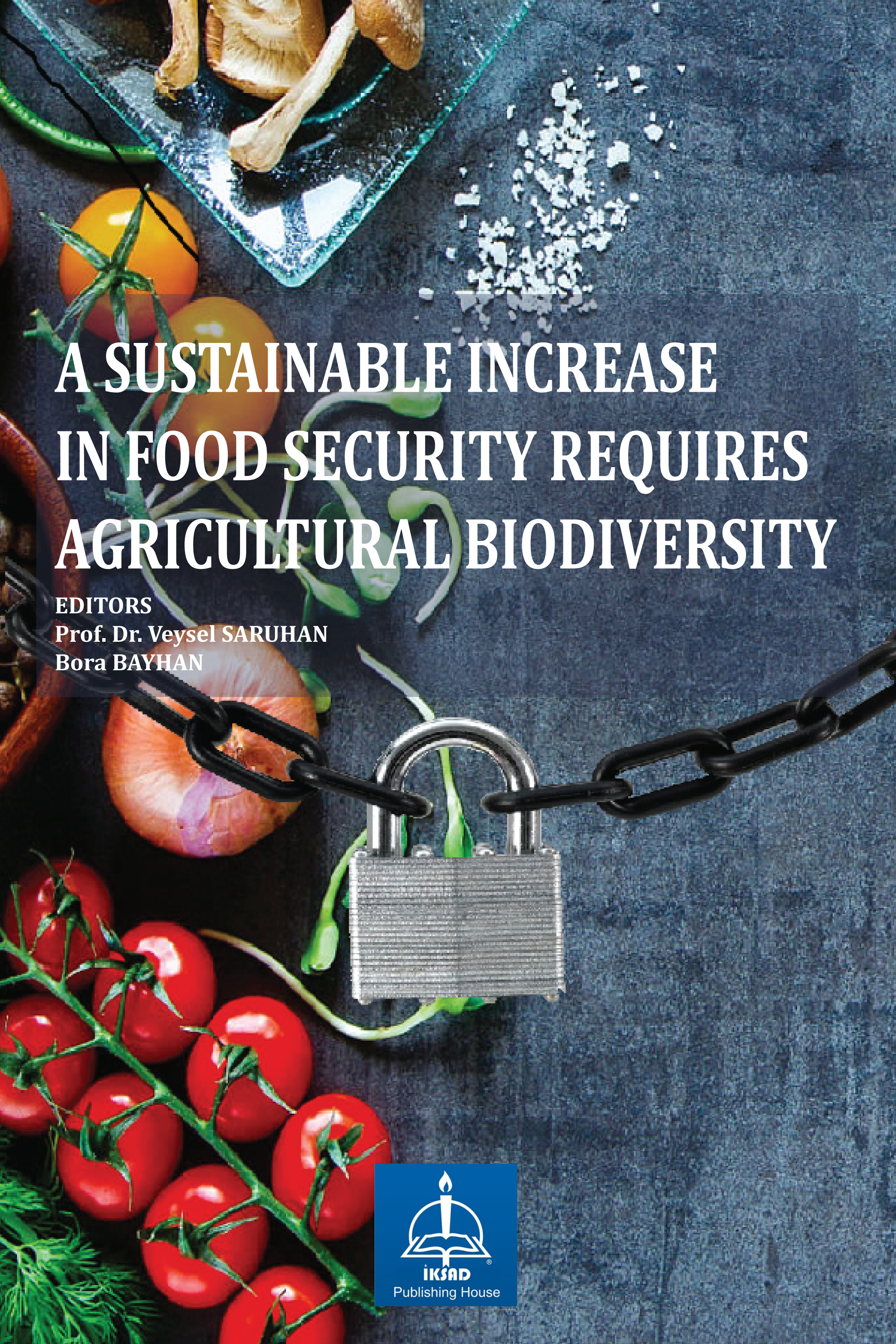

A SUSTAINABLE INCREASE IN FOOD SECURITY REQUIRES AGRICULTURAL BIODIVERSITY

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PREFACE

Millions of individuals worldwide are experiencing a change in their life due to breeding, agronomy, and transgenic techniques. The agricultural biodiversity and vital long-term sustainability are impacted by plant genetic resources with beneficial effects on nutrition, agronomy, and the economy. Our agricultural system is mainly concerned with improving grain yield and crop productivity; it is not intended to improve human health. Currently, agriculture is changing from producing more food crops in greater quantities to improving the quality of cereal, fodder, and oilseed crops. Poor people's lack of purchasing power, access to markets and healthcare systems, and lack of knowledge about the long-term health benefits are limitations. Humanity is being forced to take advantage of the chance by increasing production, converting biomass into energy and new products, intelligently intensifying marginal land, adopting tools for precise agriculture to help detect pollutant hotspots and poor soils, and evaluating specific interventions from an array of angles, including environmental, sociocultural, political, economic, ethical, and biomedical. Because the world population is rapidly growing , there is a continuing need for agricultural soils to produce more food.

CHAPTER 1

APPLİCATİONS OF BİOTECHNOLOGY TO FENUGREEK (*TRİGONELLA FOENUM-GRAECUM* L.) BREEDİNG FOR FORAGE

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1. INTRODUCTION

Using double-haploids, molecular markers (PCR-based markers, hybridization-based markers, markers based on sequencing), genome-wide selection, tissue culture applications for the genetic improvement of plants (somaclonal variation, mutagenesis, protoplast fusion, embryo rescue, production of double haploid lines, synthetic seed production, in vitro selection), development of transgenic plants are biotechnological methods used for breeding of crops worldwide.

Combining different parents throughout the hybridization process can result in hybrids with higher levels of heterosis and improved genetic profile. Assessing genetic diversity before developing hybrids can help to better utilize heterosis. An appealing alternative to the traditional diversity study, genetic variation assessment using molecular markers can also help manage and conserve biodiversity.

Fenugreek (*Trigonella foenum-graecum* L.) is an annual self-pollinating crop of *Leguminaceae* family well suited to growth in cool and temperate growing regions with low to moderate rainfall. Fenugreek is a medicinal plant, but also can be used as a forage crop for livestock feed. Application of modern techniques like molecular markers and functional genomics studies have significant potential to improve Fenugreek traits

Fenugreek (*Trigonella foenum-graecum* L.) is an annual dicot (*Magnoliopsida*) belonging to the *Leguminaceae* (*Fabaceae*) family of flowering plants, subfamily *Papilionaceae* (Petropoulos, 2002). It is a self-pollinating crop which is well suited to growth in cool and temperate growing regions with low to moderate rainfall (Thomas et al., 2011). Fenugreek is a medicinal plant that can lower blood glucose and cholesterol levels in animals, but also can be used as a forage crop for livestock feed (Thomas et al., 2011).

Fenugreek is an annual herb that grows to a height of about 50 cm. Its trifoliate leaves have oblong to lanceolate-shaped leaflets, and its flowers, which are often light yellow or white, are produced at the leaf axils. Pods have a pronounced beak and are long, straight, and flat. Small, golden-brown, oblong to rhomboidal, and grouped in batches of 10–20 within the pods are the seeds (Solorio-Sanchez et al., 2014).

Fenugreek forms symbiotic relationships with nitrogen fixing bacteria that can help replenish and maintain soil fertility on tropical agricultural lands by supplying nitrogen to the soil-plant system (Ravikumar & Anuradha, 1999).

2. Fenugreek forage

There is a long history of using fenugreek as animal feed (Petropoulos, 2002). Its scientific name, "foenum-graecum," which means as "Greek hay," suggests that the plant was used as a source of food by the ancient Greeks and Romans. Fenugreek is capable of producing excellent quality feed during all stages of its growth, according to field tests conducted in Canada. Diosgenin, a natural steroid found in fenugreek, can help calves gain weight and has the added benefit of keeping animals from bloating when fed a diet containing it. Over a 105-day period, steers fed either silage consisting entirely of alfalfa or fenugreek displayed comparable weight increase (Acharya et al. 2008).

Fig. 1. *Trigonella foenum-graecum*, A) Animal grazing on fenugreek plots in Alberta, Canada. B) Fenugreek crop (Basu et al., 2014).

Fenugreek, is a dryland adopted crop which can produce high quantity and quality forage and do not cause bloating among cattle. Both rainfed and irrigated environments are suitable for growing it as a cool season crop. In milder climates, the crop can also be cultivated in the summer. Fenugreek has the potential to reduce the use of artificial growth promoters and feed concentrates because it contains animal growth-promoting compounds that are not present in other competing forage legumes. A significant supply of protein, minerals, and vitamin C is in leaves (Billaud, 2001). Horses show a preference for feed flavored with fenugreek (Goodwin et al. 2005).

Fenugreek can be grown for direct grazing or as a fodder bank in a cut and carry system. It is palatable to cattle. In the tropics, diets for ruminant production often consist of low-quality roughages with a high proportion of neutral detergent fibers and few nitrogen or protein. Some of the problems raised on by low protein and high fiber diets can be resolved by including legumes in these animal production methods. Fenugreek can help animals increase weight consistently and produce high quantities of milk. Compared to conventional systems, using fenugreek may yield more milk per animal per hectare (Solorio-Sanchez et al., 2014). Fenugreek seed may improve beef cattle weight gain as well as dairy cattle milk production (Montgomery, 2009).

As part of its therapeutic effects, fenugreek is a significant source of soluble fiber and diosgenin (Basu et al. 2008). The occurrence of diosgenin in livestock feed can enhance carcass weight and as an annual legume it is easy to incorporate into short term rotations where perennial forage legumes do not fit. In Alberta, Tristar was the first forage fenugreek cultivar, developed for its capacity to continuously provide high biomass yields over time and space. In western Canada, this cultivar is best for producing silage and hay in large quantities, especially in areas with warm summers (Acharya et al., 2008). However, Tristar fenugreek is slow to mature under adverse conditions, making it difficult to obtain seed for ongoing commercial production. In recent years, researches has focused on use of a mutation breeding approach to identify new, early maturing fenugreek lines to help address this problem (Basu et al., 2009).

3. Toxicity of fenugreek

Sudanese chicks fed fenugreek showed pathological abnormalities in the liver and kidney, decreased body weight, and elevated uric acid concentrations in the blood, according to Nakhla et al. (1991). According to Panda et al. (1999), the inhibition of triiodothyronine synthesis in mice and rats led to a drop in body weight. Coumarin or its derivatives, which are anticoagulants that stop blood coagulation, are present in fenugreek formulations (Lambert and Cormier 2001). The use of fenugreek during pregnancy is also not advised since, according to animal research, the plant's saponins may promote uterine contractions, resulting in an early miscarriage of the fetus (Basch et al. 2003). Kassem et al. (2006) reported a potential anti-fertility effect of fenugreek in rabbits. Through a diet containing 30%

fenugreek seeds, these researchers reported a significant anti-fertility effect in female rabbits and a toxicity effect in male rabbits.

4. Biotechnology of fenugreek

The results of the first biotechnological investigation into fenugreek were published in 1945 and described how a fungicide of the triazole type called diniconazole affected fenugreek cell suspension cultures. Studies focusing on increased protein production and commercially significant metabolites including trigonelline, sapogenin, isoflavonoid pterocarpans, diosgenin, gitogenin, and tigogenin from callus, leaves, stems, and roots explants were conducted in its path employing cell suspension and callus culture. The utilization of callus, cotyloden, hypocotyls, shoot tip epicotyls, apical meristem, cotyledon node, and cotyledon leaf explants has been emphasized in studies on plant tissue culture. The majority of researchers agree that in vitro rooting is challenging. Studies on protoplasts utilizing root apices and leaf mesophyll have also been described. Root apices protoplasts exclusively produced cell colonies or roots, but leaf mesophyl protoplasts may be transformed into leafy shoots. *Agrobacterium rhizogenes* and *A. tumefaciens* are utilized in genetic transformation studies, but these studies are in their early phases, and the genes employed in the studies only show transformation with either marker or reporter genes. It appears that fenugreek plant biotechnology is still in its early stages of development and that there is still much work to be done on fenugreek breeding and enhancement (Aasim et al., 2014).

Improved Fenugreek features may come from the employing contemporary methods such in vitro artificial mutations, molecular marker characterisation, successful plant tissue culture techniques, genetic transformation methods, and functional genomics research (Aasim et al., 2018). The most widely used marker methods for studying plant diversity are RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeats), and SSR (Simple Sequence Repeat) (Tomar et al., 2014).

The most practical PCR-based markers are RAPD markers since they are quick, inexpensive, and don't involve radioactivity. RAPD is a PCR-based approach that uses a single primer with any nucleotide sequence to find sequence polymorphisms in DNA. The primary benefit of RAPD is its speed and simplicity of testing. Since PCR is involved, only small amounts of template DNA are needed for each reaction, typically 5–20 ng, and thus is more effective at producing many of markers for genomic study. The Inter-Simple Sequence Repeat (ISSR) PCR method for genotyping is quick and low-cost. ISSRs are DNA pieces of 100–3000 bp that are positioned between two neighboring microsatellite sections with opposite orientations. The main advantage of ISSRs is that no sequence data for primer construction are needed. This is mostly dominant marker. It is widely used for the characterization of genetic relatedness among populations (Tomar et al., 2014).

In the study by Shanthy & Shalini, (2011) a representative set of 61 accession of two species of fenugreek, group 1 (*T. foenum-graecum*) with 59 accessions and group 2 (*Trigonella corniculata*) with 2 accessions, were used for assessment of diversity with 18 random primers. With some modifications, the CTAB technique was used to extract the whole genome. With the aid of a master cycler gradient thermal cycler, PCR amplification was completed. 18 primers were used in total, and 141 bands were scored across all species and accession types, with 74 of those bands being polymorphic. This study demonstrated the value of RAPD markers for assessing the genetic diversity and relationships among several *Trigonella* species.

The study conducted Kumar et al., (2012) examined five common fenugreek types from India for DNA analysis. By comparing genotypes with 9 RAPD primers and performing a randomly amplified polymorphic DNA (RAPD) study, a total of 47 bands with an average polymorphism of 62.4% were generated. These bands ranged in size from 200 to 5000 bp. A total of 669 peaks in the size range of 50 to 538 bp were amplified using 17 fluorescently labeled AFLP primer combinations (PCs). Band counts ranged from 21 to 60 in total. There were a total of 25 variety-specific AFLP markers discovered in all the fenugreek cultivars. RAPD markers were shown to be more polymorphic in fenugreek than AFLP markers. RAPD markers also showed more diversity in comparison to AFLP markers, although the data generated for AFLP markers were more authenticated and reproducible than RAPD markers.

Based on SRAP markers, Amiriyan et al. (2019) examined the genetic diversity and population structure of 88 individuals from eight landraces of Iranian fenugreek. Six primers produced 72 bands, of which 56 (80.11%)

were polymorphs. Shiraz and Hamadan landraces experienced the least gene flow, while the most of it occurred between Mahallat and Varamin landraces. According to observations, SRAP is a reliable method for exposing the genetic diversity and population dynamics of Iranian fenugreek landraces.

Randhawa et al., (2012) used 186 inter-simple sequence repeat (ISSR) markers and 19 morphometric markers to determine the genetic relatedness of a sample of 49 accessions of fenugreek (*Trigonella-foenum-graecum* L.). An accession of *T. corniculata* was also assessed as an out-group. One of the accessions was brought in from Eritrea, and the others were gathered from various eco-geographical areas spread over the states of India. Data were gathered for 12 qualitative and 7 quantitative morphometric characteristics. All of the quantitative descriptors, with the exception of primary branches and seeds/pod, showed significant differences between the accessions. Only 21 out of a total of 100 ISSR primers used for first screening were determined to be polymorphic. ISSR analysis was performed with selected 21 primers to generate 186 amplicons, of which 92.4% were polymorphic. Cluster analysis put 47 accessions in a single group at ~65% similarity. Though there was no agreement between the groupings based on morphometric and ISSR markers.

For a crop like fenugreek, the development, identification, and classification of completely new sets of SSRs is a costly and time-consuming operation. Instead, transplanting the microsatellite loci of the related species into fenugreek can easily and affordably produce a fresh collection of SSRs. SSRs of two important *Apiaceae* family plants, carrot and celery, are available in the public domain for cross-species and inter-family research. Jethra et al., (2020) evaluated the transferability of 100 carrot SSR loci in fenugreek, where 19% of the SSRs amplified and 5 primers were monomorphic. 14 SSRs with bands ranging from 2 to 5 were polymorphic in character. Four monomorphic bands in all were genotype-specific for fenugreek and can be seen as the main tool for fenugreek breeding and genetic research. The most polymorphic GSSRs, however, were GSSR81 and GSSR-96, which can be used to further identify fenugreek from other seed spices. The potential of carrot SSRs to be transferred into fenugreek genotypes suggested that the found markers might also be used to further develop molecular breeding strategies for less well-researched crops.

For nine cultivars of fenugreek (*Trigonella foenum-graecum* L.), measurements of in situ nuclear DNA content, karyotype analysis, and somatic chromosome number (2n = 16) were made, revealing cultivar-specific chromosomal traits and minute structural changes in chromosomes of the genome. From 3.12 to 4.36 picograms, there was a substantial range in the amount of 4C DNA. Significant variations were also observed in total chromosome length, total chromosome volume, and total form percentage at the cultivar level. At the cultivar level, correlation coefficients revealed a relationship between chromosomal volume and length. Variations in genomic structure and the nuclear DNA content of fenugreek cultivars, despite the same somatic chromosome number, suggest a genetic drift among the cultivars.

Using the random amplified polymorphic DNA (RAPD)-PCR technique, Al-Yasiri et al. (2021) assessed the genetic polymorphisms in fenugreek plants after treating fenugreek seeds with various combinations of biotic and abiotic agents. On the development characteristics of fenugreek plants, researchers examined the impacts of two strains of the fungus *Trichoderma harzianum* (Th-1 and Th-2), methyl jasmonate (MeJA), and aloe vera gel (AVG). Fenugreek root length, shoot length, shoot fresh weight, number of true leaves, and chlorophyll content were all considerably increased by combinations of Th-1, MeJA, and AVG. On the other hand, the Th-2 isolate greatly reduced plant growth (except for root length which was not affected significantly). Contrarily, when combined with MeJA, all growth metrics showed little to no influence, however when combined with VAG, shoot height and chlorophyll content significantly decreased in comparison to unaffected growth parameters. The current study has demonstrated that 62 DNA fragments that could be scored in all genotypes were generated by the PCR amplification of DNA employing five primers for RAPD analysis. There were 26 polymorphic bands in all, with an average polymorphism percentage of 54.2%. The findings of the RAPD-PCR test demonstrated that fenugreek seeds treated with Th-1 either alone or in conjunction with MeJA and AVG resulted with polymorphisms in the plant's leaves.

Methyl jasmonate, cholesterol, and squalene were used by Ciura et al. (2018) to induce changes in the fenugreek transcriptome linked to increased steroid synthesis, which were then detected using RNA-seq. After de novo assembling of the next generation sequencing data, 112,850 unigenes were obtained and used for functional annotations. Transcripts involved in the synthesis of mevalonate, terpenoid backbones, and plant sterols were annotated in the steroidal saponins pathway. Quantitative RT-PCR was used to confirm that numerous phytosterol biosynthetic genes were overexpressed. Fatty acid hydroxylase and steroid 22-alpha-hydroxylase genes were identified in all stimulated transcriptomes as part of the diosgenin production pathway.

For representational difference analysis of cDNA, Ciura et al., (2017) used methyl jasmonate elicitation and precursor feeding (cholesterol and squalene) to increase the amount of sterols and steroidal sapogenins in in vitro grown plants (cDNA-RDA). Differential, factor-specific libraries were subjected to next-generation sequencing in order to find potential diosgenin biosynthesis candidate genes. A total of 9.9 million reads were collected, trimmed, and assembled into 31,491 unigenes, each of which had a 291 bp average length. Then, by aligning all-unigenes with public databases, functional annotation and gene ontogeny enrichment analysis were carried out. We identified novel candidate genes for diosgenin biosynthesis among the transcripts associated to sterol and steroidal saponin synthesis, and researchers verified their expression using quantitative RT-PCR analysis. Representational difference analysis of cDNA was performed and differential products were sequenced and annotated. Candidate genes involved in biosynthesis of diosgenin in fenugreek were identified. Detailed mechanism of diosgenin synthesis was proposed.

Gujarat Methi Variety 2 was chosen for the transcritome study out of the three developmental stages that were sequenced by the genome sequencing machine in the study of Shubha & Tomar, (2019) which were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS), and maturity stage (80-90 DAS). A total of 59,306 assembled reads from the vegetative stage were produced by the de novo assembly. 30,648,677 assembled readings from the reproductive stage were obtained using assembly. 16,924,096 assembled readings were produced at the maturity stage.

RAPD and ISSR are useful for determining genetic relationships between populations as well as for accessing the genetic diversity of uncommon and endemic species. Tomar et al. (2014) used RAPD (Random amplified polymorphic DNA) and ISSR (Inter-simple sequence repeat) markers to assess the genetic diversity of thirty fenugreek genotypes. To find genetic variation, a total of 30 RAPD primers and 20 ISSR primers were employed. When compared RAPD with ISSR, RAPD showed a higher percentage of polymorphic bands (76.78%) than ISSR (68.08%). Genotypes are organized into two major groups, as seen by the dendrogram formed by RAPD and ISSR analysis. The AM-329 and AFG-5 comparison showed the most similarity (94%) in the RAPD study. The minimum degree of similarity between AFG-6 and other types was discovered to be 46%. In terms of ISSR analysis, AM-326 and CO-2 as well as AM-202 and Hissarsuvarna shared the highest genetic similarity (89%). The AM-288 genotype and the other genotypes shared the least similarities. The results showed that RAPD and ISSR marker systems, either separately or in combination, can be used to identify the genetic relationships among fenugreek. Ample genetic variation among the genotypes was revealed by molecular marker study of 30 fenugreek genotypes by RAPD and ISSR, which was otherwise not achievable by morphological analysis of these genotypes.

5. Conclusions

The improvement of Fenugreek features may greatly benefit from the use of methodologies such as in vitro manufactured mutations, molecular marker characterisation, successful plant tissue culture techniques, genetic transformation techniques, and functional genomics investigations. The most practical PCR-based markers are RAPD (inexpensive and don't require radioactivity) and Inter-Simple Sequence Repeat (ISSR) PCR method for genotyping (fast and low-cost). RAPD and ISSR marker systems, either separately or in combination, can be used to identify the genetic relationships among fenugreek.

In order to study fenugreek for forage and feed (seeds) purposes, improvements in biomass production, cut number, vegetative regrowth, nitrogen fixing capacity, drought tolerance, and quality criteria including the reduction of toxicity to animals may be key priorities.

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CHAPTER 2

FOOD GRADE OİL QUALİTY OF SUNFLOWER (*HELİANTHUS ANNUUS* L.)

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1.INTRODUCTION

Sunflower (*Helianthus annuus* L.) is among the most cultivated oil crops globally (Flagella et al., 2002). Sunflower as major annual globalwide edible oil crop has also meal as a source of protein for human consumption (Haddadi et al., 2011). Sunflower is grown mainly for edible oil purpose. Its oil content and quality are desirable due to polyunsaturated fatty acids (oleic and linoleic acids) and saturated fatty acids (palmitic and stearic acid). Oils containing more unsaturated fatty acids reduce the cholesterol levels in human body. High oleic sunflower oil is highly effective in preventing cardiovascular diseases and has higher oxidative stability than many other cooking oils (Nagarathna et al., 2011). Coronary artery diseases are closely related to lipids (Vijayakumar et al., 2016). Classical sunflower varieties have high linoleic acid content in seeds and they are called low oleic (LO) varieties (Lacombe et al., 2009). High oleic sunflower oil can be used as a cooking oil for deep fat frying of potato chips and other fast foods (Urie, 1985). Demand to high oleic sunflower oil is high in the market than standard sunflower oil (Dimitrijevic et al., 2017).

Seed oil content and composition are very important subjects in sunflower breeding programmes as these traits are related to oil quality. These are complex traits determined by genotype and environment (Van Der Merwe et al., 2013). In sunflower, high oleic acid content trait is mostly controlled by dominant alleles at a major gene (Ol gene) with other modifiers (Premnath et al., 2016). Treatment of normal sunflower varieties with chemical mutagens was resulted with hydrids with high oleic acid (80%) and low linoleic acid (10%) contents (Purdy, 1986). High oleic fatty acid composition is less susceptible to oxidative changes during refining, storage, and frying and therefore, its quality is retained longer than high linoleic fatty acid containing sunflower (Miller et al., 1987).

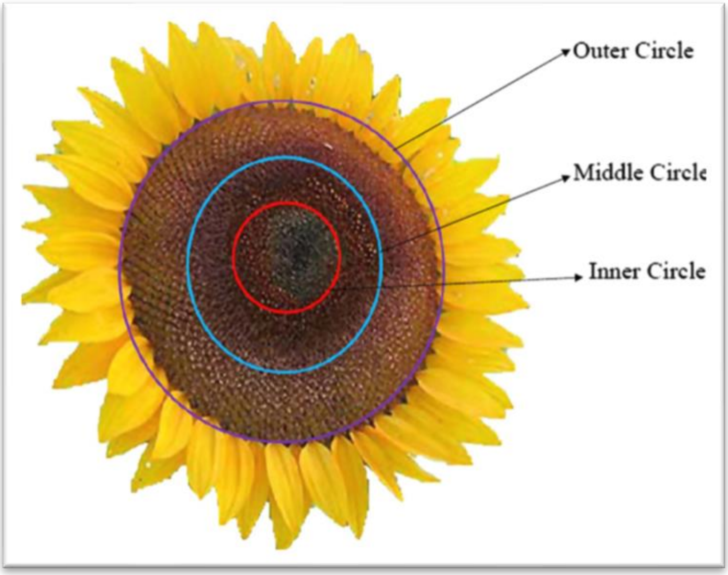

Fig. 1. Quality circles of sunflower head: The outer circle accumulates high oil and oleic acid contents which decrease to a minimum in the central circle. However, linoleic acid consistently increases from outer to central circle (Kaleem & Ahmad, 2011).

Tocopherols are valuable fat soluble antioxidants family for nutritional and technological properties of seed oils. The four naturally occurring tocopherols (alpha, beta, gamma and delta) differ for their relative in vivo vitamin E and in vitro antioxidant properties. Sunflower seeds mainly contain alpha-tocopherol (95% of the total tocopherols) (Vera-Ruiz et al., 2006). Sunflower lines that accumulate increased levels of beta- tocopherol, gamma- tocopherol and delta-tocopherol in seeds as well as lines with reduced and increased total seed tocopherol content have been developed. These lines also have increased levels of these tocopherols in leaves, roots and pollen (Del Moral et al., 2012).

Supplementation of ruminant diets with plant oils and marine lipids is an effective strategy for lowering saturated fatty acid content and increasing the concentration of cis-9,trans-11 conjugated linoleic acid and long-chain n-3 fatty acid in ruminant milk (Toral et al., 2012). Rumen microbial biohydrogenation of dietary unsaturated fatty acids has a major effect on the process of developing healthier dairy products (Belenguer et al., 2010).

2. High stearic and high oleic sunflower varieties

Palm oil is widely used in the food industry due to its lower cost, high oxidative stability index, long shelf-life and replacement of trans fats. However, palm oil production damages biodiversity (deforestation, loss of natural habitat, rendering many critically endangered species such as Sumatran orangutans, elephants, and tigers), damages ecosystem (decomposition and burning carbon-rich soil in deep peatlands is increasing carbon emissions). It also makes health risks to humans (excessive consumption of palmitic acid and other saturated fats except stearic acid increases bad cholesterol and the risk of cardiovascular diseases). Development of sunflower varieties with oils including high stearic (~18%) and high oleic (~70%) acids makes them healthy and sustainable alternatives to palm oil. High-stearic-high-oleic sunflower crops can have high grain yields (~4 t/ha), oil yield (~1,6 t/ha), oleic acid yield (~73%) and stearic acid yield (~21%). High-stearic-high-oleic oils obtained from mutant and hybrid sunflower cultivars also have higher oxidative stability index and therefore have better stability, quality, and functionality than regular sunflower oil. For example, the oxidative stability index of commercially available Nutrisun at 110°C is six times greater than that of regular sunflower oil. Recent advances made several mutant and hybrid cultivars with high grain and oil yield and high levels of stearic and oleic acids available. Natural healthy high-stearic-high-oleic sunflower oil can now be grown in a sustainable manner without damaging ecosystem as in palm oil cultivation (Anushree et al., 2017).

Fractionation of fats and oils makes generate products with specific natural fats containing different triacylglycerol species. High-oleic-high-stearic sunflower oils contain high levels of saturated fatty acids, mainly stearate, on a high-oleic background (Bootello et al., 2011). During the frying process, oxidation, hydrolysis, polymerization, isomerization and cyclization occur. Cyclic fatty acid monomers are potentially toxic and are detected in used frying oils (Romero et al., 2006). The combination of seed oil phenotypes of sunflower with high oleic acid (C18:1), which imparts a higher oxidative stability to the oil and health benefits, and high stearic acid (C18:0), which gives greater solidity, would result in a novel oil quality of great value for the food industry (Perez-Vich et al., 2000). High stearic-high oleic sunflower oil presents high thermal stability. This oil is an alternative to the hydrogenation process which produces trans fatty acids (Martinez et al., 2012).

CSIC (Instituto de la Grasa of Sevilla, Spain) and Advanta Seeds Nutrisun Business Unit (Buenos Aires, Argentina) jointly developed hybrid high-stearic and high-oleic sunflower oil to be used as fat for food applications. The main difference between fatty acid profiles of hybrid high-stearic and high-oleic sunflower oil and regular sunflower oil was in the stearic acid content, which was four times greater in hybrid high-stearic and high-oleic sunflower oil than in regular sunflower oil. Hybrid high-stearic and high-oleic sunflower oil offers significant advantages when used in different types of foods, including increased shelf life and much higher yields in fast food and industrial frying compared to any other fat. Hybrid high-stearic and high-oleic sunflower oil is a healthy alternative to partially hydrogenated oils that were rich in trans fatty acid, along with other animal and vegetable fats, such as palm, which had fatty acid contents that raised cholesterol blood levels and led to increased risk of cardiovascular disease (Dubinsky & Garces Mancheno, 2011).

Genetic control of stearic acid (C18:0) and oleic acid (C18:1) synthesis in the sunflower seed oil was studied through candidate-gene and QTL analysis by Perez-Vich et al., (2002). Results provide strong support about the role of fatty acid desaturase genes in determining fatty acid composition in the seed oil of sunflower.

The oxidation of bulk and encapsulated high-oleic sunflower oil was evaluated by Belingheri et al., (2015). Encapsulation modified the oxidation kinetics of oil exposed to heat and light. Light was the main factor determining increases of peroxide values in the oils. Encapsulation reduced the effect of light on the rise of conjugated dienes in the oil. Plating flavor oils onto porous starch was found to be a valid alternative to spray drying.

3. Effect of Agronomy

Sunflower is moderately tolerant to salt stress and currently cultivated in dry areas where salinity is a threat. In the study of Flagella et al., (2004), significant differences were observed for oleic and linoleic acids among saline treatments. Oleic acid showed an increase from 83% in the control to 87% at the highest salinity treatment. Inversely, for linoleic acid a progressive decrease from 6.9 to 2.8% with increasing salinity level was observed.

Jalilian et al., (2012) determined that the existence of a water deficit strikingly decreased the total oil, oleic acid and linoleic acid concentrations of the seeds. However, it increased the protein, palmitic acid, and stearic acid in sunflower s eeds.

Ebrahimian et al., (2019) determined that severe lack of irrigation water availability significantly reduced oil percentage in sunflower. A significant reduction in oleic acid, linoleic acid, linolenic acid and palmitic acid content due to low availability of irrigation water was also observed.

High oleic hybrids of sunflower are widely cultivated around the world. Mean oleic acid percentage and its stability across environments differed among hybrids (Angeloni et al., 2017). The oil industry demands sunflower oils with high oleic acid content. New varieties producing high oleic oils independently of the growing environment are needed as growers could receive an extra prime for offering them. Oil fatty acid composition of high oleic sunflower hybrids currently available carrying the Pervenets mutation could however be affected by the temperature during the grain filling period (Alberio et al., 2016).

4. Refining

Sunflower oil is an important ingredient due to high beneficial and nutritional value (Rai et al., 2016). The consumption of health-promoting products such as cold pressed oils may improve human health and prevent certain diseases (Ramadan, 2013).

Crude pressed sunflower oil obtained from a local oil mill was refined by Pal et al., (2015) by using chemical methods (degumming, neutralization, bleaching and dewaxing). Reduction in phosphorous content from 6.15 to 0 ppm, FFA content from 1.1 to 0.24 % (oleic acid), peroxide value from 22.5 to 7.9 meq/kg, wax content from 1,420 to 200 ppm and colour absorbance value from 0.149 to 0.079 were observed from crude to refined oil. Refining did not have significant effect on fatty acid compositions.

The changes of total and individual tocopherols were investigated during different sunflower oil processing stages by Naz et al., (2011). Total and individual tocopherol content levels decreased during the neutralization, bleaching, and deodorization processes. Total loss of total tocopherols during these stages was 37.9%, although the general reduction trend of delta, gamma, and alpha tocopherols is very similar during neutralization (35.3%), bleaching (38.3%), and deodorization (37.8%). Most parts of the tocopherols were wasted during processing.

Sunil et al., (2015) incorporated synthetic antioxidants such as tertiary butyl hydroquinone in sunflower oil to make it stable. Also natural antioxidants like oryzanol and tocopherols can be used.

5. Thin film material

There is an increasing environmental concern on the use of polymer as they tend to persist in the environment for largely being non-biodegradable and for their main dependence on petroleum resources. The 50% of plastic production are comprises of light weight, single use product and packaging materials. They are not disposed properly after use and get heaped up that are polluting the environment worldwide. Sunflower oil based biodegradable advanced materials with hyperbranched architecture has drawn tremendous attraction in recent years (Das et al., 2013).

6. Conclusions

Sunflower is among the most cultivated oil crops globally and is grown mainly for edible oil purposes. Classical sunflower varieties have high linoleic acid content in seeds and they are called low oleic varieties. Demand to high oleic sunflower oil is high in the market than standard sunflower oil. High oleic fatty acid composition is less susceptible to oxidative changes during refining, storage, and frying and is highly effective in preventing cardiovascular diseases. Sunflower seeds mainly contain alpha-tocopherol. Development of sunflower varieties with oils including high stearic and high oleic acids improved sunflower oil from health approach and added greater solidity which resulted in a novel oil quality for the food industry.

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CHAPTER 3

BIOLOGICAL SOIL REGULATORS; SOIL-FRIENDLY WORMS

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INTRODUCTION

While agriculture, which is indispensable in vital activities, was done with primitive methods in the past, with the rapid development of technology and the increase in the world population, the use of chemicals has increased, and all the possibilities of technology have been used in agricultural areas in order to get the most product with the least input. In the late 1970s, the negative effects of mechanized agriculture/traditional agriculture began to be understood among the people, and as a result of excessive use of chemical fertilizers, carcinogenic residues were found in water resources, human and animal foods (Chernyak et al., 1996). In the 1980s and 1990s, it was noticed that the intensive use of agro-chemicals and monoculture production encouraged by traditional agriculture negatively affected the natural fauna and flora balance of the soil and accelerated the infertility process of the soil (Fushiwaki, 1990; Chen et al., 2001). For this reason, research on new approaches in agricultural production that respects the natural balance and gives the opportunity to renew itself has gained momentum, and these researches have revealed new approaches in agricultural production, which are expressed in terms of "sustainable" or "organic" (Erşahin, 2007).

In addition to all kinds of food products, organic agriculture is produced and consumed with organic certificate in thousands of non-food products, from toys to furniture, from textile products to toothpaste and from cut flowers to make-up materials. While consumers used to consume organic products only to be healthy in the past, they now consume them to protect both health and the environment. People all over the world are turning to organic agriculture in order to maintain a healthy and happy life by consuming or using healthy and clean food and non-food products that do not contain chemical residues for a healthy and clean environment. It is very difficult to fight the problems caused by the consumption of chemicals. The increase in chemical consumption day by day causes great concerns for the future of humanity and the safety of limited living spaces. For this reason, people make an intense effort to create areas that can be safe for the environment and human health in the selection of settlements and food production. In this respect, it is very important to develop agricultural control techniques that are compatible with the natural environment and safe for human and environmental health (Azizoğlu et al., 2012). In the 1980s, using aerobic compost as a soil conditioner to increase soil organic matter and being an economical, sustainable and environmentally friendly alternative to the treatment of urban waste and waste, which has become an important environmental problem in parallel with the urbanization level of compost, are among the reasons for the rapid spread of compost applications. Dominguez et al., (1997) vermicompost; They stated that the use of wormy (mesophilic) compost has superior properties than aerobic compost on the basis of process and product in the recovery of urban and industrial organic waste. Vermicompost is obtained in a much shorter time than thermophilic compost. In terms of product quality, vermicompost products have physically, chemically and biologically superior qualities and economic value than thermophilic compost products. At the same time, since there are no pathogens threatening human health in the vermicompost end product, applicators can even touch the vermicompost with bare hands, even if the main material is sewage waste.

1. WHAT IS VERMICOMPOST?

Vermicomposting (vermicompost/biohumus) is the process of decomposing organic wastes of plant and animal origin into building blocks by processing by earthworms and beneficial microorganisms. Worms that live and feed in the environment they live in pass the body fluids (coelom fluid) that are on them and in their digestive systems and protect them, to the fertilizer they create. The coelom liquid that passes into the fertilizer provides a very good resistance to pathogens in plants. In the digestive systems of worms, there are micro-organisms useful for many plants, bacteria, mycorrhizal fungi, natural growth hormones and enzymes that will create an antibiotic effect. Enzymes and plant nutrients that pass into the fertilizer help plants to achieve rapid and healthy development and increase the yield of plants (Anonymous, 2016). Microbial population and activity growth are the main factors influencing the nitrogen cycle, the production of plant growth regulating substances, as well as the rate of resistance to plant pathogens and nematode attacks (Arancon et al., 2005; Figure 1).

Figure 1. Vermicompost overview (Anoniymus, 2023)

Earthworms, while improving the physical properties of the soil, also increase the microbial activity in the soil, accelerate the decomposition of organic materials, and contribute to the formation of humus. Earthworms balance the soil temperature and keep moisture at optimal levels. While it is observed that their activities increase in the spring months when there is a lot of precipitation, they go deeper and their activities decrease in the summer months when the soil is dry (Yıldız et al., 2005). Earthworms are of great importance for soil fertility. Earthworms work at night to carry out their effective activities. They feed by carrying the plant residues that have been thrown into the soil and started to rot during the night they work, to the channels they have opened in the soil at night. As a result of digesting these plant wastes, humus is formed in their feces and this humus increases soil fertility. An average of 400 earthworms in one m2 of garden soil make 2.5 kg of humus in a year. Earthworms also aerate the soil thanks to the new channels they continuously open in the soil, while allowing rainwater to accumulate (Demir et al., 2010).

Stable vermicompost, a new form of organic soil conditioner in crop production, is a fine-textured peat-like material with high porosity, aeration, drainage, water holding capacity and low C/N ratio (Edwards, 1998). Vermicompost contains nutrients such as nitrate, exchangeable calcium, phosphorus and water-soluble potassium that can be easily taken up by plants. Vermicompost has a large surface area and thus creates many microsites for microbial activities, and also makes the persistence of nutrients more strongly. It is also stated that vermicompost has superior biological properties and has a larger and more diverse microbial population compared to conventional thermophilic compost (Edwards, 1998). Worm compost (vermicomposting) is the composting process of organic waste / residues by worms. Although the term vermicompost is used for the product obtained as a result of the composting process of organic residues and/or wastes using earthworms, the vermicompost product is generally called vermicompost (worm excrement; manure) or simply cut (Edwards & Bohlen, 1996).

Nutrients contained in vermicestine, surrounded by worm mucus, are released slowly and are in a form that can be used immediately by the plant. Since these nutrients dissolve slowly, there is no loss of nutrients as a result of leakage. In addition, the porous, high aeration and water holding capacity of vermicast makes it an excellent soil "regulator". In addition to these properties, this material protects plant roots from extreme temperatures, reduces erosion and weed growth. Vermikest is odorless, does not contain pathogens or chemicals that can harm human health, and contains 100% reusable materials. Vermikest is the most perfect mixing material imaginable as greenhouse and potting soil. These positive effects were observed in both horticultural and field crops. Even the most sensitive plants do not have a burning effect and all nutrients are water soluble. When used as mulch, nutrients reach the plant root directly with irrigation. Most enriching potting mix materials lose their nutrients within 2-3 days. Vermikest, on the other hand, preserves its function as a food source in the pot for 6 times longer than the aforementioned materials. Therefore, for the same amount of potting soil, 5 times less vermix than other mixes will be sufficient. Also, vermicest is less expensive and works better than other commercial potting mixes. Since vermikest can hold 2-3 times its weight in water, there is less irrigation cost. It does not burn plant roots like chemical fertilizers (Anonymous, 1992).

2. SPECIES OF WORMS USED IN THE VERMICOMPOST PROCESS

The rapid increase in the world population has led to the pressure of agricultural areas and the importance of new approaches in plant production. The limited agricultural areas require higher yields from the unit area in order to meet the food needs of the increasing population. In agricultural areas, chemical fertilizers are mostly applied to increase the yield per unit area. The use of agrochemicals, which have been used extensively recently, which puts human health and environmental safety at risk, has led to a decrease in soil quality and an increase in pathogen resistance, which has led to serious concerns about the safety of natural resources. Recent studies have led to the development of sustainable agricultural production systems targeting the use of effective organic products as biological fertilizers and pesticides (Erşahin, 2007). In agricultural production, the amount of organic matter is an important factor in sustainability and obtaining high yield from the plant, protecting and improving soil fertility (Önal et al., 2003). At the same time, the fertility of the soil is closely related to the living things in it. One of the living species found in the soil is undoubtedly earthworms. The use of organic materials in agricultural areas improves the physical properties of the soil, while it is an environmentally friendly method for the disposal of waste (Nazli et al., 2016). Vermicompost, which is produced by worms and has become increasingly popular in our country in recent years, is an organic material used as a soil conditioner and fertilizer (Kıran, 2019).

In vermicompost technology, *Eisenia fetida* (tiger worm), *Eisenia andrei (*red tiger worm), *Dendrobaena veneta, Lumbricus rubellus* (red worm), *Perionyx excavatus* (Indian blue worm), *Eudrilus eugeniae* (African nightcrawler), *Fletcherodrilus* spp. types are generally used. *E. fetida, E. andrei, D. veneta* species are well adapted to temperate climate zones, *while L. rubellus* and *P. excavatus* are more common in warm tropical climates. These five species are the species that give the best results in vermicompost studies to reduce organic waste/residues (Edwards & Bohlen, 1996).

Among the species listed above, *Eisenia* spp. and *Lumbricus rubellus* are the most preferred species in commercial vermiculture/vermicompost enterprises (Dickerson, 2004). There are many reasons why *Eisenia* spp is the most preferred species. This species consumes food faster than other species and has higher reproduction and population growth rates, can live in environments with sufficient nutrient content, have a high capacity to consume existing food and reproduce, adapt to very different climate and environmental conditions, suitable environmental conditions and easily accessible It has been the most preferred species for reasons such as rapid population growth if sufficient food sources are available (Edwards & Bohlen, 1996). For these reasons, *Eisenia* spp is the most preferred and most cultivated earthworm species in commercial or non-commercial vermicompost businesses all over the world, especially in geographies in the temperate climate zone.

Especially Red California worms are used in compost (vermicompost) production. There are approximately 115.000 earthworms that can rework more than 1.2 tons of soil in one decare area (Özer & Elibüyük, 2006). Red California Worms are generally fed with fermented cattle manure as food on large farms. They can live an average of 5 years under favorable conditions. 1000 worms produce an average of half a kilo of fertilizer per day. The ideal temperature for the production and reproduction of worms is 20°C. Red California worms can survive at 0°C-40°C. Worms can double their numbers in an average of three to four months. They reproduce by laying eggs, and a worm lays about 1500 eggs per year, with 1 to 21 worms hatching from each egg. Their eggs resemble lemons and are the size of an average lentil grain. Adult worms can reproduce after about three to four months, increasing their numbers up to 20 times under favorable conditions.

In studies conducted by scientists to identify earthworms, it is stated that worms can be cultured and manure (vermicompost) can be obtained from their feces, and this can be used in vegetable and field agriculture. is an example. In parallel with the increase in yield and quality with the use of organic fertilizers, there is a significant increase in the organic matter and water holding capacity of the soil.

3. THE PLACE OF VERMITECOLOGY IN THE CONCEPT OF SUSTAINABILITY IN AGRICULTURE

The increase in industrialization in the world has increased the applications of chemical fertilizers and pesticides and has made the soil, environment and human health threatening as a result of their unconscious use. Vermicompost applications have become an option in order to increase the amount of soil organic matter and improve the soil structure. Plant nutrients (N, P, K) in vermicompost can be taken directly by the plant (Şimşek, 2007). Vermicompost is obtained as a result of the digestion of organic wastes by earthworms (*Eisenia Foetida, Lumbricus Rubellus*) (Mısırlıoğlu, 2011). Vermicompost, which is used as plant nutrition and soil conditioner, is a fertilizer rich in plant nutrients, microorganisms, organic matter, humic and fulvic acids (Özkan et al., 2016).

One of the most important advantages of economical, environmentally friendly and sustainable vermitechnology over traditional agricultural methods is that it supports low-input production model. In this respect, vermitechnology has a very high applicability and economic profit for small and medium-sized agricultural enterprises. These technologies, especially vermicompost, transform the waste/waste class materials formed in the agricultural production process into a product with a very high commercial value. Thus, the resources spent on agricultural fertilizers and pesticides, which are very important in traditional production, remain within the enterprise. Reducing the input cost at the beginning of production makes the producer profitable at the first stage of production. This is a very important feature that alleviates the risk of yield decline observed in the first years of transition from traditional farming to organic farming methods. Vermicompost aims to bring these functions of worms, which perform macro and micro nutrient conversion in nature, to the highest level of physical and biochemical efficiency. Today, vermicompost is the method that provides the highest economic benefit among the methods that support the sustainability feature in agriculture, but it is also very intensely applied in the processing of solid organic wastes and residues, which have become a major environmental problem with rapid industrial development and population growth. The vermicompost technique, which provides high value products both commercially and ecologically, is extensively applied all over the world (Erşahin, 2007).

4. STUDIES ON USAGE AREAS OF VERMICOMPOST PRODUCTS

The plant-feeding effect of vermicest was first reported by Fosgate & Babb (1972). Vermicompost has been described as an “excellent organic fertilizer” because it provides yield increase in many agricultural plants. In addition to the high levels of macro and micronutrient content in the emergence of the extraordinary positive effect of vermicompost, it is thought that significant amount of worm secretions is an important factor in the emergence of this effect. The use of chemical fertilizers has brought about physical, chemical and biological problems in soils. Many environmental problems that have occurred have led people away from chemical fertilizers and towards organic fertilizers by considering sustainable soil fertility. It is necessary to reduce environmental pollution, which is one of the biggest problems in the world, and to maintain the balance of the ecosystem. In addition to being a source of nutrients for plants, organic fertilizers protect the natural balance without polluting the environment, increase the yield and quality in plant production, and fulfill their soil conditioner duties. In order to protect the soil ecosystem and increase sustainable agricultural productivity, to increase the use of organic fertilizers, to ensure that agriculture is sustainable and to make accurate plans for the future, first of all, to know and know the soil very well, to determine its physical, chemical and biological properties correctly and to take into account the determined characteristics. Fertilization, which has an important place among the measures, should be done correctly (Bellitürk, 2011). Vermicompost is a material that has become very common in agricultural production in recent years and is an important environmentally friendly organic fertilizer obtained by digesting many organic materials by earthworms (Şimşek & Erşahin 2007). It has been determined that vermicompost applied at different rates has a significant effect on the development of curly lettuce placed in open field conditions and on the earliness feature (Hınıslı, 2014). It has been determined that the amount of phosphorus (P) taken by the vine seedlings applied in different doses of vermicompost and graft combination applied to the soil, peat and perlite mixture in equal amounts increases (Açıkbaş, 2016). It was determined that vermicompost applied at different doses to the sunflower plant caused significant increases on yield, and according to the plant analysis results, the amount of phosphorus increased in parallel with the increasing doses of vermicompost (Büyükfiliz, 2016). Vermicompost positively affects the development and soil properties of some plants such as onions, garlic, parsley and purslane, with increasing vermicompost applications, the amount of Mn in plants decreases and the amount of Zn increases, the amount of Ca and Mg increases up to a certain level of vermicompost, but when the dose continues to be increased. It has been determined that there is a decrease in the amount of Ca and Mg (Eryüksel, 2016). It has been reported that the yield, plant height, leaf length and width, root weight values increase significantly depending on the increase in the amount of vermicompost applied to the spinach plant by mixing the increasing doses of vermicompost into the soil environment, and the effect on the soil reaction and P coverage, which are the fertility properties of the soil, is statistically significant (Müftüoğlu et al. .2016). Azarmi et al. (2008) investigated the effect of vermicompost application on soil where tomatoes are grown, and it was stated that the application of 1.5 tons of vermicompost per decare changed the physical structure of the soil positively, and there was an increase in the amounts of N, P, K, Ca, Zn, Mn and organic carbon. (Figure 2).

Figure 2. Soil erosion and vermicompost (Anoniymus, 2023)

The capacity of a soil to form aggregates is accepted as a measure that determines the water movement, the diffusion of gases and the growth and development of roots in the soil. Worms indirectly promote the formation of water-resistant aggregates in the soil by carrying arbuscular mycorrhizal fungi. It is thought that placing worms in pots in seedling cultivation, together with increased mycorrhizal infection in three tree species, positively affects plant growth and this is probably due to the spread of this fungus in the root zone (Wright & Upadhyay, 1998). In a vermicompost application produced from urban garbage, a decrease in soil pH and an increase in dry weight of corn plant were detected (Sharma et al., 2005).

Buchanan et al. (1988) stated that the water solubility of important plant nutrients in earthworm excrement is higher than the solubility of the material that the earthworm takes in as food, and they can feed the plant for a longer time since they release these nutrients into the environment at low speed. However, the microbial activity level of vermicompost is 10 to 20 times higher than soil. It is known that this high microbial diversity enables the production of chemicals that promote plant growth, and enzymes and various compounds that suppress the growth of harmful plant pathogens (Logsdon, 1994). The nutrients surrounded by the worm mucus contained in the vermicompost are released slowly and are in a form that can be used by the plant immediately. In addition, the porous structure, high aeration and water holding capacity of vermicompost makes it an excellent soil conditioner, protects plant roots from extreme temperatures, reduces erosion and weed growth (Şimşek, 2007). In short, the vermicompost obtained at the end of the vermiculture process has many ecological, economic and agricultural benefits (Türkmen, 2016).

Incubation period is very important in revealing the effects of organic applications on N and P mineralization rates in soils. Belliturk & Sağlam (2005) determined that there was an increasing relationship between mineralization rates and lime contents of soils left to incubation for 14 days. Kara (1997) reported that after 6-7 weeks of incubation, the mineralizable N content of the soil reached its maximum and the rate of organic matter degradation of the soils decreased as the incubation period increased (Wadman & De Haan 1997). Working on a large number of mineral soil samples, Pritchettk et al. (1959) found statistically insignificant relationships between the organic matter and nitrate contents of the soils at the end of the two-week incubation; They found that there are very important statistical relationships between total N and nitrate contents. In the studies, it has been reported that the total N values, mainly organic C, increase significantly in environments that are vermicomposted by earthworms or in soils with earthworm activity, and that the properties of the organic material added to the mixture are an important criterion in determining the content of vermicompost (Kızılkaya & Hepşen, 2007; Namlı et al. ., 2014).

While vermicompost increases the germination rate and plant growth in applications made with different commercial mixtures, vermicompost also increases plant growth hormones and microbial mass. It has been reported by researchers that their application also suppresses plant diseases (Edwards & Burrows, 1988; Buckerfield et al., 1999; . Atiyeh et al., 2000, 2000a, b, c; Edwards & Arancón, 2004, 2004a; Edwards et al., 2004, Buckerfield & Webster, 1998; MBA, 1983; Masciandaro et al., 1997; Venkatesh et aal., 1998;. Vadiraj et al., 1998, Kale et al., 1992;. Arancón et al., 2003b, 2004, Edwards, 1983;. Tomati et al., 1987; Carlile & Wilson, 1993, Edwards & Burrows, 1988; Werner & Cuevas, 1996, Tomati et al., 1988; Muscolo et al., 1999; Arancón et al., 2003a). Vermicompost, which is very rich in organic matter, is an alternative fertilizer source for sustainable soil fertility. In this way, the sustainability of our lands, which we will leave to future generations, will be ensured depending on the use of organic fertilizers.

5. CONCLUSION AND RECOMMENDATIONS

Vermicompost is an easy-to-apply method that requires low cost. A properly applied and well-followed vermicompost process will result in a product with very high commercial value, effective as a bio-fertilizer and bio-pesticide. The use of earthworms in composting increases soil microbial activity, plant nutrient content of the environment and yield, while suppressing pathogens in the environment. With the increase in the use of vermicompost, the sustainability of the soil will be ensured. Vermicompost's slow release and soil conditioner (because it improves the physical, chemical and biological properties of the soil) makes it the most important organic fertilizer. The use of vermicompost not only meets the need for organic matter, but also maintains the sustainability of the soil with its cutting liquid. The slow release of vermicompost compared to inorganic fertilizers prevents harmful nitrate accumulation, especially in vegetables whose green leaves are consumed. Highly used vermicompost increases the organic matter, phosphorus and copper contents. The use of vermicompost is very important in terms of environment, health and sustainable agriculture.

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CHAPTER 4

THE USE OF GENETICALLY MODIFIED ORGANISM FEED INGREDIENTS IN ANIMAL NUTRITION

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1. INTRODUCTION

Concerns and debates about the genetically modified organism (GMO) crops have divided people and scientists worldwide. A part of society state that human beings cannot survive in the future with today's population growth and climate changes without genetically modified organisms (GMOs), whereas others think that artificially altering evolutionary processes could have currently unseen effects on the health of nature, including those of animals and humans (Von Götz, 2010). Although the dispute about GMOs is advantageous or detrimental for animals, people, and habitats, the number of GMOs is increasing daily. The first commercially transgenic plant cultivation began with the cultivation of tobacco plants with the virus resistance gene transferred in the People's Republic of China in the early 1990s (James, 1997; James & Krattiger, 1996). The most widely known transgenic plants are soybean, corn, cotton, and canola. While the cultivation area of GMO crops was 44.2 million hectares in 2000, it increased by approximately 4.3-fold to 190.4 million hectares in 2019 (Figure 1). Soybean is grown on 95.9 million hectares and is the primary GMO commodity commercially produced. This is followed by 58.9 million hectares of corn, 24.9 million hectares of cotton, and 10.10 million hectares of canola (GMO Compass, 2009).

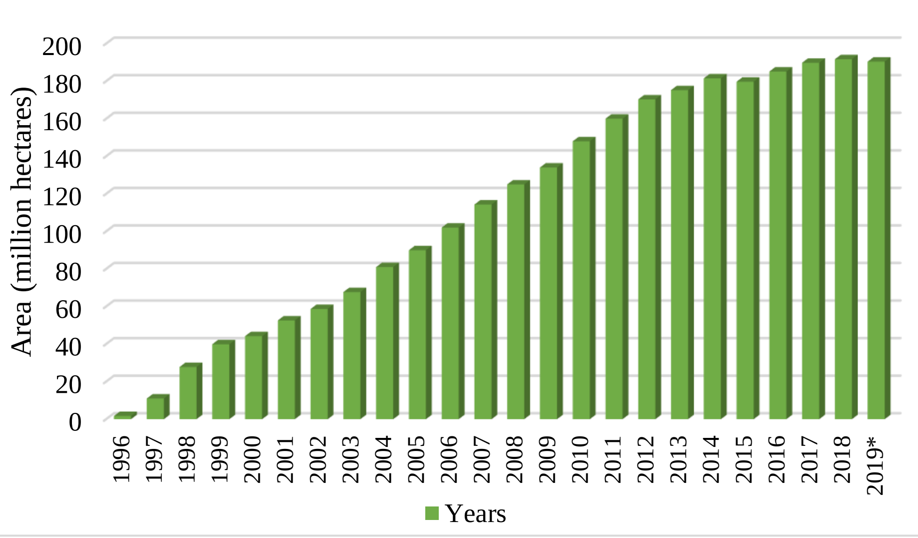

Figure 1. GMO crops production area by years (ISAAA, 2018) *(ISAAA, 2019)

Today, the cultivation of GMO crops continues to increase in 25 countries, especially in Canada (8.2 million ha), India (8.4 million ha), Argentina (21.3 million ha), Brazil (21.4 million ha), and America (64 million ha). Figure 2 shows the countries where 0.1 million hectares or more of GMO crops were cultivated in 2019. Countries where GMO crops were cultivated on less than 0.1 million hectares, include Honduras, Chile, Malawi, Portugal, Indonesia, Bangladesh, Nigeria, Eswatini, Ethiopia, and Costa Rica.

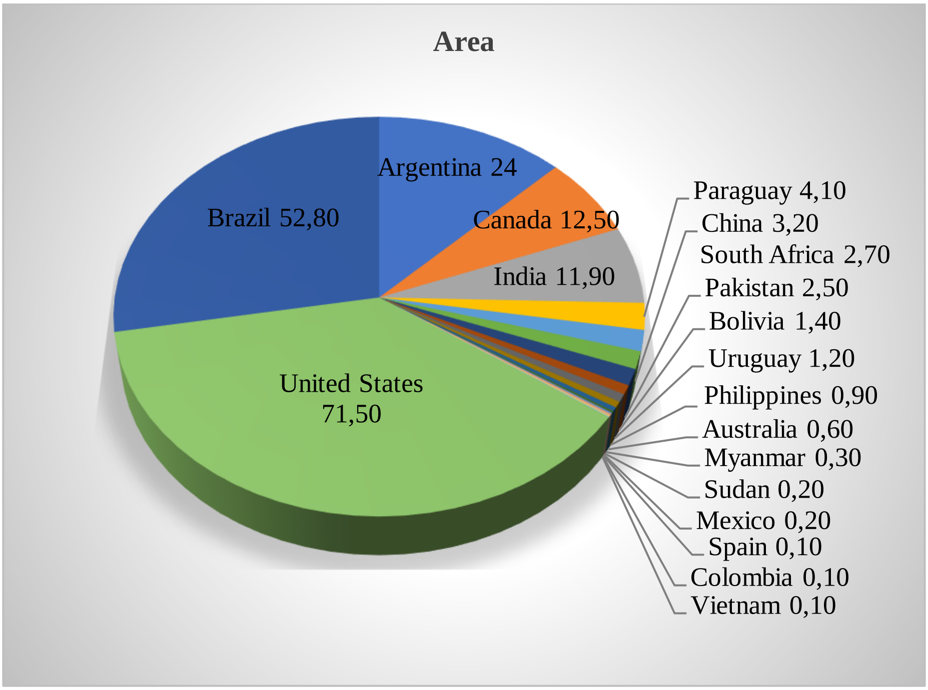

Figure 2. Production areas of GMO plants by country in 2019 (million hectares) (ISAAA, 2019)

GMO crop varieties have been developed for altered growth/yield, abiotic stress tolerance, disease resistance, pollination control system, and modified product quality (Figure 3). Today, 374 GMO plant varieties can be used in animal feeds (Table 1) (ISAAA, 2023). In Türkiye, import permits were granted to 21 GMO corn varieties and 15 GMO soybean varieties in 2022 by the Republic of Türkiye Ministry of Agriculture and Forestry (Table 2) (TBBDM, 2023).

Table 1. GMO nutrients are used directly or as additives in feeds in the world

Nutrients	**Number of GMOs**
Maize (*Zea mays* L.)	146
Cotton (*Gossypium hirsutum* L.)	57
Potato (*Solanum tuberosum* L.)	42
Canola (*Brassica napus*)	38
Soybean (*Glycine max* L.)	36
Tomato (*Lycopersicon esculentum*)	11
Rice (*Oryza sativa* L.)	6
Alfalfa (*Medicago sativa*)	5
Turnip (*Brassica rapa*)	4
Sugarcane (*Saccharum* sp.)	4
Apple (*Malus* x *Domestica*)	3
Sugar beet (*Beta vulgaris*)	3
Chicory (*Cichorium intybus*)	3
Papaya (*Carica papaya*)	2
Squash (*Cucurbita pepo*)	2
Wheat (*Triticum aestivum*)	2
Safflower (*Carthmus tinctorius* L.)	2
Bean (*Phaseolus vulgaris*)	1
Cowpea (*Vigna unguiculata*)	1
Creeping bentgrass (*Agrostis stolonifera*)	1
Eggplant (*Solanum melongena*)	1
Eucalyptus (*Eucalyptus* sp.)	1
Flax (*Linum usitatissimum* L.)	1
Plum (*Prunus domestica*)	1
Tobacco (*Nicotiana Tabacum* L.)	1
Total	**374**

*Source: (ISAAA, 2023)

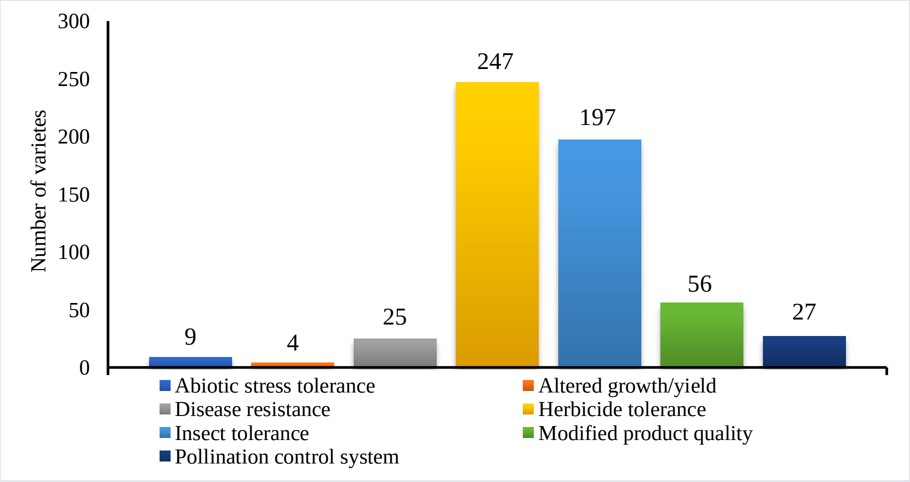

Figure 3. The number of varieties according to the development purpose of GMO crops used in animal feeds worldwide (ISAAA, 2023)

Table 2. GMO feedstuffs allowed to be imported into Türkiye in 2022

Crops	Variety	Official Gazette	Gene/Genes
Maize	Bt11 (SYN-BTØ11-1)	The Biosafety Board Decision No. 4 in the Official Gazette dated December 24, 2011, No:28152	The *cry1Ab* gene confers resistance to several Lepidoptera-order corn pests, and the pat gene confers glufosinate-ammonium tolerance.
Maize	DAS1507 (DAS-Ø15Ø7-1)	The Biosafety Board Decision No. 5 in the Official Gazette dated December 24, 2011, No:28152	The *cry1F* gene confers resistance to several Lepidoptera-order corn pests, and the pat gene confers glufosinate-ammonium tolerance.
Maize	DAS59122 (DAS-59122-7)	The Biosafety Board Decision No. 6 in the Official Gazette dated December 24, 2011, No:28152	The *cry34Ab1* and *cry35Ab1* genes confer resistance to several Coleoptera-order corn pests, and the pat gene confers glufosinate-ammonium tolerance.
Maize	NK603 (MON-ØØ6Ø3-6)	The Biosafety Board Decision No. 8 in the Official Gazette dated December 24, 2011, No:28152	The Epsps gene provides tolerance to the herbicide glyphosate
Maize	GA21 (MON-ØØØ21-9)	The Biosafety Board Decision No. 10 in the Official Gazette dated December 24, 2011, No:28152	The Epsps gene provides tolerance to the herbicide glyphosate
Maize	MON89034 (MON-89Ø34-3)	The Biosafety Board Decision No. 11 in the Official Gazette dated December 24, 2011, No:28152	Resistance to various Lepidopteran pests that attack maize is provided by the *cry1A.105* and *cry2Ab2* genes
Maize	MON88017 (MON-88Ø17-3)	The Biosafety Board Decision No. 17 in the Official Gazette dated April 21, 2012, No:28271	The *cry3Bb1* and *cp4 epsps* genes tolerate the herbicide glyphosate and resist several Coleoptera-order corn pests, respectively
Maize	MON810 (MON-ØØ81Ø-6)	The Biosafety Board Decision No. 18 in the Official Gazette dated April 21, 2012, No:28271	The *cry1Ab*, a gene that confers defense against several Lepidoptera-order corn pests
Maize	MIR604 (SYN-IR6Ø4-5)	The Biosafety Board Decision No. 23 in the Official Gazette dated July 16, 2012, No:29418	The *mcry3Aa2* gene provides resistance against harmful insects from the order of Coleoptera.
Maize	MON863 (MON-ØØ863-5)	The Biosafety Board Decision No. 24 in the Official Gazette dated July 16, 2012, No:29418	The Coleoptera order's insect species are resistant thanks to the *cry3Bb1* gene
Maize	T25 (ACS-ZMØØ3-2)	The Biosafety Board Decision No. 25 in the Official Gazette dated July 16, 2012, No:2815229418	The pat gene confers glufosinate-ammonium tolerance
Maize	Bt11xMIR604 (SYN-BTØ11-1 x SYN-IR6Ø4-5)	The Biosafety Board Decision No. 30 in the Official Gazette dated November 5, 2015, No:2815229523	The cry1Ab gene confers resistance to Lepidoptera-order insects, the cry3A gene confers resistance to Coleoptera-order insects, and the pat gene confers glufosinate-ammonium tolerance

Table 2. GMO feedstuffs allowed to be imported into Türkiye in 2022 (continued)

Crops	Variety	Official Gazette	Gene/Genes
Maize	MIR162 (SYN-IR162-4)	The Biosafety Board Decision No. 31 in the Official Gazette dated November 5, 2015, No:29523	The vip3Aa19e gene confers lepidopteran insect resistance
Maize	MIR604xGA21 (SYN-IR6Ø4-5 x MON-ØØØ21-9)	The Biosafety Board Decision No. 32 in the Official Gazette dated November 5, 2015, No:29523	The *cp4 epsps* gene, which imparts tolerance to the herbicide glyphosate, and the *mcry3A* gene, which confers resistance to insects in the Coleoptera-order
Maize	MON87460 (MON 8746Ø-4)	The Biosafety Board Decision No. 40 in the Official Gazette dated August 2, 2017, No:30142	Reduces crop loss in dry environments thanks to the *cspB* gene
Maize	MON87427 (MON-87427-7)	The decision of MAF No. 9 in the Official Gazette dated January 23, 2021, No:31373	Herbicide resistance, which is the active ingredient of glyphosate, is provided by the *cp4 epsps* gene
Maize	DAS40278-9 (DAS-4Ø278-9)	The decision of MAF No. 15 in the Official Gazette dated February 27, 2021, No:31408	The *aad-1* gene confers tolerance to phenoxy auxin herbicides and resistance to aryloxy propionate (AOPP) acetyl-coenzyme A carboxylase (ACCase) inhibitory herbicides
Maize	4114 (DP-ØØ4114-3)	The decision of MAF No. 23 in the Official Gazette dated January 7, 2022, No:31712	The *pat* gene, which confers tolerance to the herbicide glufosinate ammonium, and the *cry1F*, *cry34Ab1*, and *cry35Ab1* genes, which confer resistance against insects of the Lepidoptera and Coleoptera orders
Maize	MON87411 (MON-87411-9)	The decision of MAF No. 31 in the Official Gazette dated April 27, 2022, No:31822	The *cry3Bb1* and *DvSnf7* genes protecting against corn rootworms (*Diabrotica* spp.) and *cp4epsps* gene conferring tolerance to glyphosate herbicide
Maize	MZIR098 (SYN-ØØØ98-3)	The decision of MAF No. 32 in the Official Gazette dated April 27, 2022, No:31822	The Coleoptera order's *ecry3.1Ab* and *mcry3A* genes confer insect resistance, and the pat gene confers glufosinate-ammonium tolerance
Maize	5307 (SYN-Ø53Ø7-1)	The decision of MAF No. 35 in the Official Gazette dated October 13, 2022, No:31982	The Coleoptera order insect resistance gene (*pmi* gene)
Soybean	MON87701 (MON-877Ø1-2)	The Biosafety Board Decision No. 26 in the Official Gazette dated July 16, 2012, No:29418	Lepidoptera order insects are resistant thanks to the *cry1Ac* gene
Soybean	356043 (DP-356Ø43-5)	The Biosafety Board Decision No. 28 in the Official Gazette dated November 5, 2015, No:29523	The *Gat4601* gene, which confers resistance to the herbicide glyphosate, and the *Gm-hra* gene, which confers resistance to herbicides that block acetolactate synthesis (ALS)

Table 2. GMO feedstuffs allowed to be imported into Türkiye in 2022 (continued)

Crops	Variety	Official Gazette	Gene/Genes
Soybean	A5547-127 (ACS-GMØØ6-4)	The Biosafety Board Decision No. 29 in the Official Gazette dated November 5, 2015, No:29523	The pat gene confers glufosinate ammonium tolerance
Soybean	MON87708 (MON-877Ø8-9)	The Biosafety Board Decision No. 37 in the Official Gazette dated August 2, 2017, No:30142	The *dmo* gene confers resistance to dicamba-containing herbicides
Soybean	BPS-CV127-9 (BPS-CV127-9)	The Biosafety Board Decision No. 38 in the Official Gazette dated August 2, 2017, No:30142	The imidazolinone herbicide tolerance-granting *csr1-2* gene
Soybean	MON87705 (MON-877Ø5-6)	The Biosafety Board Decision No. 39 in the Official Gazette dated August 2, 2017, No:30142	The *FAD2-1A* and *FATB1-A* genes, which raise the level of oleic acid and reduce the concentration of linoleic acid, respectively, and the *cp4epsps* gene, which confers resistance to the herbicide glyphosate
Soybean	305423 (DP-3Ø5423-1)	The decision of MAF No. 7 in the Official Gazette dated January 23, 2021, No:31373	The *gm-hra* gene, a soybean endogenous *als* gene that confers resistance to ALS (acetolactate synthase) inhibitory herbicides and whose fatty acid profile is altered, and the soybean-derived *fad2-1* gene, which raises oleic acid content and reduces the linoleic acid concentration
Soybean	FG72 (MST-FGØ72-2)	The decision of MAF No. 8 in the Official Gazette dated January 23, 2021, No:31373	The *2mepsps* gene confers resistance to herbicides containing glyphosate as their active component, and the *hppdPf3366* gene confers tolerance to herbicides containing isoxaflutole as their active ingredient,
Soybean	A2704-12 (ACS-GMØØ5-3)	The decision of MAF No. 16 in the Official Gazette dated January 23, 2021, No:31373	The *A2704-12* soybean herbicide tolerance gene
Soybean	MON40-3-2 (MON-Ø4Ø32-6)	The decision of MAF No. 17 in the Official Gazette dated January 23, 2021, No:31373	The *MON40-3-2* soybean herbicide tolerance gene
Soybean	MON89788 (MON-89788-1)	The decision of MAF No. 18 in the Official Gazette dated January 23, 2021, No:31373	The *MON89788* soybean herbicide tolerance gene
Soybean	DAS-44406-6 (DAS-444Ø6-6)	The decision of MAF No. 19 in the Official Gazette dated January 23, 2021, No:31373	The 2mepsps, aad-12, and pat genes confer tolerance to glyphosate and glufosinate herbicides

Table 2. GMO feedstuffs allowed to be imported into Türkiye in 2022 (continued).

Crops	Variety	Official Gazette	Gene/Genes
Soybean	SYHT0H2 (SYN-ØØØH2-5)	The decision of the MAF No. 24 in the Official Gazette dated January 7, 2022, No:31712	The *pat* gene confers resistance to glufosinate ammonium, and the *avhppd-03* gene confers tolerance to the active component mesotrione and other HPPD-inhibiting herbicides.
Soybean	MON87751 (MON-87751-7)	The decision of MAF No. 29 in the Official Gazette dated April 27, 2022, No:31822	The *cry2Ab2* and *cry1A.105* provide lepidopteran insect resistance genes
Soybean	DAS-81419-2 (DAS-81419-2)	The decision of MAF No. 30 in the Official Gazette dated April 27, 2022, No:31822	The *cry1Ac, cry1F,* and the selective marker *pat* gene confer resistance against insects of the Lepidoptera order

*Source: (TBBDM, 2023)
**MAF: Republic of Türkiye Ministry of Agriculture and Forestry

While 14 million farmers worldwide were farming using GMO plants in 2009 (GMO Compass, 2009), this number reached 17 million farmers in 2019. Moreover, it has been reported that more than 65 million people, including their family members, benefited from GMO crop production (ISAAA, 2019). Over 6 million Indian farmers cultivated Bt cotton on 11.9 million hectares in 2019.

The commercial release of GMO plants has been based on producing specially developed hybrid seeds with better tolerance to herbicides, viruses, and insect pests (ILSI Research Foundation, 2017). It has been used in plants with modified nutrient content, such as soybean (Wilson, 2012) and maize (Zhang et al., 2019). Today, some GMO plants contain high levels of oleic fatty acids (Do et al., 2019)

Gene modification studies have also been carried out on animals. For example, The humanized Caenorhabditis elegans gene, which produces omega-3 fatty acids, has been transferred to pigs (Lai et al., 2006), and even genetically modified breeding pig strains have been developed that produce phytase enzyme with saliva and utilize plant phosphorus 50-75% better, and consequently reducing phosphorus excreted with manure (Anonymous, 2010). However, commercial breeding of genetically modified animals has not been carried out so far.

2. USING GMO FEEDS in ANIMAL NUTRITION

Although many scientific studies have reported that GMO products are safe, there are debates about the risks arising from their consumption. More evidence is needed that GMO consumption with animal feed is safe (Prescott et al., 2005). Approximately one-third to one-half of the world's total plant production comprises plant nutrients for animal feed. On a worldwide scale, 90% of the total soybean produced is used as animal feed, and a large part of this production is exported between the countries. Only European Union+ member states imported soybean oil, soybean, and soybean meal (0.263, 15.54, and 18 million tons) in 2018 (IDH, 2020). GMO crops have been planted for over 20 years and are used in animal feed at a rate between 70% to 90% and consumed by animals (Flachowsky et al., 2012; Van Eenennaam & Young, 2014). Considering that less than 5% of livestock raised in the USA in 2011 were organically raised, it was estimated that most animals consumed feed containing GMO crops (Van Eenennaam & Young, 2014). According to this estimation, it has been reported that more than 100 billion farm animals were fed with GMO feeds between 2000 and 2011 in the USA.

The transgenic products the plants use directly or by-products for animal feeding are corn, soybean, canola, cotton, alfalfa, rice, and sugar beet. The largest share among all varieties belongs to soybean, corn, cotton, and canola. Soybean and corn are used in feed as feed nutrients. Especially in poultry feeds, these two ingredients make up 60-90% of the total ration and are produced using GMOs. Poultry meat and eggs are products based on direct GMO feed consumption. In the USA, it has been reported that a typical feed in dairy cattle consists of 10% dehulled soybean meal, 20% maize grain, and 50% corn silage, and also other GMO feed materials in cow rations are fuzzy cottonseed (no processing other than lint removal), ground maize grain, alfalfa straw, roasted full-fat soybeans, sugar beet pulp, cottonseed meal, processed corn by-products, soybean husks, and canola meal (Van Eenennaam & Young, 2014). According to the feeding period and feed prices in beef cattle, 80-85% of the ration consists of foods with high energy content (grain, processed grain products, and other energy/starch sources), and 10-15% of it consists of straw, silage or other forage (Mathews & Johnson, 2013). The other part of the diet includes protein sources, such as soybean or cotton meal which have probability of being GMOs. Although genetically modified corn feeds raw material has been approved in many countries, doubts remain regarding the safety of foreign gene transfer with recombinant DNA (rDNA) technology in European countries and Japan (Hino, 2002).

3. RESEARCH on the USE of GMO FEEDS in ANIMAL NUTRITION

According to the reports, there is no difference in the nutritional content of GMO and non-genetically modified (nGMO) feeds and their effects on animal performance (FAO/WHO, 2000). However, there are insufficient and even contradictory scientific reports regarding the fate of rDNA in the digestive system, tissue accumulation, and transmission to milk, eggs, and fetuses after consuming genetically modified feed by animals. Some scientific studies report that rDNA was not found in eggs, milk, and meat of farm animals fed with GMO feeds (Ash et al., 2003; Faust, 2000; Flachowsky & Aulrich, 2001; Guertler et al., 2009; Jennings, Albee, et al., 2003; Jennings, Kolwyck, et al., 2003; Phipps et al., 2003; Weber & Richert, 2001). However, some studies have reported that GMO-DNA fragments were found in some components of the digestive system of ruminants (Einspanier et al., 2001; Faust, 2000; Klotz et al., 2002; Phipps et al., 2003; Reuter & Aulrich, 2003). Even Agodi et al. (2006) showed that corn-GMO-DNA sequence in 15 samples (25%) and soybean-GMO-DNA sequence in 7 samples (11.7%) of 60 milk samples that were taken from 12 different milk brands commercially sold in Italy. However, it is interesting that DNA fragments were found even in organic milk in the study by Even Agodi et al. (2006) mentioned above. The authors explained that this could be due to fecal contamination or during milking. This study also revealed that pasteurization could not break down the GMO-DNA sequence.

According to Chowdhury et al. (2003), when pigs were fed with genetically modified maize (Bt11), 110 bp and 437 pb *cry1Ab* DNA fragments were detected in their digestive tracts. However, pigs fed with nGMO maize had no traces of GMO-DNA fragments in their digestive system. Finamore et al. (2008) determined that an immune response developed against GMOs in the intestinal and peripheral tissues of 21-day-old suckling mice and 18-19-month-old adult mice fed with feed containing nGMO or GMO (*cry1Ab* DNA) soybean meal for an experimental 90-day-period. It was determined that glyphosate-tolerant soybeans caused RNA alterations in the base tissues of young, adult, and aged mice (Malatesta et al., 2003; Malatesta et al., 2008; Malatesta, Caporaloni, Gavaudan et al., 2002;

Malatesta, Caporaloni, Rossi, et al., 2002; Vecchio et al., 2004) (Malatesta et al., 2002a, 2002b and 2003; Vecchio et al., 2004; Malatesta et al., 2008). Cisterna et al. (2008) reported that the embryos of mice fed with GMO soybean were adversely affected compared to embryos of mice fed with nGMO feeds.

Einspanier et al. (2001) and Klotz et al. (2002) reported that transgenic feedstuffs were used in broiler and layers feed, and transgenic DNA fragments were found in the kidneys, liver, and spleen of these animals but not in eggs. Phipps et al. (2003) established that GMO fragments were found in the rumen fluid, small intestine contents, milk, blood, and manure of dairy cattle fed with feeds containing GMO maize grain (*cry1Ab* gene) and GMO soybean meal (*cp4epsps* gene).

Shahid et al. (2020) fed albino rabbits with rations containing 0, 20, 30, and 40% GMO cottonseed meal (Cry1Ac, Cry2Ac, and cp4 epsps) for 180 days. GMO-DNA fragments were not found in rabbits' blood, liver, kidney, heart, and intestinal tissues when fed with GMO cottonseed meal.

Lin et al. (2022) fed rats with feed containing GMO soybean or nGMO soybean variety for 90 days. It has been reported that GMO soybeans did not cause pathological side effects in rats.

5. CONCLUSION

Although the future fate of animals fed with GMO-containing feed is not known precisely, it has been scientifically accepted that some GMOs have toxic and allergenic properties, and some have developed antibiotic resistance. It has been suggested that the transmission of genetic material resistant to antibiotics found in GMO foods, especially to bacteria in the human intestine, can create a hazardous case. Because of such a transfer, antibiotics used to treat many diseases today may become ineffective (Meseri, 2008). The American Medical Association (AMA) advocates that it should be noted that consumer safety for genetically modified foods is not yet apparent and that labeling these products should be mandatory.

In Türkiye, GMO products are banned based on the laws on which the "Regulation on Import, Processing, Export, Control, and Inspection of Genetically Modified Organisms and Products for Food and Feed Purposes" came into force after being published in the Official Gazette dated

26.10.2009. However, with the amendment to the regulation on April 28, 2010, the importation of 16 different GMO corn varieties, 6 GMO cotton varieties, 3 GMO rapeseed varieties, and H7-1 sugar beet varieties was allowed. Today, the Republic of Türkiye Ministry of Agriculture and Forestry has authorized the use of 21 genetically modified corn varieties, 15 soybean varieties, and 2 enzyme varieties in animal feed. For more than 20 years, GMO crops have been developed and marketed for commercial use, and millions of humans and billions of animals have eaten food made from them. In many feeding studies conducted on GMO feed raw materials such as corn (Appenzeller, Malley et al., 2009; Appenzeller, Munley, et al., 2009; Delaney et al., 2013; Hammond et al., 2004; Hammond et al., 2006; He et al., 2008; He et al., 2009; Healy et al., 2008; Liu et al., 2012; MacKenzie et al., 2007; Malley et al., 2007; Zeljenková et al., 2014; Zhu et al., 2013), rice (Cao et al., 2011; Momma et al., 2000; Zhuo et al., 2004), soybean (Appenzeller et al., 2008; Delaney et al., 2008; Hammond et al., 1996; Qi et al., 2012; Wang et al., 2016), canola (Delaney et al., 2014), and cottonseed meal (Dryzga et al., 2007), none of the GMO feed raw ingredients have been shown to have any indication of negative impacts on animals (Delaney et al., 2018) health.

GMO feeds facilitate the constant development of an economic part of the global market. However, the debate on this topic in Türkiye and worldwide is still underway. Further scientific research must be carried out on using GMOs in animal nutrition and their effects on human health. Nevertheless, it is foreseen that the discussions on this subject will not end soon.

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CHAPTER 5

SOIL REGULATORS AND SOILLESS AGRICULTURAL TECHNIQUES

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INTRODUCTION

Despite the increase in world food supply with the increasing population, it is not possible to increase the lands on which agriculture can be made, and agricultural lands are shrinking day by day due to reasons such as urbanization, drought, salinity, alkalinity and erosion. In addition, with the aim of making maximum use of the scarce resources with the developing industry and technology, the use of heavy agricultural tools and machinery has been ensured to increase the efficiency obtained from the unit area. However, these technological possibilities, which make it necessary to regulate agricultural production in a rational way, change the physical properties of the soil negatively in the same proportion, especially by compressing agricultural lands, causing a decrease in production and an increase in costs. For this reason, although there are natural resources such as soil, climate and topography for agricultural production, the use of these resources and especially the management of water and soil are of great importance.

The cultivation of plants in stagnant or flowing nutrient solutions, in organic or inorganic solid media enriched with nutrient solutions is called soilless agriculture (soilless culture). This method has been accepted as alternative agriculture all over the world. With this system, aquaculture is grouped under two headings: growing plants in nutrient solution is called aquaculture (hydroponic), and growing them in solid media is called medium (substrate) culture. The fact that water culture requires technical knowledge and equipment has led to the development of more ambient culture. (Winsor & Schwarz, 1990; Eltez et al, 2002; Sevgican, 2003).

However, considering that it would not be practical and economical to abandon the soil, it was found more scientific to try to improve the physical and chemical properties of degraded soils. For this purpose, many soil improvers and physical environment conditioners have been tried.

Conscious soil management requires determining the most efficient use of soils by determining their physical, chemical, microbiological and hydrological properties. Again, irrigation, fertilization, agricultural struggle, use of quality seeds, mechanization and appropriate land cultivation are of great importance in increasing the product taken from the unit area. Compaction changes the structural properties and functions of the soil. Soil compaction (Kok et al., 1996; Jones et al., 1997) affects the physical, chemical, biological properties of the soil and plant production. With proper mechanization and land tillage techniques or less tillage, the biggest problem of the lands, compaction is tried to be minimized. For these reasons, in order to feed the increasing world population, it is inevitable to get the most economical efficiency from the unit area available or to search for the possibilities of finding production environments that can be an alternative to the soil.

Today, one of the biggest reasons for the rapid spread of soilless agriculture in greenhouse cultivation is soil-based problems. Soilless culture has advantages such as the absence of problems such as soil fatigue, diseases and pests arising from the soil, controlling plant growth by controlling the fertilizer and water relationship, removing the factors that reduce the quality of the soil and increasing the yield (Jones, 1997).

In our country, some organic media are widely used by agricultural companies in greenhouse cultivation and seedling production alone or in combination with inorganic media. In the Mediterranean region, where greenhouse cultivation is intense, ready-made seedlings are produced in soilless mixtures consisting of 100% peat, peat-perlite or mostly peat-perlite-vermiculite. Although the ratio of peat - perlite - vermiculite in the mixture varies according to the company producing the seedling and the growing season, less vermiculite is used in the mixtures (Sevgican, 2003).

Since intensive irrigation, fertilization and soil cultivation are done in greenhouse agriculture, greenhouse soils lose their properties quickly, resulting in hard, salty, chemically contaminated soils. Apart from this, pests such as nematodes and fungi, which are very difficult to remove once infested with the soil, are also found in greenhouse soils. For this reason, greenhouse soils are completely changed every few years, or they are moved outside and ventilated, bringing great costs to our farmers. In recent years, soilless agriculture has gained importance in order to eliminate salinity, soil pests and other negative problems. Among the soilless agricultural practices, environments with many raw materials such as perlite, rock wool, peat have been tried. The short-lived nature of these raw materials, their production and availability of different qualities, and the importation of some of them into our country have led to difficulties in use.

1. SOILLESS AGRICULTURE FROM PAST TO PRESENT

It is known that soilless agriculture, which has an average of 40-50 years of commercial history, is a fairly new type of cultivation, but experiments on soilless cultivation began in the 1600s, and the composition of plant growth materials and plants was determined. The hanging gardens of Babylon and the floating gardens of the Aztecs and Chinese can be given as the first examples of soilless agriculture. At the same time, there are records that describe the cultivation of plants in water by the Egyptians a few centuries before Christ.

In the 1600s, studies began to determine plant components, and in the mid-1800s it was proven that plants could be grown in an inert environment irrigated with a solution containing the minerals necessary for their development. Plant cultivation in nutrient solution was carried out by German researchers (Sachs and Knop) in the 1860s. The nutrient solution formula developed by Knop is still used today. Between 1925 and 1935, soilless aquaculture began to be implemented in the United States. In the first study conducted outside the laboratory in 1930 by Gericke at the University of California, tomatoes were grown in nutrient solution. This technique is called "hydroponics" (hydroponics), which consists of two Greek words meaning water (hydro) and work (ponos).

Figure 1. Hydroponic agriculture general view (Gül, 2019)

The first major application of hydroponic farming was carried out by the American army, which grew vegetables with water and gravel culture in order to provide fresh vegetables to its soldiers stationed on the islands in the Pacific Ocean during World War II. Thus, in soilless agriculture, after water culture, solid media other than soil began to be used, and in addition to hydroponics, the terms aggregate culture, soilless culture, nutriculture or chemical culture (chemiculture) began to be encountered in the literature.

Since soil disinfection with steam is a very expensive practice due to the energy crisis, soilless agriculture has become commercially widespread in greenhouse production after the 1970s. The availability of soilless environments as an alternative to soil disinfection has allowed soilless agriculture to become widespread in commercial greenhouse production (Figure 1).

The term hydroponic, which is defined as aquaculture only in nutrient solution (water culture), is also defined as aquaculture and aquaculture in inert (chemically inactive, does not react chemically with the nutrient solution) environments. For this reason, the terms Hydroponics (hydroponics) and soilless agriculture (soilless culture) are used synonymously by some researchers. However, today it is accepted that the terms "hydroponic" and "soilless agriculture" are not synonymous and that hydroponics is a special form of soilless agriculture.

Since sand and gravel were used in the first large-scale cultivation studies in solid media, this cultivation method was called "aggregate culture" and the materials used were called "aggregate". However, later, with the use of media such as pumice and sawdust, it has been discussed how accurate the term aggregate is used for solid growing media. Since the term aggregate has the meanings of sand, gravel, aggregate or all, it is not appropriate to use this term to describe organic or inorganic media. Therefore, soilless growing media are called "substrate", and the cultivation using these media is called "substrate culture".

2. SOILLESS AGRICULTURAL CULTURES

Soilless agriculture is based on the principle of giving the necessary amounts of water and nutrients necessary for plant life to the root environment and is divided into two as water culture and substrate culture. In water culture, which is classified depending on the application of the nutrient solution, the plant roots develop in the nutrient solution (still water culture) or the nutrient solution is flowed along the plant roots (running water culture) or the nutrient solution is applied to the plant roots in the form of mist (aeroponics). In substrate culture, plants are grown in organic, inorganic or synthetic materials. The water and nutrient requirements of the plants are met with the nutrient solution given with the drip irrigation system, with some exceptions.

2.1. Water Culture

2.1.1. Still water culture

In stagnant water culture, which is the oldest soilless farming technique used in plant cultivation outside the laboratory, the nutrient solution is placed in opaque dark colored containers with a depth of 15 cm. In the simplest form, jars covered with aluminum foil can be used. The tops of the containers are covered with a material that is opaque and will help to keep the plants standing. For good plant growth, aeration of the solution is necessary and an aquarium pump is used for this. A vent stone can be attached to the end of the vent tube so that the solution can be aerated effectively(Figure 1).

By controlling the level of the nutrient solution, the decreased amount is completed by adding water and stock solution. Depending on the plant size and solution volume, the solution should be changed at regular intervals (7-14 days). In the first development period of the plants, the nutrient solution is used without changing for a longer time, the nutrient solution is changed more often as the plants grow.

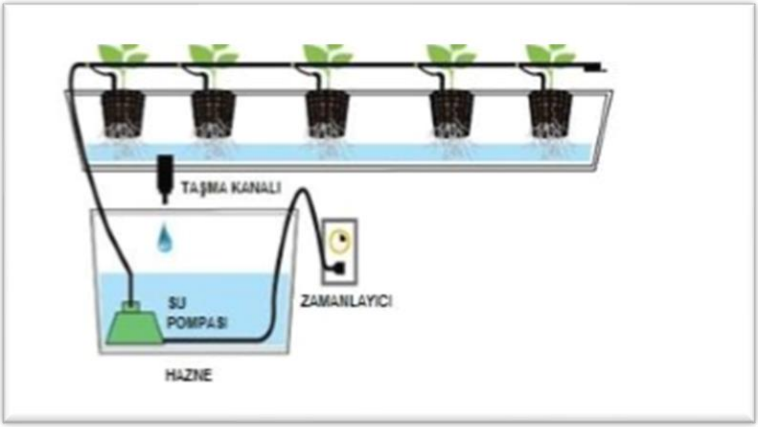

Figure 1. Hydroponic system (Drip system) (Anoniymus, 2015)

Developed in the 1930s, the system could not become commercially widespread due to the decrease in the oxygen content of the solution, but it was adopted as a hobby for plant breeding at home. Commercially, stagnant water culture is used in the production of greens such as salad and lettuce, which have a short growing period. Plants are grown on foam boards that are released in nutrient solution. This method is called "floating water culture".

This system developed in Italy; It consists of 4x70x30 breeding ponds. Production is carried out with a similar system in Alaşehir-Manisa in Turkey.

Differences have been made to solve the problem of solution aeration in stagnant water culture systems. The Tidal (Ebb-Flow) system is one of these developed systems. In the tide system, the pump works for a certain time and is disabled for a certain time. During the operation of the pump, the nutrient solution is pumped into the growing ponds to the desired level. Then the pump is disabled and the solution in the growing ponds returns to the tank, while an oxygen-rich environment is created for the roots.

2.1.2. Flowing water culture

The problem of not being able to aerate the solution in stagnant water culture has led to the development of running water culture. Flowing water culture is divided into three classes depending on the depth of the nutrient solution. These are Feeder Film Technique, Deep or Semi-Deep Flowing Water Culture and Layered Flowing Water Culture Systems.

2.1.2.1. Nutrient film technique

The Nutrient Film Technique, originally called the Nutrient Film Technique (NFT), was developed in England in 1965 to monitor the root development of tomatoes. The name Nutrient Film Technique "Nutrient film technique" was chosen to emphasize that the depth of the solution flowing through the plant roots must be really thin so that the upper surface of the root system is in the air to provide enough oxygen to the roots. The basic principle of the system; circulating a thin layer (less than 1 cm) of nutrient solution along the roots of plants to provide adequate water, nutrients and aeration. This technique is based on the use of the channels in which the plant roots develop and the nutrient solution tanks in which the nutrient solution is fed into the system and returned to the system, and the nutrient solution used is changed when necessary. The NFT system consists of plant growth tanks, nutrient tanks and pipes, control equipment and nutrient solution flow.

In the NFT system, plants are grown in cultivation channels. In order for the nutrient solution to flow in the channels, there must be a suitable slope. Mostly, slopes of 1/50 and 1/75 are suitable. The channel slope should not be less than 1/100. Channels can be placed either directly on the greenhouse floor or on the frame. Inclining the cultivation channels is easier in skeletal systems. At the same time, a warmer environment is provided for plant roots in this way. In the canal preparation, attention should be paid to pitting and that the length of the canal does not exceed 20-30 meters. Channels with a base width of 25-30 cm are suitable in the cultivation of vegetable species whose fruit is consumed, and channels with a width of 10 cm in the cultivation of salad-lettuce. The seedlings are placed in the channels with pots filled with an inert substrate such as perlite, pumice, or rockwool blocks with a lattice bottom and sides.

In the NFT system, there is a nutrient solution tank below the channel level. Although a single tank is sufficient, commercially, two nutrient solution tanks are generally used, one of which is placed below the channel level (collection tank) and the other at the top of the slope (feeding tank). The nutrient solution is pumped from the feeding tank to the plant growth channels and flows in the channels depending on gravity and returns to the collection tank. After the chemical properties of the collected solution are checked, it is pumped to the feeding tank and the solution continues to be circulated in the system again. The use of tanks made of concrete or hard plastic is preferred. The nutrient solution is delivered to the upper end of the channels by 1-inch pipes. These pipes should be hung on the channels over an average of 5 cm. The reason why the pipe is high is to provide aeration of the nutrient solution and to easily check whether the holes are blocked. Tank capacity should be determined by taking into account the plant water consumption. The water consumption of plants varies depending on the species, age of the plant and climatic conditions.

The electrical conductivity, pH and temperature of the nutrient solution should be controlled. In small scale aquaculture for experimental or hobby purposes, electrical conductivity and pH can be measured daily with portable instruments and necessary corrections can be made. But in large systems, automatic control is more suitable. If for any reason the nutrient solution flow is interrupted, the plants may wilt within a few hours. For this reason, it should be checked whether the nutrient solution flows in the channels. In NFT systems, nutrients are added according to the chemical analysis or electrical permeability of the nutrient solution. Periodic chemical analyzes of micro elements in 4-6 weeks and macro elements in 2-3 weeks are required. However, for this purpose, only the control of the electrical permeability of the solution is sufficient.

In the NFT system, the circulation of the nutrient solution is continuous or intermittent. In intermittent flow application; It saves electricity, extends the life of the pumps, enables plants to bear fruit early in tomato cultivation, and increases the oxygen content of the root zone.

Solution flow; It is programmed using a timer or solar integrator. When the timer is used, the pump starts and stops for a certain period of time (like every 30 minutes). When a solar integrator is used, the flow is programmed based on the solar radiation value. The use of solar integrator provides more precise control, but since it is an expensive application, the use of a timer is preferred in programming the solution flow.

2.1.2.2. Deep or semi-deep flowing water culture

The basic principle of the deep or semi-deep running water culture developed in Japan is the vigorous aeration of the nutrient solution. In this system, the nutrient solution reaches the growing beds through an air mixer. Each cultivation unit is 1 m wide and 3.15 m long. In the system called "M system", the solution in the growing units is sucked by the circulation pump, and the air is given back to the growing unit by passing through the mixers.

2.1.2.3. Layered flowing water culture

Layered systems to increase the number of plants per unit area are used for hobby plant growing in homes and offices or commercially for green or berry fruit growing. In these systems, care must be taken to ensure that the sun's rays are homogeneous.

2.1.3. Aeroponics

In this system, as the nutrient solution is given directly to the plant root in the form of mist, oxygen and water, which limit plant growth, are provided sufficiently in the aeroponic system. In this system, the root zone of the plant is controlled at the maximum level. The feature of the fog and the frequency of fogging are important in practice. In order to apply the system, an opaque container, a nutrient solution tank and a fogging system are needed (Figure 2).

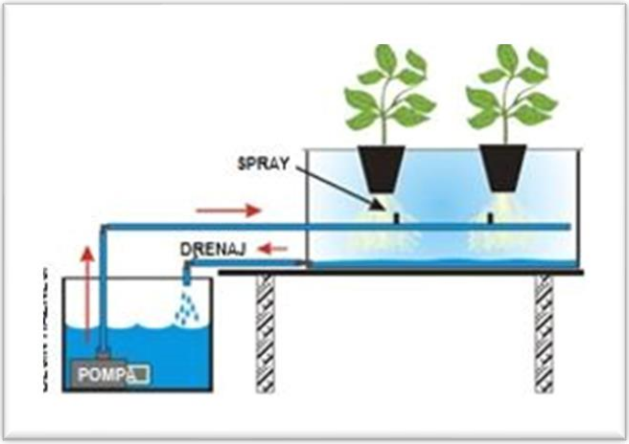

Figure 2. Aeroponic system (Anoniymus, 2015)

The aeroponic system consists of 3 shelves placed at an angle of 120° on a 0.75 inch diameter, 2.10 m long pipe. The distance between the shelves is 60 cm. Growing pots with a length of 100 cm and a depth of 30 cm are mounted on each shelf. Pipes with fogging heads pass through these containers with lids. It is reported that 200-250 units can be placed in one decare. Studies have shown that this technique has important advantages such as increasing the number of plants per unit area, reducing the use of water and nutrients, rapid plant growth, earliness and high total yield. As mentioned above, this technique is suitable for rooting cuttings as well as for the production of different vegetables and ornamental plants. It can be used in different ways to increase the number of plants per unit area.

2.2. Substrate Culture

Substrate culture is more widely used commercially in plant breeding. Substrates (growing media) used in soilless agriculture, organic media (peat, coconut fibers, sawdust, bark, rice husk, peanut shell, etc.), inorganic media (natural sand, gravel, volcanic tuff, etc.), treated media (It consists of four groups: perlite, vermiculite, expanded clay, rock wool, etc.) and synthetic media (polyurethane foam). They are also classified as inert substrates and chemically active substrates. Inert substrates are chemically inert. Chemically active substrates can retain or give nutrients to the environment. Most inorganic materials are chemically inert. However, some inorganic media such as zeolite and vermiculite with high cation exchange capacity are known to be chemically active. Organic media are more or less chemically active.

The role of hydroponic growing media is to provide plants with water and nutrients, helping plants to survive. For a successful aquaculture, physical (such as volume weight, porosity, water holding capacity) and chemical (such as pH, composition, KDK) properties of the growing medium should be known.

Substrates are used by placing them in beds-boats, bags or pots. Rockwool is used in pressed form and covered with plastic. Coconut fibers, the use of which has increased rapidly in recent years, are sold as compressed open or in bags, and are inflated with water in the operation before use. The soilless growing media used vary depending on the countries. For example, while the use of rock wool is common in Northern European countries, perlite and rock wool are mostly used in Mediterranean countries, but pumice and volcanic tuff are also used due to their cheapness. Sand-gravel, which is the oldest medium used in soilless agriculture, has lost its importance due to its heavy weight.

2.2.1. Substrates

2.2.1.1. Organic substrates

Sawdust and shavings are a by-product of the forest industry, which is abundant. Nitrogen-added wood fibers are produced in some countries, especially as a growing medium. Sawdust and chips should be used as a plant growing medium by composting. The quality of sawdust and chips, which decompose more quickly than bark, varies depending on the type of tree and the composting process.

Bark, a by-product of the forest and paper industries, is crushed and screened to obtain the desired particle size. It can be used composted or fresh, depending on the type of tree. While the bark of softwood trees such as pine, fir and spruce can be used without composting, the bark of hardwood trees such as maple and oak should not be used without composting. The quality of the compost obtained from the bark varies according to the type of tree, particle size and composting process. Nitrogen fertilizer should be added during composting. Some tree bark contains substances such as phenols that can have a poisonous effect on the plant, this effect is eliminated by composting. If the bark is to be used without composting, the bark must be thoroughly wetted before use. Fresh tree bark is used in the cultivation of ornamental plants such as orchids and anthuriums and in nurseries. There is no waste problem of tree bark.

Cocopeat (Coconut Peat) is a type of palm cultivated in the tropics. Cocopeat is a fibrous organic medium derived from coconut. Long fibers can be used as rope, mat, etc. used in the manufacture of products. The remaining parts are composted and cocopeat is produced. Cocopeat, which has become widespread in soilless agriculture all over the world, is mostly sold in compressed blocks. Coconut peat, which is inflated with water before use, can be sold covered with plastic such as rock wool to be used in soilless agriculture. The properties of cocopeat, which is sold in plastic packaging, differ according to the source of the product and the processes it sees during production.

Peat, as is known, is obtained from peat beds. Peat consists of the debris left by peat plants, which grow rapidly under water and in swamps, during their long vegetation, and accumulate in heaps under anaerobic and airy conditions. In our country, there are peat beds in the provinces of Bolu, Denizli, Van, Kahramanmaraş, Kayseri, Erzurum and Kars. Our Kars-Göle and Bolu-Yeniçaga peats are suitable for agricultural use (Sevgican, 2003). Companies producing ready seedlings import peat from Germany, Lithuania, France and Belgium and use it in seedling production (Varış et al. 2004). Due to the uncontrolled use of our domestic peat resources, the lack of quality control and standards, and the increasing demand for this material in our country, a large amount of peat is imported every year.

Peat can be used alone or mixed with other materials in the growing medium. Peat consists of partially decomposed organic materials. It is the material of geological origin, which is formed as a result of the accumulation of plant residues under limiting conditions such as excess water and oxygen deficiency. Regions suitable for peat formation are regions with high precipitation, high humidity and cool summer months. Peat is used as a cover soil in nurseries and greenhouses, in making pillows, in vegetable production, in ornamental plants, in potting mortar, in seed germination, in medium mortar mixture, in rooting cuttings, in cultivated mushroom production. Peat has the feature of constantly having water in the soil, fertilizing at the desired level because it is poor in nutrients, having a positive effect on the growing environment in physical and chemical terms, not containing weed seeds and pathogens, and bringing the pH to the desired level by applying alkaline fertilizer, which is one of the low acidity values, Since it has a low volume weight, its ease of transportation is the benefit it provides to the growing environment.

Other organic wastes used in soilless culture can be used alone or added to soilless growing media. Different vegetable wastes such as rice husk, peanut shells, plant and fruit juice wastes, vegetable or seafood wastes can be given as examples of other organic wastes that can be used in soilless agriculture.

2.2.1.2.Inorganic substrates

Pumice has been tried in many greenhouses and different agricultural plants in scientific institutes in Italy and Spain, apart from Turkey and Turkey, and has proven many times that it has no alternative in soilless agriculture. In addition to its high water retention and heat storage properties, it also eliminates drainage problems while providing aeration due to its porous structure. Pumice improves the physical properties of the environment as well as its chemical properties for the benefit of plants. In addition, it does not contain heavy metals that can harm human and plant health. Pumice stone (pumice) is used for very different purposes not only in the construction sector, but also in industrial areas such as the agriculture sector, the chemical sector, the textile sector, and the abrasive industry. However, experimental and observational studies on topics such as the usage criteria and characteristics of pumice stone and its applicability in industrial areas have not yet reached a sufficient level by the institutions dealing with pumice. In this respect, due to the fact that pumice is an industrial raw material with a significant reserve potential, adequate investigations should be made on it. There is no doubt that a high level of added value will be provided to the country's economy by expanding the usage areas.

Pumice is one of the options used as a remedy for drought in agriculture in most of the European countries. Pumice has an important place among the substances that improve the properties of the soil. Improving the water-holding property of the soil (hydroculture) is very important, especially in areas with water problems. Pumice is an important raw material (input) for plants in agriculture in terms of both its cheapness and its properties. In order for the pumice to be used in this area by adjusting the water/moisture it holds according to the environment it is in and giving it to the plant as needed, its suitability is selected by passing some tests (water retention factor, nutritional property, pore status, apparent density of the granules, etc.). Pumice minimizes the loss of fertilizer in the liquid fertilization system and prevents the contamination of groundwater in agricultural areas (Güngör &Tombul., 1997).

Perlite; It is an aluminum silicate of volcanic origin, which is converted into a white, light and granular structure by heating up to 1000 0C after grinding and has positive properties as a plant growing medium (Varış & Eminoğlu, 2003; Varış, 1998; Munsuz et al., 1982). . There is a perlite reserve approaching 6 billion tons in Western Anatolia (İzmir, Manisa, Balıkesir), Central Anatolia (Ankara, Eskişehir, Nevşehir) and Eastern Anatolia (Munsuz et al., 1982; Sevgican, 2003). . The greatest advantage of perlite as a rooting medium is that the root zones of the rooted cuttings are removed without damage and there is no root loss during diversion. It can be used for years as it is sterile and preserves its properties. Perlite used as rooting medium should be super coarse. It is known that perlite is a very good rooting medium in terms of its properties. However, researchers report that there is a lot of pumice in our region and in many parts of our country. Pumice is easier to obtain than perlite.

Perlite is widely used in greenhouse cultivation, ornamental plants, seedlings, vegetable growing and cut flower production in the world. In the Netherlands and developed countries in this field, production is also carried out in growing environments created entirely by using perlite. There are companies that produce in this way in Turkey as well. The leaf area expands and the number of leaves and roots increases with the mortars prepared using perlite or the growing media created entirely using perlite. In soils mixed with perlite, there is no hardening and the contact between root and soil increases. Due to its inorganic structure, it does not carry weed seeds and diseases. For this reason, with the use of perlite, it is possible to ensure that the producers benefit economically by producing high quality, fast and low-waste products, as well as ensuring that the end consumer buys high quality and long-lasting products.

Peat, perlite, pumice etc. In the studies carried out to determine the effects of different plants grown using media on yield in greenhouse and climate room conditions; plant height, number of stems and leaves per plant, number of nodes on the main stem, colour, vitality, general appearance, leaf area, weight, total product per plant, average fruit weight, average fruit number, product received per unit volume of solution (1 liter), The amount of solution applied during the vegetation period, the number of leaves until the first cluster, the diameter of the plant stems, the pH in the juice, the titratable acidity, the vitamin-C parameters were examined, and as a result, it was stated that the environments in which the soil conditioners used in the research were alone or in a mixture were recommended. (Özkaynak & Samancı, 2004; Kütük, 2000., Dura et al. 2000., Şahin et al.1998., Çinkılıç, 2008., Başayiğit, 1994).

Sand and Gravel are soil minerals whose main component is quartz (S_iO_2). Those with a particle diameter of 2-0.2 mm are classified as "coarse sand", and those between 0.2-0.02 mm are classified as "fine sand". The part with a particle size of 20-2 mm is separated as "fine gravel". It is used as a substrate in desert areas where sand is abundant and coarse sand is preferred. These materials, which were widely used in the first years of soilless agriculture, are not preferred today. It is a heavy material, its water holding capacity is low. Its bulk weight is around 1.48 g/cm^3 in fine sand and 1.80 g/cm^3 in coarse sand. It is not chemically active. It is a material that can be used for many years, it can be disinfected with steam. There is no waste problem, it can be used in soilless post-agricultural landscaping.

Expanded clay is a material with limited use. It is obtained by expanding the clay at 1100°C. Those with a particle size of 3-10 mm are used in agriculture. The volume weight is 0.28-0.69 g/cm^3. Its pH is around 7.0. It is considered chemically inert. This material, which is sterile at the beginning, can be used for a long time by being sterilized when necessary. There is no waste problem.

Vermiculite is a material added to the seedling production environment in our country. Its raw material is a natural clay mineral. Similar to perlite, it is produced as a result of crushing, screening and expansion with 1000°C heat treatment. It is produced in different sizes (0-2, 2-4, 4-8 mm), it is a light and highly porous material. Its pH is around 7.0-7.5, its cation exchange capacity is high (150-210 mmol/kg). It also has the ability to retain phosphate ions. In cases where the ambient pH is low, there is a risk of aluminum being released at a level that will create a toxic effect. It is a sterile material. Not suitable for steam sterilization and reuse. Since it does not cause environmental pollution, there is no waste problem.

Rockwool, which was started to be used in agriculture in Denmark in 1969, is obtained from a mixture of 60% basalt (diabase), 20% limestone and

20% coke. This mixture is melted at a temperature of about 1500°C, which is converted into fine fibers by passing through very rapidly rotating rollers. The length and thickness of the fibers are very important as they determine the porosity of the rockwool to be obtained and are adjusted under the control of the rotational speed of the rollers, the temperature of the mixture and other factors. The additions made during the production phase to the rockwool used for insulation and agriculture are different, the rockwool produced for insulation purposes does not hold water.

Rockwool production used in soilless agriculture is in the form of slices or blocks. Rockwool slices are generally produced 90-100 cm long, 15-45 cm wide and 5-10 cm high; It is sold covered with plastic or open. The height of the rockwool cubes used in seedling production varies between 6.5-10 cm, the surface area of those used for seed planting is approximately 4 cm^2 and the height is generally around 4 cm. In addition, granules are produced to add to the environment, especially in the cultivation of potted plants. The direction of the fibers in the rockwool slices is determined during the production phase and is important in terms of the physical properties of the product. The fibers in the rockwool slices are generally horizontal, so that the capillary action of the nutrient solution in the rockwool slice is facilitated and the upper part of the rockwool slice is prevented from drying out after irrigation. In rockwool cubes used in seedling production, the fibers are generally vertical. The production stage affects how many years the rockwool can be used and its suitability for steam disinfection.

Seedling production and soilless plant cultivation in rockwool must be of the same quality, different quality products should not be used in the same greenhouse as irrigation may need to be programmed differently. It is a light material, its volume weight is around 0.07-0.1 g/cm^3. On average, 5% of dry rock wool slices are fiber and 95% are air voids. After irrigation, the water:air ratio in the drained rockwool slices is 65:30. Plants can easily take up to 90% of the water in the rockwool. Excess water drains easily, so plants are not adversely affected by overwatering. It is not chemically active. However, rockwool can slightly increase the pH of the nutrient solution, this feature is observed in the first year of use, this effect decreases in the following years. For this reason, it is necessary to reduce the pH of the nutrient solution applied to the plants so that the pH of the medium is 5.5-6. It is sterile, can be sterilized for reuse. There is a waste problem.

Polyurethane can be given as an example of synthetic organic substrates obtained by the reaction of di-isocyanates with substances such as glycol. Its use as a substrate is found in Belgium and the Netherlands. It is very light, its volume weight is 0.078 g/cm^3. It has high aeration capacity and low water holding capacity. Element content is not significant; It does not release any element except Fe, Zn and B. It can be used easily for ten years and can be disinfected with steam. Its wastes are used in the production of foam for construction purposes or are incinerated.

2.2.2. Effects of substrates on soil properties

Soil health and existence, which is necessary for a safe and quality food, is only possible with a sustainable agriculture system. With the sustainable agriculture system, the integration of many activities such as recycling organic wastes, strengthening the soil with biological fertilizers, biological control methods and cleaning the pollutants of agricultural ecosystems by biological means is provided. It is an ecological environment and a necessary system for production. Many toxic and dangerous chemical inputs are used in agricultural ecosystems and these are carried by plants, soil, underground and surface waters and mixed into foods. In order to provide ideal conditions for plant growth in the soil environment, inorganic materials such as pumice, perlite, volcanic ash can be used as a regulator of physical and chemical properties of the soil, and organic materials such as peat, peat, sawdust can be used separately or in mixtures as a plant growing medium directly or indirectly. A quality growing environment; It should have good aeration and drainage, appropriate bulk density, moisture characteristics, reaction, electrical conductivity, cation exchange capacity, balanced and optimum nutritional element and the level of providing these elements. The growing environment should have properties that will reduce the frequency of irrigation as well as providing good drainage conditions. Different organic and inorganic materials are used to regulate the physical and chemical properties of the soil in order to provide ideal conditions for plant growth in the soil environment.

For this, inorganic materials such as pumice, perlite and organic materials such as peat are used as plant growth media. Pumice is a material used to improve the water holding property of the soil. Adding pumice to the soil is an important application in order to improve the physical conditions in heavy textured soils. By mixing this material with the soil, suitable soil physical conditions are developed in the prepared environments and the air and water balance is brought to optimum levels (Cabrera, 2003; Şahin et al. 2004; Kuşlu et al., 2005; Şahin & Anapalı, 2006). Perlite, which is used in agriculture, has different advantages because it improves aeration and drainage, provides useful moisture and availability of nutrients to plants, acts as an insulator in preventing excessive soil temperature, and is clean, odorless and light in plant development studies (Bunt, 1988; Varış & Eminoğlu, 2003; Örs, 2004; Çinkılıç, 2008). Peat is generally used as an organic soil conditioner because it contributes positively to the agronomic characteristics of plant growing environments in terms of chemical and physical properties. With its suitable physical structure, peat, which creates an environment with high water holding capacity and sufficient aeration of the roots in the root zone of the plant, can be used alone or mixed with other materials (Carlile, 2009; Sönmez et al., 2010; Demirkıran & Cengiz, 2011). The positive contribution of the application of organic materials to the soil on the physical properties such as volume weight, porosity and aggregate stability can cause an increase in the infiltration rate of the soil (Hanay, 1991). It is important to apply it to the soil and to prepare healthy growing environments.

2.2.3. Substrate culture techniques, reuse and nutrient solution delivery methods

Places where sand is plentiful, especially developed for desert areas, cultivation areas called bed, tub or canal, cultivation in beds that allow many plants to be grown together, unlike bags and pots, bags in which a single plant can be planted in substrate culture (fruit sapling bags) or horizontal bags in which more than one plant can be planted (pillow-shaped bags), growing in pots and growing in vertical bags developed for the purpose of increasing the number of plants per unit area.

The repeated use of substrates for a long time is a waste substrate problem and is economically very beneficial. Inorganic substrates can be reused by washing (sand, gravel, etc.) or they can be used by changing them every 4-5 years (pumice, etc.). Since soilless agricultural greenhouses gain importance as an environmentally friendly sustainable form of agriculture, disinfection is carried out with steam. It is tried to prevent diseases and pests by stimulating the natural formation of beneficial microflora or adding certain antagonists to the environment. For this purpose, root bacteria (PGPR) that increase plant growth are generally used. PGPRs are naturally occurring soil microorganisms and increase plant growth by settling in the roots.

In the substrate culture, the water and nutrient requirements of the plants are usually provided by the nutrient solution given by the drip irrigation system. The nutrient solution is applied once or several times a day, depending on the characteristics and volume of the medium used, in such a way that approximately 20% of the given solution is drained. According to the application of the nutrient solution, substrate culture is divided into open and closed systems. In the open system, the solution that drains from the root zone of the plant is discarded, while in the closed system, the solution that drains is collected and recirculated in the system. In the substrate culture, nutrient solution is applied with drip irrigation and capillary system. In the drip irrigation system, the nutrient solution is given from the top, while in the capillary system, the nutrient solution is given from the bottom.

3. ADVANTAGES AND DISADVANTAGES OF SOILLESS AGRICULTURE

The most important advantages of soilless agriculture are that it enables production in places where the soil is not suitable for plant production, increases water use efficiency, feeds plants in a controlled manner, increases yield and quality, requires less labor, is easy to irrigate, sterilization is not required or is easy. On the other hand, the cost of soilless agriculture and the need for technical knowledge are the disadvantages of the system compared to traditional cultivation in soil.

4. THE IMPACT OF SOILLESS AGRICULTURE ON THE ENVIRONMENT AND SOILLESS AGRICULTURE IN THE FUTURE

Agricultural policies prioritized increasing the yield per unit area with high input use in the 1960s-1970s, but 20 years later it was understood that these practices caused environmental and health problems. As a result of this, sustainable environmentally friendly production techniques have been started to be researched. Soilless farming has undoubtedly been adopted and accepted as an environmentally friendly practice. However, as in every application, soilless agriculture can have negative effects on the environment. In soilless agriculture, discarded nutrient solutions, discarded plastics (plastics used as ground cover, used in the preparation of growing places, etc.) and discarded growing media (rock wool) can cause environmental pollution. The formation of pollution elements varies depending on the soilless farming technique used. For example, there is no waste substrate problem in aquaculture. The amount of nutrient solution excreted in the substrate culture also varies depending on whether the system used is open or closed. Also, there are differences between substrates in terms of recycling. Although the negative effects of soilless agriculture on the environment are not yet seen in our country, it is possible that negative effects will occur with the increase in the greenhouse area where soilless agriculture is made. For this reason, urgent measures should be taken on waste management in soilless agricultural enterprises.

Standards developed to ensure consumer confidence in terms of traceability, health and environment in national and international platforms; The fact that water has become a limited resource and the necessity of farming in places where it is not possible to grow plants with traditional techniques are signs that soilless agricultural areas will increase in the future. Today, soilless agriculture can be successfully carried out commercially in greenhouse cultivation. Soilless cultivation can be carried out in different ways, from the very simple to the advanced. Experimentally, successful cultivation is possible in most hydroponic farming systems, but when considering commercial production, it is important to choose the system that is suitable for the farm type and pay attention to details. In terms of the development of soilless agriculture in our country, geothermal areas offer attractive opportunities to investors. Soilless agriculture investments that started in Antalya turned to geothermal fields in the 2000s. The most important climatization event affecting production in greenhouses in our country is heating. Since the share of heating costs with fossil fuels in greenhouses in general expenses reaches 40-60%, the use of alternative energy sources is important. The most important alternative energy source that can be used in greenhouse heating in our country is geothermal energy. Turkey ranks seventh in the world in terms of geothermal resources. For these reasons, it is thought that geothermal fields will maintain their attractiveness for soilless agriculture investments in the future. Investors who are considering establishing a greenhouse business in these areas should first make surveys about the presence and quality of irrigation water when choosing the location of the business.

Entrepreneurs who want to establish a new soilless agricultural business or start soilless agriculture in their existing greenhouses should not forget that soilless agriculture is a form of cultivation that requires knowledge-experience and attention to detail compared to traditional cultivation in soil. Since the mistakes made at the beginning will be difficult to correct after starting production, meticulous attention should be paid at every stage (such as land selection, operating facility, preparation of greenhouses for soilless cultivation). Success in commercial production depends on marketing as well as mastery in breeding. For this reason, manufacturers should strive to find specific markets for their products.

In addition to 15 years of commercial experience in soilless agriculture in our country, there is an important scientific knowledge. In recent years, "Soilless Agriculture" courses are given at the undergraduate level in the Faculties of Agriculture. In soilless agriculture, synergy should be created by combining the existing knowledge in the private and public (university, ministry) sectors. In addition to commercial production, one of the most important future uses of soilless agriculture will be its hobby use at home.

5. CONCLUSION AND RECOMMENDATIONS

Due to the increase in greenhouse areas in our country, the increase in our production has enabled the introduction of new modern technologies to our country and the intensification of the use of these technologies by making them optimally suitable. At the beginning of these technologies, cultivation with soilless culture has an important place. Because the use of this technique, according to conventional cultivation, providing optimum plant health conditions, eliminating the salt problem in the soil, providing optimum plant root development, etc. Having advantages contribute to the increase of cultivation and increase the country's economy.

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CHAPTER 6

WHEAT GROWING IN HUMID OR IRRIGATION AREAS AND SOME RISKS

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1. INTRODUCTION

Wheat is the most cultivated species in the Triticum genus and is consumed by humans in many areas. Wheat is an important cultivated plant grown as tall and annual (Konopatskaia et al., 2016). It is noted that the annual precipitation amount of wheat growing areas in the world varies between 250-1750 mm, but the main wheat belt receives 350-1000 mm precipitation per year and 600-700 mm annual precipitation regularly distributed during the development period is sufficient for maximum yield (Kara vd., 2008).

Wheat can be grown in many different climates and conditions. There are many varieties that can be suitable for any environment, depending on how many days the plant can withstand humidity and heat (Palosuo vd., 2011). Wheat, which has been cultivated sustainably for tens of thousands of years around the world, is the field crop with the largest cultivation area (217 million ha) and the highest production amount (729 million tons) after corn and paddy rice (Atak 2017). The geographical regions suitable for growing wheat in the world are the United States, Canada, Russia, China, Northern Europe and Australia (Yue vd., 2019).

In general, humid regions are not suitable for wheat cultivation. The negativities that occur during germination, seedling development and earing time are the main reasons for this situation. In humid or irrigated areas, wheat is susceptible to many diseases. Three of the most important are stem rot caused by Oidium, brown stem rot caused by Puccinia, and strip rust caused by Ustilago maydis. If the seed is not properly sprayed before sowing, germination problems may occur in the seeds. These problems cause poor germination rates and consequently heavy yield losses (Bisen vd., 2015).

Based on the statements given above, in this study, the general adaptation of wheat, the main diseases seen in wheat grown in humid regions and grown by irrigation are discussed in line with the relevant literature.

2. GENERAL ADAPTATION OF WHEAT

Wheat is a grain that can be easily cultivated in a wide range of climatic zones, especially in tropical and subtropical regions, while requiring fertile soil and regular sunlight. Wheat is known to grow in practically every climatic zone on the earth. It can survive drought, frost, heat and rain. Wheat also grows well in acidic soil, which is a nice change from most other crops (Freebairn vd., 2006). This wide adaptation ability has been further increased by breeding studies over time. Therefore, the growing areas have expanded.

The wheat plant can grow in a variety of climates, but it is most productive in temperate areas. The growing season in temperate zones is long enough to fully develop the plant and produce seed, but short enough to avoid extremely hot or cold weather (Zwart vd., 2010).

Wheat is a globally important crop and is grown on every continent except Antarctica, with the exception of some isolated island nations where climatic conditions do not allow its successful cultivation. The regions of production are determined by the temperatures that wheat seeds can tolerate, which are generally between 14°C to 27°C (Birch vd., 2012).

Wheat is a staple food for humans. It is the most widely grown grain and is grown in more than 105 countries. The most important wheat producing countries are China and India, followed by the USA, Russian Federation, Germany and France. There are three main types of wheat (Zaharieva vd., 2010).

Wheat is an important crop worldwide, not only as a source of food and animal feed but also in providing raw material for the textile and paper industries. Cereal crops are characterized by high leaf biomass production, which makes them vulnerable to temperature extremes. Wheat therefore occupies a climatic niche between annual crops such as corn and perennial plants such as sugarcane, which have fairly wide latitude for germi-nation and flowering but tend to be less productive than wheat (Devi vd., 2017). The optimum temperature for wheat growth has been estimated at about 15-20°C, with growth inhibited below this temperature (cold acclimation) and above 30°C (heat stress). This latter temperature can be encountered at various parts of the world during periods of drought or heavy rainfall followed by heat waves during summer (Song vd., 2005).

Wheat has a wide geographic distribution, and grows throughout the world in a range of climates. While it has adapted to most environments where it is economically grown, wheat has certain climates that it prefers. In particular, it does not like cold weather or hot arid conditions. Wheat is a crop grown in the temperate and tropical zones. In temperate regions, the crop grows well on soils with good drainage. Tropical climates are characterized by an abundance of rainfall, but do not allow for grains to mature sufficiently for harvest (You vd., 2009).

Wheat is grown in most climatic zones of the world, provided appropriate varieties are chosen. The highest yields are achieved at temperatures between 20 and 24 degrees °C, with humidity levels of about 85%. The wheat yield will be higher if a rainfall of 900 to 2000 mm per year is received (Sultana vd., 2009).

THE MAIN DISEASES SEEN IN WHEAT GROWN IN HUMID REGION

The major diseases affecting wheat grown in humid regions include leaf rust, leaf blotch, stem rust and wheat diseases caused by viruses. These diseases are caused by various fungi, oomycetes and viruse (Sutela vd., 2019).

It is stated that there are many diseases in wheat and rust is the most damaging one. In this context, the main disease-causing problems in wheat in humid regions can be expressed as waterlogging, soil salinity and potassium deficiency (Timsina & Connor, 2001).

Wheat is susceptible to disease as it does not have an effective immune system. One solution to these problems was expressed as using higher yielding varieties and deep plowing. It is stated that this application reduces the disease pressure on the product and increases the quality. Stem rot is caused by fungi that attack the stem while developing, and it has been explained that the resulting air circulation will help prevent rot (Singh vd., 2016).

It is reported that the main diseases seen in wheat grown in the humid region are generally leaf blight (early infestation), rust and powdery mildew (Gautam vd., 2013).

It is known that air temperature changes and humidity can cause important diseases in wheat grown in humid regions of the world. In this context, it can be stated that three main types of pathology have been developed. These species can be explained as root and root rot rot, gray leaf spot and Septoria leaf spot diseases. It is explained that if these diseases are not controlled in time, they can cause 10-15% product loss (Séguin vd., 2009).

Wheat is one of the most produced and consumed cereals in the world and in our country. It is known that the losses of wheat produced every year by affecting wheat agriculture in a negative way vary between 3-50% at the end of the harvest and most of these losses are caused by root and root collar fungal disease agents. Among these factors, it has been reported that Fusarium species cause crop losses up to 17% due to severe infections in the root and root collar of wheat as a result of seed/soil borne infections (Köycü and Özer, 2019).

Brown rot is another major disease affecting wheat grown in humid regions. This disease factor is explained as cold, rainy weather and high humidity, which reduces the soil temperature below normal levels for fungi that grow in warm soils (Khrieba, 2020).

MAJOR DISEASES OBSERVED IN IRRIGATED WHEAT

Water stress conditions, water density, soil types and soil chemical composition can affect seed grain quality for germination and germination ability, seedling vigor and growth rate. In this context, in addition to low crop yields, pre-harvest growth defects are observed. These diseases are caused by abnormal development of the crop, both before and after harvest. (Koike vd., 2007).

In irrigated or humid regions, the most important characteristics for high yield are resistance to lodging, while drought resistance is in arid areas. Zeleny, who examines the inheritance of the bread quality of wheat, considers the sedimentation value, protein ratio and thousand grain weight as important quality criteria. In addition to the high protein rate in wheat, the quality of the protein is also important. One of the important methods used to determine the quality of wheat protein is sedimentation value. All these factors are affected by the growth environment of wheat (Mut, 2005).

Irrigated wheat is susceptible to many diseases. Three of the most important are stem rot caused by Oidium, brown stem rot caused by Puccinia, and strip rust caused by Ustilago maydis. (Grewal vd., 1996).

To meet the increasing demand for wheat, farmers irrigate their wheat fields. This can be done by sprinkling or furrow irrigation. In this context, the fact that the sprinkler system is cheap and easy to maintain increases its preferability (Timsina & Connor, 2001). Due to this preference, disease factors arising from the irrigation method easily emerge.

Irrigated wheat, grown under stress in water-deficit situations over large areas of the world, faces challenges related to water use efficiency that differ in the types of fungal pathogens infecting them. Corticosteroid-induced hypersensitive response can affect multiple elements of wheat breeding programs (Singh vd., 2020).

Brown spot is described as a disease that affects grain crops in some parts of the world and is characterized by shedding marks on the leaves or spikes of plants. The disease causes brown streaks, pale green or yellow spots on leaves, or diseased-looking margins. Rust is caused by fungi and requires a lower temperature to initiate fungal growth. At lower temperatures, water stress may delay symptom development in a crop, however, at higher temperatures, disease symptoms may be observed at a much earlier stage than crop quality improvement (Streets, 1972).

3.CONCLUSIONS

The results obtained from this study, which was created in order to discuss the general adaptation of wheat, the main diseases observed in wheat grown in humid regions and irrigated in line with the relevant literature, are listed below:

Wheat is the most important cereal crop in temperate regions and has been found to constitute the basic foodstuff for most of humanity. It has been determined that the climate in which wheat is grown affects the characteristics of the plant and grain, including protein content, gluten content, yield potential and harvest maturity. It has also been determined that wheat can be grown in a wide variety of climatic conditions, but most varieties will develop best in temperate climates with sufficient rainfall at important stages of development.

It has been determined that leaf rust, leaf spot, stem rust and wheat diseases caused by viruses occur in wheat grown in humid regions. Similarly, it has been shown that various diseases such as fascia leaf spot, brown spot, rust and stem cancer can occur in wheat grown by irrigation.

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CHAPTER 7

CHANGES OF THE INTERNAL TEMPERATURE IN BALE SILAGE WITH DIFFERENT APPLICATIONS*

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INTRODUCTION

Silage is one of the roughage preparation methods in which water enriched green forages mass is left to ferment in an airless environment (İpsaş et al., 2009). Factors such as silo shape, silo size, quality of plant materials used, mechanization infrastructure, numbers of animals, human resources, preliminary wilting, shredding, compacting, providing airless environment, using additive materials limit traditional silage production for small enterprises. Furthermore, these factors directly affect silage quality. Bale silage, a method of preparing a different roughage other than hay and traditional silage, was used for the first time in the UK where there is a humid climate in the 1950s (Çakmak and Yalçın, 2005). This method is applied by packaging grass having a dry matter (DM) ratio of 40 - 60% with a special film material and keeping them in a kind of silage for a long time. It has been reported that this roughage also known as bale silage is more valuable in terms of DM and nutrient losses than dry hay and traditional silage (Noller and Thomas 1985; Ball et al. 1996; Schroeder, 2004; Kılıç and Galipoğlu, 2008).

It is important that all stages are determined and followed up from sowing to storage for good quality silage. Silage microbiology is under the influence of three interrelated factors. These are plant materials, silage preparation and fermentation. Fermentation occurs under the influence of very different microorganisms, both aerobic and anaerobic (Basmacıoğlu and Ergül, 2002). Lactic acid bacteria (LA) from anaerobic microorganisms are more effective in quality silage. These bacteria become active at certain temperatures and maintain the quality of silage (Ipsaş et al., 2009). The internal temperature when silage microorganisms are most active is 37°C. The internal temperature must be between 15°C - 30°C for the growth of LA and for active operation. 45°C - 62°C is an undesirable internal temperature for silage and is a suitable ambiance for the growth of harmful microorganisms (Kılıç, 2010). Similarly, Uygur (2015) emphasized that internal silo temperature must be 15°C - 25°C and that an internal temperature of up to 35°C - 40°C was an indication that fermentation had not taken place as desired. Pitt (1990) reported that heat production in silage could be used as a marker of aerobic stability. He stated in his study that this value should be 35°C at the most. It has been reported that in the aerobic period when LA which have the task of protecting silage, loss of energy and nutrients will increase with the increase in internal temperature and when the internal temperature of silage reaches 42°C - 44°C the Maillard and Browning Reactions occurs and spoil the roughage (Filya, 2001). Butter acid bacteria (BA), are undesirable bacteria in silage. These bacteria compete with the carbohydrates used by LA and multiply rapidly due to the increase in the temperature in the silo. The optimal temperature range for these bacteria is 37°C - 50°C. At high temperatures such as 42°C the formation of BA occurs in roughage plants with low DM content and at 20°C and below the formation of LA. The LA starts to dominate (Basmacıoğlu and Ergül, 2002). In studies conducted under laboratory conditions, it has been reported that when the internal temperature of silage is 8°C - 12°C higher than the ambient temperature, approximately 1.5 - 3.0% of DM is lost per day (Filya, 2001). Therefore, determining how the internal temperature of traditional silage or bale silage changes in relation to the applications in silo operations is an important issue in terms of obtaining quality feed and reducing losses.

Monitoring the internal temperature gives information about the status of the silages feed. In a study carried out by Kılıç (2010), he proposed that with the increase of the internal temperature it would be a good solution immediately to use the silage as feed to prevent the silage from spoiling. Active fermentation in silage occurs within the first 7 - 21 days which is the most critical period. During this period, the pH decreases and the fermentation ends because sugar is depleted. The type of fermentation formed during this period is directly related to the quality of silage. Forages containing legumes need more sugar, namely, LA (Filya, 2001). The remaining oxygen in the silo causes temperature increase of 2oC in the silage. Loss under these conditions should be kept under control because it can cause 10% of the DM to be lost within 10 days (Kutlu, 2009). It has been determined that as the ambient outdoor temperature increases, LA production decreases. As a result, this forage mixture can be maintained at the best silage quality without addition of organic acid when the temperature of the storage medium is 20^{o}C (Koç et al., 2010).

There are studies determined internal temperature changes during fermentation in traditional silage; however studies on how this change takes place in bale silages are limited. Fermentation characteristics of traditional silage and bale silage are different because of the different DM content and plants material related applications.

The aim of this study was to determine the changes of the internal temperature of the bale silages made with two different forages mixtures according to the plant material, the harvest machinery used and the number material wrapping layers. Furthermore, by comparing the changes of quality parameters according to the applications, the best quality silage packaging and storage method has been determined. The data obtained from the baled silages were also compared with the traditional silage.

2. MATERIAL and METHOD

2.1. Material

The study was conducted in the farming fields of Eastern Mediterranean Agricultural Research Institute for two years in the 2014-2015 and 2015-2016 growing season, in Adana province, Turkey. According to the measurements the soil contains values of pH 7.86 - 7.75, lime 16.3 - 15.9%, phosphorus 5.16 - 4.37% and organic matter content 2.41 - 2.28% at a soil depth of 0 - 30 cm and is suitable for forage crops cultivation. The highest temperature in Adana province is around 45.6°C in August and the lowest is around -8.1°C in January. The most rainfall is observed in December, with the least rainfall in July. The number of days with relative humidity in excess of 90% is numerous.

The harvesting machines used were disc mower with conditioner and mower machine. The technical characteristics of machines were also tabulated in Table 1.

Table 1: Some Technical Characteristics of Machines

Disc mower with conditioner	**Number of disc**	**5**
	Working width (cm)	240
	Tractor power (Hp)	50 - 80
	PTO (min)	540
	Weight (kg)	500
Mower	Number of drums	2
	Working width (cm)	165
	Number of cutting blade	6
	PTO (min)	540
	Weight (kg)	360
Wrapping machine	Electrical requirements (V)	12
	Film width (mm)	200 / 250
	Film stretching ratio (%)	70
	Dimensions LengthxWidthxHeight (cm)	123 x 158 x 164
	Weight (kg)	350

Round baler	Feed Circle (mm)	500 x 700
	Weight (kg)	540
	Dimensions LengthxWidthxHeight (cm)	240 x 152 x 155
	Bale weight of dried forage (kg)	25 - 30
	Bale weight of green forage / hay bale (kg)	35 – 40 / 15 - 20

Two forages mixtures namely, vetch + triticale (Vicia sativa L. - Triticasecale wittmack) and caramba + berseem clover (Lolium multiflorum cv Caramba - Trifolium alexandrinum L.) were used as plant material in the experiment. Mixture rates were 70% and 30% for vetch + triticale and 50% and 50% for caramba + berseem clover, respectively. Plants were harvested at the end of the flowering stage. During growing period, the sowing was carried out in November and harvesting was carried out in the last week of April. 150 kg ha-1 20:20 fertilizer was used with the sowing. The dough stage of the wheat grains in the blends was taken into consideration in the determination of the harvesting date. This is when the DM content is 25 - 30%. Forage harvested with an average of 30% DM was left to wilt to reach 40 - 60% DM content. The wilting process took an average of 18 hours during the first year and 48 hours during the second year depending on the temperature and humidity of the harvesting season. Forages reaching the desired level were wrapped with 4, 6, 8 layers of white polyethylene material (PE) in the form of round small bales each weighing 40 - 50 kg (0.025 mm thick, 25 cm wide and white color PE coating material was used in the wrapping process). The bales were kept in a shaded covered environment with side walls for over 60 days. Traditional silages were harvested on the same day as bale silages. The DM content of these silages was 25 - 30%. The traditional silage as well as the mixtures were compressed into layers in trenches. After being covered with a silage cover, it was left to ferment for 60 days.

2.2. Method

The study was carried out on randomized blocks according to split trial design for two years as three replications. Bale silages that two forage mixtures (FM): vetch + triticale (FM1) and caramba + berseem clover (FM2) were assigned to the main plots, two harvesting machines (HM) for the sub plots: disc mower with conditioner (HM1) and mower (HM2), and three wrapping layers (WL) for the sub sub plots: 4(WL1), 6(WL2), 8(WL3) were established. The average values of the data obtained from the traditional silage (TS): vetch + triticale (TS1) and caramba + berseem clover (TS2) applied in both varieties as witness samples were used for comparison with bale silages. The samples of roughage obtained after the 60 day fermentation were analyzed. The DM content of roughage was determined by drying to constant weight at 105oC according to the ASAE standards (AOAC, 1990). Acid detergent fiber (ADF%), neutral detergent fiber (NDF%) were determined as suggested Van Soesst et al. (1991) by using ANKOM fiber analyzer. Crude protein (CP%) was determined according to AOAC (1990). The Relative Feed Value (RFV) was calculated according to Mayouf and Arbouche (2014). The pH of samples was measured using a pH meter Chen et al. (1997). The densities of roughages were taken as the volume fraction of the weight. The quality class of the roughage was determined using "Quality Standards for Wheat and Leguminous Grain Crops" (Ball et al. 1996; Mayouf and Arbouche, 2014; Russell, 2014) which provides standards for the quality assessments of roughage. Templog-Ig150 (Figure 1) data recorder was installed to measure the average internal temperature of 15 daily (IT15) and 30 (IT30) daily before the bales were covered with PE material. The data on the device which was acquired by measurements every 9 minutes was interpreted into a daily average. The ambient temperature and the warehouse temperatures were also recorded using the same measuring device during the same period. The trial was carried out to determine the impact of plant material, harvesting machines and the number of wrapping layers on bale silage in terms of 15 day and 30 day average internal temperature change. The data collected were analyzed statistically. Data were subjected to analysis of variance for FM, HM, WL. The LSD test at P = 0.05 was used to compare the treatment means. In additional, the data obtained from the bale silages were also compared with the traditional silage data.

Figure 1: Templog-ıg150 data recorder used to store internal temperature values in the trials

3. RESULTS and DISCUSSION

The data obtained for two years in the experiment were tested for homogeneity according to years and it was determined that the data for 2014 and 2015 were homogenous according to Std - Dev values (Table 2).

Table 2: Homogeneity test of data according to years

Parameter / Y	Std - Dev		F Ratio	DFN	Prob>F	p value (F test)
	Y1	Y2				
DM (%)	7.63	3.02	0.858	1	0.3590	<.0001**
pH	0.52	0.35	13.73	1	0.0005*	0.0201*
Density (kg m^{-3})	58.02	32.07	0.087	1	0.7682	0.0007*
ADF (%)	2.54	3.02	130.67	1	<.0001**	0.260
NDF (%)	2.46	2.34	44.94	1	<.0001**	<.0001**
CP (%)	2.59	1.24	18.87	1	<.0001**	<.0001**
RFV	8.04	6.73	106.54	1	<.0001**	0.3011
IT_{15}(°C)	1.71	0.64	25.64	1	<.0001**	<.0001**
IT_{30}(°C)	0.91	0.40	9.22	1	0.0039*	<.0001**

Y (Y1: First year and Y2: Second year), DM: dry matter, pH: acidity, ADF: acid detergent fiber, NDF: neutral detergent fiber, CP: crude protein, RFV: relative feed value, IT_{15}: internal temperature change of 15th days, IT_{30}: internal temperature change of 30th days

The evaluation of the data for 2014 and 2015 was presented with separate and combined analyses (Table 3, Table 4 and Table 5). In Table 3 ADF, NDF and CP values were examined in bale silages according to the applications. Statistically it was determined that HM ($p<0.001$) was effective on ADF. It has been found that the ADF value of roughage harvested with the disc mower with conditioner (HM1) is higher than that of harvested with mower (HM2). The FM and WL parameters were not statistically significant on the ADF. According to the ADF value, the roughages obtained from all applications were found to be 2nd quality according to the standards determined by Mayouf and Arbouche (2014). The FM x HM ($p<0.05$) interactions for ADF was determined.

Table 3: ADF, NDF and CP variance analysis according to applications in bale silages.

Parameters		ADF (%)			NDF (%)			CP (%)		
		Y1	Y2	Ave.	Y1	Y2	Ave.	Y1	Y2	Ave.
Y	Y1	-	-	33.7[b]	-	-	51.0[b]	-	-	11.3[a]
	Y2	-	-	41.3[a]	-	-	54.9[a]	-	-	9.3[b]
FM	FM_1	32.1	42.7[a]	37.3	50.3[b]	55.2	52.7	9.1[b]	8.5	8.8[b]
	FM_2	35.3	39.9[b]	37.6	51.8[a]	54.5	53.2	13.6[a]	10.0	11.8[a]
HM	HM_1	33.5	39.5[b]	36.4[b]	51.4	54.7	53.0	11.6	9.5[a]	10.3
	HM_2	33.9	43.2[a]	38.5[a]	50.7	55.0	52.9	11.1	9.0[b]	10.3
WL	WL_1	32.5[b]	43.2[a]	37.8	49.6[b]	55.9[a]	52.8[ab]	11.4	9.3	10.3
	WL_2	34.7[a]	40.4[b]	37.5	52.5[a]	54.9[ab]	53.7[a]	11.1	9.4	10.2
	WL_3	33.9[ab]	40.3[b]	37.1	51.0[ab]	53.7[b]	52.4[b]	11.6	9.1	10.3
CV(%)		5.3	3.6	4.3	3.6	3.3	3.6	10.6	10.8	10.9
p value										
Y		-	-	**	-	-	**	-	-	**
FM		ns	*	ns	*	ns	ns	*	ns	**
HM		ns	*	**	ns	ns	ns	ns	*	ns
WL		*	**	ns	**	*	*	ns	ns	ns
FM x HM		ns	ns	*	ns	ns	ns	ns	ns	ns
WL x FM		ns	ns	ns	*	*	ns	ns	ns	ns
WL x HM		ns	ns	ns	ns	ns	ns	ns	*	ns
FM x HM x WL		ns	ns	ns	ns	ns	ns	ns	ns	ns
Average values for conventional silage										
TS_1		41.77	49.40	45.59	57.90	66.28	62.09	10.81	8.70	9.76
TS_2		39.87	48.05	43.96	45.33	63.08	57.21	13.77	12.88	13.33

The differences between the meanings indicated by different letters in the same column are significant. * $P<0.05$, ** $p<0.01$ is important within error limits, ns is not important. Y: year, FM: forage mixture (FM_1: vetch + triticale bale silage, FM_2: caramba + berseem clover bale silage), HM: Harvesting machine (HM_1: disc mower with conditioner, HM_2: mower), WL: Number of wrapping layers (WL_1:4, WL_2: 6, WL_3:8), TS: Traditional silage (TS_1: vetch + triticale traditional silage, TS_2: caramba + berseem clover traditional silage)

It was obtained that the WL ($p<0.05$) was effective on NDF statistically. It has been reported that when the NDF value decreases, the feed intake level of animals increases (Van Soest et al. 1991; Van Soest, 1994; Tekce and Gül, 2014). Similarly, in this study, it was also determined that the NDF value is the lowest in WL3 (53.75%) compared to other wrapping layer numbers (WL1, WL2). A similar result has been reported by O'Kiely et al. (2002) in bale silages made with Perennial Ryegrass. In their study, Keller et al. (1998) examined the effect of 4, 6, 8 layers of wrapping on quality and determined that less mold was formed in bales wrapped with 6 or 8 layers of wrapping compared to those wrapped with 4 layers. Yaman and Sönmezler (2011) reported that their study revealed that wrapping with 4 and 6 layers had no effect on quality however; bales wrapped with 6 layers of wrapping generated less mold.

The FM, HM parameters had no statistical effect on the NDF. However, according to the NDF value, it is determined that all the parameters were 2^{nd} quality. When the mean values of the mixtures in the bale silages were compared with the traditional silage, FM1 was found to have a lower ADF value by 17.99% than TS1 and FM2 by 14.33% compared to TS2. The same is valid for the NDF value, which was achieved with low NDF values of 15.03% and 6.96%, respectively. Budak et al. (2014) reported that the best roughage was obtained with the highest protein and lowest ADF and NDF. Polak and Jancovo (2006) studied the CP ratio of forage harvested with two different harvesters and determined that the protein value was slightly higher for harvester with the plastic conditioner than the metal conditioner despite not significant statistically. It has also been reported that the CP ratio tends to decrease with the increase of DM content (Müller et al. 2007). A similar result was obtained in this study. Traditional silages have low DM contents but high CP rates. In bales silage, the DM ratio increases while the CP ratio decreases compared to traditional silages (Table 3 and Table 4).

Table 4 shows the combined variance analysis values of DM, pH and Density. It was determined that FM ($p<0.001$) on DM and HM ($p<0.001$) was effective on pH ratio statistically. It has been found that the pH ratio of the bale silages (5.15) of the roughages harvested with the disc mower with conditioner was higher than the pH value (5.58) of harvested with the mower.

Table 4: DM, pH and Density variance analysis according to bale silage applications

Parameters		DM (%)			pH			Density (kg m^{-3})		
		Y1	Y2	Ave.	Y1	Y2	Ave.	Y1	Y2	Ave.
Y	Y1	-	-	48.97[b]	-	-	5.56[a]	-	-	293.8
	Y2	-	-	50.24[a]	-	-	5.17[b]	-	-	297.1
FM	FM_1	55.8[a]	50.59	53.24[a]	5.65	5.18	5.41	245.1[b]	279.4[b]	262.2[b]
	FM_2	42.0[b]	49.88	45.97[b]	5.47	5.16	5.31	342.5[a]	314.9[a]	328.7[a]
HM	HM_1	47.8	51.55	49.69	5.25	5.04[b]	5.15[b]	311.9	324.2	313.1[a]
	HM_2	50.1	48.92	49.51	5.87	5.29[a]	5.58[a]	275.7	280.0	277.9[b]
WL	WL_1	48.5	49.28	48.93	5.69	5.09[b]	5.39	283.3[b]	294.0	288.7
	WL_2	48.4	51.27	49.84	5.42	5.39[a]	5.41	294.6[ab]	300.8	297.7
	WL_3	49.9	50.16	50.04	5.56	5.02[b]	5.29	303.6[a]	296.5	300.0
CV (%)		4.80	4.99	5.24	6.95	4.45	6.37	6.05	6.53	7.61
p value										
Y		-	-	*	-	-	**	-	-	ns
FM		**	ns	**	ns	ns	ns	**	*	**
HM		ns	ns	ns	ns	*	**	ns	*	**
WL		ns	ns	ns	ns	**	ns	*	ns	ns
FM x HM		ns	ns	ns	ns	*	ns	ns	ns	ns
WL x FM		ns	ns	ns	ns	*	ns	**	ns	*
WL x HM		*	ns	ns	ns	ns	ns	**	ns	ns
FM x HM x WL		ns	ns	ns	*	ns	ns	ns	ns	ns
Average values for conventional silage										
TS_1		29.6	29.54	29.61	4.26	4.17	4.22	310.2	412.3	356.8
TS_2		28.3	24.20	26.24	4.28	4.73	4.51	695.8	627.0	661.4

The differences between the meanings indicated by different letters in the same column are significant. * P<0.05, ** p<0.01 is important within error limits, ns is not important. Y: year, FM: forage mixture (FM_1: vetch + triticale bale silage, FM_2: caramba + berseem clover bale silage), HM: Harvesting machine (HM_1: disc mower with conditioner, HM_2: mower), WL: Number of wrapping layers (WL_1:4, WL_2: 6, WL_3:8), TS: Traditional silage (TS_1: vetch + triticale traditional silage, TS_2: caramba + berseem clover traditional silage)

The pH ratios of traditional silage are higher compared to those of bale silage. Huhnkle et al. (1997) reported that the pH ratio in bale silages could go up to 6.5. In a study conducted in Turkey the pH ratio for mixtures cereal + legume small bale silage was found to be 5.9 (Yaman and Sönmezler, 2011). The pH ratio increases with the proportion of legumes in the mixture (Konca et al., 2005). Therefore, it is not possible to reach a correct conclusion about the quality of roughages by looking at the pH ratio. Statistically FM ($p<0.001$) and HM ($p<0.001$) were found to be effective on Density. It has been determined that other parameters have no statistical significance on DM, pH, and Density. While the DM value of the bale silage was 53.24% and 45.97% for the FM1 and FM2 respectively, this value was 29.61% and 26.25% for the traditional silage respectively. While the pH value of the bale silage was 5.41 and 5.31 respectively, this value was 4.22 and 4.51 in the traditional silage. The density of bale silage was 262.29 and 328.75 kg m-3, whereas the density of the traditional silage was 356.83 and 661.46 kg m-3, respectively. The WL x FM interaction was also significant effect on density (p<0.05). Furthermore, regression coefficient (R2) value between DM and Density, DM and pH, pH ratio and density was calculated as 69.55%, 82.13% and 43.13%, respectively (Table 4 and Table 7). Similarly, Bilgel et al. (1997) compared the pH ratio of loose and tight bale silages in their studies and found that the pH ratio level increased as the density decreased. The variance analysis results for IT15, IT30 and RFV were tabulated in Table 5.

Table 5. IT_{15}, IT_{30} and RFV variance analysis according to applications

Parameters		IT_{15} (°C)			IT_{30} (°C)			RFV		
		Y1	Y2	Ave.	Y1	Y2	Ave.	Y1	Y2	Ave.
Y	Y1	-	-	24.9^{a}	-	-	22.9^{b}	-	-	114.3^{a}
	Y2	-	-	23.4^{b}	-	-	23.4^{a}	-	-	96.28^{b}
FM	FM_1	23.4^{b}	22.9^{b}	23.2^{b}	22.1^{b}	23.2^{b}	22.6^{b}	118.5^{a}	94.04	106.29
	FM_2	26.4^{a}	23.8^{a}	25.1^{a}	23.7^{a}	23.7^{a}	23.7^{a}	110.1^{b}	98.51	104.32
HM	HM_1	25.24	23.42	24.3^{a}	23.07	23.47	23.27	113.8	98.9^{a}	106.4^{a}
	HM_2	24.68	23.41	24.0^{b}	22.86	23.47	23.17	114.7	93.6^{b}	104.1^{b}
WL	WL_1	24.70	23.48	24.09	22.84	23.54	23.19	119.5^{a}	91.9^{b}	105.7^{ab}
	WL_2	24.97	23.44	24.21	22.95	23.48	23.22	109.5^{c}	97.2^{a}	105.4^{b}
	WL_3	25.21	23.33	24.27	23.11	23.38	23.25	113.8^{b}	99.6^{a}	106.7^{a}
CV (%)		2.91	1.84	2.40	1.62	1.47	1.53	4.29	4.72	4.42
P value										
Y		-	-	**	-	-	**	-	-	**
FM		**	*	**	**	*	**	*	ns	ns
HM		ns	ns	*	ns	ns	ns	ns	*	*
WL		ns	ns	ns	ns	ns	ns	**	**	*
FM x HM		ns	ns	**	ns	ns	*	*	ns	ns
WL x FM		ns	ns	ns	ns	ns	ns	*	*	ns
WL x HM		ns	ns	ns	*	ns	ns	ns	*	ns
FM x HM x WL		ns	ns	ns	ns	ns	ns	ns		ns
Average values for conventional silage										
TS_1		35.88	33.36	34.62	32.04	33.03	32.54	90.96	70.84	80.90
TS_2		31.01	29.10	30.06	28.84	29.96	29.40	118.67	75.88	97.28
Warehouse and outdoor environment temperature										
WT		21.74	22.19	21.96	21.44	23.03	22.23			
ET		21.52	21.00	21.26	21.61	23.41	22.51			

The differences between the meanings indicated by different letters in the same column are significant. * P<0.05, ** p<0.01 is important within error limits, ns is not important. Y: year, FM: forage mixture (FM_1: vetch + triticale bale silage, FM_2: caramba + berseem clover bale silage), HM: Harvesting machine (HM_1: disc mower with conditioner, HM_2: mower), WL: Number of wrapping layers (WL_1:4, WL_2: 6, WL_3:8), TS: Traditional silage (TS_1: vetch + triticale traditional silage, TS_2: caramba + berseem clover traditional silage), WT: Warehouse temperature, ET: Outdoor environment temperature; RFV=(88.9-(0.779×ADF%))×((120/NDF)/1.29%)

It was determined that FM ($p<0.001$) and HM ($p<0.05$) were effective on IT15 according to statistically data. However, it has been obtained that WL has no statistically significant effect on IT15. It was found that only FM ($p<0.001$) was effective on IT30 statistically. The FM x HM interaction for IT15 and IT30 values was found to be effective at 1% and %5 significant levels. Also, it was determined that HM and WL had a statistically significant on RFV ($p<0.05$). This roughage was obtained as 2nd quality. In terms of RFV, it has been determined that the highest quality roughage silage was achieved by harvesting disc mower with conditioner and wrapping with 8 layers (WL3) of PE material.

IT15 and IT30 values were given as an average for bale silage and traditional silage in Table 6. The IT15 difference between the bale silage and the traditional silage was 11.41°C and 4.89°C, respectively, according to the two year mean values in the mixture of vetch + triticale (FM1) and caramba + berseem clover (FM2).

Table 6: IT_{15}, IT_{30} values in bale and traditional silage according to mixtures (°C)

Application	IT_{15}			IT_{30}			
	Y1	Y2	Ave.	Y1	Y2	Ave.	[IT_{15}-IT_{30}]
FM_1	23.45	22.97	23.21	22.16	23.21	22.68	-0.53
TS_1	35.88	33.36	34.62	32.04	33.03	32.54	-2.08
Difference	12.43	10.39	11.41	9.88	9.82	9.86	-1.55
FM_2	26.47	23.86	25.17	23.77	23.73	23.75	-1.42
TS_2	31.01	29.10	30.06	28.84	29.96	29.40	-0.66
Difference	4.54	5.24	4.89	5.07	6.23	5.65	+0.76
Warehouse and outdoor environment temperature							
WT	21.74	22.19	21.96	21.44	23.03	22.23	+0.79
ET	21.52	21.00	21.26	21.61	23.41	22.51	+1.25

FM_1: vetch + triticale bale silage, FM_2: caramba + berseem clover bale silage, TS_1: vetch + triticale traditional silage, TS_2: caramba + berseem clover traditional silage, WT: Warehouse temperature, ET: Outdoor environment temperature

In traditional silages, the IT30 ranged from 35.88°C to 28.84°C in both mixtures. In bale silages this value was measured as 26.47°C and 22.16°C, respectively. On average, 34.62°C and 30.06°C internal temperature values were determined in the traditional silages made from the mixture of vetch + triticale (TS1) and caramba + berseem clover (TS2), respectively. The average ambient temperature measured during this interval was 21.26°C. It has been reported that an increase in the internal temperature of silage which makes it 8°C - 12°C warmer than the ambient temperature will occur a loss of approximately 1.5 - 3% of DM per day (Filya, 2001). Similar results have been found in this study. It was determined that the difference between traditional silage and the ambient average temperature is 13.36°C for FM1 and 8.80°C for FM2. It is thought that this result may have affected negative losses as well as RFV.

Figure 2 and Figure 3 show changes in the internal temperature of bale silage and traditional silage mixtures until the 15th day of fermentation. The internal temperature change in the first and second years was the same and the internal temperature value in traditional silages was above 28°C in both mixtures. Figure 2 shows that the days in the first year that the internal temperature value of traditional silage (TS1) of vetch + triticale and traditional silage (TS2) of caramba + berseem clover fermentation peaked were on the 4th (41.2°C) and 3rd (36.1°C) days. Pitt (1990) reported that the internal temperature of the silage exceeded 35°C in 7 or more days. During 15 days, the internal temperature of traditional silage (TS1) vetch + triticale did not fall below 29.5°C, and it did not decrease below 26.8°C for the caramba + berseem clover (TS2). It was determined that in the bale silages (FM1 and FM2) during this period the internal temperature fell below 25°C on the first 5 days of fermentation and the internal temperature of both mixtures was stabilized between 19.62°C and 23.97°C in the next few days.

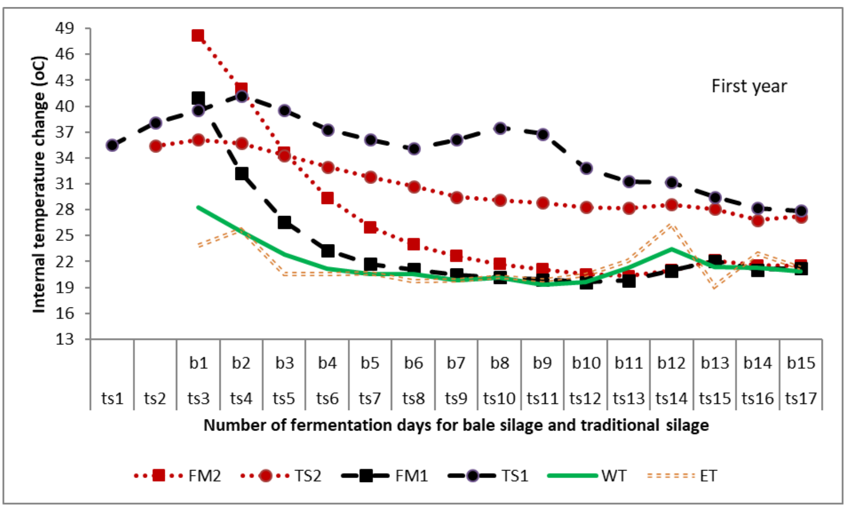

Figure 2: Change of internal temperature until the 15th day of fermentation (1st year) (FM1: vetch + triticale bale silage, FM2: caramba + berseem clover bale silage, TS1: vetch + triticale traditional silage, TS2: caramba + berseem clover traditional silage, WT: Warehouse temperature, ET: Outdoor environment temperature, b: fermentation day for bale silages, ts: fermentation day for traditional silages)

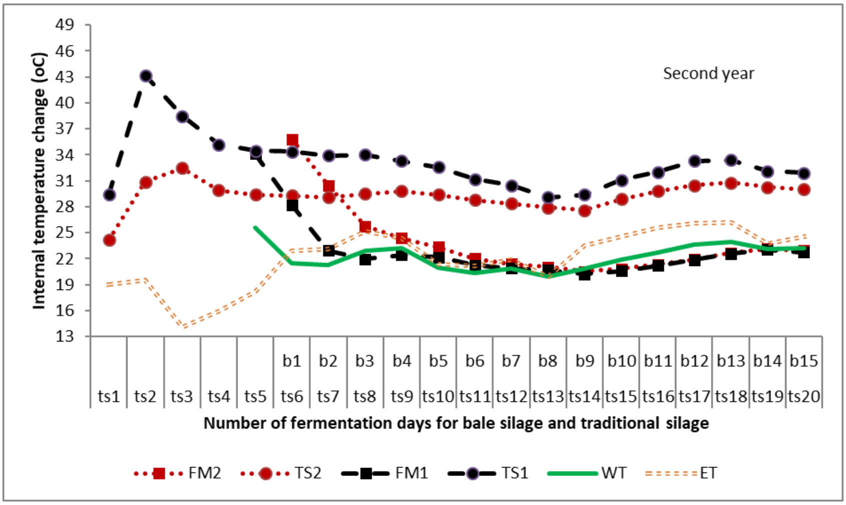

Figure 3: Change of internal temperature until the 15th day of fermentation (2nd year) (FM1: vetch + triticale bale silage, FM2: caramba + berseem clover bale silage, TS1: vetch + triticale traditional silage, TS2: caramba + berseem clover traditional silage, WT: Warehouse temperature, ET: Outdoor environment temperature, b: fermentation day for bale silages, ts: fermentation day for traditional silages)

The internal temperature value of the conventional vetch + triticale mixture silage (TS1) and the caramba + berseem clover traditional silage (TS2) peaked on the 2nd (43.2°C) and 3rd (32.5°C) days of fermentation in the second year. For 15 days, the internal temperature value of the traditional silage (TS1) with vetch + triticale did not fall under 29.1°C, while the caramba + berseem clover traditional silage (TS2) did not fall below 27.6°C. During this period it was determined that the internal temperature rapidly decreased below 25 °C in the bale silages (FM1 and FM2) as of the first three days of fermentation and the internal temperature was stabilized between 20.22°C and 23.03°C in the subsequent days for both mixtures (Figure 3). In general, LA bacteria are reported to proliferate at temperatures between 15-25°C (Basmacı and Ergün, 2002; Uygur, 2015). On average, bale silages have an appropriate internal temperature for LA bacteria to function. It was obtained that the internal temperatures of traditional silages were higher than the 15-25°C reported in literature and the internal temperature values of bale silages were within this range during both years. This situation is thought to affect RFV.

In a study conducted by Filya (2001), it was reported that the active fermentation of silage occurred within 7 - 21 days. It is asserted that in the bale silages (FM1 and FM2), the permeability of O2 decreased with the wrapping of the mixtures and the internal temperature value between 15 - 25°C which LA bacteria require was achieved within the first 3 - 5 days and the fermentation was completed rapidly. Furthermore, Filya (2001) also emphasizes that if the silage feed contains 35% of DM the fermentation rate is rapid while the fermentation rate is slow if the roughages contains less than 50% DM. All the plant materials to be silaged were reported to have a moisture content between 55 - 75%, in other words if the DM is between 25 - 45%, the fermentation period was between 7 - 14 days. In conclusion of the study it can be said that the internal temperature of the bale silages decrease below 25°C within a few days and that the fermentation of FM1 and FM2 could be completed and that this process continues for a while in traditional silage due to its high internal temperature. As a result and like examples in literature, it is argued that fermentation is faster in bale silage and slower in traditional silage.

Holmes (2006) reported that some oxygen is required between feed piles and that oxygen supports aerobic activity. Aerobic microorganisms use oxygen in the environment to release heat, water, and carbon dioxide, leading to the destruction of sugar. This leads to an increase in DM losses and internal heat. When the ambient oxygen is depleted, the internal temperature increase stops and stabilizes. For this the study, the bale silages were stabilized below 25°C in 3 - 5 days. This interpretation for this situation can be explained that the oxygen in the bale silage was exhausted, the sugar was released. Basmacıoğlu and Ergül (2002) reported that BA formation forage with low DM was observed at high temperatures such as 42°C, while the domination of SA, ie., LA was reported under 20°C.

Table 7 displays the relationship level between the IT15 and IT30 values and the parameters according to the mixtures. Accordingly, the R2 calculated between DM and IT15 and IT30 values in the mixture of vetch + triticale and caramba + berseem clover are 75.6 - 86.8% and 75.1 - 69.4% respectively. It was also determined that the R2 value between the Density and IT15 and IT30 of the caramba + berseem clover mixture was 83.4% and 66.3%, respectively. According to this data, it can be said that internal temperature values change according to DM and Density.

Table 7: Relationship level between parameters and internal temperature values (%)

	Parameters	IT_{15}^* (°C)	R^2	IT_{30}^* (°C)	R^2
FM_1	DM (%)	y = -0.4017x + 44.877	75.6	y = -0.3675x + 42.42	86.8
	Density (kg m^{-3})	y = 0.0369x + 14.674	22.8	y = 0.0403x + 12.983	37.3
	pH	y = -5.5071x + 53.729	54.6	y = -5.1093x + 50.893	64.4
	ADF (%)	y = 0.243x + 15.474	13.3	y = 0.3191x + 11.793	31.5
	NDF (%)	y = 0.4471x + 0.6567	30.4	y = 0.4833x + 2.0476	48.8
	CP (%)	y = 1.4716x + 11.695	9.3	y = 0.8432x + 16.561	4.1
	RFV	y = -0.11345x + 36.489	20.4	y = -0.1347x + 37.92	39.5
FM_2	DM (%)	y = -0.2295x + 35.775	75.1	y = -0.2012x + 33.245	69.4
	Density (kg m^{-3})	y = 0.0147x + 19.034	83.4	y = 0.0144x + 20.465	66.3
	pH	y = -1.2847x + 32.552	11.8	y = -1.7014x + 33.412	24.9
	ADF (%)	y = 0.0175x + 25.192	0.1	y = 0.2666x + 14.281	29.5
	NDF (%)	y = -0.1445x + 33.581	6.3	y = 0.0901x + 19.751	2.9
	CP (%)	y = 0.5332x + 19.517	27.5	y = 0.0341x + 24.154	0.1
	RFV	y = 0.0453x + 21.192	5.1	y = -0.0514x + 29.867	7.8

*x=internal temperature, y=parameters, FM_1: vetch + triticale bale silage, FM_2: caramba + berseem clover bale silage, DM: dry matter, pH: acidity, ADF: acid detergent fiber, NDF: neutral detergent fiber, CP: crude protein, RFV: relative feed value, IT_{15}: internal temperature changes of 15th days, IT_{30}: internal temperature changes of 30th days

Figure 4 shows the association between internal temperature change and RFV in a graph according to the application. It has been determined that there is an inverse relationship between IT15 and IT30 values and RFV. As IT15 and IT30 values decrease, RFV is determined to increase. Based on the lowest internal temperature value, the highest RFV was obtained from the bale silages with FM1 and FM2 mixtures. This internal temperature value was between 25.17°C and 22.68°C regardless of the mixtures. This value ranged from 34.62°C to 32.54°C in the traditional silage (TS1) with the vetch + triticale mixture with the lowest RFV. Pitt (1983) reported that the internal temperature of non-compact tightly wrapped silages (320 kg m^{-3}) increased with the increase of DM while the internal temperature of tightly packed traditional silages (961 kg m^{-3}) tended to decrease with the elimination of DM. It was also determined that there is an inverse, mostly linear relationship between the DM and the internal temperature in both mixtures for this study.

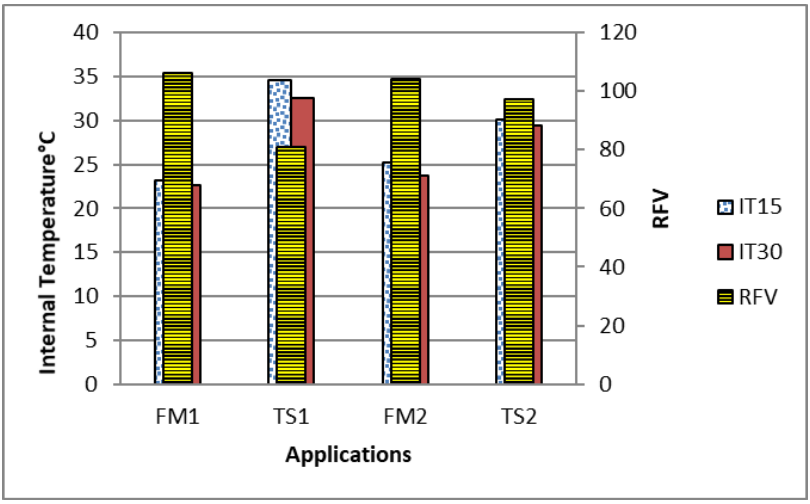

Figure 4: IT15 and IT30 values and RFV variation according to traditional silage (TS) and bale silage (FM) applications. (FM1: vetch + triticale bale silage, FM2: caramba + berseem clover bale silage, TS1: vetch + triticale traditional silage, TS2: caramba + berseem clover traditional silage, RFV: Relative feed value, IT15: internal temperature change of 15^{th} days, IT30: internal temperature change of 30^{th} days)

4. CONCLUSION

In this study, changes in internal temperature up to the 15th and 30th day of fermentation after bale silage and some chemical parameters analyzed after 60 days of fermentation have been evaluated. Two forage mixtures (vetch + triticale and caramba + berseem clover) were assigned to the main plots, two harvesting machines for the sub plots (disc mower with conditioner and mower) and three wrapping layers for the sub-sub plots (4, 6, 8) were established. The study was carried out to determine the impact of the plant material, harvesting machine and number of wrapping layers regarding on the internal temperature of 15th and 30th days in bale silage.

In roughage studies, it is necessary to evaluate the effects of mechanization applications on the roughage. All the mechanical applications from the harvesting of the green material have an effect on the quality of roughages. Mechanized applications in traditional and bale silage differ from each other and these differences change the quality level of roughage. In conclusion, it has been determined that as the internal temperature values decreased on the 15th and 30th days, RFV increased. It has been determined that bale silages have lower internal temperature than traditional silage. It was obtained that the internal temperature of the roughages having the highest RFV, regardless of the plant material, varied between 25.17°C and 22.68°C. The number of wrapping layers used for bale silage does not affect the internal temperature value in both mixtures. Furthermore, the internal temperature value of the traditional silage for the vetch + triticale mixture with the lowest RFV was between 34.62°C and 32.54°C. In both mixtures, it was determined that the internal temperature value changed with the amount of DM, the pH value in the vetch + triticle mixture and the density in the caramba + berseem clover mixture. In order to be able to make the highest quality bale silage, it is necessary to make bales with a disc mower with conditioner and 8 layers of wrapping regardless of the mixtures.

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Conflict of Interest: All author declared that there isn’t any conflict of interest

Data Availability Statement: All data used to support the findings of this study are included in the article.

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CHAPTER 8

PHYTOESTROGENIC PLANTS and EFFECTS on RUMINANTS

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1. Phytoestrogens

The term phytoestrogen originates from the combination of the Greek words 'phyto' and 'estrogen,' which signifies plant and female reproductive hormone, respectively (Tepavčević, 2013; Kˇrížová et al., 2019). Phytoestrogens are phenolic, plant-derived compounds activating several functions in plants, including defense mechanisms against pathogens (Adler et al., 2015). Although phytoestrogens are widely known as compounds that classically exert estrogenic effects on the central nervous system, induce oestrus, and stimulate the development of the female reproductive system, they are the elements naturally found in plants and initiate the E2 (17β-estradiol)-like effects in animal tissue (Tucker, 2009; Soldamli and Arslanoglu, 2019). Numerous studies also reported that phytoestrogens constitute additional effects that may help lower cancer risk, including the ability to function as antioxidants, inhibit tyrosine kinase, reduce cholesterol, and inhibit cell proliferation and DNA synthesis (Akiyama et al., 1987; Mitchell et al., 1998; Tikkanen et al., 1998; Anthony et al., 1998; Pan et al., 2001). In plants, phytoestrogens function as phytoalexins instead of hormones; in other words, they are low molecular weight compounds synthesized and accumulated in plants during stress and microbial attacks. These active defense compounds pose fungistatic, antibacterial, antiviral, and antioxidant properties, and thus they are critical substances in fighting against malignity and preventing angiogenesis (Kˇrížová et al., 2019). Several clinical or epidemiological studies revealed that phytoestrogens have a positive impact on helping avert hormone-dependent cancers, cardiovascular diseases, osteoporosis, and the alleviation of menopausal symptoms in animal, clinical, or epidemiological studies (Sumien et al., 2013).

The first study indicating the estrogenic activity of several plants and their extracts dates back to 1927 (Loewe et al., 1927). Bennetts et al. (1946) initially described that plants retain natural estrogens, and Curnow et al. (1948) reported that estrogens are associated with infertility in grazing sheep; consequently, these compounds gained great significance in the literature. The following studies in 1951 documented that isoflavone genistein, naturally found in estrogenically active mice, was also isolated from the subterranean clover *(Trifolium subterraneum)*. Subsequently, estrogenically active isoflavones such as formononetin, biochanin A, daidzein, and pratensein were discovered in subterranean clover and red clover *(Trifolium pratense)*. Alfalfa *(Medicago sativa* L.*)* and white clover *(Trifolium repens)* were among the non-estrogenic forage crops until 1953. However, experiments in the Western Utilization Research and Development Division (WURDD) and reports by Cheng et al. at Iowa State, Engle et al. at Ohio State, and Pieterse and Andrews at Purdue explicitly revealed that both alfalfa and white clover plants retain significant estrogenic activity. Consequently, WURDD initiated research on the estrogen content of the white clover plant in 1955 and discovered a benzo-furocoumarin derivative that belongs to a brand-new class of substances termed coumestans (Bickoff et al., 1969).

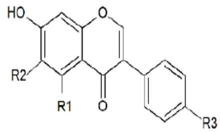
2. Classification of Phytoestrogens

Phytoestrogens are a large family of chemical compounds with two primary classes of flavonoids and lignans. Flavonoids have comparatively higher estrogenic activity than lignans (Whitten and Patisaul, 2001). In this context, phytoestrogens are also grouped as flavonoids (Isoflavones, Coumestans, and Prenylated Flavonoids) and non-flavonoids (Lignans) (Kˇrížová et al., 2019). Isoflavones, a type of flavonoid, and non-flavonoid lignans are the sources of phytoestrogens that have been the subject of most studies (Soldamli and Arslanoglu, 2019). Isoflavones are the most extensively studied group of phytoestrogenic compounds (Coward et al., 1993; Cassidy, 2004). Contrarily, lignans are less studied phytoestrogens since isolation and analysis of these compounds are challenging, despite their existence in numerous sources (Oomah and Hosseinian, 2008). Yet, lignans are a more favorable source of phytoestrogenic compounds for western diets than isoflavones since most fiber-rich foods contain them abundantly (Cassidy, 2004). Systematically, the phytoestrogen group comprises over 100 molecules categorized into isoflavones, flavones, coumestans, stilbenes, and lignans based on their chemical compounds (Wocławek-Potocka et al., 2013) (Table 1). Isoflavones and coumestans are occasionally referred to as structural estrogens since they are synthesized spontaneously in the plant (Ozer, 2006).

Table 1. Classification of phytoestrogens (Cos et al., 2003; Konar et al., 2011; Wocławek-Potocka et al., 2013; Wyse et al., 2022)

Phytoestrogens				
Isoflavones	Flavonoids	Stilbenes	Coumestans	Lignans
Genistein	Flavones	Resveratrol	Coumestrol	Secoisolariciresinol
Daidzein	Flavanones		4'-methoxy-coumestrol	Matairesinol
Glycitein	Chalcones		3'-methoxy-coumestrol	Pinoresinol
Formononetin			Trifoliol	Lariciresinol
Biochanin A			Sativol	
			Repensol	
			Lucernol	
			Medicagol	
			11,12-dimethoxy-7-hydroxycoumestan	

2.1. Isoflavones: As one of the most well-recognized phytoestrogens, isoflavones are a class of chemical compounds most particular to the Leguminosae family and yet highly frequent in the plant kingdom (Wiseman, 2000; Mazur, 1998; Cos et al., 2003). They are also one of the secondary metabolites mediating the fundamental interactions between plants and microorganisms (legume/rhizobium symbiosis). The amount and number of isoflavones simultaneously retained by a single plant species might vary depending on many factors (Wyse et al., 2022). This group comprises genistein, daidzein, glycitein, biochanin A, and formononetin. Besides, although some literature (Kamiloglu et al., 2002; Wyse et al., 2022) includes 'equol' in this group, Setchell et al. (2002) reported that equol is not a phytoestrogen since it is not a naturally synthesized plant chemical. Figure 1 depicts the chemical structure of isoflavones categorized in this group.



Aglycon	R1	R2	R3
Daidzein	H	H	OH
Genistein	OH	H	OH
Glycitein	H	OCH_3	OH
Formononetin	H	H	OCH_3
Biochanin A	OH	H	OCH_3

Figure 1. Chemical structure of isoflavones (Kˇrížová et al., 2019)

2.2. Flavonoids: This group consists of flavones (luteolin, apigenin, quercetin, chrysin, kaempferol, and wogonin), flavanones (hesperidin and naringin), and chalcones (Hashem and Soltan, 2015; Kiyama, 2023). Many species, including alfalfa and white clover, contain numerous flavones (Reed, 2016).

2.3. Stilbenes: Stilbenes are synthesized via phenylpropanoid acetate-like flavonoids. Resveratrol is one of the significant stilbenes (Figure 2), and it functions as a phytoalexin. Unlike flavonoids, the plant kingdom does not contain resveratrol abundantly. It is typically found in grapes *(Vitis vinifera)*, peanuts *(Arachis hypogaea* L.*)*, and pineapples *(Ananas comosus)* (Ozer, 2006).

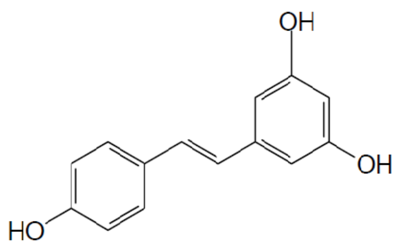


Figure 2. Chemical structure of resveratrol (Ozer, 2006)

2.4. Coumestans: It is another group of compounds derived from plant phenols potentially displaying estrogenic activity (Table 1). As the most well-known representative of this class, coumestrol is characterized as the predominant phytoestrogen in alfalfa, white clover, and some other annual clover species (Bickoff et al., 1969). Figure 3 depicts the structure of typical coumestans found in legume species. Of all phytoestrogens, coumestrol has the highest estrogenic activity. It has 30-100 times more estrogenic activity than isoflavones and may display 10-20 times lower activity than endogenous estrogen estradiol (Konar et al., 2011). The 4'-methoxy-coumestrol considered the second most significant coumestan after the coumestrol is found in higher concentrations than coumestrol in certain annual alfalfa species such as *Medicago littoralis* (Wyse et al., 2022).

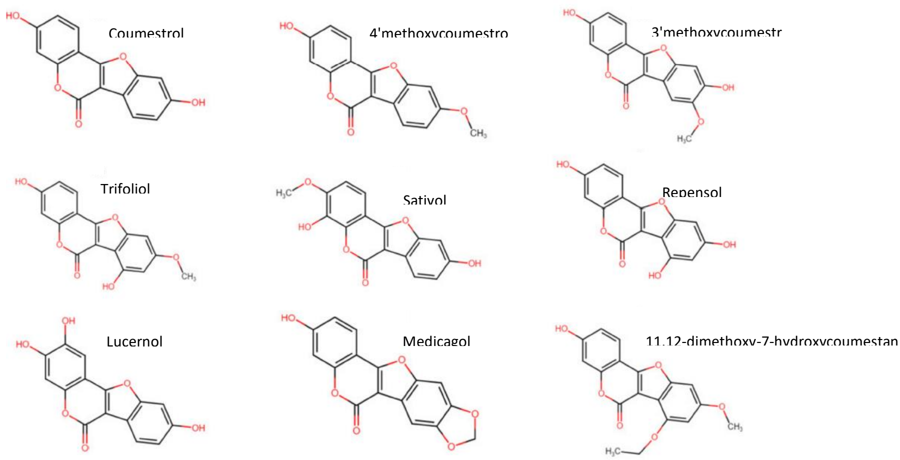


Figure 3. Common coumestans structures in legumes (Wyse et al., 2022)

2.5. Lignans: The initial study on lignans, the most significant representative of the non-flavonoid phytoestrogen group and a constituent for plant cell wall structure in lignin synthesis, was published in the 1980s. As the byproducts of plants' secondary metabolism, they have essential functions in human nutrition and in protecting plants from environmental stressors (Soldamli and Arslanoglu, 2019). Enterodiol and enterolactone are the most significantly identified lignans (Axelson, 1982). They are the metabolic byproducts of plant lignan precursors. The most significant enterolignan precursors were identified to be secoisolariciresinol, matairesinol, pinoresinol, and lariciresinol (Raffaelli et al., 2002). With their current form in plants, herbal lignans are not active estrogens. The estrogenic activity is generated only by being metabolized through the intestinal flora (Konar et al., 2011).

3. Forage Crops with Phytoestrogenic Effect

3.1. Red clover

Formononetin, biochanin A, daidzein, and genistein are the primary isoflavones in red clover. Studies revealed that the concentrations of isoflavones in a plant are determined genetically, whereas their concentrations are also influenced by environmental factors (Tsao et al., 2006; Tucker, 2009; Yatkin and Daglioglu, 2011). Red clover is naturally high in phytoestrogens; however, Australian plant breeders have developed low-estrogen varieties (Redwest and Redquin) that are much safer for livestock use (Hamilton, 2001). The phytoestrogen content of red clover typically ranges from 10-25 mg g^{-1} DM (Saloniemi et al., 1995). The amounts of isoflavones in this plant vary greatly depending on the cultivars and plant organs (leaf, stem, petiole, and flower), with the leaf typically having the highest total concentration, followed by the plant stem, the petiole, and flower (Tsao et al., 2006). Accordingly, in their research with 13 distinct clover cultivars, Tsao et al. (2006) identified total isoflavone concentration as 52.42 mg g^{-1} DM at the early bud stage and 54.54 mg g^{-1} DM at the late flowering stage (2006). Correspondingly, Vetter (1995) found the isoflavone concentrations in the stem, leaf, and flower in the red clover as 1.070 mg g^{-1} DM, 0.740 mg g^{-1} DM, and 1.210 mg g^{-1} DM, respectively. Andersen et al. (2009) reported 11.40 mg g^{-1} DM formononetin in red clover. As a comprehensive study, however, Hloucalová et al. (2016) determined 3.697 mg g^{-1} DM biochanin A, 4.315 mg g^{-1} DM formononetin, 0.177 mg g^{-1} DM genistein, 0.943 mg g^{-1} DM ononin, 0.462 mg g^{-1} DM sissotrin, 0.232 mg g^{-1} DM daidzein, and 0.185 mg g^{-1} DM daidzin concentrations in red clover. It is necessary to note here that the percentage of estrogenic clover in the pasture defines estrogenic potency.

3.2. Subterranean Clover

The phytoestrogen content of the subterranean clover varies depending on whether it is green or dry. In dry grass, the total phytoestrogen content is 0.82 mg g^{-1} DM, whereas this ratio in green form is 0.79 mg g^{-1} DM. Additionally, the green plant contains 0.71 mg g^{-1} DM genistein, 0.02 mg g^{-1} DM biochanin A, and 0.03 mg g^{-1} DM formononetin, while these concentrations are 0.78 mg g^{-1} DM, 0.01 mg g^{-1} DM, and 0.02 mg g^{-1} DM for the same compounds in dry grass, respectively (Pace et al., 2004). Deficiencies in nutrients (such as low phosphorus and sulfur), diseases (such as red leaf virus in clover), waterlogging, droughts, or moisture stress may result in a phytoestrogen content rise in the plant (Hamilton, 2001). In this context, Ramírez-Restrepo and Barry (2005) found that the total isoflavone content in red clover ranged from 7 to 14 mg g^{-1} DM. De Rijke et al. (2001) reported that red clover contained 0.33 mg g^{-1} DM biochanin-A, 0.60 mg g^{-1} DM formononetin, 0.54 mg g^{-1} DM sissotrin, 0.97 mg g^{-1} DM ononin, and 0.042 mg g^{-1} DM daidzin. Some studies also reported that subterranean clover retained low quantities of coumestrol (Bickoff et al., 1969).

3.3. White clover

Vetter (1995) reported 0.027 mg g^{-1} DM, 0.119 mg g^{-1} DM, and 0.094 mg g^{-1} DM isoflavone concentrations in the white clover leaves, stem, and flowers, respectively. Similarly, Andersen et al. (2009) found 0.41 mg g^{-1} DM formononetin in white clover. White clover also retains small quantities of coumestrol (Bickoff et al., 1969).

3.4. Other clover species

Vetter (1995) reported that the isoflavones concentrations in the red clover (*Trifolium incarnatum*) plant were 0.474 mg g^{-1} DM in its leaf, 0.221 mg g^{-1} DM in its stem, and 0.164 mg g^{-1} DM in its flower. Hloucalová et al. (2016), on the other hand, identified the biochanin A, formononetin, genistein, ononin, sissotrin, daidzein, and daidzin concentrations in Persian clover (*Trifolium resupinatum* L.) as 0.072 mg g^{-1} DM, 0.202 mg g^{-1} DM, 0.011 mg g^{-1} DM, 0.218 mg g^{-1} DM, 0.057 mg g^{-1} DM, 0.051 mg g^{-1} DM, and 0.004 mg g^{-1} DM, respectively. The same researchers also reported the concentrations for these chemicals (mentioned above) in Berseem clover (*Trifolium alexandrium* L.) as 0.314 mg g^{-1} DM, 0.106 mg g^{-1} DM, 0.033 mg g^{-1} DM, 0.129 mg g^{-1} DM, 0.186 mg g^{-1} DM, 0.161 mg g^{-1} DM, and 0.016 mg g^{-1} DM, respectively. Finally, Butkutė et al. (2018) remarked that the isoflavone content in the *Trifolium medium* ranged between 23.2-28.7 mg g^{-1} DM.

3.5. Alfalfa

Recently, alfalfa has drawn attention as a potential source of secondary metabolites. Similar to other legumes, alfalfa is also a rich source of phytoestrogens. These phenolic non-steroidal compounds resemble steroidal estrogens in terms of their steric structure; as a result, they have the capacity to bind to estrogen receptors and potentially exert estrogenic and/or anti-estrogenic functions. The most typical phytoestrogens are isoflavones, lignans, and coumestans (Tucak et al., 2020). The Alfalfa plant is also a significant source of isoflavone phytoestrogens, especially formononetin, genistein, and biochanin A (Konar et al., 2011). Additionally, isoflavone content in alfalfa varies between 0.054-0.212 mg g^{-1} DM (Butkutė et al., 2018). Both annual and perennial alfalfa species contain coumestans, and their concentration increases in the presence of fungi infections (Hamilton, 2001). Alfalfa plant also retains modest concentrations of formononetin and biochanin-A; however, it contains 25-65 ppm of coumestrol in its dry matter (Saloniemi et al., 1995). Tucak et al. (2020) identified 30.33% genistein, 26.84% kaempferol, 25.02% coumestrol, 14.84% glycitein, and 2.97% daidzein in their study on 20 different alfalfa populations. Andersen et al. (2009) also reported 0.1 mg g^{-1} DM formononetin in the alfalfa plant.

3.6. Some annual medics species: Studies have categorized an order of *Medicago littoralis* > *Medicago truncatula* > *Medicago scutellata* = *Medicago polymorpha* when taking the coumestrol content as a basis among annual clover species (Reed, 2016). Francis and Millington (1971) also reported that *Medicago littoralis* and *Medicago truncatula* stems contained 737 mg kg^{-1} and 576 mg kg^{-1} of coumestrol, respectively.

3.7. Birdsfoot trefoil (*Lotus corniculatus* L.)

This species contains low concentrations of formononetin and biochanin A (Sarelli et al., 2003).

3.8. Certain forage grasses: Timothy (*Phleum pratense* L.) and meadow festuca (*Festuca pratensis* Huds.) are two examples of forage grasses with low or undetectable isoflavone concentrations (Kallela et al., 1987; Mustonen et al., 2009).

3.9. Soybean

The initial studies documenting the isoflavone content in soybean date back to the 1940s, and genistin was the first isoflavone isolated from this species (Tucker, 2009). Soybean is rich in isoflavones such as genistein and daidzein. Moreover, albeit in low concentrations, this plant also contains glycitein. The content of isoflavones (particularly daidzein, genistein, and their conjugates) in soybean is 1.2-4.2 mg g-1 DM (Kurzer and Xu, 1997). There are 111 mg and 84 mg of genistein and daidzein in 100 grams of soybeans, respectively (Anderson et al., 1999).

4. Factors Affecting Phytoestrogen Content in Plants

4.1. Species and cultivar differences: Phytoestrogen concentrations in plants greatly vary depending on the species and cultivar (Francis and Millington, 1971; Kallela et al., 1987; Saloniemi et al., 1995; Vetter, 1995; Kurzer and Xu, 1997; Hamilton, 2001; Pace et al. et al., 2006; Tsao et al., 2006; Saviranta et al., 2008; Andersen et al., 2009; Mustonen et al., 2009; Reed, 2016; Butkutė et al., 2018; Kˇrížová et al., 2019; Tucak et al., 2020; Wyse et al., 2022). For instance, in his study to quantify the amount of four estrogenic isoflavones found in the leaves, stems, and flowers among seven clover species, Vetter (1995) identified a high isoflavone content in subterranean clover and red clover. Pace et al. (2006) also determined that the total isoflavones contents of subterranean clover varied significantly in cultivars (Figure 4).

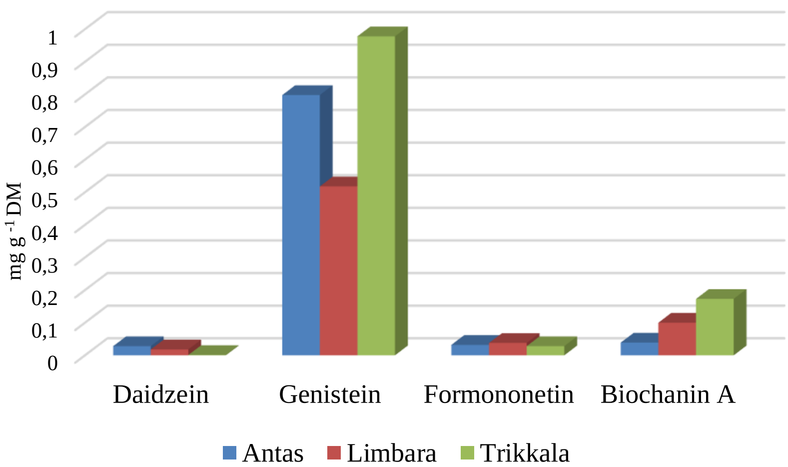


Figure 4. Daidzein, genistein, formononetin, and biochanin A concentrations in subterranean clover cultivars (mg g^{-1} DM)

4.2. Plant organ: The phytoestrogen content varies greatly depending on the plant component sampled. For instance, Tsao et al. (2006) reported that total isoflavone concentrations were the highest in leaves, followed by stems, petioles, and inflorescences. Saviranta et al. (2008) also identified the highest levels of daidzein and genistein in the stem region harvested in August, whereas the highest formononetin and biochanin A concentrations were in young leaves harvested in June. According to studies, roots contained almost as high formononetin concentration as leaves, albeit they retained low concentrations of daidzein, genistein, and biochanin A. Vetter (1995), on the other hand, reported that the highest content of genistein, daidzein, and biochanin A was in the flowers of *Trifolium subterraneum*, *Trifolium pratense*, and *Trifolium alpestre* species, respectively.

4.3. Plant development stage: The development stage is another factor influencing the level of phytoestrogen in plants. For instance, the total isoflavone concentration in red clover leaves increased by nearly 30% between the early budding and late flowering stages, whereas its concentration in the stem and inflorescence decreased as the plant matured (Tsao et al., 2006). Sarelli et al. (2003) contrarily indicated that while the isoflavone content was 9.41 g kg^{-1} DM during the budding stage in red clover, it declined to 6.85 g kg^{-1} DM in the flowering stage; correspondingly, these concentrations were 0.04 g kg^{-1} DM and 0.05 g kg^{-1} DM in the birdsfoot trefoil within the same periods, respectively. Studies also reported that formononetin and biochanin A compounds are primarily responsible for a decline in isoflavone levels in these species, particularly during the budding and flowering stages. Similar to previous studies, Kallela et al. (1987) also identified a considerable decline in the concentration of total isoflavones with the advancing maturation in red clover. The coumestrol is at its lowest level during the early flowering period of the alfalfa plant (Seguin et al., 2004a). The isoflavone content in plantlets is 1.2–5.6 times higher than that of fully flowered plants in various perennial legumes, including *Trifolium pratense* L., *Trifolium medium* L., *Medicago sativa* L., *Medicago lupulina* L., *Onobrychis viciifolia* L., and *Astragalus glycyphyllos* L. (Butkut et al., 2018). However, while the isoflavone level declines as the subterranean clovers mature, the coumestrol level, which is low until the flowering stage in annual medics, increases concurrently with plant development (Eroglu, 1993). Seguin et al. (2004a) also found that, during the flowering stage, different parts of the alfalfa plant contained higher luteolin, quercetin, and apigenin contents than the stem and leaves.

4.4. Changes in climate and environmental conditions: The phytoestrogen content of plants varies greatly depending on the climate conditions of the region where they are grown, the regional altitude, cultivation conditions (field or greenhouse), the environment (light, temperature, precipitation, etc.), and stressors (drought, low and high temperature, etc.). In red clover plants harvested at two-week intervals throughout the growing season, for instance, while June-July is the best period when the highest daidzein and genistein contents were detected, it is September (early September) when the highest formononetin and biochanin A levels were determined (Booth et al., 2006). Saviranta et al. (2008) reported that daidzein, genistein, formononetin, and biochanin A levels and the plant sections in which these compounds are synthesized significantly varied when growing the red clover in the greenhouse or field conditions. Accordingly, the researchers discovered that the highest daidzein and genistein levels were in the petioles (0.11-0.28 and 0.30-0.54 mg g^{-1}, respectively), and the highest formononetin and biochanin A levels (5.57-9.05 and 10.94-14.59 mg g^{-1}, respectively) were in greenhouse-grown plants. Considering the field-grown plants, however, the maximum daidzein and genistein levels were in the stems collected in August (0.24 and 0.55 mg g^{-1}, respectively), whereas the highest formononetin and biochanin A contents were in the young plantlet leaves collected in June (7.47 and 9.69 mg g^{-1}, respectively). Caldwell et al. (2005) found negative relationships between temperature and phytoestrogen concentrations in dwarf soybean seeds cultivated under controlled conditions, indicating that drought stress positively influenced the concentrations. Furthermore, Tsukamoto et al. (1995) also stated that extreme temperature negatively affected the isoflavone concentrations in soybean seed and hypocotyls. Correspondingly, the phytoestrogen contents of alfalfa and soybean also vary depending on the regions in which they are cultivated (Seguin et al., 2004b; Seguin and Zheng, 2006; Zhou et al., 2011). Seguin et al. (2004b) reported that drought stress and rising daily average temperatures caused an increment in soybean isoflavone levels. Mustonen (2015) indicated that rainy and cold weather during the growing season increased isoflavone levels in red clover. Again, precipitation positively influenced the phytoestrogen levels, albeit simultaneously stimulating the fungi growth (Eroglu, 1993). The regional altitude in which the plant is cultivated is also a significant factor affecting the phytoestrogen levels. For instance, the coumestrol content of alfalfa plants grown in humid and foggy weather at 1500 m above sea level in Hawaii was 99 mg kg^{-1}, whereas it was 32 mg kg^{-1} at 600 m altitude (Reed, 2016).

4.5. Diseases and pests: Studies on the coumestrol content of alfalfa and white clover have revealed that their coumestrol content is greatly varied by disease and insect infestation (Bickoff et al., 1969). As the literature explicitly specified, the coumestrol concentration of 0-100 mg kg^{-1} DM in the alfalfa increased more than 180-fold in response to pathogen-induced stimuli (Bickoff et al., 1966). Barbetti (1995) reported that the coumestrol content of annual medics altered in a disease state; as a result, the coumestrol content in sick plants elevated to 230–500 mg kg^{-1} in the stem and 30-130 mg kg^{-1} in the pods when compared to the fungicide-treated plants. Certain necrotrophic fungal infections (*Phoma medicaginis*) in annual medics (*Medicago* spp.) also stimulated the synthesis of phytoestrogenic substances (coumestrol) (Tivoli et al., 2006). Similarly, fungal infections in the white clover leaves significantly increased the coumestan level. The rise in concentration figures might even multiply 100-fold depending on how severe the disease infection is.

4.6.Post-harvest processing: The phytoestrogen levels vary considerably depending on whether the plant is analyzed as fresh (green), dry grass, or silaged after harvest. The green plant retains more formononetin than canned silage or hay (forage) (Sivesind and Seguin, 2005). In addition, artificially dried alfalfa had more estrogenic activity in post-harvest plants than naturally dried alfalfa (Eroglu, 1993). There is less phytoestrogen in hay than in silage since the drying process reduces some estrogenic activity (Kallela, 1980; Saloniemi et al., 1995; Sarelli et al., 2003). In this context, Eroglu (1993) reported that the silaging process increased the estrogenic activity 3-5 times. Sivesind and Seguin (2005) also identified 9.02 mg kg^{-1} DM, 6.47 mg kg^{-1} DM, and 7.22 mg kg^{-1} DM formononetin in green grass, dry grass (field-dried) and silage of red clover, respectively. A study conducted on mixtures of legume/grass silages revealed that white clover/grass silage had contentwise lower isoflavone and higher lignans and coumestrol than red clover/grass silage (Steinshamn et al., 2008).

4.7. Plant nutrients: The content of isoflavones in subterranean clover plants increased dramatically when the soil was deficient in phosphorus, sulfur, and nitrogen, whereas the potassium, copper, and zinc deficiencies resulted in no significant concentration difference (Eroglu, 1993). McMurray et al. (1986) reported that a high soil phosphorus level lowered the formononetin content in red clover. Additionally, since a considerable amount of estrogen is excreted naturally into the soil by the urine, studies concurrently reported that if liquid manure and the manure itself are applied to meadows and pastures, plants will uptake these additives, increasing the estrogen level (Eroglu, 1993).

5. Metabolism of Phytoestrogens in Ruminants

The putative functions of phytoestrogens are based on their structural similarities to estrogen (especially estradiol-17β) and their potential to bind to estrogen receptors (Hashem and Soltan, 2015). In other words, thanks to their phenolic ring structure, they potentially compete with endogenous estrogens such as estradiol-17β and inhibit the estrus cycle of numerous mammalian species by binding to the estrogen receptors of cells (Mostrom and Evans, 2011). Furthermore, they can also emulate the biological activity of estrogens due to their ability to pass through cell membranes and interact with cell enzymes and receptors when ingested. In this way, they can bind to estrogen receptors and generate biologically detectable effects. Since these compounds contend with endogenous steroids when consumed by farm animals, the balance between estrogenic and antiestrogenic activity relies on their potential to antagonize natural steroid estrogens and their own estrogenic activity (Folman and Pope, 1966). Therefore, this condition may explain why the estrogenic effects of phytoestrogens tend to be predominant, given the comparatively modest plasma estradiol concentrations (15 pg/ml) in livestock. Phytoestrogens differ not only in their biological activity; they also have structural differences, as they come from various chemical classes that will exert diverse effects on tissues and receptors (Lieberman, 1996). The vast diversity of phytoestrogens drives each compound in its class to affect the estradiol-17β-mediated response to act in several ways (Cornwell et al., 2004). The ability of phytoestrogens to function as estrogen agonists or antagonists contributes to their wide range of biological actions. In other words, as estrogen agonists, phytoestrogens cause estrogenic effects by emulating endogenous estrogens, while as estrogen antagonists, they yield antiestrogenic results by blocking estrogen receptors or inhibiting their activity (Brzezinski and Debi, 1999).

Commonly used animal feeds retain isoflavonoids such as genistein, daidzein, formononetin, and biochanin A (Cos et al., 2003; Ososki and Kennelly, 2003). Isoflavones are typically in the form of biologically inactive glycoside conjugates containing glucose or carbohydrate moieties; subsequently, these conjugates become active compounds either as hydrolyzed glycosides when the sugar residue is removed in the gut by bacteria or as aglycones resulting from the hydrolysis of glycosides in the gastrointestinal tract (Cornwell et al., 2004). Therefore, phytoestrogens, found predominantly in the form of glycosides in plant material, are not estrogenically active, but demethylation, methylation, hydroxylation, chlorination, iodization, and nitration of phytoestrogens processes occur after their consumption by farm animals (Hashem and Soltan, 2016). Once ingested, they are quickly metabolized and absorbed; consequently, they mostly reach systemic circulation as conjugates with limited bioavailability (Patisaul and Jefferson, 2010). Lundh et al. (1990) reported that the absorption and dispersal of isoflavones vary in dairy cattle and sheep. They stated that, within the first hour after feed consumption, formononetin (530 mg/kg feed) and daidzein (12.6 mg/kg feed) are rapidly absorbed in the blood plasma, reaching up to approximately 90 and 50 g L^{-1} concentrations, respectively. They also emphasized that these values were three times higher than the concentration identified in sheep fed with the same isoflavone concentration. Yet, sheep's concentration levels equaled those of cows within three hours, and the equol content in sheep was half as high as in dairy cows after 16 hours, indicating that sheep metabolized isoflavones more quickly than cows. However, sheep are more sensitive to equol because estrogen receptors in the uterus are expressed 2-4 times more in sheep than in cows (Lundh et al., 1990). Markiewicz et al. (1993) documented that the relative estrogenic potency of equol was 0.061% of that of 17-β-estradiol and that free equol in sheep might reach 20 ng concentration per 100 mL, thus creating an estrogenic effect 100 times higher than 17-β-estradiol during estrus. According to Johnston (2003), phytoestrogens indirectly stimulate the synthesis of steroid hormone-binding globulin, reducing the amount of biologically active estradiol in the serum and may influence the bioavailability of sex hormones (Taspinar et al., 2018). This results in weak antiestrogenic effects in the absence of estrogen, whereas isoflavones have substantial antiestrogenic effects in the presence of estrogen (Burton and Wells, 2002). Some phytoestrogens may have an inhibitory effect on steroidogenic enzymes. For instance, isoflavonoids and lignans potentially influence the metabolism of steroid hormones by inhibiting the activity of 5α-reductase and 17ß-hydroxysteroid dehydrogenase enzymes (Evans et al., 1995). The demethylation process converts biochanin A to genistein; however, ring cleavage transforms it into para-ethylphenol and organic acids. Formononetin is predominantly demethylated to daidzein. Ruminants are the typical equol producers. Biochanin A and genistein metabolites are estrogen-inactive substances. However, the formononetin metabolism leads to the formation of more estrogenic metabolite, which is the chemical equol (Lundh et al., 1990). Metabolic processes catalyzed by microorganisms in the rumen may last six to ten days after ingestion (Adams, 1995). In red clover, biochanin A is the chief estrogen, while formononetin is the inactive estrogen. Given the relatively low genistein and daidzein concentrations and the inactivity of formononetin, red clover's estrogenic activity primarily depends on biochanin A (Wong, 1962). Biochanin A and genistein in animal feeds are converted into p-ethyl phenol in the rumen, whereas formononetin and daidzein are transformed into equol (Figure 5) (Hashem and Soltan, 2016). The concentration of daidzein and genistein began to drop within one hour after feeding, whereas equol and p-ethyl-phenol remained in animals' blood for several hours after feeding. Biochanin A and genistein in the rumen are almost, probably completely, converted to para-ethylphenol and organic acids without estrogenic action, even after 6–10 days, when the rumen microbes are better accustomed to the phytoestrogens in the feed. Therefore, phytoestrogens and their resultant metabolites are primarily found in plasma in both conjugated and sulfo-conjugated forms and are converted into more estrogenically active unconjugated forms by metabolism. Phytoestrogens are excreted from the body through conjugated urine and unconjugated feces (Njåstad et al., 2014). For instance, after absorption, isoflavones are primarily converted to glucuronic acid in the liver (Lundh, 1995). Although isoflavones are degraded substantially in the gut, plasma equol concentrations (20 mmol L^{-1} in sheep) are significant, indicating the pathophysiological effects on reproduction. In sheep, formononetin and biochanin A are bioconverted by ruminal bacteria to the demethylated intermediates daidzein and genistein and then to the estrogenic isoflavone equol. In this context, Setchell et al. (2002) stated that equol is just a non-steroidal estrogen from the isoflavone class, a metabolic byproduct of gut bacterial metabolism. He further emphasized that it is not a phytoestrogen, albeit incorrectly classified by many researchers since it is not a naturally synthesized plant derivative. They also emphasized that equol production solely depends on the intestinal microflora and that the absence of equol in animals devoid of bacteria and the plasma of infants fed with infant formula indicates the requirement of an active microflora for equol production. Coumestrol, unlike other phytoestrogens, is considered estrogenic without being metabolized (Njåstad et al., 2014). Phytoestrogens are involved in several regulatory processes by binding to estrogen receptors. Coumestrol also binds to estrogen receptors, inducing the expression and regulation of numerous growth-related activities. Coumestrol also affects the expression of androgen, glucocorticoid, progesterone, and thyroid receptors (Nehybová et al., 2014). The estrogenic activity of these compounds depends on many factors, including the chemical structure of the substance, the animal species, the feeding style, the animals' physiological state, age, exposure duration, the concentration, estrogen receptor subtypes (α or β), and their metabolites resulting from fermentation and digestion (Hashem ve Soltan, 2015). WocBawek-Potocka et al. (2013) also reported that the critical factor in determining the effects caused by phytoestrogen is the time of exposure to phytoestrogen.

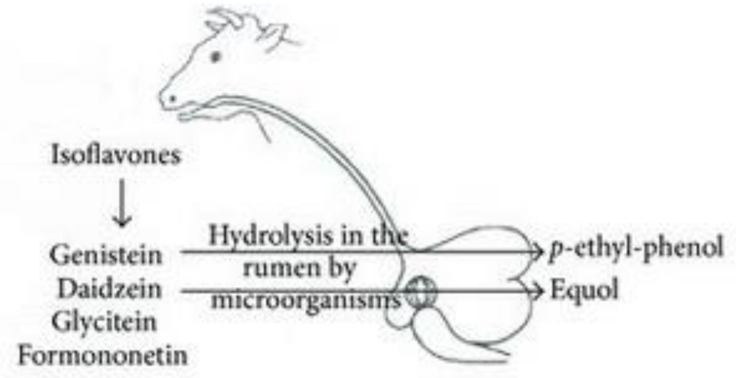


Figure 5. Metabolism of phytoestrogens (Woclawek-Potocka et al., 2013)

6. Effects on Reproduction in Ruminants

Numerous phytoestrogens are now considered endocrine disruptors; in other words, natural or artificial substances capable of altering hormonal function through various mechanisms (Patisaul and Jefferson, 2010). Each phytoestrogen type at concentrations of 25 mg kg-1 DM influences the reproductive attributes of pasture-grazing animals, although the isoflavones, lignans, and coumestans are species-specific (Wyse et al., 2022). These phytoestrogens function as endocrine-disrupting compounds by either directly stimulating or inhibiting the endocrine system, emulating or blocking the body's response to endogenous steroid hormones, or inhibiting the secretion and transport of endogenous hormones responsible for sustaining homeostasis and reproduction (Dickerson and Gore, 2007). Pool et al. (2022) reported that, in addition to the endocrine-disrupting functions of estrogenic compounds, they potentially alter the epigenome in many tissues; and especially considering that pregnancy is a critical period of epigenetic remodeling, intrauterine exposure is likely to impair reproductive outcomes as a result of leakage of estrogenic compounds across the blood-placental barrier into fetal tissues, including the brain. The mid-1920s witnessed a rise in interest regarding plants' ability to induce animal estrus (Bradbury and White, 1954). In the 1940s, however, scientists began to recognize that the alfalfa plant induced adverse effects n the fertility of sheep grazing in pastures due to its hyperestrogenic activity; consequently, it was named alfalfa disease. Feeding farm animals with rations rich in phytoestrogen disrupts the hormonal balance of the animals and leads them to display a calm estrus period (Zduńczyk et al., 2005), progesterone deficiency (Piotrowska et al., 2006), embryonic losses (Wocławek-Potocka et al., 2005), minimal lambing rate, uterine prolapse, severe metritis, pyometra, and hydrops uteri-like consequences (Lightfoot and Wroth, 1974).

Phytoestrogens typically inhibit endogenous estrogen synthesis, causing disorders in follicular development and preventing estrus (Rosselli et al., 2000). Furthermore, they potentially disrupt estrus and ovulation patterns through their effects on the central nervous system (Wocławek-Potocka et al., 2005). Nynca et al. (2009) reported that daidzein affects the ovaries by reducing progesterone and 17β-estradiol synthesis and causing the expression of estrogen receptors. According to Mustonen et al. (2014), however, formononetin and daidzein are metabolized to equol, a more estrogenic compound rapidly absorbed by the rumen wall, and high levels of equol synthesis appear to be the primary factor causing sheep to have fertility issues. Therefore, the rate of estrogenic clover in pastures is very critical. While grasslands (pastures) with 0.3% or less formononetin in dry matter (DM) are safe for sheep, concentrations above 0.8% in DM may result in fertility problems (Marshall, 1973). When sheep consume excessive amounts of clover, they may experience abnormalities such as infertility, abnormal mammary gland development, lactation, uterine prolapse (for high levels of isoflavones), and maternal dystocia due to inability to fully dilate the cervix (dysfunctions related to motor power leading to birth). In 43% of sheep fed lucerne (coumestrol) over a prolonged period, Cantero et al. (1996) observed explicit uterine alterations, cervical folds developed more rapidly than usual, and cystic formations with varying eosinophilic content. Hashem et al. (2018) also reported that late pregnant ewes fed with Berseem clover from two months before birth until estrus induction had longer estrus intervals, shorter estrus duration, lower progesterone concentration, and smaller litter size compared to ewes fed with corn silage. Adams (1998) also noted that phytoestrogens raise the risk of uterine prolapse and that exposure to long-term isoflavone ingestion in sheep might result in irreversible infertility. After synchronizing estrus, when ewes in seasonal anoestrus are fed alfalfa during mating season and early pregnancy, they display an increase in calm estrus and a decrease in conception rate (Hashem and Sallam, 2012). Sheep subjected to estrogen for a long time might become permanently infertile in their maturity (Adam, 1995). Coskun and Izmir (1991) indicated that the reason for such infertility is that spermatozoids experience difficulty in passing into the cervix due to impaired sperm transport function of the cervical mucus during mating and the deterioration in the structure of the cervix. Adams (1995) also reported that especially formononetin and coumestrol cause reproductive abnormalities. Coumestrol, on the other hand, is a potent coumestan that might lower the laying rate of grazing sheep. Coumestans affect fertility in sheep by delaying estrus and reducing the ovulation rate. As a result, multiple births reduce, yielding a lower lambing rate on average. The presence of coumestans in forage plants in high concentrations (1000 ppm) inhibits both estrus and ovulation, while only the ovulation rate is affected if the level is 400 ppm or below (Smith et al., 1979). However, the effects of coumestan on fertility are temporary; thus, if the feed does not retain it, fertility quickly recovers to normal. Reed (2016) reported that coumestrol also negatively affects fertility in cows. However, avoiding high-quality alfalfa grazing before and during the mating season may reduce the potential benefits of high ovulation due to body-weight gain (Rattray et al., 1980; Thompson et al., 1990). Consequently, identifying when and why coumestrol levels rise is a critical issue for improving alfalfa grazing management.

There are similar situations identified among phytoestrogen-dominated cows. Woclawek-Potocka et al. (2005) reported that cows fed a soy-containing diet had a lower pregnancy rate than cows fed a soy-free diet, and these cows required more artificial insemination to become pregnant. The pregnancy stage in cows involves isoflavone absorption, biotransformation, and metabolism differently; hence, abortions might ensue due to high concentrations of active metabolites, such as equol and para-ethyl-phenol, when cows are fed with isoflavone-containing feeds for a long time, especially in the early stages of pregnancy (Woclawek-Potocka et al., 2008). Red clover silage retaining isoflavones and alfalfa containing coumestan also result in behavioral anomalies in cows, such as irregular estrus, nymphomania, and anoestrus (Kallela et al., 1984). In this context, by only measuring the phytoestrogens in the diet or monitoring their effects on the reproductive healthiness and fertility of the animals, Adams (1995) asserted that isoflavone-induced infertility could be detected before it even occurs. Hashem et al. (2016) also reported a decrease in the fertility of heifers fed with berseem clover. They further emphasized that berseem fed negatively affected progesterone secretion in heifers fed with clover, leading to hormonal imbalance associated with the development of early embryonic losses in early pregnancy and a decline in the progesterone estradiol ratio.

Rochira et al. (2005) revealed that estrogens play a critical role in the male reproductive system, and subsequently, phytoestrogens become popular in male fertility. Pool et al. (2022) conveyed that phytoestrogens binding to estrogen receptors in male animals potentially lead to jeopardizing the dynamics of fluid resorption in the efferent ducts, increasing the secretion of chloride ions into the seminal fluid, generating an abnormal sperm morphology, inhibiting sodium transport in the testicles, and ultimately reducing fertility due to altered testicular function, sperm production, and sperm quality. Glover and Assinder (2006) also documented that animal-feeding diets rich in phytoestrogens reduced sperm quality in male animals. Gunnarsson et al. (2009) remarked that 3-month-old goat offspring fed a phytoestrogen-rich diet had significantly higher testosterone and free T3 concentrations at five months of age (37.5 nmol l^{-1}) compared to control animals (19.1 nmol l^{-1}). They also reported that isoflavones might stimulate testosterone synthesis by increasing T3 secretion during puberty in male goats. Pool et al. (2021) stated that ram spermatozoa's in vitro exposure to physiological equol concentrations reduced sperm motility, albeit increased reactive oxygen species, membrane fluidity, and DNA fragmentation. Yet, some studies revealed that feeding with phytoestrogens favorably affected reproduction. For instance, Mustonen et al. (2014) discovered that feeding sheep with red clover silage retaining high phytoestrogens, especially formononetin (0.68% in DM), for a total of 5 months before, during, and after the breeding season resulted in pregnancy in all sheep; however, the same silage with ample amount of phytoestrogens did not change sheep fertility. Pace et al. (2011) divided female lambs into three groups and fed alfalfa, oats (*Avena sativa*), and subterranean clover with phytoestrogen content ranging from 10.21mg g-1 DM at the beginning to 0.90 mg g-1 DM at the end of the experiment for 60 days. Accordingly, they found no substantial difference among the three groups regarding the development of the reproductive system, fertility, fecundity, and breeding performance in lambs. Feeding with subterranean clover also aided the lambs in reaching sexual maturity earlier and improving their growth rate. Austin et al. (1982) reported that feeding heifers with red clover silage before and during the insemination period boosted the pregnancy rate, and 76% of them became pregnant at first service. Speijers et al. (2005) also revealed that lambs fed red clover silage had higher DM and metabolizable energy intake than lambs fed alfalfa or ryegrass silage, resulting in a faster growth rate and higher fitness score.

7. Effects on Growth and Performance

Isoflavones have been proven to stimulate growth in grazing and farm animals by minimizing the time required to reach target weights. Moorby et al. (2004) reported no significant difference between carcass quality of lambs fed by red clover retaining either a high formononetin (HF) or low formononetin (LF) concentration; however, lambs fed by red clover with a high formononetin concentration gained higher carcass weight. Since the plasma concentrations of growth hormone and insulin-like growth factor-1 were highest in lambs fed by red clover with a high formononetin concentration, scientists opined that the growth rate in these lambs was associated with these chemicals. Pace et al. (2006) reported similar findings indicating that male and female lambs fed by subterranean clover with low formononetin (10% in DM) content gained significantly more body weight than those sustained by Italian ryegrass (non-estrogenic roughage). They further emphasized that such a significant gain was attributable to the fact that subterranean clover increased body weight in sheep and lambs by providing a higher metabolizable protein intake. Zhao et al. (2017) also documented that daidzein was beneficial in stimulating the digestion of dietary proteins and improving the secretion of growth hormones, thereby increasing productivity. Liang et al. (2018) remarked that adding daidzein to the diet improved rumen fermentation and lipid synthesis due to its estrogenic activity, and free fatty acids reached high levels in the serum. Speijers et al. (2005) stated that the body weight gain of the lambs fed with red clover was higher than that of lambs grazing on alfalfa or perennial ryegrass pastures. Vanhatalo et al. (2007) also discovered that dairy cows fed red clover silage had higher DM intake and milk yield than those fed grass silage, as well as a more desired polyunsaturated fatty acid mil composition.

8. Effects on Antioxidant Activity

In addition to numerous studies documenting either positive or negative effects of isoflavones on animal health in many animal species, a significant amount of literature also reports the favorable influences of isoflavones on growth-promoting, anti-inflammatory, and antioxidant properties (Rodríguez-Roque et al., 2013). Assuming the concentrations of these compounds in plants ingested by animals, these properties are unlikely to cause a critical impact on the total cellular redox state. However, it is well-established that phytoestrogens significantly increase the expression and activity of the antioxidant defense enzymes catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase, which may have a critical effect on the accumulation of oxidative stress and oxidative damage (Borras et al., 2006). These effects are potentially attributable to the potency of genistein or daidzein to bind estrogen receptors preferentially and up-regulate antioxidant defenses (Borras et al., 2007; Raschke et al., 2006), which are comparable to those effects induced by estrogens (Borras et al., 2007). Dykens et al. (2003) reported that genistein and daidzein, two phenolic compounds, might function as antioxidants directly by donating hydrogen and electrons through hydroxyl groups, a feature they share with estrogens. Djuric et al. (2001) indicated that dietary isoflavones increase resistance to low-density lipoprotein oxidation, minimizing oxidative DNA damage by directly affecting free radicals or antioxidant-scavenging enzymes. Genistein is regarded as the best-known isoflavone compound with the highest antioxidant activity. High genistein levels inhibit tyrosine kinases and hinder macrophage and natural killer cell numbers and phagocytosis rates (Steele and Brahmi, 1988, cited by Zhengkang et al., 2006). Rahman-Mazumder and Hongsprabhas (2016) reported that genistein functions as an antioxidant and antiproliferative in several processes, including scavenging reactive oxygen species, blocking oxidative and photodynamic damage in DNA, and inhibiting tyrosine protein kinase. Lephart et al. (2005) and Lee et al. (2005) concurrently suggested that the ability of phytoestrogens to reduce modifications in cellular redox state, improve redox signaling, or inhibit the accumulation of molecular oxidative damage through an up-regulation of cellular antioxidant defenses might elucidate why phytoestrogenic plants enhance cognitive performance in female animals. Elsayed et al. (2019) also indicated that genistein acts as an antioxidant at critical concentrations by easing oxidative stress.

9. Conclusions

Although phytoestrogens have species-specific biological activities, their effects potentially differ based on numerous aspects, including chemical form, delivery method, current estrogenic state, age, exposure duration, and dosage (concentration). Estrogenic grasslands are still used in livestock breeding, although the exact mechanisms by which phytoestrogens put animals grazing in pastures at risk for health and reproductive issues are yet unclarified. Accordingly, considering the aspects outlined above, further and well-designed studies on farm animals are necessary to more accurately evaluate the advantages and risks of phytoestrogen ingestion and determine physiological and genetic underpinnings of individual sensitivity. Therefore, Such an objective will be much more feasible to curb fertility losses from phytoestrogenic effects and develop future strategies to ensure farm animals' healthy growth and development.

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CHAPTER 9

Fatsia japonica AS A POTTED ORNAMENTAL PLANT (*JAPANESE ARALİA*)

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1. INTRODUCTION

Fatsia japonica (Thunb.) Decne. & Planch. (Syn: *Aralia japonica* Thunb.) (Syn: *Aralia sieboldii* hort. ex K.Koch) is in Genus: *Fatsia*, Family: *Araliaceae*; Order: *Apiales*; Clade: *Asterids* (Anonymus, 2022).

Fatsia genus is a small genus of three species. These species are indigenous to Taiwan, the Bonin Islands, the Ryukyu Archipelago, and Japan. *Fatsia* is one of few plant genera distributed in both continental and oceanic islands. *Fatsia oligocarpella* is indigenous to the Bonin Islands, while *Fatsia polycarpa* is common in midelevations of Taiwan. *Fatsia japonica* is native to southern Japan and the Ryukyu Archipelago (Chiang et al., 2014). In Taiwan's middle elevational evergreen woods, *Fatsia polycarpa* serves as a representative of the genus' southern locational limit (Huang et al., 2008). It is a herbal medicine with a history for treating a number of conditions linked to inflammation (Cheng et al., 2013).

2. *Fatsia japonica*

Fatsia is a foliage plant (Garibaldi et al., 2004) and a subtropical species native to southern Japan and southern China. The plants of this species have potential medical use in addition to being frequently utilized as a beautiful ornamental tree (Shi et al., 2017). With branched stems, *Fatsia japonica* can reach heights of 3-6 m. (El-Sayed et al., 2015). It is a tiny tree or evergreen shrub. It is an attractive foliage plant noted for its palmately lobed glossy dark green leaves. Black berries appear in the fall, after which there are tiny creamy umbel-shaped blooms. However, indoor plants hardly ever bloom or bear fruit. Can be pruned to shape. Aphids, mealybugs, scale, thrips, and spider mites are a few potential issues. Root rot may develop in soils that are too wet (Missouri Botanical Garden, 2022).

Fatsia Japonica blooms in autumn and fruits ripen in mid to late winter. It has umbel-shaped flowers with cream/tan and white petals. Drumstick-like cream-white blooms on the terminal umbel have a white stalk and are glabrescent. Excellent, long-lasting cut greenery for floral arrangements is simple to prepare. Leaf surface is glossy and leathery. The plant can withstand salt, polluted soils, long shadows, and drought. Common names are Formosa rice tree, Figleaf Palm, Glossy-Leaved Paper Plant, Japanese Fatsia, Japanese Aralia and Paper Plant (NC State Extension, 2022).

Ornamentals and cut foliage are the primary crops grown on Italy's northwest coast. Markets in Italy, Europe, and North America are very interested in these crops. Among the cut greens, *Fatsia japonica* is one of the most important.(Sacco et al., 2000). Because it grows naturally in nature, Fatsia japonica is easy to obtain.With wide and huge leaves divided into seven or eight portions, it has a strong air purifying effect, notably in the reduction of toluene (Song, 2012).

A humus-rich potting mixture is ideal for growing *Fatsia japonica*. Plant likes cool temperatures. During the growing season, it is possible to water often, but from fall to late winter, irrigation must be significantly reduced (Missouri Botanical Garden, 2022). Fatsia japonica should be planted in a covered area because the leaves will turn brown if exposed to direct sunlight or wind. Grows best in acidic, well-drained organically rich soils but tolerates sandy and clay soils. It can withstand drought, air pollution and a little salt stress after it is well rooted. It can be pruned at any time of the year to shape the plant, except during the winter months when it is dormant (NC State Extension, 2022).

Fig. 1. Habitat of *Fatsia japonica* in Yuwan Amami–Oshima (Japan) (Kobayashi et al., 2013)

El-Sayed et al., (2015) was conducted a study in Cairo University to investigate the effect of different pulsing and holding solutions treatments and their interactions on keeping quality and extending vase life of *Fatsia japonica*. When compared to distilled water, pulsing solutions containing benzyladenine (BA) and citric acid were the most successful treatments for extending vase life, improving overall appearance, and increasing the concentration of chlorophyll a and b in both non-stored and stored leaves (control). In contrast to room temperature storage, cold storage reduced the longevity, general look, and chlorophyll a and b levels of cut foliage. The interaction of holding and pulsing solutions revealed that the combination of 20 g/l sucrose + 10 mg/l BA + 200 mg/l CA + 2 ml/l Clorox as a holding solution and BA and citric acid as pulsing solutions offered the best results in all parameters.

Fig. 2. Fatsia japonica (Anonymus, 2020)

The plant *Fatsia japonica* is high in triterpenoid glycosides and has anti-inflammatory properties, is used to treat rheumatoid arthritis (Ye et al., 2014). There are some variances in the composition and content of the essential oils from various regions of *F. japonica* (Liang et al., 2012). The seeds can be harvested at any time because they are abundant in the tree and have a lengthy maintenance period, emerging from October to May of the following year. There is little known research on the *Fatsia Japonica* (Ye et al., 2014). The essential oils isolated from the roots, leaves, and fruits of the *Fatsia Japonica* was found containing total 97 different chemicals, primarily monoterpenoids and their oxides as well as semiquinones and their oxides. Thus, the seeds, with have potential as medicines, are rich in other elements besides carbon, which could result in a decrease in cyclic stability due to the provision of a reacting active site (Aokia et al., 1981).

By Lee et al., (2010) chemicals such as "Dehydrotriferulic Acid," "Roseoside," and "Oxyresveratrol" were discovered for the first time in the family *Araliaecae,* while "Protocatechuic Acid" was reported for the first time from the genus *Fatsia*. *F. japonica* contains large amounts of "Glucopyranosyl-hederagenin" and "Arabinopyranosyl-hederagenin." It is extremely unusual for the family *Araliaceae* to have the chemicals "Dehydrotriferulic Acid," "Roseoside," and "Oxyresveratrol," and this could serve as an index for the chemotaxonomic categorization of the genus.

Fig. 3. Japanese Aralia (Anonymus, 2023)

In Japan, leaves of *F. japonica* have been showing virus-like ringspot symptoms for decades, but prior efforts to determine the cause have been fruitless. Using RNA sequencing research, Kitazawa et al. (2022) discovered an orthotospovirus-like sequence that they have named "Fatsia japonica ringspot-associated virus" (FjRSaV) in symptomatic *F. japonica* plants.

2. Health effects

F. japonica is the third species of plants to be known to possess baccharane glycosides, and "Fatsioside A" is the first baccharane glycoside discovered in the *Araliaceae* family. The development of human glioma cells and rat glioma C6 cells was reduced by Fatsioside A. Further research revealed that fatsioside A inhibited the cell cycle and caused apoptosis and necrosis in glioma cells (Yu et al., 2014).

Everygreen *Fatsia japonica* can be found in the areas surrounding the Black Sea. An ethanolic extract of the leaves of *Fatsia japonica* was obtained at the Institute of Pharmacochemistry, Tbilisi State Medical University (TSMU), in the Laboratory of Terpene Compounds. This extract was converted into the paramedical preparation Fatsiphloginum™. Its active ingredient has anti-rheumatic, anti-inflammatory, and analgesic properties, and Georgia uses it as a unique, nonsteroidal paramedical medicine (Getia et al. 2017).

Numerous Fatsia japonica trees have been planted in metropolitan areas in China, south of the Yangtze River. At Sichuan Agricultural University, leaf-spot symptoms were seen on 120/200 of *Fatsia japonica* plants. On the leaves, there were initially yellowish dots that aged to become white. With time, the dots grew larger, forming irregular edges before eventually engulfing the entire leaf. Eventually, diseased leaves became curled and died. According to field observations, this disease primarily affects weaker and old leaves. A genomic DNA extraction revealed 100 percent homology with *Colletotrichum karstii* sequences. There have been reports of *Colletotrichum fructicola* causing anthracnose on *F. japonica* in China (Shi et al. 2017). But this is the first record of anthracnose on *F. japonica* carried on by *Colletotrichum karstii*. This disease affects the aesthetic appearance of the plants, reducing their appearance as an ornamental plant.

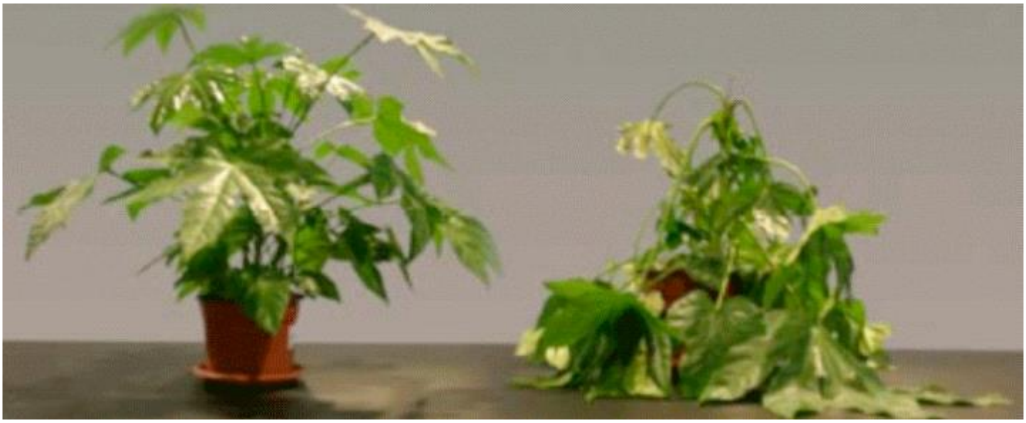

Fig.4. *Fatsia Japonica* wilting without damage (Salem et al., 2008)

Fatsia japonica, often known as Japanese aralia, is a potted houseplant indigenous to southern Japan and South Korea but can also be found in other countries, such as the south and southwest of Iran. The diseases that affect this plant include root rot (*Phytophthora* and *Pythium* spp.), leaf blight (*Phytophthora cactorum*), Alternaria leaf spot (*A. panax*), leaf wilt (*Botryosphaeria dothidea*), and leaf blight (*Phytophthora cactorum*) (Kim et al. 2005; Li et al. 2018). In February 2020, Japanese aralia in a glasshouse in the Iranian region of Khuzestan were discovered to have a stem disease. Based on appearance and phylogenetic analysis, four similar isolates were taken from symptomatic tissue and identified as *Botryosphaeria dothidea*. The pathogenicity of the isolates was confrmed by inoculating healthy shrubs of F. japonica (Mehrabi-Koushki et al., 2021).

3. As a source of functional material

The *Fatsia japonica* seed, which mostly contains glucose, has the potential to serve as a porous carbon matrix precursor for high-value supercapacitors. By annealing at a high temperature, cost-effective hierarchical porous carbon materials were produced from *Fatsia japonica*. Due to its straightforward manufacturing technique and exceptional electrochemical performance, this hierarchical porous carbon from biomass would make a viable candidate for use in supercapacitors' electrodes (Li et al., 2020).

Widely available *Fatsia japonica* is ideal for the architecture and construction industry due to its combination of environmentally friendly and long-lasting corrosion resistance (Wang et al., 2023).

4. Propagation Methods

The fatsia plant can be quickly multiplied through seed or cuttings that root. Scale or mealybugs might occasionally irritate fatsia. To germinate from softwood, you need 21–24°C. The techniques used for propagation range from seed germination to clonal techniques including cuttings and air layering. Both technique has advantages, with clonal methods being the greatest at accurately reproducing the original plant's foliage and seeds being the simpler. All methods result in a lush, green plant that can be grown either indoors or outdoors in U.S. Department of Agriculture plant hardiness zones 8 to 10. Ideal for foliar propagation is leaves with semi-mature growth at the base or an 8-inch stem with a few small leaves at the tip. Cut just below the leaf group with a sterile pruning shears.. The lower portion of the cutting should be leaf-free. The leafless part can be dipped in powdered rooting hormone to promote rooting. Plants are placed in plastic bags with drainage for rooting. After planting, the plants are pressed tightly and then covered with a clear plastic bag. Place the plastic bag close to a window with good light but no direct sunshine. The majority of *Fatsia japonica* cuttings take one to two months to root. After it has rooted, take the plastic bag off and cultivate the plant for at least a year or two in a bright area before moving it into a shady area of the open air and into a permanent planter filled with nutrient-rich potting soil. The simplest way to grow fatsia japonica is from seeds, albeit the offspring might not look exactly like the parent. When the fruit turns completely black, the seeds are collected and the dark outer layer is peeled off. A pot with a drainage hole is selected and seeds are planted in 4-inch plastic pots filled with moist potting soil. A thin layer of vermiculite should be placed on top of the seeds after they are placed in the soil.

Air layering is another clonal technique for growing Fatsia japonica. Although some more difficult than rooted cuttings, the procedure is just as successful at growing a new plant. Air layer in Fatsia japonica can be make when the stems are still green throughout the active growing season. Measure back 4 to 6 inches from the tip of a stem that has one or two tiny leaves at the end. Girdling is the procedure of removing a 1-inch-wide strip of bark all the way around the stem with a sterilised knife. A piece of wet sphagnum moss the size of a golfball should be wrapped around the girdled section of the stem. To keep the sphagnum moss tightly wrapped around the stem, cover it with a piece of black plastic and secure it with twist ties. Every few days, remove the plastic and add water. In one to two months, the majority of *Fatsia japonica* will begin to sprout roots. At this point, you can cut the stem below the roots and transplant the plant into a pot with potting soil. For a year or two, you can cultivate the *Fatsia japonica* indoors or outdoors before deciding on a long-term growing area (Hearst. (2021).

In vitro a propagation for *Fatsia japonica* using axillary bud explants were established. Cultures were initiated from axillary bud explants on MS medium supplemented with IAA (1-3 mg/l), 2,4-D (1-3 mg/l) or NAA (1-3 mg/l) in combination with BA (0.5 mg/l). The maximum shoot bud formation was obtained in MS medium supplemented with 0.5 mg/l BA and 2 mg/l IAA after 4 weeks culture. The microshoot rooted within 4 week in MS medium containing 1 mg/l IBA.

5. Conclusions

Southern Japan is home to the subtropical foliage plant species known as *Fatsia japonica*. It is frequently grown as an ornamental plant and has therapeutic benefits. This small, evergreen tree or shrub has branched stems, lobed, glossy, dark green leaves, and tiny, creamy flowers. It can reach a height of 3–6 meters. Although it can withstand sandy and clay soils, it grows in acidic, well-drained, organically rich soils. The essential oils isolated from the roots, leaves, and fruits of the *Fatsia japonica* included a total of 97 different chemicals, primarily monoterpenoids and their oxides as well as semiquinones and their oxides. Studies are very limited on this ornamental plant species.

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