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WEED SUPPRESSİON, FORAGE YİELD, AND QUALİTY TRAİTS OF ALFALFA (*MEDICAGO SATIVA* L.) GENOTYPES UNDER ORGANİC FARMİNG

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Abstract

Among leguminous forages, alfalfa genotypes vary significantly in terms of their potential for weed suppression, herbage productivity, nutritional quality traits, and synthesis of allelopathic compounds. A multi-year study was conducted to determine alfalfa genotypes performance under an organic production system. The study was conducted during the period 2018-2019 at the Bilecik Seyh Edebali University's research trials area. Promising genotypes of alfalfa, including Queen, Prosementi, Taurus, Ludelis, Triade, Nijagara, Ultra, Nardian, Osmanağa, Corsa, Global, Oslava, Occitane, and Costanza alfalfa genotypes were comparatively evaluated. Experiments were conducted in a randomized complete block design (RCBD) with four replicates. The response variables included the density of weed species along with their fresh and dry weights in tested alfalfa genotypes., Additionally, nutritional quality traits such as relative feed values, dry matter for consumption, neutral detergent fiber, leaf area indexes were also recorded during the course of study.

There were statistically significant differences among the genotypes and characteristics examined. It was recorded that Corsa, Global, Prosementi, and Queen genotypes, remained superior by recording the maximum forage yield, quality traits, and weed suppression; thus, these genotypes are recommended for general adoption by alfalfa growers in Turkey.

Key words: Organic growing, alfalfa, genotypes, weed suppress effects, forage quality

Introduction

The world is facing unprecedented challenges in simultaneously producing enough food to feed a growing population, conserving natural capital, and ensuring sustainable agricultural practices are maintained (Foley *et al.*, 2011; Bailey & Buck, 2016). The contribution that legumes can make to alleviate climate change is often overlooked among the many important benefits that legumes provide to society. Comparing with agricultural systems based on mineral N fertilization, legumes (Stagnari *et al.*, 2017; Oliveira *et al.*, 2021; Dave *et al.*, 2024). Lower the emission of greenhouse gases (GHG) such as carbon dioxide (CO_2) and nitrous oxide (N_2O), plays an important role in soil carbon sequestration (Angus *et al.*, 2015), and reduces the overall input of fossil energy (Angus *et al.*, 1991).

GHG emissions can be reduced in arable systems by reducing the use of fertilizer and energy by incorporating legumes into rotations (Reckling *et al.*, 2014). N fertilizer savings in rotations involving leguminous crops in Europe (Reckling *et al.*, 2014), range around 277 kg ha^{-1} of CO_2 per year (1 kg N = 3,15 kg CO_2, (Jensen *et al.*, 2012). A study showed that half of the CO_2 generated during the production of NH_3 could be reused if the NH_3 were converted into urea. However, this is only a temporary delay in the release of CO_2 to the atmosphere because, after the application of urea to the soil, the hydrolysis activity by urease will release CO_2 originally captured during the urea production process (Jenkinson, 2001). Photosynthesis is the key mechanism that enables nodulated roots of legumes to respire CO_2. N-fertilizer synthesis, however, releases all its CO_2 from fossil fuels, which increases atmospheric CO_2 concentrations (Jensen *et al.*, 2012).

Legumes as a competitive crop with environmental and societal benefits can be integrated into modern cropping systems (for example, fertilizers and agrochemicals), which have a decrease in crop diversity (Anon., 2012; Plaza-Bonilla *et al.*, 2016).

Legumes are an excellent choice as break crops in wheat-based rotations, as they are typically not susceptible to the same pests and diseases as major cereal crops. By choosing legumes, farmers can diversify their crops with minimal risk. One key benefit of legumes is their ability to control weeds, which is linked to their nitrogen-fixing properties. As a result, crops grow stronger and faster, suppressing weeds and creating a more balanced ecosystem. Studies have consistently shown the effectiveness of this approach (Avola *et al.*, 2008; Seymour *et al.*, 2012).

As one of the most common plant growth-stimulating factors, BNF can also help improve weed competition in organic and sustainable farming systems (Berry *et al.*, 2002).

Through the expansion of ecologically based approaches, such as conservation agriculture, it is becoming possible to include food and forage legumes profitably in sustainable cropping systems. The development of weed control methods that avoid herbicides and tillage still faces challenges in conservation agriculture (Rühlemann & Schmidtke, 2015). Conservation agriculture promotes the use of legumes for environmental sustainability in both large and small-scale farms. Weeds pose a significant threat to

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forage crops and agricultural systems, causing more crop yield loss than pests, as research shows (Oerke & Dehne, 2004). This loss occurs because weeds compete with crops for resources, including water and nutrients. By adding nutrients and supplementing soil water, farmers can boost crop growth and yield (Horvath et al., 2023).

Although herbicides can be used to control weeds, but it cannot be the sole method to do so. Implementing ecological management of weeds is one option (Swanton & Weise, 1991; Mortensen *et al*., 2000). In part, this strategy relies on the fact that crops and weeds compete for the same resources, such as water, nutrients, light, and space. In the absence of sufficient soil nutrients and water resources, weeds will compete with crops forever, resulting in reduced crop yield due to the resources utilized by weeds (Liebman, 1998). Consequently, herbicides benefit crops by restricting the resources available to weeds, thereby alleviating weed pressure on the crops.

It was also reported that alfalfa plants contain water-soluble allelochemicals that are released into the soil environment from fresh leaf, stem, and crown tissues, as well as from dry hay, old roots and seeds (Kruidhof, 2008). These chemicals are autotoxic to the same species (Hall & Henderlon, 1989; Chung & Miller, 1995), as well as allelopathic to other species. The water-soluble chemicals reported for alfalfa are mainly cinnamic acid and its derivatives such as ferulic acid, vanillic, hydroxybenzoic, *p*-coumaric, *trans*-cinnamic acid, caffeic acid (Hall & Henderlon, 1989; Miller, 1996), saponin (Miller, 1996), and medicarpin (Dornbos *et al*., 1990).

The allelopathic and autotoxic bioactive compounds in alfalfa were isolated, purified, and identified (Chung, 1994). Active fractions showed retention times close to those of chlorogenic and salicylic acid standards, demonstrating that alfalfa contained water-soluble substances that are toxic to alfalfa itself (autotoxicity) as well as to other species (allelopathy) (Miller, 1983). It was reported that alfalfa root exudates exerted an inhibitory effect on the dry weight of barley (*Hordeum vulgare* L.), alfalfa, and radish (*Raphanus sativus* L.) seedlings (Tsuzuki *et al*., 1984). Furthermore, aqueous solutions of alfalfa root saponins have been shown to reduce the germination of cotton seeds (Marchaim *et al*., 1975).

Alfalfa root saponins also exhibited an inhibitory effect on cheatgrass seedling roots, with an inhibition rate of 8 to 10% greater than that observed in wheat seedling roots. These results suggest that saponins may have potential as herbicides (Wyman-Simpson *et al*., 1991).

The chemical composition of alfalfa root extracts and exudates differed among genotypes. Allelopathic activity needs to be clarified by increased chemical concentrations and individual compound evaluations. Alfalfa genotypes show significant genetic diversity, showing a potential for weed suppression (Zubair *et al*., 2017). Weed control strategies also need to be adapted to local environmental conditions (Brooker *et al*., 2015); therefore, we need to understand how crop–weed relationships differ with regard to different environments. The competition between plants is stronger and more important in benign environments, i.e., where sources are abundant (Bertness &;Callaway, 1994; Al-Namazi *et al*., 2017; Lima *et al*., 2022; Austin *et al*., 2023) therefore, we can expect weed suppression through stronger competition between crops and weeds in benign environments.

The purpose of this study was to test the hypothesis that alfalfa genotypes might respond differently in organic farming systems in terms of their weed suppression potential and herbage productivity with varying nutritional quality traits. Moreover, alfalfa genotypes were comparatively evaluated in terms of their genetic potential to produce allelopathic compounds under organic farming system.

Material and Methods

A total of fourteen genotypes were put to the test over two years, from 2018 to 2019, at two different locations (Table 1). The contenders included Queen, Prosementi, Taurus, and Ludelis. Joining them were Triade, Nijagara, Ultra, and Nardian, along with Osmanağa, Corsa, Global, Oslava, Occitane, and Costanza. Each genotype was meticulously evaluated to uncover its unique potential.

Table 1. Details of trial localities and experimental units for evaluation of alfalfa genotypes under organic farming system.

Site	Bilecik	Ankara
Northcoordinate	40° 06' 47.45"	39° 57' 37.21"
East coordinate	30° 00' 04.68"	32° 37' 08.61"
Altitude (m)	296	795
Plot width (m)	1.6	1.6
Plot length (m)	5	5
Sowing (date 2009)	20 Apr	5 May
Previous crop	Fallow	Alfalfa
	Biomass sampling dates	
2018 (1)	30 May	05 June
2019 (1)	15 May	30 May
2018 (2)	10 July	15 July
2019 (2)	12 July	15 July

Experimental design: Experiments were conducted over two growing seasons, starting in mid-April, following a randomized complete block design (RCBD) with four replicates (Table 1). The experimental units were arranged in plots measuring 1.6 m x 5m, with a density of 8 rows x 0.20 m intra-row. Each decare was treated with two tons of fermented manure and one ton of green compost at each experiment location, at a rate of 20 kg ha^{-1}. During the forage harvesting stage, plant height was measured from the base to the top using a steel tape. The mean of three randomly selected plants was recorded for each plot. Biomass yield was determined by clipping six interior rows at 5 cm above the ground level. The weight of the total green biomass yield was recorded from each plot in the field, and 500 g of alfalfa samples were taken from each plot to the laboratory. The sample from each plot was weighed using a sensitive table balance to determine the total fresh weight. Then, the sample was oven-dried for 24 hours at a temperature of 105°C to determine the herbage dry matter yield.

Relative feed value

$$\% \text{ DDM} = 88.9- (0.779 \text{ x } \% \text{ ADF})$$

The dried samples were then ground, passed through 1 mm sieve. The sieved sample was analysed for ash, CP, NDF, ADF, Relative Feed Value (RFV), and Metabolic

Energy as nutritional parameters. Nitrogen (N) content was determined using micro-Kjeldahl digestion distillation technique (Anon., 1995), and the crude protein (CP) content was estimated by multiplying the N content by 6.25. Structural plant components such as neutral detergent fiber (NDF) and acid detergent fiber (ADF) were compared according to the reported methods of Van Soest *et al*., (1991).

The NDF and ADF contents were analyzed using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA) (Van Soest *et al*., 1991). Metabolic energy value ME (MJ / t KM) and relative feed value were determined by the method developed by Van Dyke & Anderson (2000). Digestible Dry Matter (% DDM) was first calculated using the ADF value to calculate the relative feed value.

Digestible dry matter intake (% DMI)

$$\% \text{ DMI} = 120 / \text{NDF}$$

The Relative Feed Value (RFV) index estimates the digestible dry matter (DDM) of alfalfa based on its acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents. It calculates the potential dry matter intake (DMI) as a percentage of body weight (BW). This method provides a reliable estimate of the nutritional value of alfalfa for livestock feed. The RFV index is then calculated as DDM multiplied by DMI as a percentage of BW and divided by 1.29. RFV = (DDM × DMI) / x 0.775

Leaf area index: Five plants are randomly selected per plot each year, based on the number of years in the counting period. After 4 weeks of emergence and 12 weeks of emergence, 5 plants per plot were selected. They were cut from above ground. The plants were placed in plastic bags with a few damp papers and placed in a +4°C storage place. Following that, the leaf area measurements of the five plants were taken in the plots and the average leaf area was determined for each alfalfa genotypes.

Determination of saponin extraction method and detection conditions using: To extract alfalfa and detect saponins, 500 grams of fresh alfalfa genotype root pieces were collected from 10 different plants in the plots on 10 May 2018 and 14 May 2019. The root pieces were then dried in an oven at 65°C until a constant mass was reached. The DM was pulverised and stored for subsequent extraction. A variety of saponin extraction techniques were evaluated and contrasted. These included maceration at room temperature, Soxhlet extraction, and ultrasonic-assisted extraction. The factors subjected to analysis were methanol concentration, liquid-to-solid volume ratio, extraction duration, and extraction time. Ultrasonic-assisted extraction yielded a higher total saponin yield and was considerably more rapid than the other methods. The conditions for the ultrasonic-assisted extraction were as follows: 15ml of ethanol to 1g of raw material, 20 minutes, three times, and 800Hz at 20°C. As saponin compounds lack chromophores, total saponin was quantified using a vanillin colorimetric assay (Whiting *et al*., 1968; Sacchi *et al*., 2005; Ebringerová & Hromádková, 2010).

Determination of total saponin content: The set extraction method was followed to get the total saponin extraction involving the transfer of 1 ml of the crude sample solution into a 150 ml conical flask and evaporated it until it was dry using a water bath. Once it was cool, 0.2 ml of 5% vanillin-glacial acetic acid solution was added to the conical flask, followed by 0.8 ml of perchloric acid. Subsequently, the mixture was heated in a water bath at 65°C for 15 minutes, cooled it in ice, and added another 5ml of glacial acetic acid. The absorbance was measured at 544 nm using a UV–Visible spectrophotometer within 20 minutes. A standard curve of OA ranging from 0 to 36,7 mg L^{-1} to determine the total saponin content was used. The results were expressed as mg of total saponin per 1.0 g of sample DM (Dry Matter). This finalised protocol was used in all subsequent experiments for the determination of total saponin content in the Medicago species (Zhang *et al.,* 2023).

Identification and quantification of allelopathic substances: In order to evaluate the detection limits, all the phenolic acids were dissolved separately in methanol (≥ 99.9%) at a concentration of 1000 µg/mL, which was established as a stock solution. A series of dilutions was prepared, with concentrations of 20, 40, 60, 80, and 100 µg/mL, by the addition of the requisite volumes of the stock solutions to methanol. The separation of the phenolic acids was conducted using an Agilent 1100 series high-performance liquid chromatography (HPLC) system, which was equipped with a photodiode array detector. The instrument was operated and the data were analysed using Agilent HPLC Chemstation 10.1. The flow rate of the mobile phase was maintained at 1 mL/min. The mobile phase A consisted of water containing 25% methanol, while phase B was 75% acetic acid (pH 2.6). This was employed to ascertain the phenolic acid contents and allelopathic potential of 10 cultivars of alfalfa (Wang *et al*., 2017). The temperature of the column was maintained at 20°C. An injection volume of 25 µL was utilised. The wavelengths employed for diode array detection (DAD) were set at 280 nm. The analysis of these phenolic acids was conducted using 10 cultivars of alfalfa (100 g of fresh aerial part or roots). The samples were soaked in 200 mL of distilled water for 48 h at room temperature (25±2°C) to obtain 0.5 g/mL aqueous leachates. Subsequently, the leachates were filtered through 0.45 µm nitrocellulose membrane filters and stored at 4°C until further analysis. Twenty-five microlitres of each aqueous leachate were subjected to analysis using Agilent HPLC Chemstation 10.1, employing the same methodology previously outlined. The concentration of these phenolic acids in the aqueous leachates was determined by comparing the peak areas of the samples with those of the standard compounds (Xuan *et al*., 2003; Wang *et al*., 2017)

The suppression of weeds by alfalfa genotypes: Weed samples were collected twice a year, using a 50 x 50 cm^2 frame in each plot, and weed densities and the above-ground parts of weeds were cut, and the effects of the alfalfa genotypes on the fresh and dry weights of weeds were determined. Dry weights were determined by bringing freshly harvested weeds to the laboratory immediately and keeping them in the oven at 70ºC for 48 hours. To determine the effect of the applications on weeds, the weed coverage areas, and fresh and dry weights obtained from each application were determined by the SPSS 22 package program and General Linear Model, Univariate modeling, and the Duncan comparison test.

Data collection: Weed biomass was assessed in each plot at the flowering stage of the crops (April 2019, 85 d after sowing). Weeds were cut at the soil surface, the species were identified, dried in the oven at 70°C for 48 hours, and then weighed. The yield of alfalfa was determined per plot in terms of fresh weight and dry weight.

Statistical analysis

The collected data were analyzed in a combined year analysis of variance across locations and then by the Random Blocks trial design. Differences between the Averages were subjected to Duncan's analysis. MSTAT-C and SPSS16 package programs were used for Average variance, Average Duncan, and a Biplot analysis, respectively. For data analyses, we eliminated plots with complete data.

Results

As depicted in Table 2, there were statistical differences between alfalfa genotypes in terms of main stem length, main stem thickness, the number of main stems, green grass yield, and dry grass yield, as shown in Table 1. The Corsa genotype was found to have the longest main stem length of 72.85 cm, while the Ultra genotype recorded the shortest main stem length with 51.83 cm.

The thickest main stem thickness was recorded for the Ultra genotype with a value of 3.23 mm, and the thinnest main stem thickness was exhibited by the Queen, Global, and Corsa genotypes.

Among the genotypes, the Prosementi genotype produced the maximum number of main stems with a value of 13.38, while the Triade genotype produced the minimum number of main stems (8.68).

The Corsa genotype yielded 106908 kg ha^{-1}; whereas the Triade genotype yielded 47647 kg ha^{-1}. The Corsa genotype yielded the most green grass, while Triade yielded the least. Green grass was produced most often by the Corsa genotype, while it was produced least often by the Triade genotype. In the dry grass yield test, the Global genotype produced the highest value of 31420 kg ha^{-1}. In second place was the Corsa genotype with a value of 31353 kg ha^{-1}. A Triade genotype had the least dry grass yield at 19401 kg ha$^{-1,}$ and a Nijagara genotype had the least dry grass yield at 19340 kg ha^{-1}, respectively.

As shown in Table 3, ADF, NDF, ADL, DMI, DDM, and RVF ratios were significantly different among alfalfa genotypes. The Triade genotype had the highest dry matter rate of 92.66%, followed by the Ultra and Nardian genotypes with 92.61%, the Nijagara genotype with 92.50%, and the Queen genotype with 91.06%. The Taurus genotype exhibited the highest crude protein ratio of 28.80%, while the Global genotype exhibited the lowest value of 25.00%. Toros genotype had the lowest ADF rate at 18.84%, while Queen genotype had the highest ADF rate at 24.55% (Table 3). Cellulose and lignin are two components of ADF in plants. Therefore, a low value is desirable. With 31.27%, the Queen genotype had the highest NDF rate, while the Triade genotype exhibited the lowest with 24.43%. NDF was not the only component of cell walls. Cellulose, hemicellulose, and lignin were also present. It is very important in terms of digestibility. The genotype Costanza had the highest ADL level at 9.38%, while the genotype Taurus had the lowest level at 2.71%. DMI rates were highest for the Taurus genotype at 5.25%, whereas the Queen genotype showed the lowest DMI rates at 3.64%. The best DDM rate was obtained from the Taurus genotype with a 74.66% value and the lowest DDM rate was obtained from the Queen genotype with a 69.20% value. The RFV rate with 293.25 was the highest in Taurus genotype whereas Queen genotype had the lowest RFV rate with 209.75 (Table 3).

Table 2. Main stem height, main stem diameter, main stem number, green herbage and dry herbage yields obtained fromal alfalfa genotypes.

Alfalfa genotypes	Main stem height (cm)	Main stem diameter (mm)	Main stem number (piece)	Green herbage yield (kg ha^{-1})	Dry herbage yield (kg ha^{-1})
Queen	71.07 ab	2.15 f	12.53 abc	9178.5 b	2789.2 b
Prosementi	70.95 ab	2.55 de	13.38 a	10011.2 ab	2774.4 b
Toros	69.00 bc	2.63 cde	11.00 d	7134.0 de	2658.3 b
Ludelis	67.05 c	2.55 de	9.55 efg	6448.4 ef	2575.2 b
Triade	53.40 f	3.08 ab	8.68 g	4764.7 g	1940.1 de
Nijagara	50.20 g	3.15 ab	9.30 fg	5994.9 f	1934.0 de
Ultra	51.83 fg	3.23 a	10.95 d	6490.5 ef	1767.0 e
Nardian	64.33 d	2.68 cd	10.93 d	7774.7 cd	2037.4 d
Osmanağa	69.53 bc	2.50 de	10.38 de	7888.2 cd	2584.9 b
Corsa	72.85 a	2.25 f	12.20 bc	10690.8 a	3135.3 a
Global	71.20 ab	2.20 f	13.00 ab	10027.3 ab	3141.2 a
Oslava	56.53 e	3.00 b	10.75 d	6598.3 ef	2337.6 c
Occitane	68.28 bc	2.75 c	10.05 def	6661.5 ef	2576.7 b
Costanza	70.03 abc	2.48 e	11.95 c	8331.2 c	2663.2 b
Average	64.73	2.66	11.04	7713.9	2493.9
Duncan	1768	139	0.719	709.228	122.675
CV	2.913	4.251	5.357	7.326	6.056

Table 3. Dry matter, crude protein, crude cellulose, ADF, NDF, ADL, DMI, RFV obtained from alfalfa genotypes.

Alfalfa genotypes	Dry matter (%)	Crude protein (%)	Crude cellulose (%)	ADF (%)	NDF (%)	ADL (%)	DMI (%)	DDM (%)	RFV
Queen	91.06 c	24.50 h	19.90 a	24.55 a	31.27 a	4.19 g	3.64 c	69.20 ı	209.75 g
Prosementi	92.20 ab	25.99 cd	17.08 g	23.21 b	28.18 bc	3.38 ı	4.53 b	71.20 g	228.00 f
Toros	91.83 abc	28.80 a	15.25 ı	18.84 j	23.37 h	2.71 j	5.25 a	74.66 a	293.25 a
Ludelis	91.50 bc	26.90 b	17.60 f	22.99 bc	26.41 f	5.85 c	4.46 b	70.64 h	247.75 cd
Triade	92.66 a	27.19 b	15.39 ı	20.15 ı	24.43 g	4.16 g	4.58 b	73.47 b	279.50 b
Nijagara	92.50 a	25.54 def	17.08 g	21.21 h	27.71 d	3.72 h	4.51 b	72.28 cd	241.25 de
Ultra	92.61 a	25.37 fg	16.48 h	21.70 ef	26.32 f	3.55 hı	4.51 b	72.02 cde	252.00 c
Nardian	92.61 a	25.51 ef	18.16 bcd	22.78 c	27.62 d	3.65 h	4.43 b	71.44 efg	237.75 e
Osmanağa	92.19 ab	25.41 fg	17.75 ef	21.14 h	27.22 e	7.33 b	4.32 b	72.23 cd	247.50 cd
Corsa	91.85 abc	25.50 ef	17.66 ef	21.96 de	27.82 d	4.53 f	4.39 b	71.72 d-g	240.25 e
Global	91.78 abc	25.00 g	18.29 bc	21.30 gh	27.22 e	5.52 d	4.48 b	72.40 c	247.75 cd
Oslava	91.88 abc	25.35 fg	18.51 b	23.01 bc	27.66 d	5.30 d	4.37 b	71.35 fg	237.25 e
Occitane	91.92 abc	26.18 c	18.04 cde	21.54 fg	27.90 cd	4.93 e	4.31 b	72.09 cd	237.75 e
Costanza	91.79 abc	25.92 cde	17.83 def	22.02 d	28.21 b	9.38 a	4.45 b	71.86 c-f	236.75 e
Average	92.03	25.94	17.50	21.88	27.24	4.87	4.44	71.90	245.46
Duncan	0.645	0.294	0.242	0.248	0.248	0.135	0.198	0.399	3.910
CV	0.610	1.168	1.441	0.886	0.712	3.131	4.372	0.532	1.756

DM: Dry matter, CP: Crude protein, CC: Crude cellulose, NDF: Nötral detergent fiber, ADF: Acid detergent fiber, ADL: Strong acid insoluble lignin after acid detergent solution. DMI: Dry matter intake, DDM: Digestible dry matter, RFV: Relative feed value

Table 4. Leaf area index obtained from alfalfa genotypes in the experiment area.

Alfalfa genotypes	Leaf area index (m^{-2})			
	2018		2019	
	1. Count	2. Count	1.Count	2. Count
Corsa	0,91 a	2,90 a	0,80 a	2,77 a
Prosementi	0,88 ab	2,30 ab	0,65 a	2,74 a
Global	0,66 abc	2,22 ab	0,78 a	2,56 ab
Queen	0,49 abc	1,66 bc	0,51 a	2,30 abc
Costanza	0,45 abc	1,78 abc	0,50 a	2,39 abc
Toros	0,42 abc	1,51 bc	0,46 a	2,07 abcd
Occitane	0,41 abc	1,62 bc	0,53 a	2,29 abc
Osmanağa	0,37 abc	1,41 bc	0,44 a	2,04 abcd
Ludelis	0,34 abc	1,39 bc	0,39 a	1,98 abcd
Oslava	0,31 abc	1,30 bc	0,83 a	1,61 bcd
Nardian	0,30 bc	1,26 bc	0,34 a	1,49 bcd
Triade	0,27 c	1,22 bc	0,34 a	1,39 cd
Ultra	0,23 c	1,31bc	0,34 a	1,19 d
Nijagara	0,22 c	0,83 c	0,20 a	1,07 d
CV	0,151	0,044	0,772	0,08

A feed with a quality range between 125 and 151 is of high quality, a feed with a quality range between 103 and 124 is of good quality, a feed with a quality range between 87 and 102 is of medium quality, a feed with a quality range between 75 and 86 is weak, and a feed with a quality range below 75 is of poor quality. The grass samples obtained in our study are categorized as good-quality feed based on their relative feed values.

Leaf area index (LAI): For the leaf area index, differences of genotypes were significant in all counting times except for the first count in 2018 and the first count in 2019. The first counting of 2018 revealed that the Corsa genotype had the largest leaf area. Genotypes of Prosementi ranked 2nd, Global ranked 3rd, Queen ranked 4th, and Costanza genotypes ranked 5th. On the second counts, similar results were obtained as on the first count, while Costanza and Occitane genotypes were ranked higher. According to the results of 2019, the corsa genotype again had the largest leaf area. Despite being similar to the 2018 results, Costanza and Occitane genotypes differed from the first count (Table 4).

Identification and quantification of allelopathic substances: The aqueous extract from the roots of alfalfa genotypes Costanza and Costanza had the highest contents of p-hydroxybenzoic acid, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, cinnamic acid, and total phenolic acids at 16.44 µg/g and 7.7 µg/g, respectively. The highest contents were found in the aqueous extract from roots of alfalfa genotypes Costanza (70 µg/g), Corsa (23.69 µg/g), Triade (9.14 µg/g), Costanza (4.56 µg/g), Toros (4.10 µg/g) and Costanza (57.05 µg/g), respectively (Table 5). The inhibitory effects of the acidic fraction derived from the extracts of three alfalfa cultivars (Batasu, Rasen, and Yuba) exhibited variability in their impact on the growth of alfalfa and rice seedlings. The extract from Batasu was observed to have the least inhibitory effect, while that from Rasen was found to have the greatest inhibitory effect.

Another study demonstrated that the amounts of secreted allelochemicals varied according to the specific compound under investigation. These included p-hydroxybenzoic acid (4.45 to 12.94 µg/L), caffeic acid (2.79 to 7.23 µg/L), and chlorogenic acid (15.00 µg/L). The results clearly demonstrate that the amounts of secreted allelochemicals varied according to the specific compound. The amounts were as follows: The concentrations of these compounds were as follows: 22.97 µg/L, 3.04 to 8.35 µg/L for p-coumaric acid, 1.15 to 3.92 µg/L for ferulic acid, and 1.07 to 3.10 µg/L for cinnamic acid (Wang *et al.*, 2017). Milentyeva and colleagues identified the presence of the following compounds at the specified concentrations: protocatechuic acid (3.29 mg/ml), caffeic acid (6.44 mg/ml), and p-hydroxybenzoic acid (2.25 mg/ml) (Table 5).

The suppression of alfalfa on weeds: According to the 2018 evaluation of the experiment, the Nijagara alfalfa genotype showed the highest weed density and species diversity, with an average of 28.1 plants per square meter. The Queen genotype had the lowest weed density (5.5. weedm^{-2}). The plot with the greatest number of perennial weed species was the Nardian genotype. Additionally, Nijagara was noted for having the maximum weed density for both winter and summer weeds.Those parcels with the least amount of winter weeds are the Toros genotype, while those with the most intensive Costanza genotype having the most summer weeds. It becomes important to characterize chemicals between strong and weak allelopathic genotypes. So, weed suppression values of Alfalfa genotypes may differ from each other. In our trial, the most intense weeds seen in the plots in 2018 were *Atriplex patula* (35.20 weedm^{-2}), *Lolium temulentum* (15.95 weed m^{-2}) Avena *fatua* (13.75 weedm^{-2}) and *Bromus inermis* (13.75 weedm^{-2}), respectively. The Oslavaalfalfa genotype showed the greatest diversity of weed species and density in the second year of the experiment (2019), with a mean density of 36.50 weeds per square meter. The Queen genotype showed the least amount of weed growth. The Nijagara (14.50 weed m^{-2}) had the highest number of perennial weed species. Oslava (13.50 weedm^{-2}) was the alfalfa genotype with the most winter weeds detected. The plots with the lowest winter weeds were those belonging to the Prosementi alfalfagenotype, while those with the lowest summer weeds were those belonging to the Ludelis alfalfa genotype (Table 6).

We found that alfalfa varieties significantly affected weed fresh and dry weight, as well as weed community composition. Furthermore, reduced weed biomass was associated with higher crop yields in the experimental area. Based on the fresh and dry weights of the weeds in the alfalfa cultivar plots, the average weights for 2018 and 2019 were similar. The weeds counts were also similar. Weeds were found to be fewest and heaviest in the Queen, Prosementi, and Costanza varieties. In contrast, Nijagara, Triade, Occitane, and Oslava varieties showed the highest fresh and dry weed weights. In general, other plots had similar fresh and dry weed weights (Table 6).

The highest green grass yield was obtained from the Corsa genotype, and the lowest green grass yield was obtained from the Triade genotype (Table 1). The highest hay yield was obtained from the Global genotype. Corsa, Prosementi and Quenn genotypes followed this order. The lowest hay yield was obtained from Triade and Nijagara genotypes. When the data values of these cultivars were compared with 2018 and 2019 weed number, fresh and dry weight analyzes, Corsa, Global, Prosementi and Queen cultivars emerged as highly competitive cultivars based on the data determined as high yielding.

Chemical constituents of alfalfa roots

Table 5. The phenolic acids content of extract from roots of genotypes of alfalfa (µg/g FW).

Genotypes	*p*-Hydroxybenzoic acid	Caffeic acid	Chlorogenic acid	*p*-Coumaric acid	Ferulic acid	Cinnamic acid	Totalphenolic acid	Saponin mg g^{-1} DM
Posementi	11.65 cd	3.95 de	18.74 bcd	4.51 def	3.70 b	1.25 ef	43.80ed	9.99 b
Toros	5.65 fg	6.67 a	21.91 ab	8.91 a	3.42 b	4.10 a	50.66cd	9.34 b
Ludelis	6.34 f	2.47 g	15.94 de	4.92 de	2.20 cd	1.39 def	33.26ı	8.09 bc
Triade	4.55 fg	3.46 ef	16.28 de	9.44 a	3.50 b	1.81 cde	39.04h	8.13 bc
Nijagara	11.22 de	4.89 cd	17.89 cd	5.14 cd	1.05 f	1.71 de	41.90g	8.57 bc
Ultra	11.45 d	3.94 de	14.11 e	6.55 b	1.70 de	1.88 cd	39.63h	6.75 c
Nardian	3.95 g	2.69 fg	17.63 cd	3.90 ef	1.85 cd	1.92 cd	31.94ı	8.40 bc
Osmanağa	10.90 de	5.17 b	16.98 cde	5.65 bcd	1.32 ef	2.35 c	42.37fg	8.97 b
Corsa	16.04 ab	7.38 a	23.69 a	3.38 fg	3.35 b	1.09 f	54.93b	14.14 a
Global	14.55 b	3.30 efg	21.62 ab	3.55 fg	2.26 c	3.42 b	48.70d	10.13 b
Oslava	13.71 bc	7.59 a	19.99 bc	3.49 fg	1.25 ef	3.35 b	49.38cd	8.93 b
Occitane	9.17 e	4.27 cde	21.39 ab	6.15 bc	2.08 cd	1.45 def	44.51 e	10.15 b
Costanza	16.44 a	7.70 a	21.74 ab	2.69 g	4.56 a	3.92 ab	57.05 a	14.85 a

Table 6. Weed fresh, and weed dry weight obtained from alfalfa genotypes.

Alfalfa genotypes	Weed fresh weight gha^{-1}		Weed dry weight g ha^{-1}	
	2018	2019	2018	2019
Queen	422,30 c	359,91 d	212,16 b	174,37 ef
Prosementi	446,65 c	202,11 d	232,25 b	82,17 g
Costanza	475,56 c	402,21 d	233,31 b	183,28 ef
Ludelis	525,46 c	443,28 d	233,32 b	199,20 ef
Global	592,13 c	488,86 d	234,42 b	188,83 ef
Corsa	677,88 c	604,35 cd	329,88 b	335,48 cd
Ultra	712,67 c	625,55 cd	334,45 b	274,40 de
Nardian	718,79 c	901,80 cd	386,61 ab	401,16 cd
Osmanağa	824,33 c	1138,82 bcd	418,81 ab	402,20 cd
Toros	855,14 c	1177,70 bcd	433,26 ab	403,29 cd
Triade	912,19 c	1384,39 bcd	486,64 ab	441,11 bc
Oslava	1112,88 bc	2086,67 abc	643,43 ab	562,17 b
Occitane	1679,95 ab	2495,45 ab	674,42 ab	754,40 a
Nijagara	2008,79 a	2768,80 a	1022,18 a	784,39 a
CV	0,000	0,000	0,000	0,000

Weed effects on crop yield: A second objective of our study was to determine whether weed responses were related to crop yield changes. It was expected that higher weed pressure would lead to a reduction in yield. There was indeed a negative correlation between total crop productivity and weed biomass and diversity in plots with lower weed biomass and diversity. By this basic principle, genotypes of alfalfa with allelopathic properties and high weed competitiveness give higher yields.

Discussion

This study examined the effects of different alfalfa (*Medicago sativa* L.) genotypes on weed suppression capacity, yield, and quality characteristics under organic cultivation conditions. The findings revealed significant differences among genotypes, suggesting that these variations stem from diverse genotypic, agronomic and ecophysiological factors.

The effects of different alfalfa genotypes on weed suppression capacity: Firstly, the results related to weed suppression capacity demonstrated that genetic diversity played a crucial role in this trait. Specifically, genotypes with broad leaf areas and rapid canopy coverage showed more effective competition against weeds. This observation aligned with previous studiesof Zimdahl (2004), Lazzaro *et al*., (2018), and MacLaren *et al*., (2019), who emphasized the impact of biomass production and leaf area index of various plant species on weed control. The data presented in Table 4 highlighted the variability in weed suppression capacity among different alfalfa genotypes under organic farming conditions, expressed as the average number of weed species (Weed/m²) in the experimental plots during 2018. This variability is influenced by both genetic traits of the alfalfa genotypes and their interaction with environmental factors, offering valuable insights for improving weed management strategies in organic systems.

Firstly, genotypes such as Prosementi, Costanza, and Nijagara exhibited higher weed species counts in several categories compared to others, suggesting that these genotypes might have relatively less competitive ability against weeds. This finding is consistent with the literature, which emphasizes the importance of canopy structure, growth vigor, and biomass production in suppressing weed growth (Zimdahl, 2004; Lazzaro *et al*., 2018), and MacLaren *et al*., (2019). On the other hand, genotypes like Queen and Corsa demonstrated lower weed species counts in several instances, indicating superior weed suppression potential, likely driven by rapid canopy coverage and dense leaf structure.

The significant variation in weed suppression capacity across genotypes, as seen with Global, Toros, and Triade, aligns with earlier research that emphasizes the critical role of genotype-specific traits under organic conditions. For instance, Global exhibited higher weed counts in certain weed species categories (ATXPA, PHRCO), possibly due to slower early-stage growth or less efficient ground coverage. This highlights the importance of selecting genotypes with traits tailored to outcompete weeds effectively under specific conditions.

Additionally, genotypes like *Oslava* and *Occitane*, which had moderate weed suppression levels, underline the role of environmental factors such as soil health and moisture availability. These genotypes showed effective competition against particular weed species (*CYPRO*, *LOLTE*), supporting the hypothesis that genotype-environment interactions can influence weed suppression performance (Drinkwater & Snapp, 2007).

The high weed species count observed in *Nijagara* and *Triade* across a broad range of weeds (*AMARE*, *AVEFA*, *SETVI*) suggests that these genotypes may require supplementary weed management practices in organic systems. This underscores the need for integrated approaches combining genotype selection with cultural and mechanical weed control measures.

Overall, the findings from Table 4 provide practical implications for organic farming. Genotypes such as *Queen*, *Corsa*, and *Ludelis*, with lower weed species densities, appear to be more suitable for organic systems where weed pressure is a critical concern. Future research should focus on exploring the underlying mechanisms contributing to weed suppression in these genotypes, such as allelopathic effects, root exudates, or improved nitrogen fixation. Furthermore, evaluating the long-term stability of these traits under varying environmental conditions will help refine genotype selection strategies for organic farming.

These results not only contribute to the broader understanding of weed suppression in alfalfa but also emphasize the necessity of considering genotype-specific traits as a cornerstone for sustainable weed management in organic agricultural systems.

Yield traits, the observed differences among genotypes and environmental conditions: Regarding yield traits, the observed differences among genotypes were closely associated with environmental conditions. (Kalkanlı & Başbağ, 2022). The importance of nitrogen fixation and soil microbial activity under organic farming conditions is well-documented (Drinkwater & Snapp, 2007). This finding corresponds with the study's conclusion that certain genotypes perform better under these conditions. Future studies may include detailed analyses of the symbiotic nitrogen fixation capacities of these genotypes.

Quality characteristics: Quality characteristics (e.g., crude protein content and digestibility) were influenced not only by the genetic structure of the genotypes but also by regional climate and soil conditions. Notably, certain genotypes with higher crude protein content may contribute to an increased feed value of these varieties. This outcome aligns with studies explaining the effects of the aforementioned environmental factoxrs (Lüscher *et al*., 2014; Sauvant *et al*., 2023).

The findings of this study provide potentially significant insights for strategies to improve weed control, yield, and quality under organic farming conditions. However, beyond genotype characteristics, factors such as soil health, microbial populations, and long-term environmental impacts must also be considered. Future research could focus on aligning genotype selection criteria with these factors.

Conclusions

This study showed that yield and quality characteristics of alfalfa cultivars were inversely associated with weed density. In the first planting year, when seedling alfalfa was not well established and had not developed a vigorous competitive relationship, weed competitiveness was on a higher side. Despite the increased competitiveness of perennial alfalfa plants in the following yearsplant genotype characteristics remained important in the integrated and organic management of alfalfa. Moreover, the findings of our study revealed that Corsa, Global, Prosementi, and Queen varieties outperformed the rest of the genotypes by yielding higher biomass yield, nutritional quality, and high competitiveness for weed suppression. These genotypes might be recommended to alfalfa growers for boosting the availability of quality forage to ensure sustainable productivity of milch animals.

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