

Competition indices

Two competition indices of milk thistle, ability to compete (AC) and ability to withstand competition (AWC), were calculated as described by Vasilakoglou and Dhima (2012) using the following formulas:

$$AC = 100 - [(f_w/f_t) \times 100]$$

where fW_w is the fresh weight of weeds and fW_t is the total fresh weight (milk thistle and weeds combined), and

$$AWC = 100 \times (BY_{wp}/BY_{wfp})$$

where BY_{wp} and BY_{wfp} are the biomass yields from weedy and weed-free subplots, respectively.

Milk thistle's allelopathic potential

Extract preparation

In late March during the first two years, when milk thistle leaves reached 90% of the maximum size (BBCH code 49) (Martinelli et al., 2015), stems and leaves were harvested for allelopathic potential evaluation. The chopped parts were air-dried at 28±4 °C for 72 h and ground using a 1-mm screen in a Wiley mill. Extracts were prepared following the method described by Tsiaousi et al. (2019), using water extract concentrations of 1.25, 2.50 and 5.00 g per 100 mL. The extracts were stored at 4 °C for one day before use. Three replicates (glass jars) were used for each treatment (plant material from weed-free or weedy subplots × extract concentration).

Bioassay procedure

A bioassay was conducted to assess the possible allelopathic effects of milk thistle extracts on the germination and root elongation of rigid ryegrass and wild mustard, two of the most serious winter weeds (which threaten all winter crops) that were used as bioindicators. The method described by Tsiaousi et al. (2019) was followed, using perlite-based Petri dishes. In particular, 50 rigid ryegrass (weighting about 1.8 g per 1000 seeds) or 100 wild mustard seeds (weighting about 1.5 g per 1000 seeds) were placed in each Petri dish (8.5-cm diameter) and covered with 5 g of perlite. Each Petri dish was then moistened with 10 mL of milk thistle water extracts. Deionized water was used as the control. Each combination (milk thistle biotypes × weed competition × extract concentration) was replicated three times, with two Petri dishes per replicate. Finally, the Petri dishes were placed in shallow trays, covered with plastic bags to maintain moisture and incubated at 19±2 °C and in dark conditions for 12 days in a growth chamber. After this period, germination rate and root elongation (for germinated seeds) were measured and expressed as percentages of the control. The experiment was conducted twice using plant material harvested in 2018 and 2019.

Statistical analyses

Regarding the milk thistle parameters (stand, fresh weight, height, head number, total dry biomass and seed yield), an across-year split-plot factorial (milk thistle biotypes × weed competition) analysis of variances was conducted. For weed data and competition indices (AC and AWC), a one-factor randomized complete block design was used. Bartlett's test examined homogeneity of variances, and data were transformed when necessary. In particular, square-root (x + 1) transformation was applied to weed plant numbers and log (x + 1) transformation was applied to both weed and milk thistle fresh weight data before ANOVA, but the results present the back-transformed values.

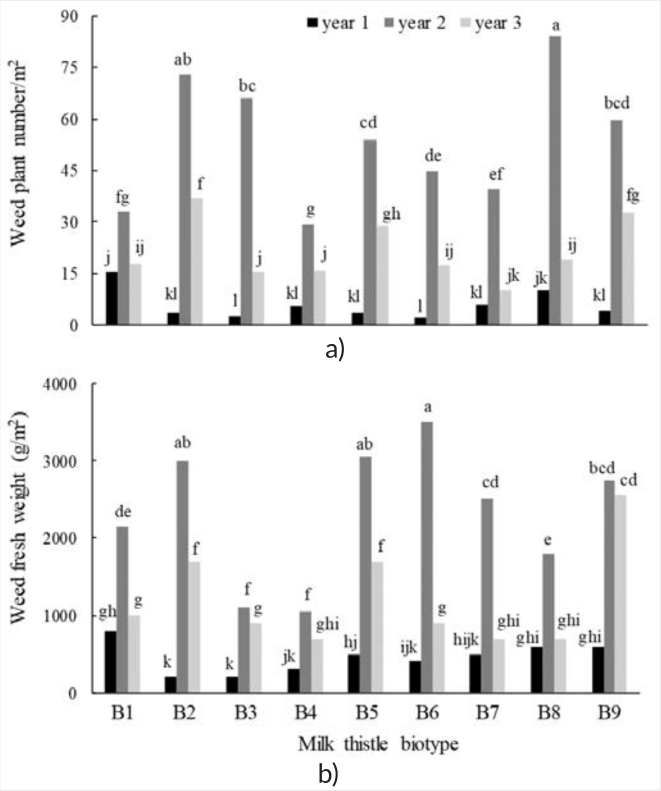
Data received from the bioassays were analyzed separately for each weed species across both years, using a 9 × 2 × 3 [9 milk thistle biotypes × 2 weed competition conditions (weed-free and weedy) × 3 extract concentrations] factorial design. The phytotoxic dose-response of milk thistle extracts on rigid ryegrass and wild mustard germination and growth was assessed using the whole-range method (An et al., 2005), as described by Tsiaousi et al. (2019).

The MSTAT program (MSTAT-C, 1988) was used to conduct ANOVAs. For means' comparison, Fisher's protected least significant difference (LSD) test was used at $P = 0.05$.

RESULTS

Weed presence

Regarding the data received in late March (weed density and fresh weight at 22 WAS), the ANOVA indicated a significant year × milk thistle biotype interaction ($P<0.001$). Thus, these interaction means are presented in Figure 2. Generally, the lowest weed density and fresh weight were recorded in year 1, while the highest values (approximately nine and five times, respectively) were observed in year 2. In particular, in milk thistle biotypes B1 and B4, weed density was lower than that in the other biotypes, as averaged across years (Figure 2a).



In each figure, means followed by the same letter did not significantly differ according the Fisher's Protected LSD test at *P* = 0.05

Figure 2. Density (a) and fresh weight (b) of weeds in milk thistle weedy treatments during their competition period (22 WAS)

The highest values were observed for the B2, B3 and B8 biotypes. Regarding the fresh weight of weeds, lower fresh weights were recorded in milk thistle biotypes B3 and B4, as averaged across years (Figure 2b). The highest values were recorded for the biotypes B2, B5 and B6. The fresh weight recorded in B9 was intermediate.

Milk thistle growth

At 6 WAS, the emergence of milk thistle plants was completed. In particular, 4.2, 9.3 and 7.2 plants/m^2 were recorded in year 1, year 2 and year 3, respectively (Figure 3). In both year 1 and year 3, the milk thistle density was lower than the applied seed density (10 plants/m^2).

Regarding the milk thistle data received in late March (height and fresh weight), the ANOVAs indicated a significant year × milk thistle biotype × weed competition interaction (*P*<0.001). Thus, these interaction means are presented in Figures 4 and 5, respectively. In particular, milk thistle achieved the greatest height in year 2, whereas no differences were observed in most cases between years 1 and 3 or between weed-free and weedy treatments (Figure 4). Among the biotypes, the B3, B4 and B7 achieved the greatest plant height, averaged across years.

Averaged across years and biotypes, the greatest fresh weight was observed in the B4, B6 and B7 biotypes, with greater fresh weight in weed-free subplots than in weedy ones (Figure 5). In particular, a 32% reduction in milk thistle fresh weight (due to weed competition) was recorded. In weed-free subplots, the highest fresh weight was observed in the B1, B4, B6, B7, and B9 biotypes (Figure 5a), averaged across years. In the weedy subplots, the B4, B6 and B7 biotypes provided the greatest fresh weight (Figure 5b). In addition, the highest fresh weight, averaged across biotypes and weed competition, was observed in year 2 (4,472 g/m^2), while the lowest was observed in year 1 (2,556 g/m^2).

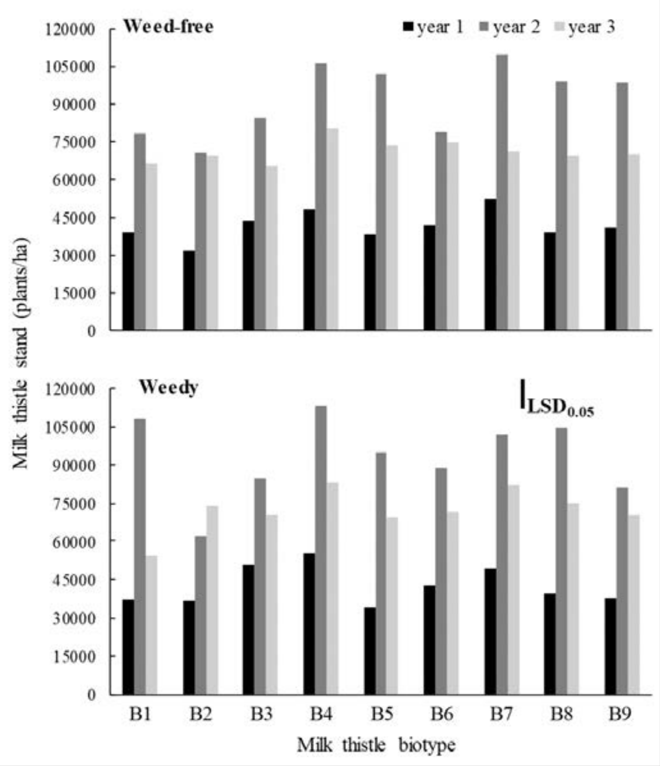


Figure 3. Milk thistle stands at 6 WAS grown in weed-free and weedy conditions

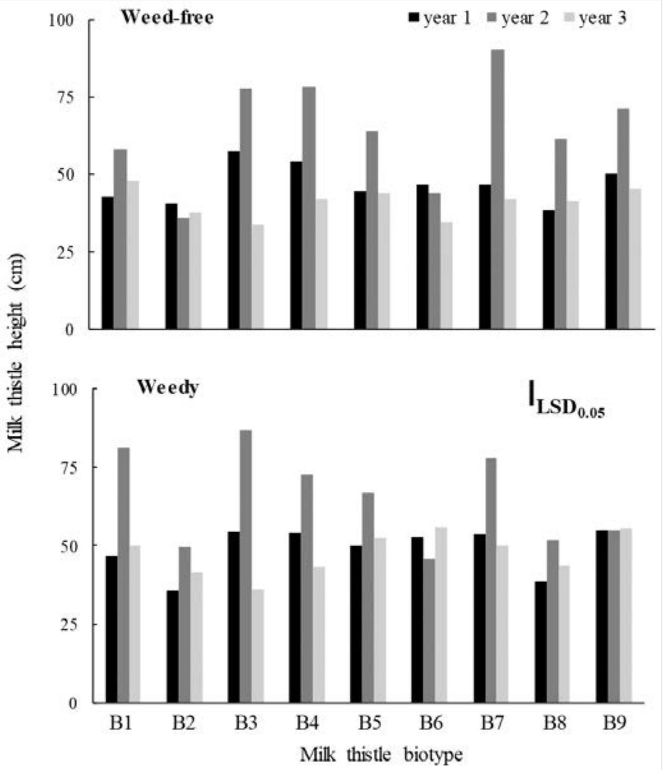
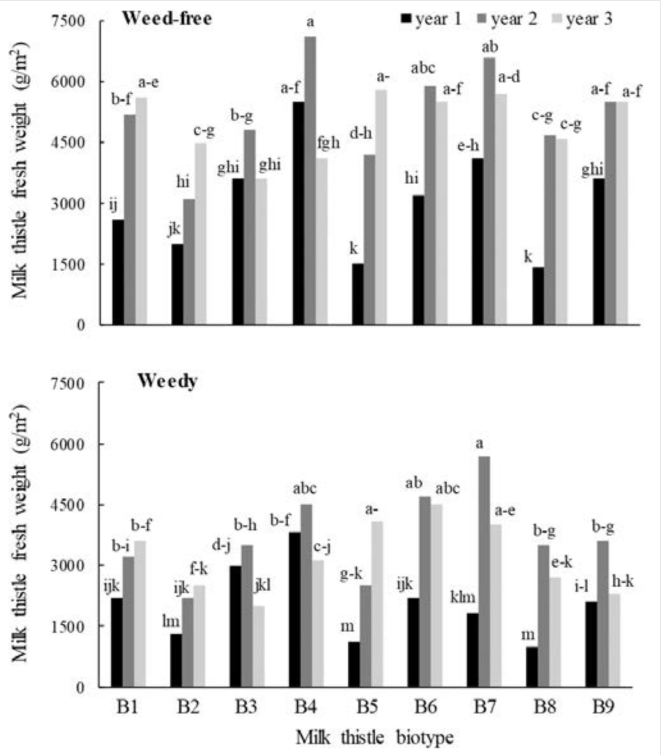


Figure 4. Milk thistle height at 22 WAS grown in weed-free and weedy conditions



In each figure, means followed by the same letter did not significantly differ according the Fisher's Protected LSD test at $P = 0.05$

Figure 5. Milk thistle fresh weight during their competition period with weeds (22 WAS)

Competition indices

Significant year × biotype interactions ($P<0.001$) were indicated by the ANOVAs conducted for milk thistle AC and AWC. In particular, the lowest AC was achieved in year 2 (48% as averaged across biotypes), whereas the highest was observed in year 1 (73%) (Figure 6a). The greatest AC was achieved by the B4 biotype (72.9%), whereas the lowest was achieved by the B9 biotype (51.3%). In contrast, no significant difference was observed in most cases among milk thistle biotypes or among years regarding the AWC, which averaged (across years and biotypes) 81.4% (Figure 6b).

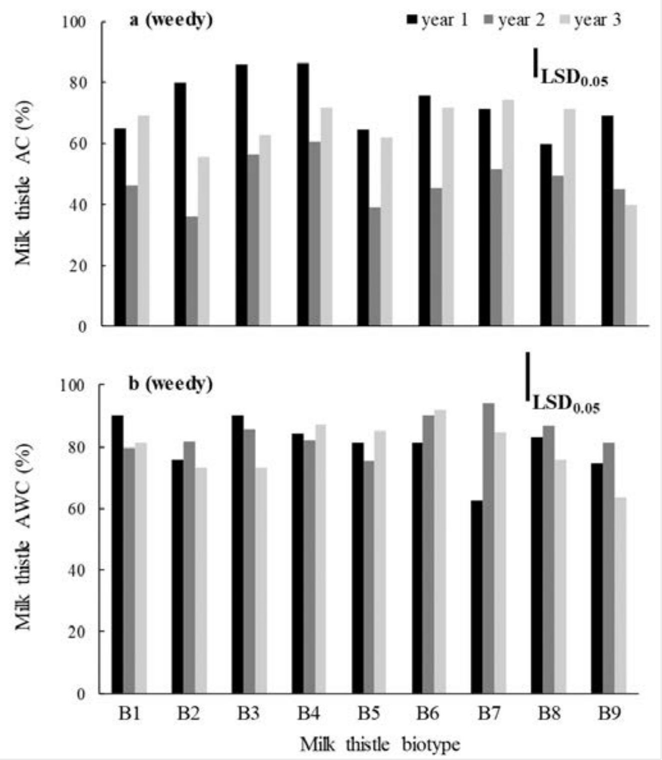


Figure 6. Milk thistle ability to compete (AC) and ability to withstand competition (AWC) during their competition period with weeds (22 WAS)

Biomass and seed yields

Significant year × biotype × weed competition interactions ($P<0.001$) were indicated by the ANOVAs conducted for milk thistle yield components (dry biomass, head number/m² and seed yield). Thus, these interaction means are presented in Figures 7, 8 and 9.

In the weedy subplots, milk thistle provided approximately 21% lower dry biomass than the weed-free ones, as averaged over the years and biotypes (Figure 7). The lowest dry biomass was produced in year 1 (6.6 and 5.9 t/ha, in weed-free and weedy subplots, respectively), whereas the greatest dry biomass was produced in year 2 (8.9 and 8.1 t/ha, in weed-free and weedy subplots, respectively).

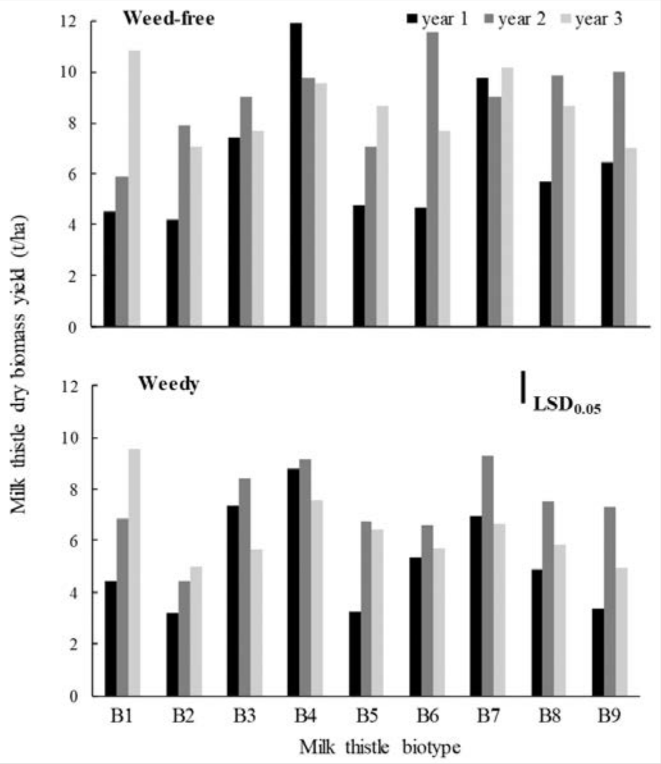


Figure 7. Dry biomass yield of nine milk thistle biotypes as affected by weed competition

As averaged across years, the greatest dry biomass was provided by the B4 biotype (10.4 and 8.5 t/ha, in weed-free and weedy subplots, respectively), while the lowest one by the B2 biotype (6.4 and 4.2 t/ha, in weed-free and weedy subplots, respectively). Totally (for 3 years), the biotype 4 produced 31.2 and 25.6 t/ha dry biomass in weed-free and weedy treatments, respectively.

Similarly, milk thistle biotypes produced approximately 17% fewer heads in weedy subplots than the weed-free ones, as averaged over years and biotypes (Figure 8). The fewest heads were produced in year 1 (41.2 and 30.8 heads/m^2, in weed-free and weedy subplots, respectively), whereas the most were produced in year 3 (56.3 and 46.4 heads/m^2, in weed-free and weedy subplots, respectively). The highest head number was provided by the B4 biotype (64 and 53.6 heads/m^2, respectively, in weed-free and weedy subplots), while the lowest one was provided by the biotype B9 (43.7 and 30.6 heads/m^2, in weed-free and weedy subplots, respectively), averaged across years. Regarding head number, differences among years were only observed in biotypes B1, B4, B7 and B8.

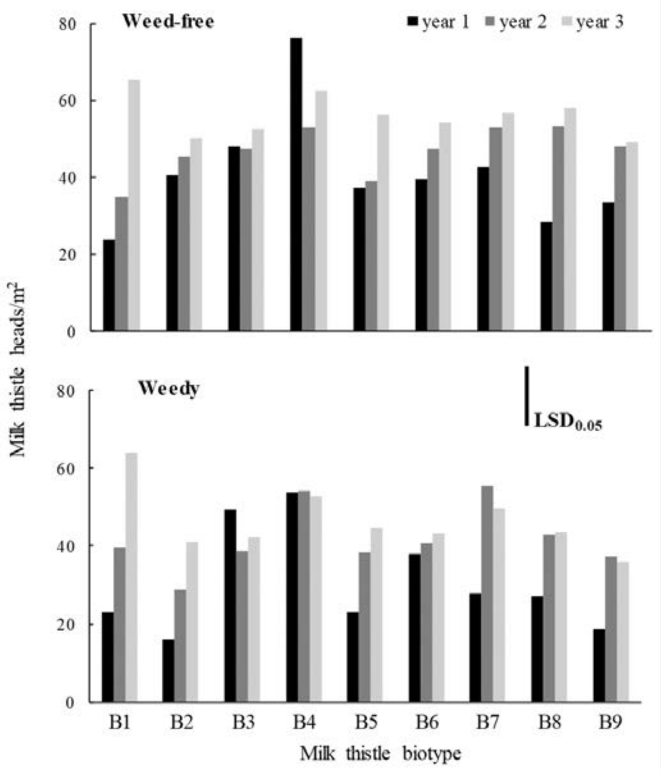


Figure 8. Head number of nine milk thistle biotypes as affected by weed competition

According to the dry biomass and head number data, the milk thistle fruit yield produced by the nine biotypes was 38% greater in weed-free plots than in weedy subplots (Figure 9). The lowest seed yield was recorded in year 2 (1.04 and 0.87 t/ha, in weed-free and weedy subplots, respectively). The greatest yield was recorded in year 3 (1.80 and 1.24 t/ha, in weed-free and weedy subplots, respectively). The biotypes B4 and B7 provided the greatest fruit yields (1.57 and 1.64 t/ha, respectively, in weed-free conditions and 1.35 and 1.29 t/ha, respectively, in weedy conditions and thus were the most productive.

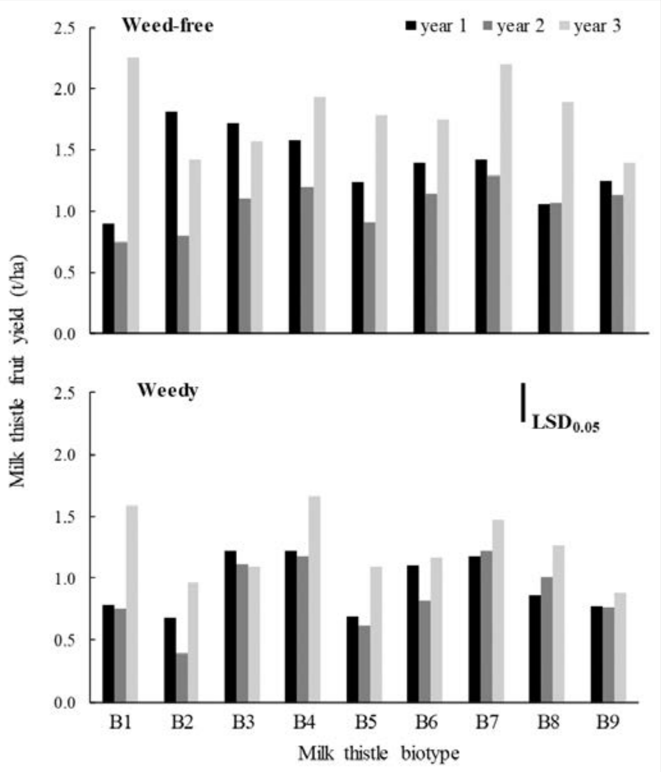


Figure 9. Seed yield of nine milk thistle biotypes as affected by weed competition

Allelopathic potential

No significant year × biotype × extract concentration interactions were recorded by the ANOVAs performed for both weeds (rigid ryegrass and wild mustard) germination and growth. In contrast, significant biotype × extract concentration interactions (*P*<0.001) were recorded; thus, the means of these interactions are presented in Figures 10 and 11.

Generally, the increase in milk thistle extract concentration caused further reduction in both weed germination and root elongation, with no significant differences in most cases between weed-free and weedy subplots (Figures 10 and 11).

Wild mustard was more sensitive to the milk thistle extracts than rigid ryegrass. In particular, the extracts of 1.25% of nine milk thistle biotypes grown in weed-free subplots caused 0 to 19.9% and 0 to 22.2% reduction in rigid ryegrass germination and root length, respectively (Figure 10). The extracts of biotypes grown in the weedy subplots caused 5.8 to 25.8% and 0 to 21.2% reduction, respectively. In the same way, the 5% extract concentration received from the weed-free subplots caused 28.9 to 71.6% and 21.0 to 81.7% reduction, respectively, while the extracts received from the weedy subplots caused 37.9 to 55.7% and 23.0 to 64.3% reduction, respectively. The extracts of the biotypes B6, B7 and B8 were the most phytotoxic (inhibition indices from 31.24 to 46.36%) (Table 2).

Regarding the phytotoxicity on wild mustard, the extracts of 1.25% of nine milk thistle biotypes grown in weed-free subplots caused a reduction in germination and root length that ranged from 31.1 to 74.5% and 0 to 40.3%, respectively (Figure 11). The corresponding reductions caused by the biotypes grown in the weedy subplots range from 37.4 to 71.7% and from 0 to 24.1%. The 5% extract concentration of the nine milk thistle biotypes grown in the weed-free subplots caused 94.9 to 100.0% and 21.2 to 100.0% reduction in wild mustard germination and root length, respectively. The extracts obtained from the weedy subplots caused 92.3 to 100.0% and 67.7 to 100.0% reduction, respectively, with the extract of the biotypes B2, B3, B4 and B5 being the most phytotoxic (inhibition indices from 51.63 to 78.05%) (Table 2).

DISCUSSION

Weed presence

The higher weed density and fresh weight were recorded in year 2 than in years 1 and 3 could be attributed to the lower rainfall recorded from October to December 2018, the period of milk thistle emergence and establishment (Figure 1). This lower rainfall resulted in slower emergence and establishment and consequently, slower canopy development of the milk thistle (Zimdahl, 2004). Similarly, Tsiaousi et al. (2019) reported slower milk thistle and cardoon (*Cynara cardunculus* L.) establishment and growth when lower rainfall was recorded during the autumn.

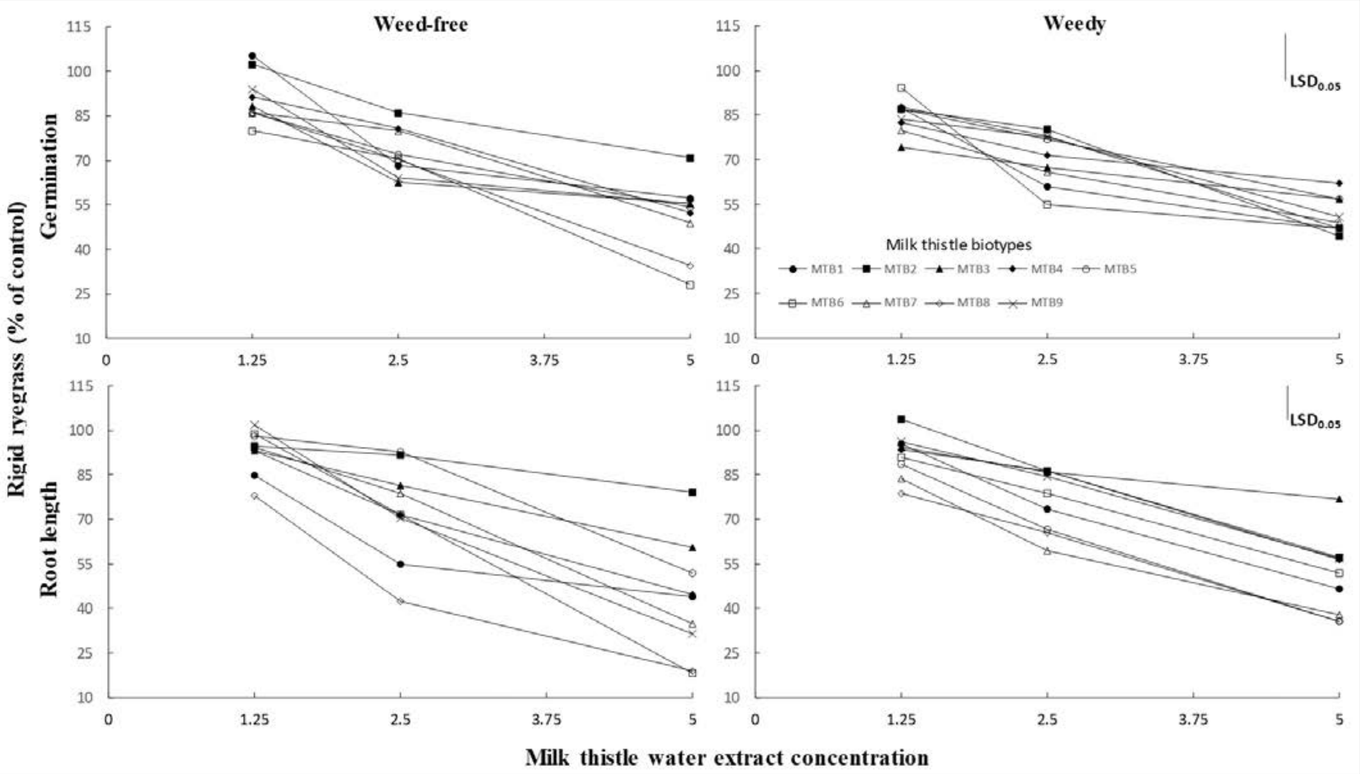


Figure 10. Rigid ryegrass germination and root length as affected by weed competition and milk thistle water extract concentration

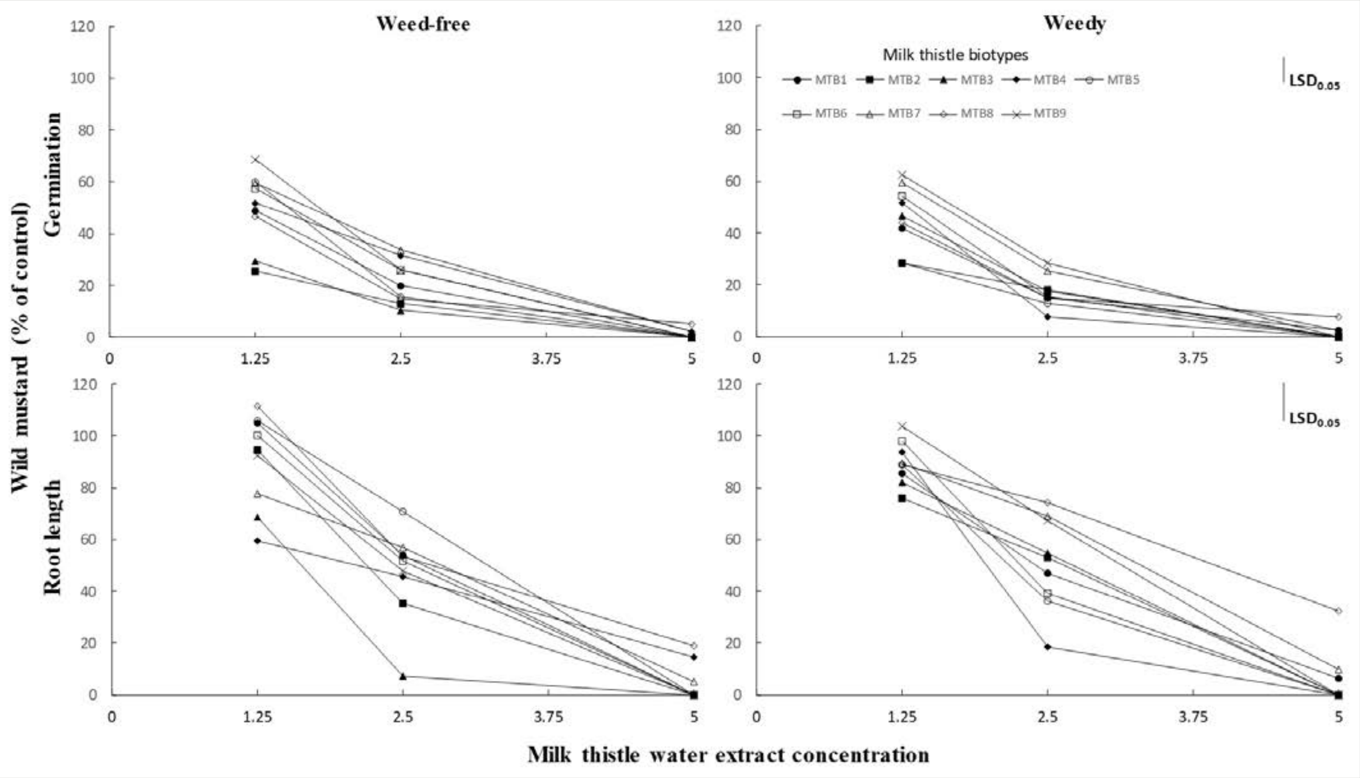


Figure 11. Wild mustard germination and root length as affected by weed competition and milk thistle water extract concentration

Table 2. Rigid ryegrass and wild mustard germination and root length inhibition indices, as affected by nine milk thistle biotypes' extracts

Milk thistle	Germination		Root length	
Biotypes	Weed-free	Weedy	Weed-free	Weedy
		Rigid ryegrass inhibition index (%)		
B1	22.2	30.4	33.5	24.7
B2	12.7	24.2	9.8	16.1
B3	27.0	30.2	18.5	12.3
B4	20.8	25.3	26.1	17.2
B5	25.6	22.8	14.0	31.2
B6	33.8	30.7	31.2	22.0
B7	23.5	31.4	24.6	34.3
B8	30.8	24.3	46.4	34.8
B9	25.5	25.0	29.2	17.6
Mean	24.7	27.2	25.9	23.4
		Wild mustard inhibition index (%)		
B1	70.2	72.0	43.5	46.6
B2	78.1	75.5	51.6	48.5
B3	78.0	71.8	69.6	46.0
B4	63.2	73.9	52.9	56.4
B5	68.7	77.2	35.1	51.9
B6	65.2	70.5	44.8	49.8
B7	60.8	64.2	45.2	35.7
B8	70.9	70.5	38.5	28.9
B9	58.9	62.6	46.8	36.4
Mean	68.2	70.9	47.6	44.5

In year 2, despite the high weed number recorded in B3, the corresponding fresh weight was lower than expected. This fact could be attributed to the faster canopy closure (due to the sufficient stand and higher growth rate) of this biotype this year, resulting in greater competition against weeds.

Milk thistle growth

The lower milk thistle stand recorded in year 1 than in years 2 and 3 could be attributed to fewer seeds placed in each hole (2 instead of 3) and to milk thistle seed dormancy (Moradi et al., 2024). Generally, weed presence did not affect the emergence of milk thistle. By contrast, Vasilakoglou and Dhima (2014), Scavo et al. (2018) and Tsiaousi at. al. (2019) found that the presence of weeds slightly reduced the establishment and growth of milk thistle and cardoon.

The higher milk thistle height recorded in year 2 than in years 1 and 3 could be attributed to stronger weed competition for light owing to their greater density and growth. Similarly, the lower milk thistle fresh weight recorded in weedy subplots was due to weed competition for light and nutrients (Zimdahl, 2004).

Competition indices

The greater weed presence in year 2 may have resulted in a slightly lower ability of milk thistle to compete. However, the lack of significant differences in the ability of milk thistle to windstand weeds' competition, recorded in most cases among biotypes, could be attributed to the rapid growth of milk thistle during March. Tsiaousi et. al. (2019), in a previous experiment on milk thistle competitive ability, found a range of 82.73 to 99.97% and 58.30 to 90.13% for milk thistle AC and AWC values, respectively.

Biomass and seed yields

In year 1, the milk thistle biotypes produced lower dry biomass yields than those produced in years 2 and 3. Difficulties in establishment during year 1, which resulted in a lower stand, probably caused this lower production. However, in years 2 and 3, milk thistle biotypes provided greater dry biomass yields that averaged 8.7 t/ha in weed-free and 7.8 t/ha in weedy subplots, agreeing with the results reported by Tsiaousi et al. (2019), who found that the milk thistle (biotype originated from Larissa) during the three years of cultivation produced a total of 20.89 t/ha under weed-free conditions and 15.49 t/ha under weedy conditions. Among the milk thistle biotypes, commercial B4 and B7 (originating from Bulgaria) were the most productive with respect to dry biomass, indicating better adaptability to reduced inputs.

Regarding the fruit yield, the biotypes B4 and B7 were also the most productive, averaging 1.6 and 1.32 t/ha in weed-free and weedy conditions, respectively. This fact could be attributed to the more uniform genetic material of these biotypes, since they were commercial seeds, as well as to their high allelopathic potential (Zimdahl, 2004). Generally, weeds' competition caused a slight reduction in milk thistle productivity, indicating the ability of milk thistle to windstand weeds' competition.

Although the silymarin content of the milk thistle fruits is not presented in the current study, the determination of silymarin concentrations is in progress.

Allelopathic potential

Generally, the increase in milk thistle extract concentration caused a further reduction in both weed germination and root elongation. The presence of allelopathic substances in the extracts may have been responsible for this inhibition. In previous studies, Tsiaousi et. al. (2019) found that the growth of rigid ryegrass and littleseed canarygrass (*Phalaris minor* Retz.) was reduced by 47.4 to 81.7% and 76.2 to 78.1%, respectively, because of the effect of milk thistle extract at 5% concentration. According to Ibrahim et al. (2007), the presence of four flavonolignans (silychristin, silydianin, silybin and isosilybin) known as 3-oxyflavone silymarin may be responsible for the allelopathic potential of milk thistle extracts. In addition, La Iacona et al. (2024) reported that the polyphenols, flavonoids, flavons and phenolic acids contained in milk thistle leaf water extracts caused significant germination and root growth reduction in weeds common purslane (*Portulaca oleracea* L.), common dandelion (*Taraxacum officinale* F.H. Wigg.) and scarlet pimpernel (*Anagalis arvensis* L.). According to the calculated I indices, the phytotoxicity among the extracts of the nine milk thistle biotypes was significantly different. Although no substances in the extracts were determined, differences in the total amount of allelochemicals produced among the biotypes could be responsible for the differences (Hossen et al., 2020).

The most phytotoxic extracts in the rigid ryegrass were not simultaneously the most toxic to wild mustard. The different chemical profile in terms of accumulation and exudation of the allelochemicals, as well as different sensitivity between weeds to milk thistle allelochemicals, could explain the differences among biotypes' phytotoxicity (González-García et al., 2025).

The seeds of wild mustard were more sensitive to milk thistle water extracts than the rigid ryegrass seeds, maybe due to the presence of the lodicules surrounding the rigid ryegrass seeds (Scavo et al., 2018). The higher extract penetration on wild mustard seeds and its greater sensitivity of its embryo to milk thistle allelochemicals could explain the fact that the milk thistle water reduced the germination of wild mustard extracts more than its root length. At the same time, this was not the case for the rigid ryegrass. Similarly, Kumar et al. (2025) found that the germination of soybean [*Glycine max* (L.) Merr.] was more sensitive than its root length to stinging nettle (*Urtica dioica* L.) leaf water extracts, but not the same for flax (*Linum usitatissimum* L.). Jeddi et al. (2025) found that the germination of alfalfa (*Medicago sativa* L.) was less sensitive than its root length to leaf aqueous extracts of orange wattle [*Acacia saligna* (Labill.) H.L. Wendl.] and Italian cypress (*Cupressus sempervirens* L.)

In most cases, slight differences among weedy and weed-free treatments were observed regarding the allelopathic effect on the two weeds, indicating that the weed competition in most cases did not significantly affect the allelopathic potential of milk thistle biotypes. In particular, the 1.25% extracts from the weedy subplots caused slightly higher germination inhibition on rigid ryegrass than those from weed-free subplots. According to Belz (2007) and Merkle et al. (2025), both biotic and abiotic stresses increased the allelopathic potential of various plants. However, the 5% extracts of four biotypes from the weed-free subplots caused slightly higher inhibition of rigid ryegrass than those from the weedy subplots. Although the allelopathic substances were not quantified in this study, plants grown in the presence of weeds may have activated more or different allelopathic substances that are more phytotoxic at low concentrations. This may have a stronger effect at low extract concentrations, where these metabolites remain unchanged and active. In contrast, plants of these biotypes grown without weed competition may have produced fewer but more stable or potent allelopathic agents that act more clearly and toxically at high concentrations (Gao et al., 2017; González-García et al., 2025). This emphasizes the complexity of allelopathy in agricultural ecosystems, and further research should prioritize thorough investigation of the potential of these compounds. Similarly, the differences among biotypes in terms of phytotoxicity against weeds can be attributed to variations in their allelopathic profiles.

CONCLUSION

The results of this study clearly indicate that there are great differences among milk thistle biotypes regarding their productivity and competitiveness, which are enhanced by allelopathic potential. Indeed, the commercial biotypes B4 and B7, originating from Bulgaria, were more productive than the native Greek biotypes. Although reduced inputs (no fertilization, irrigation, or weed control) were applied, most biotypes achieved satisfactory productivity (expressed as dry biomass for energy purposes or seed yield for silymarin production). Consequently, some milk thistle biotypes could be used as an alternative energy source and medicinal crop in Mediterranean environments.

REFERENCES

An, M., Pratley, J.E., Haig, T., Liu, D.L. (2005) Whole-range assessment: A simple method for analysing allelopathic dose-response data. Nonlinearity in Biology, Toxicology and Medicine, 3, 245-260. DOI: https://doi.org/10.2201/nonlin.003.02.006

Andrzejewska, J., Sadowska, K., Mielcarek, S. (2011) Effect of sowing date and rate on the yield and flavonolignan content of the fruits of milk thistle (*Silybum marianum* L. Gaertn.) grown on light soil in a moderate climate. Industrial Crops and Products, 33, 462-468. DOI: https://doi.org/10.1016/j.indcrop.2010.10.027

Arampatzis, D.A., Karkanis, A.C., Tsiropoulos, N.G. (2019) Silymarin content and antioxidant activity of seeds of wild *Silybum marianum* populations growing in Greece. Annals of Applied Biology, 174, 61-73. DOI: https://doi.org/10.1111/aab.12470

Belz, R.G. (2007) Allelopathy in crop/weed interactions - an update. Pest Management Science, 63, 308-326. DOI: https://doi.org/10.1002/ps.1320

Cherlet, M., Hutchinson, C., Reynolds, J., Hill, J., Sommer, S., von Maltitz, G. (Eds.). (2018) World atlas of desertification. Luxembourg: Publication Office of the European Union.

Commission of the European Communities (2006) An EU Strategy for Biofuels. SEC 142, Brussels, Communication from the Commission. Available at: https://publications.europa.eu/en/publication-detail/-/publication/be8e2853-075b-4ed3-a0ca-ef3991690714/language-en [Accessed 20 September 2018].

Gao, Y., Li, G.P., Shi, H., Liu, H., Ren, A.Z., Gao, Y.B. (2017) Allelopathic effect of endophyte-infected Achnatherum sibiricum on Stipa grandis. Acta Ecologica Sinica, 37, 1063-1073 (in Chinese). DOI: https://doi.org/5846/stxb201604160704

González-García, E., Sánchez-Moreiras, A.M., Vieites-Alvarez, Y. (2025) Alelopathic potential and chemical profile of wheat, rice and barley against the herbicide-resistant weeds *Portulaca oleracea* L. and *Lolium rigidum* Gaud. BMC Plant Biology, 25, 624. DOI: https://doi.org/10.1186/s12870-025-06634-3

Hossen, K., Das, K.R., Okada, S., Iwasaki, A., Suenaga, K., Kato-Noguchi, H. (2020) Allelopathic potential and active substances from *Wedelia chinensis* (Osbeck). Foods, 9, 1591. DOI: https://doi.org/10.3390/foods9111591

Ibrahim, M.M., Ottai, M.E.S., El-Mergawi, R.A. (2007) Self-mating effect on growth traits and silymarin production of some selected lines among milk thistle (*Silybum marianum* L.) varieties. World Journal of Agricultural Sciences, 3, 97-104. DOI: https://doi.org/10.1055/s-2007-987394

Jeddi, K., Reguei, A., Galal, T., Siddique, K.H.M., Hessini, K. (2025) Potential allelopathic effect of two multipurpose trees on germination, seeding performance, and antioxidant activity of alfalfa in Mediterranean arid lands. Russian Journal of Plant Physiology, 72, 1. DOI: https://doi.org/10.1134/S1021443724606852

Karkanis, A., Bilalis, D., Efthimiadou, A. (2011) Cultivation of milk thistle (*Silybum marianum* L. Gaertn.), a medicinal weed. Industrial Crops and Products, 34, 825-830. DOI: https://doi.org/10.1016/j.indcrop.2011.03.027

Kumar, N., Kumar, S., Rana, K., Kumari, A., Rawat, S. (2025) Allelopathic influence of *Urtica dioica* L. weed on seed germination and seedling growth of *Glycine max* (L.) Merr. and *Linum usitatissimum* (L.). Allelopathy Journal, 64, 179-190. DOI: https://doi.org/10.26651/allelo.j/2025-64-2-1525

La Iacona, M., Lombardo, S., Mauromicale, G., Scavo, A., Pandino, G. (2024) Allelopathic activity of three wild Mediterranean Asteraceae: *Silybum marianum*, *Cynara cardunculus* var. *sylvestris*, *Galactites tomentosus*. Agronomy, 14, 575. DOI: https://doi.org/10.3390/agronomy14030575

Lucini, L., Kane, D., Pellizzoni, M., Ferrari, A., Trevisi, E., Ruzickova, G., Arslan, D. (2016) Phenolic profile and in vitro antioxidant power of different milk thistle [*Silybum marianum* (L.) Gaertn.] cultivars. Industrial Crops and Products, 83, 11-16. DOI: https://doi.org/10.1016/j.indcrop.2015.12.023

Martinelli, T., Andrzejewska, J., Salis, M., Sulas, L. (2015) Phenological growth stages of *Silybum marianum* according to the extended BBCH scale. Annals of Applied Biology, 166, 53-66. DOI: https://doi.org/10.1111/aab.12163

Merkle, M., Petschenka, G., Belz, R., Gerhards, R. (2025) Enhancing weed suppression in plants by artificial stress induction. Journal of Crop Health, 77, 21. DOI: https://doi.org/10.1007/s10343-024-01075-8

Moradi, M., Ghaderi-Far, F., Sadeghipour, H.R., Siahmarguee, A., Torabi, B., Baskin, C.C., Soltani, E. (2024) Dormancy-break and germination of buried milk thistle (*Silybum marianum* (L.) Gaertn.) seeds. Journal of Applied Research on Medicinal and Aromatic Plants, 43, 100581. DOI: https://doi.org/10.1016/j.jarmap.2024.100581

MSTAT-C (1988) A microcomputer program for the design, management, and analysis of agronomic research experiments. East Lansing, Michigan, USA: Michigan State University, Crop and Soil Sciences Department.

Scavo, A., Restuccia, A., Pandino, G., Onofri, A., Mauromicale, G. (2018) Allelopathic effects of *Cynara cardunculus* L. leaf aqueous extracs on seed germination of some Mediterranean weed species. Italian Journal of Agronomy, 13, 119-125. DOI: https://doi.org/10.4081/ija.2018.1021

Sultana, S., Asaduzzaman, Md. (2012) Allelopathic studies on milk thistle (*Silybum marianum*). International Journal of Agricultural Research, Innovation and Technology, 2, 62-67. DOI: https://doi.org/10.3329/ijarit.v2i1.14007

Tran, G.H., Lee, H.-D., Shim, J., Lee, S., Nguyen, D.D., Lee, S. (2024) Quantitative analysis of silymarin in *Silybum marianum* seeds collected from different countries during three year cultivation. Scientia Horticulturae, 335, 113358. DOI: https://doi.org/10.1016/j.scienta.2024.113358

Tsiaousi, A., Vasilakolgou, I., Gravalos, I., Koutroubas, S. (2019) Comparison of milk thistle (*Silybum marianum*) and cardoon (*Cynara cardunculus*) productivity for energy biomass under weedy and weed-free conditions. European Journal of Agronomy, 110, 125924. DOI: https://doi.org/10.1016/j.eja.2019.125924

Vasilakoglou, I., Dhima, K. (2012) Leafy and semi-leafless field pea competition with winter wild oat as affected by weed density. Field Crops Research, 126, 130-136. DOI: https://doi.org/10.1016/j.fcr.2011.10.003

Vasilakoglou, I., Dhima, K. (2014) Potential of two cardoon varieties to produced biomass and oil under reduced irrigation and weed control inputs. Biomass and Bioenergy, 63, 177-186. DOI: https://doi.org/10.1016/j.biombioe.2014.01.052

Zimdahl, R.L. (2004) Weed-Crop Competition. A Review. 2nd edition. Iowa, USA: Blackwell Publishing.

Zrar, R.M., Ali, K.A. (2023) The allelopathic potential of milk thistle (*Silybum marianum*) and Syrian thistle (*Notobasis syriaca*) on germination percentage, some growth characteristics and allelopathy index of wheat and barley. ZANCO Journal of Pure and Applied Sciences, 35, 18-31. DOI: http://dx.doi.org/10.21271/ZJPAS.35.SpD.3