Innovations in Sustainable Agriculture and Aquatic Sciences

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We thank all of the authors with whom we collaborated to publish their books across disciplines.

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Chapter 1

AN ALTERNATIVE HONEYBEE PRODUCT FOR THE TREATMENT OF MASTITIS: PROPOLIS

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INTRODUCTION

Mastitis is a common disease affecting dairy cows, causing significant harm to their health and profitability (1). Mastitis in dairy animals could be determined as the inflammation of the mammary glands/udder caused by infectious pathogens, affecting animal welfare and economic losses (2). Mastitis in dairy animals leads to economic losses due to reduced milk production, increased treatment costs, and culling of infected cows (3). Mastitis in dairy animals is an inflammation of the udder tissue caused by bacteria, leading to pathological changes in glandular tissue and abnormalities in milk (4). Mastitis is usually caused by the interaction between microbial infections and the host in the udder and its response. In other words, its effect is on milk yield, quality, animal health, and welfare (5). Although mastitis is usually seen locally, it may rarely become chronic in animals with weakened immune systems. The incidence of the disease in the herd may vary depending on environmental factors such as the age and lactation period of the animal. Mastitis can be seen in three different forms: clinical, subclinical and acute (6). Apart from these types of mastitis, there are also structural disorders of the breast caused by infection due to mastitis. Since the quality of milk secreted from such udders is different from normal, the picture is shaped towards clinical

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mastitis. In 10-15% of cows with mastitis, there may be a subclinical condition that decreases milk yield and increases the bacterial content of milk. As a result, all these conditions lead to a decrease in the quality characteristics of milk (7).

In the treatment of mastitis, many methods and various herbal products are also used. One of these products is propolis. Propolis which is a honeybee product, means "guardian of the city" and is also known as "Russian Penicillin" in some sources (8). Honeybees uses a resinous material called propolis to smooth walls, seal cracks in the hive, and regulate the temperature and humidity within. Honeybees collect the sticky substance known as propolis from the resin found in flowers, trees, and plant leaves, and then mix it with their saliva (9). Although there are alternative techniques, such as ultrasonic and microwave, propolis extracts are typically made by continuously soaking in different solvents (10). Due to its many biological qualities, propolis has been employed extensively in human health since ancient times and has recently generated a lot of interest. According to certain research, propolis significantly reduces inflammation in macrophages and fortifies the body's antioxidant defense system. (11). It is also stated that propolis may prevent the development of some antibiotic-resistant S. aureus species other than several different bacterial species known to cause mastitis. There is limited information on how propolis elicits responses to mastitis in mammary epithelial cells in cows and studies on this subject are ongoing (12). In this study, the possibilities and effects of propolis as an alternative product to classical mastitis methods in dairy animals were investigated.

TYPES OF MASTITIS

Mastitis occurs when harmful microbes enter the teat of a milking animal and cause infection. Many microbial strains can cause mastitis such as *Streptococci, Staphylococci*, Coliforms (*E. coli*) and *Corynebacterium* (13). Mastitis can spread rapidly in the herd and can greatly affect milk production and put the welfare and health of animals at risk. For these reasons, attention should be paid to mastitis species and the symptoms caused by them (Table 1).

Table 1. Features of various mastitis types			
Types of Mastitis			
Subclinical	**Clinical**	**Acute**	**Chronic**
Most common and difficult to diagnose	Animals with swollen, heated, or sensitive udders	The symptoms appear suddenly and rapidly	Long-lasting symptoms (months to years)
There is little to no change in the appearance of the udder or milk however slightly reduced milk production	Watery milk, clots, flakes, discolouration, and decreased production	Animal symptoms include weakening, fever, decreased intake of food or drink, swelling, redness, and soreness in the udder.	Animals with mastitis may alternate between clinical and sub-clinical states or remain sub-clinical forever.
Animals that are infected can quickly spread the infection to other animals.	The onset of symptoms may happen hours or days after the infection and days to weeks may pass between symptoms.	Dramatically reduced milk production, very abnormal milk appearance	Several infection cycles in a lactation period or calendar quarter

MASTITIS AND ITS IMPORTANCE IN DAIRY COWS

Mastitis in dairy cows is classified as clinical or subclinical mastitis as well as being defined as inflammation of the mammary gland, which is usually contagious (14). Environmental and infectious pathogens are the two main groups of pathogens that cause mastitis in cows (15). The primary source of the causative agent in mastitis caused by environmental variables is typically the animal's shelter. Concurrently, the classification of infectious mastitis considers the microorganisms' ability to survive in the mammary gland, their discharge from the body, and their capacity to spread to other animals (16,17,18). The classification of bacteria causing mastitis is given in Table 2.

Table 2. Major mastitis pathogens	
Classification	**Identified bacterium**
Environmental mastitis	*Eschericha coli* *Klebsiella spp.* *Streptococcus uberis* *Streptococcus dysgalactiae* *Citrobacter spp.* *Streptococcus spp.* *Enterobacter spp.* *Proteus spp.* *Enterobacter aerogeens* *Streptococcus bovis* *Enterococcus faecium* *Enterococcus faecalis* *Bacillus spp.*
Contagious mastitis	*Staphylococcus aureus* *Streptococcus agalactiae* *Streptococcus dysdalactiae* *Mycoplasma spp.*

Although there is a great variation among pathogens causing mastitis in dairy cows, about 80% of the disease state in herds is caused by *S. aureus, S. uberis, S. dysgalactiae, S. agalactiae* and *E. coli* (19). One of the most prevalent bacteria, particularly in cases of mammary infections, is *S. aureus* Schlegelová et al. (20). This bacterium was identified as the primary cause of mastitis in cows from various herds in Brazil, where isolation rates ranged from 8.3% to 49.23% (21).

A few strategies to lower the incidence of mastitis include employing antimicrobials, removing potential infection sources from the herd, and practicing good hygiene when milking. However, long-term and improper use of antimicrobials causes the emergence of resistant strains and reduces the effectiveness of the treatment. This situation, in addition to causing trace amounts of antibiotics to appear in milk, can negatively affect human health. Due to this situation, breeders sometimes resort to alternative searches for the treatment of mastitis.

MASTITIS RISK FACTORS

Although the origin of mastitis in dairy cows depends on pathogen, host and environmental factors, there are several known risk factors (22,23) (Figure 1). These risk factors are summarized respectively.

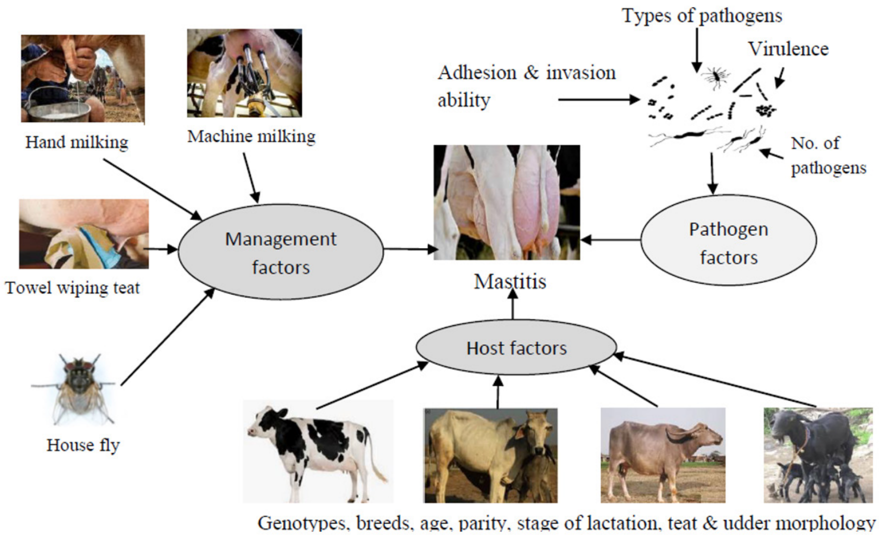

Figure 1. Risk factors of mastitis in ruminant farm animals (24)

PATHOGENIC FACTORS

Mastitis in dairy cows is thought to be mostly caused by bacterial intramammary infections. Numerous bacterial species have been identified as the cause of mastitis in cows. Based on where they originate, these bacterial illnesses are divided into two categories: infectious and environmental (17). Mastitis that can spread from cow to cow, particularly during milking, is referred to as infectious mastitis (25). The udder and teat surface of cows are home to common and uncommon bacterial species like *Corynebacterium* and *Mycoplama bovis*, as well as infectious pathogens like *Streptococcus agalactiae* and *Staphylococcus aureus* that colonize and expand into the udder duct (26). These have the ability to cause subclinical infections, frequently accompanied by a rise in the number of somatic cells. Leukocytes and epithelial cells make up the somatic cell count, which is a helpful predictor of intramammary infection (27).

Reducing interaction between reservoirs and uninfected cows can help reduce transmissible diseases. In order to minimize contagious diseases, it is crucial to maintain milking equipment properly and to treat dry period mastitis, cull cows, and disinfect teats after milking (28). Unlike infectious pathogens, environmental pathogens usually do not live on the udder and teat skin of the cow but are found in animal stalls and within the shelter. Another way to characterize them is as opportunistic infections that are seeking an opportunity to infect someone. Reducing the exposure of teats to pathogens and strengthening the cow's resistance to intramastitis through vaccination and antibiotic intervention can help control environmental infection.

Staphylococcus aureus

The most prevalent gram-positive bacterium linked to a variety of clinical and subclinical mastitis types is *S. aureus.* (29). The main source of *S. aureus* is the infected mammary gland, which is chronic due to this bacteria. Therefore, ensuring udder and milking hygiene can reduce potential infection by protecting the healthy cow from the infected cow (30). Since *S. aureus* does not stimulate as strong an immune response in cows as E. coli or endotoxin, hence Staph infection, aureus always causes chronic mastitis, which is milder and lasts for several months (31). *S. aureus* creates deteriorating enzymes and toxins that permanently harm the alveoli and decrease milk production, despite the fact that infection does not result in mammary deformities or mortality. Staph. aureus infections are treated with antibiotics. Such strains of *S. aureus* known as methicillin-resistant *S. aureus* (MRSA) has a mecA gene that confers resistance (7). The peculiarity of this bacterium is that *S. aureus* produces biofilms, providing a favourable environment for itself in the host, making it an even more difficult target for the treatment of such infections (32,33).

Streptococcus agalactiae

Gram-positive *S. agalactiae* is the pathogen that causes infectious mastitis. It is present in shelters and the digestive systems of cattle. It can be transmitted through milking machine manure, especially contaminated drinking water. *S. agalactiae* does not cause structural problems in milk, however it does induce subclinical mastitis with a high somatic cell count and decreased milk supply. Through attachment to cow mammary glands and the formation of a biofilm that keeps it there, it can survive and become more resistant to nutrient and host deprivation (34).

Escherichia coli

E. coli is the most commonly identified gram-negative bacterium. In dairy cows, the bacterium spreads to the udder via the teat, where it multiplies and initiates inflammation. It can be found in the litter material used as bedding, and shelter conditions in which the animal is kept, especially in humid environments (4, 35). *E. coli*-induced mastitis is typically clinical and episodic. The udder will swell and get red, and there may be a mild to high fever as symptoms. Profound clinical mastitis brought on by *E. coli* can cause the dairy cow to lose its ability to produce milk, suffer permanent tissue damage in the mammary gland, and occasionally even die. Since *E. coli*'s pathogenicity is not dependent on a single, distinct virulence factor, it is categorized as an opportunistic pathogen with several virulence factors. Because it may develop biofilm at different levels, this bacteria can settle in the mammary gland and cause chronic mastitis infections that are challenging to treat (36).

Streptococcus uberis

An environmental pathogen bacteria called *S. uberis* is linked to both clinical and subclinical infections and recurrent mastitis. Milk's α-and β-caseins encourage the formation of biofilms, which aggravates strep throat. According to Zhang et al. (37), *S. uberis* continues to thrive in harsh environments and exhibits resistance to antibiotic therapy and it has been found in various animal parts.

ENVIRONMENTAL FACTORS

Environmental conditions and care and feeding practices play an important role in animal health and welfare. Mastitis can be less prevalent if the udder is kept clean and in good condition. High settling frequency, unclean floors, wet litter, poor ventilation, and a hot, humid environment can all contribute to the development of mastitis pathogens and raise cows' exposure to them, which raises the prevalence of mastitis (38).

METHODS FOR DETERMINING MASTITIS

When a cow's udder becomes inflamed, many affected signs are observed as a result of changes in the milk (39). These signs are expressed as test indicators for mastitis. Numerous testing methods exist, such as molecular techniques, somatic cell counts, the California mastitis test, and other advanced approaches (40). Additionally, several tests are employed in both laboratory and field settings to identify mastitis in its early stages. (Figure 2).

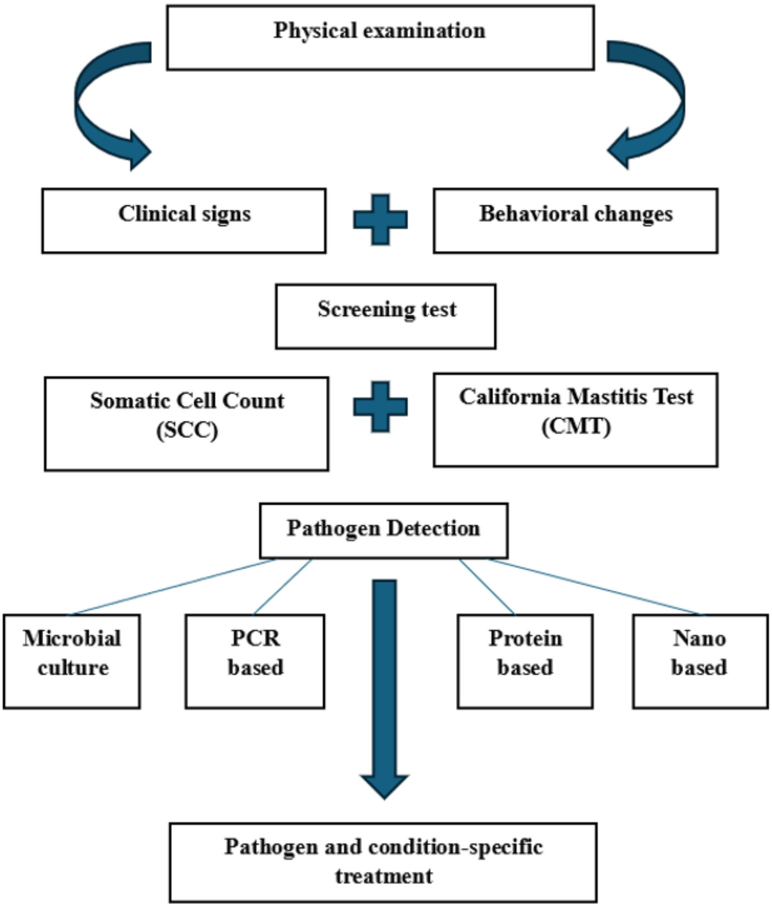

Figure 2. Methods for determining mastitis (41)

TRADITIONAL SCREENING TECHNIQUES

Traditional screening techniques for mastitis aim to detect infections in the mammary gland early to minimize economic losses and ensure cow health. These techniques rely on evaluating physical, chemical, or microbiological changes in milk or the udder. Microbiological analysis of milk is regarded as the gold standard for the diagnosis of mastitis in cattle, despite several drawbacks such as increased costs and breeding time. Nonetheless, during the inflammatory phase, a sizable portion of leukocytes move to the site of inflammation (42). As a result, the cell count of milk can be used to assess the udder health of the animal. Techniques including somatic cell count (SCC) in milk, California Mastitis Test (CMT) and Somaticell R are other methods to identify mastitis.

SOMATIC CELL COUNT

Somatic cell count (SCC) is a widely used method to screen for mastitis in dairy cattle. The SCC measures the number of somatic cells (mainly white blood cells) present in the milk, which increases in response to infection in the mammary glands. SCC is a key indicator of udder health and inflammation. When the somatic cell count exceeds 200,000 cells/mL, it generally indicates subclinical or clinical mastitis. A higher count suggests a more severe infection. SCC is an indirect but effective method for detecting mastitis (43). Changes in SCC levels during lactation can indicate susceptibility to mastitis. Research shows that maximum and standard deviation of SCC during lactation are reliable predictors of clinical mastitis (44). SCC can be performed with the help of an automated system or by visualization of microscope slides with appropriate reagents.

CALIFORNIA MASTITIS TEST (CMT)

The California Mastitis Test (CMT) is a simple, rapid diagnostic tool used to detect subclinical mastitis in dairy cows. Subclinical mastitis is an infection of the udder that does not show visible signs but can significantly impact milk production and quality The test involves mixing a reagent with a small amount of milk, which causes a reaction based on the somatic cell count (SCC) in the sample. Increased somatic cell counts indicate an inflammatory response to infection, providing a visual indication of mastitis. The test provides a quick, on-site method for farmers to assess udder health. It is commonly used to identify subclinical mastitis during milking. Identifying mastitis can be done more accurately with this test.

The CMT is sensitive enough to detect subclinical mastitis, which does not show outward symptoms but can still negatively affect milk production and quality. This early detection allows farmers to intervene before the infection progresses to clinical mastitis. A milk sample is collected in a CMT palette and an indicator is applied. Positive results include leukocyte counts over five million per milliliter and a robust gel formation. A weakly positive result shows precipitate precipitation with paddle movement, no gel formation, and a leukocyte count of 400,000-1,500,000 per milliliter. Negative results have precipitate-free structures and leukocyte counts (45).

SOMATICELL®

A quantitative assessment method comparable to the Wisconsin Mastitis control. The test needs to be taken from tank milk to determine whether the method also meets current legal limits. The test utilizes a single-use graduated plastic bottle with a preset SCC scale (46).

AUTOMATED DIGITAL DIAGNOSTICS

This is a newer test method for examining mastitis in cattle. It is very easy, fast and can be applied in the field. This technique assesses the quantity of mastitis-related biomarkers or the physical, chemical, and biological alterations in milk. Among the techniques are the Afimilk mastitis detector, the DeLaval cell counter, and the Draminski mastitis detector (47). The DeLaval cell counter can be used to count somatic cells. Proteomic applications, infrared thermography, and sensor-based systems are some more novel methods in the diagnosis of mastitis. Large businesses commonly use sensor-based detection systems. Magnetic nanoparticles are also used in a Portuguese study to identify different staphylococci, including *Staphylococcus aureus* (48).

ECONOMIC IMPORTANCE OF MASTITIS

Mastitis in cows is an inflammatory condition resulting from physical trauma or microorganism infections of the mammary gland tissue (41). It is also considered the most common disease in the dairy industry, leading to significant economic losses due to reduced yield and poor quality (49) (Figure 4).

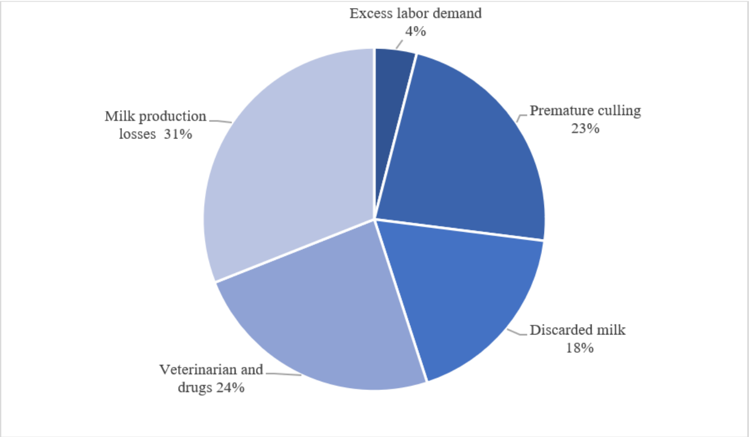

Figure 5. Total by approximate cost for clinical mastitis (50).

Bovine mastitis costs an average of $150 per cow annually, accounting for 11% to 18% of the annual gross margin per cow (51). Roughly 70% of all milk loss is caused by damage to the bladder's tissue, which lowers milk production (52). While milk with a somatic cell count (SCC) of around 100,000 is considered normal and healthy, when this value exceeds 200,000, it is considered subclinical mastitis (53,54). Although this situation is not seen as a problem in some farms, it gradually affects the cost of milk production negatively (55,56). In the case of mastitis in dairy animals, a decrease of 10-30% in total lactation yield is observed, which may cause significant losses in the milk to be obtained. Kumari et al. (57) determined milk loss ranging from 100-500 kg per cow due to subclinical mastitis (SCM). In addition to the losses in quality due to mastitis, it was found that the prevalence of SCM varied between 20-85% in cows in different. Mastitis not only affects the quantity and composition of milk, but also results in significant economic losses due to the reforming or treatment of dairy animals The annual economic loss caused by mastitis in India is estimated to be 1500 million USD (27). When this value is compared to clinical mastitis, the loss caused by subclinical mastitis accounts for approximately 60% to 70% of the total loss (58). In Argentina, the losses due to clinical and subclinical mastitis caused by *S. aureus* in Black German Gaelic cows are estimated to be more than USD 400,000 (59). A detailed economic analysis is needed to determine the prevalence and economic impact of mastitis.

CHEMICAL STRUCTURE OF PROPOLIS

Some of the benefits provided by the correct use of propolis have made it a subject of increasing research in recent years. The reason for this is the polyphenolic compounds produced by *Apis mellifera* and contained in propolis (60). The chemical composition of propolis is given in Figure 5.

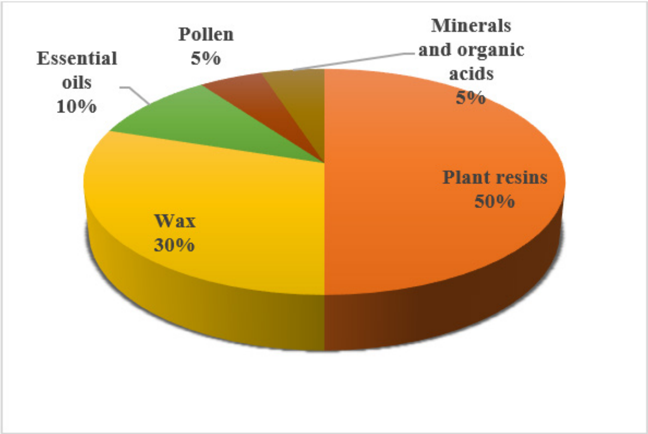

Figure 5. Chemical composition of propolis

Propolis contains over 300 compounds, including phenolic compounds, aromatic acids, essential oils, waxes, and amino acids (61). Besides, propolis contains diverse chemical classes such as flavonoids, terpenenes, phenylpropanoids, lignans, coumarins, stilbenes and their prenylated derivatives (62).

The primary polyphenols in propolis; flavonoids, are impacted by the ecological conditions ofthe honeybee's habitat (63). Among the substances that honeybees use to make propolis are lipophilic materials on leaves and buds, pitches, glues, gums, and substances secreted by plants in case of injury or cut (64). Composition of propolis is determined by the synthesis of its plant-based component, which is dependent on the location of the plant (65). Because so many different plant species grow surrounding the hive, where honeybees gather the required secretions, propolis' composition varies significantly (66). The season, altitude, lighting, and bee-feeding sites all have a significant impact on the composition of propolis. Propolis gathered from different geographical places has been shown to include over 300 distinct compounds, according to numerous research conducted on its chemical and biological structure (Freires et al., 2016).

Propolis's diverse biological properties and pharmacological effects are due to its intricate chemical composition. The use of propolis extract in integrative medicine for the treatment of neurological disorders, cancer, microbes, and diabetes is supported by its enhanced antitumor, antibacterial, and antifungal properties as well as its antioxidant properties (68). Studies conducted on human participants reveal a correlation between telomere length and regular and extended use of bee products, such as propolis (69). In addition, propolis could dramatically lower the levels of hTERT expression, which may have an impact on the telomere length of cancer cells (70).

ANTIBACTERIAL EFFECT OF PROPOLIS

Honeybees collect propolis which is a resinous material, from a variety of plant sources. Propolis is rich in polyphenols, which support its many health advantages, such as its antibacterial, anti-inflammatory, and antioxidant capabilities. Understanding the main polyphenols present in propolis is crucial for its effective use in therapeutic applications. Flavonoids such as quercetin, pinocembrin, chrysin, galangin, and pinobanksin are consistently identified as major components in various propolis samples while phenolic acids including ferulic acid, syringic acid, ellagic acid, p-coumaric acid, and gallic acid are frequently found in propolis (71). Propolis's chemical structure varies greatly depending on the bee species, geographic area, and plant sources (62). Propolis is composed of resin (50%), wax (30%), essential and aromatic oils (10%), pollen (%5) and and balsams, with other minor trace substances (72). Common compounds found in propolis include benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate, and cinnamoylglycine, along with fatty acids, terpenoids, esters, alcohols, hydrocarbons, and aromatic acids (73). Because so many different plant species grow surrounding the hive, where honeybees gather the required secretions, propolis' composition varies greatly (66). Propolis composition is highly dependent on altitude, light levels, time of year, and bee feeding grounds. Propolis gathered from different geographical places has been shown to include over 300 distinct compounds, according to numerous research conducted on its chemical and biological structure (67). Propolis mostly contains flavonoids, phenolics, and aromatic compounds.

Most of the time, propolis's stronger antibacterial activity against Gram-positive bacteria appears to be caused by the outer membrane structure of these organisms. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is effectively inhibited by artepillin C, one of the many phenolic combinations included in propolis. Similarly, Polish propolis was found to slow bacterial growth and affect biofilm formation (74). Research has indicated that while propolis from Brazil and Iran has little effect on Gram-negative bacteria, it is highly effective against spores, infections, bacterial growth, and Gram-positive microorganisms (75). Remarkably, flavonoid phytochemicals readily target different parts and components of the bacterial cell (76). *S. aureus* skin infections are treated with kaempferide, an ethanol extract of propolis. In addition, kaempferide in propolis extract have antibacterial and antioxidant activity (77). According to Meccati et al. (78), kaempferide in Brazilian green propolis has bactericidal and antibiofilm action against multidrug-resistant strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Quercetin, another flavonoid found in propolis, reduces bacterial activity by binding to the subunit of *E. coli* DNA gyrase. Propolis is believed to cause fractional bacterial lysis and potentially impact bacterial proteins. Research shows the synergistic interaction between propolis and anti-infective drugs. It has found that the combination of fluconazole and four Brazilian red propolis was efficient against *Candida sp*., and that the presence of propolis, chloramphenicol, and honey act synergistically against *Salmonella typhi* (79). Quercetin in propolis exhibits strong antimicrobial and antibiofilm activities against a wide range of bacteria, including drug-resistant strains. It works through multiple mechanisms, including membrane damage and inhibition of essential bacterial processes. Additionally, quercetin can enhance the effectiveness of antibiotics, making propolis a promising complementary treatment for bacterial infections. Propolis, including its quercetin content, can enhance the effectiveness of conventional antibiotics like gentamicin when used in combination, showing a synergistic effect against *E. coli* (80). Besides, propolis samples exhibit significant antibacterial properties and can work in conjunction with antibiotics to effectively combat *Salmonella typhi* (81). Also, quercetin in propolis have strong inhibitory effects against NF-β B activation and increased Nrf2-ARE transcriptional activity, suggesting potential benefits in mastitis control (12). Like flavonoids, apigenin and ceftazidime work together to provide a synergistic antibacterial action against MRSA and ceftazidime-resistant *Enterobacter cloacae* (67). Cinnamic acid, which is abundant in propolis, has potent antibacterial properties. According to Galeotti et al. (82), cinnamic acid is known to damage and impair the activity of the bacterial cell membrane, which in turn prevents ATPases from working, bacterial binary fission, and the capacity to build biofilms.

Research on Chilean propolis indicates that flavonoids like pinocembrin and apigenin have antibacterial properties against *S. mutans* (83). When pinocembrin was exposed to antibiotic action against Klebsiella, it responded similarly. Like flavonoids, apigenin and ceftazidime when combined also showed a synergistic antibacterial activity against Enterobacter cloacae, which is resistant to ceftazidime (84); apigenin and b-lactam also showed this effect against MRSA (85). Cinnamic acid, which is abundant in propolis, has potent antibacterial properties. According to Przybyłek and Karpinski (86), propolis possesses antibacterial capabilities that can effectively combat bacteria, viruses, protozoa, and fungi. In some studies Iranian and Brazilian propolis have been found to be effective against Gram-positive microbes, bacterial growth, spores and infections, but have limited effects against Gram-negative bacteria (75).

BIOCHEMICAL EFFECT OF PROPOLIS

The biochemical effects of propolis, a resinous substance produced by honeybees, have been the subject of various studies. Propolis has been shown to affect hematological parameters and blood chemistry in rainbow trout. Higher concentrations of propolis increased glucose, triglyceride, and total cholesterol levels, while reducing certain enzymes like aspartate aminotransferase (AST) and alkaline phosphatase (ALP) (87). In a study conducted on rats revealed that propolis does not induce significant changes in seric cholesterol, HDL-cholesterol, triglycerides, or aminotransferases (AST, LDH). Long-term administration of propolis seems to have no negative cardiac or biochemical effects, indicating its potential safety for extended use (88).

Propolis contains cinnamic and flavonoid components, which disrupt the bioenergetic status of bacterial membranes, showing bactericidal effects. This property enhances its antimicrobial capabilities, particularly against certain gram-positive and gram-negative bacteria (89). Long-term administration of propolis in fish did not significantly alter serum biochemical parameters, confirming the non-toxic nature of propolis even at higher doses (90). Besides, propolis inclusion in the diet of chickens enhanced their antioxidant status and significantly increased glucose levels and glutathione peroxidase activity, demonstrating propolis's protective biochemical effects in various biological systems (91).

ANTI-BACTERIAL MECHANISM OF PROPOLIS

Propolis demonstrates antibacterial properties through several mechanisms. Cinnamic acid in propolis causes bacterial cell membrane damage with stress on pH intracellular inhibition homeostasis, which is one method by which it inhibits ATPase synthesis, cell division, and biofilm growth (92). Propolis's biological activities are primarily due to its flavonoid compounds, which are believed to target various bacteria. Propolis disrupts bacterial cell membranes and cell walls, leading to leakage of intracellular contents and ultimately causing bacterial death. This mechanism has been observed in various strains, including *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) (93). Propolis inhibits important bacterial enzymes like lipase and coagulase and prevents biofilm formation, making it harder for bacteria like *Staphylococcus spp.* to adhere to surfaces and develop resistance. Additionally, it enhances the efficacy of certain antibiotics like ampicillin and gentamicin (94).

Propolis enhances the effectiveness of conventional antibiotics. When combined with antibiotics like honey or various compounds, the antibacterial activity of propolis shows a synergistic effect, improving its ability to inhibit the growth of multidrug-resistant bacteria (95). Propolis exhibits stronger activity against Gram-positive bacteria like *Staphylococcus aureus*, compared to Gram-negative bacteria such as *Escherichia coli*, largely due to the structural differences in their cell walls (86). Propolis increases the permeability of bacterial cell membranes, enhancing its antibacterial effects. This action has been particularly noted in Gram-positive and Gram-negative bacteria (96). Propolis's capacity to prevent protein synthesis is another way that it could be utilized in combination with other antimicrobial drugs to enhance their effects. When Brazilian and Bulgarian propolis are treated with tetracycline, neomycin, and chloramphenicol, they work synergisticly against Salmonella typhi (97). Flavonoids can also cause the activation of several important protein kinases responsible for the regulation of various intracellular signalling pathways (98). Another flavonoid, quercetin, is known to inhibit TBK1 kinase, which is present in TRIF-dependent TLR activation (99). Besides in a study propolis generated by honeybees and stingless bees was tested for bactericidal activity using the microdilution technique. The results showed that propolis from honeybees was more efficient than that from stingless bees against pathogenic bacteria. In terms of its chemical composition, the main elements present in honeybee propolis are flavonoids, 3-prenyl p-coumaric acid, p-coumaric 3-5diprenyl, and caffeic acid. Differentially polar components such flavonoids, phenylpropanoid, and phenolic acids have been discovered in stingless bee propolis. Since the primary components found in honeybee propolis are known to be effective against bacteria, the chemical structure is similar to the results of antibacterial activity (100). It has been stated that the composition and effect of propolis differs depending on the plant source and geographical origin from which it is taken. It has been proven by studies that propolis obtained from different regions have antibacterial effects on different bacteria (Table 3).

Table 3. Studies on different types of propolis and antibacterial effects on specific bacteria (80, 130, 131, 132, 133, 134, 135, 136, 137)

Type of propolis	Bacterial strains
Brazilian red propolis	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*
Peruvian propolis	*Streptococcus gordonii, Fusobacterium nucleatum*
Turkish propolis	*Escherichia coli, Staphylococcus aureus*
Algerian propolis	*Bacillus cereus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*
Tribal propolis	*Lactobacillus acidophilus, Streptococcus mutans*
Kenyan propolis	*Bacillus subtilis, Staphylococcus aureus*
Chilean propolis	*Salmonella enteritidis, Escherichia coli, Yersinia enterocolitica, Pseudomonas sp.*
Saudi and Egyptian propolis	*Staphylococcus aureus, Escherichia coli*
Iranian propolis	*Staphylococcus aureus, Staphylococcus epidermidis*

POSSIBILITIES OF USING PROPOLIS IN RUMINANTS

There are studies on the positive effects of propolis on general animal health, performance and yield parameters in ruminants. Yücel et al. (101) was found that administering 2 mL (500 mg/mL) of ethanol extract propolis orally improved various body measures and reduced diarrhea and clinical cases in calves. Besides, it was stated that administering red propolis supplements may enhance the well-being of calves, lower the frequency of diarrhea, and subsequently minimize the need for antibiotics in systems for raising calves (102). According to Sheded et al. (103), Under drought conditions, supplementing Barki ewes with 5 g/day of crude Chinese propolis enhanced milk output, milk composition, antioxidant enzymes, immunological function, growth performance, and antioxidant status in lambs. Giving ethanol extract propolis to calves prior to weaning was found to have a good impact on their performance and fecal structure, while also reducing diarrhea and fecal fluidity (104). In a study conducted by Morsy et al. (105), it was stated that in Sana Ines ewes in late pregnancy, supplementation with red propolis extract increased apparent digestibility and microbial protein synthesis and reduced methane emission. It also improved animal health and reduced stress effects. Also, propolis administration to milk (150 µL/kg BW/day) was found to improve antibacterial, antioxidant, and immunological markers as well as performance in another trial with breastfeeding lambs (106).

However, recent studies particularly have focused on the unique antibacterial properties of propolis. According to Dezmieran et al. (107), natural formulations which contain propolis and essential oils show potential therapeutic potential in treating bovine mastitis, offering an alternative to conventional antibiotics. Both in vitro and in vivo, the 1% intramammary propolis formulation demonstrated strong antibacterial and antioxidant activities. When administered in place of traditional antibiotics to treat and prevent subclinical mastitis in dairy cows, it might be a useful option (108). Voitenko and Voitenko (109) stated that propolis effectively treats puerperal mastitis and endometritis in high-producing dairy cows, improving their overall well-being within 5 days of treatment.

The prevalence of mastitis in dairy cows has led to the widespread use of antibiotics (104). However, the effectiveness of antibiotic treatment during lactation also leads to increased residues found in milk samples (110). On the other hand, dry period mastitis treatment applied immediately after the last milking of lactation helps to cure recurrent infections and prevent new infections (111). The biggest risk in such cases is the development of antibiotic-resistant bacteria, which is a problem in organic milk production. The search for antimicrobial products with a wide range of biological properties for mastitis is directly related to the fact that bacterial infection is accompanied by an inflammatory response (112, 113). Therefore, therapeutic substances with antiinflammatory, antioxidant and immunostimulating properties are needed to improve the overall health of not only the mammary gland but also the animal. For this purpose, magnolol, thyme, macela and cranberry plant extracts have been investigated in the treatment of mastitis (114). Despite the evidence that mastitis was treated with intramammary propolis, Romvary et al. (115) reported that inflammation of the gland occurred after its use.

The conflicting results on the use of propolis are because the product has a highly variable chemical composition as a result of the flora and season in the region where propolis originates. It has therefore been suggested that the therapeutic properties of propolis may vary (116). In trials with Lacaune ewes, green propolis showed potential in reducing bacterial growth in mastitis cases caused by *Staphylococcus spp.* However, the tested dose was not effective in completely curing mastitis, indicating the need for further optimization of dosage and delivery methods (117). In a study using propolis extract, growth inhibition in *S. aureus* and *Corynebacterium bovis* strains was 100%, while in *S. agalactiae* and *E. coli* it was 90% and 91%, respectively (118). Propolis had strong antibacterial activity against about 41% of microorganisms that were isolated from cases of mastitis. Gram-positive bacteria, except for *C. bovis* and *E. faecalis*, were the most sensitive to propolis, whereas Gram-negative bacteria, such as *Pasteurella spp.* and certain isolates of *E. coli*, were the least sensitive (119). Propolis ethanolic extract has shown strong antibacterial activity against *Staphylococcus aureus*, a common mastitis-causing pathogen in both ewes and cows. The antibacterial effects of propolis were confirmed both in vitro and in vivo, significantly reducing bacterial counts and promoting recovery from subclinical mastitis (120). The ethanolic extracts of propolis have demonstrated bactericidal effects against *Staphylococcus aureus* isolated from mastitic cows. Propolis also inhibits the formation of biofilms by *Staphylococcus spp.* isolated from goat mastitis. This is crucial because biofilm formation by bacteria contributes to antibiotic resistance and chronic infection. Propolis disrupted biofilm formation, showing its potential as an alternative to antibiotics in treating mastitis (121). In addition, high rate of calf losses are one of the biggest issues facing the global and Turkish cattle industries. It is crucial to ascertain the efficacy of propolis in calves as a natural feed additive substitute for antibiotics to address this issue (122). Besides, propolis has been used as a natural antiseptic in organic dairy farms for pre-dipping and post-dipping treatments to prevent bacterial infections, showing similar effectiveness to iodine-based products in reducing bacterial growth. This makes propolis a viable option for producing organic milk (123). Machado et al. (124) have been developed propolis nanoparticles (PNP) to treat bovine mastitis. These nanoparticles were shown to be stable and effective in inhibiting *Staphylococcus aureus* growth, with minimal cytotoxic effects on bovine mammary epithelial cells. This suggests that propolis could be used as a high-concentration antimicrobial treatment with moderate toxicity. Skliarov et al. (125) stated that ozone therapy (ozonated corn oil + alcohol solution of propolis) could effectively replace antibiotics in treating goats with mastitis, offering a cost-effective, environmentally friendly, and safe alternative without reducing therapeutic and cost-effectiveness.

NEW APPROACHES IN THE TREATMENT OF MASTITIS

Nanotechnology

For the past 40 years, a variety of antibiotics have been utilized to treat bovine mastitis. Unfortunately, overuse of antibiotics has led to antibiotic resistance and a low likelihood of mastitis cure. This is caused by three factors: (a) very poor cellular retention; (b) absence of diffusion of acidic antibiotics across the lysosomal membrane at neutral extracellular or cytoplasmic pH; and (c) inadequate intracellular uptake of frequently used medications. Antibiotics administered as aqueous solutions are not anticipated to have extremely long-lasting effects for all of these reasons (126). Consequently, more specialized dosage forms must be created in order to treat *S. aureus* bovine mastitis. According to nanotechnology, scientists can now create nanoparticles and employ them for a variety of purposes, most notably medication delivery. Compared to traditional micron particles, nanoparticles are more efficient against germs because of their larger surface area. Compared to micron particles, nanoparticles have a far higher chance of entering cells. In summary, compared to their micro counterparts, nano-antibacterial particles exhibit more potent effects on bacteria.

Mastitis vaccines

Mastitis vaccines have been developed to reduce the incidence of mastitis in dairy farming and increase industry profits. Mastitis pathogen vaccination is just one of the many infectious disease management strategies in the dairy cattle industry that make use of killed whole-cell vaccines. Many studies have been conducted to develop a vaccine against mastitis, but few have shown positive results (100). Mastitis brought on by numerous infections and each of their unique mechanisms of pathogenesis cannot be prevented by a single vaccination. Every vaccination ought to be given in accordance with field data and professional advice. Numerous studies have shown that immunizing cows and heifers against *S. aureus* and *Coliform* is beneficial. Coliform vaccinations can lower the frequency, intensity, and length of infections in heifers and nursing cows. Vaccines against *S. aureus* can lower the rate of new intramammary infections and boost the rate of spontaneous cure; therefore, they should be applied to herds where these organisms pose a serious threat.

Bacteriophage treatment for Mastitis

One alternative treatment for mastitis is the use of pathogen-specific bacteriophages to treat a bacterial infection. Some studies suggest that phage therapy may be beneficial against mastitis infection caused by E. coli and S. aureus (127). However, further scientific studies are needed to determine the therapeutic potential of bacteriophages in treating bacterial infections associated with clinical and subclinical mastitis.

Cytokines

It is unclear how different immune system elements contribute to the mammary gland's defense against infection. Nonetheless, during bacterial infection, leukocyte populations and cytokine production are crucial for host defense and pathophysiological processes. They can boost the bactericidal activity of hagocytes and have chemotactic activity that is responsible for leukocyte activation and recruitment (128). Numerous experiments have demonstrated that cytokine infusion, whether administered with or without antibiotics, improves the prognosis for mastitis caused by *S. aureus*. Ultimately, cytokine therapy appears to be a promising strategy, but additional thorough research is needed to verify its therapeutic applicability.

Recombinant Mucolytic Protein

These days, recombinant protein technology is crucial to the management of animal illnesses. A recombinant mucolytic protein, such lysostapine, may be used as an alternative to antibiotics for *S. aureus* mastitis. In experimentally generated *S. aureus* mastitis, the potential benefit of intramural lysostaphin treatment has been assessed (129). For bovine mastitis, the researchers propose that an enhanced recombinant formulation of lysostaphin could serve as a substitute for antibiotic treatment. Its benefits include minimal toxicity, targeted selectivity, and the ability to dispose of milk without leaving biologically active residues behind.

CONCLUSION

The dairy industry faces large financial losses as a result of mastitis. Animal welfare and health are negatively impacted by mastitis in dairy cattle. On the other hand, promising outcomes are being obtained from the use of bacteriophages, vaccinations, dry period treatments and products, appropriate feeding practices, genetic selection of mastitis-resistant animals, and bacteriophage-based products in herd management. Recent in vitro research with natural antioxidant compounds originating from plants and animals has demonstrated noteworthy outcomes in the prevention and management of mastitis. Mastitis's characteristics and prevalence point to the illness' ongoing presence as a concern going forward. On the other hand, new insights into the behavior of certain pathogens, quick disease detection, and efficient herd management techniques are producing increasingly encouraging outcomes.

Mastitis has a significant and negative impact on the health of ruminant animals, the quality of dairy products and the economy of the enterprise. Propolis is a product that can be used as a health promoter in cases of mastitis. When propolis is used in appropriate doses; it is a natural alternative that is free from the effects of synthetic antibiotics. In vitro and in vivo studies show that propolis has a preventive effect on bacterial, fulgal and viral diseases. Considering the high number of side effects of synthetic drugs, propolis is a good alternative as it has no reported side effects in scientific studies. In addition, propolis offers a natural and economical preventive and complementary treatment opportunity compared to synthetic drugs.

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Bölüm 2

ENHANCED CHARACTERISTICS AND EVALUATION PARAMETERS OF VERMICOMPOST

Fevziye Şüheda HEPŞEN TÜRKAY[1]

INTRODUCTION

Understanding the standards for organic materials in soil conditioners, fertilizers, and growth mediums is essential. These widely accepted standards ensure the effectiveness of the materials. Organic materials for these uses must be composted following specific guidelines. The most widely used composting method is thermophilic composting, also called traditional composting. There are standards for thermophilic composting processes. Globally, the beneficial impacts of thermophilically composted organic materials are well-documented and recognized (1, 2). Considering the benefits of organic matter for soils, the very important disadvantages of thermophilic composting from an agricultural perspective are ignored. However, information on vermicomposting, a new, alternative and advantageous composting method, is not detailed and widespread (3, 4). Vermicomposts do not have the disadvantages of thermophilic composts, in addition to their advantages. Although scientific studies on vermicomposting have increased in recent years, more scientific studies on quality parameters and mechanisms are needed (5, 6).

The primary ecological roles of earthworms vary based on their environmental needs and dietary habits. Epigeic earthworms, which are used in vermicomposting, inhabit the soil's surface layer, just above the mineral horizon. They thrive beneath the debris layer, consuming organic matter found on the surface (7-9). Earthworms naturally thrive in organic waste piles like barnyard manure, where multiple species often coexist, leading to the nickname "dung worms" (10). In this group, *Eisenia foetida* has been preferred by many, given its high reproductive efficiency, adaptability, and resistance to different environmental conditions (11). *E foetida* is distributed globally with exceptions made for extreme deserts and

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polar regions, occurring in most countries; it has adapted to natural and ruderal habitats. (3).

Significant differences exist between the vermicomposting and thermophilic composting technologies: the outcome, as well as the process involved. The use of earthworms, especially *E. foetida*, through the process called vermicomposting, has been under recent research to be an effective way to attain better decomposition of organic material, with an essential effect on nutrient enrichment of the final compostThe most important difference between the thermophilic composting process and the vermicomposting process is that the organic material passes through the worm digestive system. This ensures that the process is mesophilic and therefore the beneficial microorganism population does not decrease. During the vermicomposting process, organic materials undergo humification, decomposition, stabilization and sanitation very quickly. In addition to this, several studies demonstrated that thermophilic and vermicomposting may work in a synergic manner for some types of wastes, i.e., dairy manure and waste paper mixture, enhancing the nutrient content and pathogen reduction (12). Moreover, amending the compost with microbial inoculants and additives, such as water hyacinths, also helps ameliorate biodegradation and consequent compost quality (13). Vermicomposting is less sturdy but has high microbial activity and better essential characteristics for good compost (14). The technologies currently being used for producing composts have been further enhanced by adding biochar and black soldier flies to provide additional enhancement to the efficiency as well as the quality of the products derived from composts (15). Molecular studies have also shown that vermicomposting increases nitrogen preservation and the oxidation of organic matter as opposed to thermophilic composting (16).

Vermicomposts are very important, relative to conventional composts, in aspects of crop production, sustainable agriculture, organic farming, and soil health. With physicochemical improvement credited to vermicomposts, this helps improve soil health, hence the cause of increased plant growth and crop yields. Vermicomposts made from different biowastes were found to dramatically improve soil nutrient availability, reduce pest infestations, and enhance microbial activity (17). In addition to that, vermicomposting is an environ-friendly waste managing approach that changes bio-wastes into neoteric organic fertilizer, which matriculates the bio-diversity of soil and plant health maintenance (18). Vermicompost, along with N_2-fixing bacteria, enhanced the microbial activity, nutrient uptake, and grain yield in rice production by increasing soil fertility status with increased macro and micro-nutrient accumulation (19). Vermicomposting also reduces soil degradation and increase the water use efficiency of crops; thus, it offers a choice for sustainable crop-production systems, especially in semi-arid areas (20).

Vermicomposts offer numerous soil and fertilization benefits that extend well beyond enhancing total plant nutrient content. They boost the activity of beneficial soil microorganisms, enhance the soil's nutrient-providing capabilities, and suppress plant diseases and pests. Additionally, vermicomposts produce plant growth regulators and contribute to overall plant health improvement . The high levels of beneficial microorganisms in vermicomposts, compared to thermophilic compost, are mainly linked to the high populations of beneficial organisms that are enhanced during the vermicomposting process. The use of earthworms enhances microbial activity in vermicompost because they enrich the vermicasts with a wide diversity of microorganisms; thus, the end product is marketed and valued for its benefits (8, 21). Furthermore, the amounts of total phosphorus, micronutrients, and humic acid substances in vermicomposted materials are substantially higher than those of their original organic matter. This thus places vermicomposts as being superior in terms of microbial content and nutrient richness (22).

More and more these days, the process of organic waste conversion by earthworms continues to be applied in producing plant-growing media because of multilateral advantages. The virtues of this kind include enhanced regulation of growth, diseases, and pest resistance; increased content of microelements; reduced losses of nutrients, in particular, of mobile nitrate forms; slow-releasing nutrients. Quality criteria of vermicompost are crucial for considering the expected efficiency of growth media, which is essential for manufacturers, consumers, sellers, and even regulatory bodies (23-27). It is the elevation of growth performance in vermicompost, attributable to more nutrient release, hormone-like effects, and enhanced mineral nutrition through the stimulation of microbial populations, which become a valuable resource for sustainable agriculture.

FOUNDATIONAL CONCEPTS AND PRINCIPLES OF COMPOSTING AND VERMICOMPOSTING

Numerous sources can define compost as any end product obtained from the microbiological decomposition of any organic matter. It is said to play a vital role in nurturing plant growth mainly because the end product that results from the microbial breakdown of such organic material amends soil with essential plant nutrients and improves the physical properties of the soil. The Ministry of Agriculture and Forest in Türkiye defines compost simply as the decomposition and mineralization of organic substances by organisms. In the United States Department of Agriculture, much focus is put on controlled aerobic decomposition, temperature increase, and pathogen reduction while looking into improvements in the management of organic matter and promotion of properties. These definitions underscore the importance of composting to sustainable agriculture practices and soil health (8, 28-30).

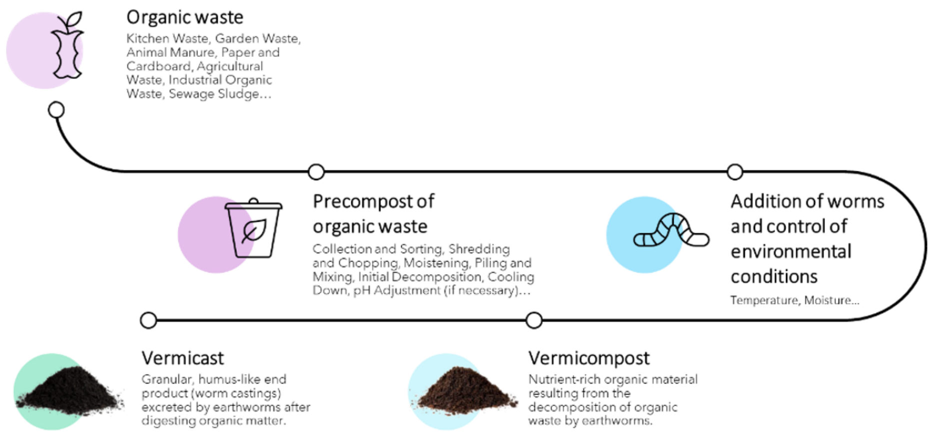

Figure 1. Transforming Organic Waste into Available Nutrient-Microbial Activity -Rich Vermicompost through Earthworm Activity

During the composting process (Figure 1), a temperature of at least 55 °C must be reached and the process must continue for at least 3 days. Thermophilic composting of organic waste practically begins with the mesophilic process (10-40.5 °C). In order to achieve the minimum temperatures required for heating the raw material, the compost heaps should be aerated by stirring. In terms of chemical components, thermophilic composts are complex, and their content varies according to the decomposition conditions and the content of the original raw material. Carbon is the most abundant element in composts and accounts for 50% of the total mass of the compost; Nitrogen, on the other hand, accounts for only 1-2%. The C:N ratio is a common parameter used to examine the maturity, stability and condition of the raw material of all composts used. In a completed compost, C:N ratios would be between 12:1 and 20:1, but the ideal C:N ratio should be between 14:1 and 18:1. Microbial stability is a prerequisite for the maturation of compost. During the healing process, the compost particles become smaller and the total volume of the original raw material is usually reduced by 30-50%. The composting process ensures that the compost is sterilized from pathogens, purified from weed seeds, broken down many toxic substances and stabilized nutrients. European countries, America, Australia and many countries continue to work on determining compost quality criteria (31, 32).

Vermicomposting (Latin vermes = worms) is a process similar to traditional composting. In the production of vermicompost, the composting process is accompanied by the addition of certain epigenic species of worms to the environment, which are used to enrich the aerobic waste recycling process and produce a better end product. Vermicomposting differs from composting in several important ways. Basically, vermicomposting is a mesophilic process that makes use of microorganisms and worms that are active in the temperature range of 10–32.2 °C. It should be taken into account that the process is faster than thermophilic composting, since organic materials pass through the worm intestine. Organic materials decompose and humify rapidly as they pass through the worm digestive system in a mesophilic process. When the temperature rises too high, there is no loss of nutrients, especially N, and even the ratio of useful nutrients increases within the total amount of elements presentThe mesophilic process eliminates the disadvantage of decreasing the population of useful microorganisms, and on the contrary, it becomes advantageous by increasing the populations.

However, this still does not mean that the organic material is completely transformed. With the defecation of the worm, there is an unusual increase in microbial activity and plant growth regulators, in which plant pest suppressants are added to the environment. Therefore, in the next stage, microbial decomposition is supported and increased (33, 34). Many methods show that worms play the role of an ecological engineer, consuming organic waste, breaking down even very small particles, reducing the C:N ratio and rapidly stabilizing organic matter. Many vermicompost manufacturers and operators prefer to describe their products differently from conventional compost by simply using the term worm excrement or vermicompost when describing and marketing their products. This term means that this end product, which has passed through the worm intestine, consists entirely of organic matter. In fact, when incomplete vermicompost is examined, it may not be possible to distinguish between organic matter that has passed through the worm intestine and matter that has not.

However, the advanced stabilization and humification characteristic of vermicompost is under the influence of physical and biochemical transformations during vermicomposting, and this is an indicator of quality. In short, the process of worm composting is a dominant process that causes the stabilization and humification of organic matter that has been mixed, digested and fragmented by worms and microorganisms and is defined as "vermicomposting". However, the vermicomposting process is part of vermiculture technology (35).

"Vermiculture" studies are the process of culturing earthworms for waste processing, soil detoxification, regeneration and sustainable agricultural practices . This definition refers to worm farming or worm farming. The concept of vermiculture includes vermicompost, vermicompost tea and liquid, ceulomic fluids of worms, obtaining biomass and vermistabilization processes. Although vermicompost and its products come to the fore in worm farming in terms of increase in plant production and soil fertility, compliance with sustainable agricultural principles, organic farming activities and transformation of organic matter, earthworms are also a valuable source of protein with their own biomass. The body of the worm is like two intertwined tubes. The "ceulom" fluid covering their bodies is located just under the epidermis between the 2 tubes and is of vital importance for the worms. Because it repairs wounds by providing a high regeneration ability against all injuries caused by the worm's living environment. In addition, in an environment rich in microbial populations, the worm forms a strong protective barrier against all pathogens and prevents all contamination. Ceulom fluid is also involved in the mating of worms that have a form of hermaphrodism, which contains both sperm and eggs but exchanges sperm with mating. Therefore, ceulom fluid obtained with vermiculture systems is used for medical and cosmetic purposes due to the cell regenerative and restorative structure it contains, apart from its agricultural benefits. Vermiculture systems enable worms to continue their lives in nature in environments created from organic wastes and residues from rural and urban life (36).

The vermicomposting process is a rapid humification and detoxification process as well as a sanitization process. There are scientific studies showing that the final product, vermicompost, is a sanitary product free from human pathogens (8). The process of sanitizing worm cast is related to the microorganism groups living in the digestive system of the worms. Microorganisms living in the worm's intestine digest pathogenic microorganisms. Partial sanitation in other composting methods results from thermophilic conditions, which have the disadvantage of reducing the population of beneficial microorganisms. In vermicomposting, the main composting process takes place as the organic material passes through the worm digestive system, so the process is mesophilic. Additionally, it does not harm the beneficial microorganism populations in the environment. As organic material passes through the worm digestive system, a process that takes about a day, it undergoes humification and is sanitized as pathogens are digested. Based on similar methods used for the development of thermophilic compost standards that mature due to pathogen and weed seed removal and stability, the vermicomposting process or vermicompost product (or worm droppings) needs to be categorized so that it can be classified (37).

Acceleration of humification and stabilization happens under mesophilic temperatures in vermicomposting, while fragmentation of the organic material is pronounced with earthworm involvement. Such simulations from the scientific literature show that 0.5 kg worms can readily consume around 0.5 kg of organic material in a day for reasonable rates to occur, with a practical and optimum population of 9–18 kilograms per square meter. Other factors that influence the decomposition rate in vermicomposting are earthworm stocking density, material type, earthworm species, and the duration of the vermicomposting process. Categorization of the various product types of earthworm vermicompost is vital for quality classification and also to ensure pathogen removal and stability (38). About this, successful vermicomposting must be characterized by the particular criteria that have to be adhered to, mainly including stabilization and humification of organic material as the primary mechanisms and their processes of degradation, fragmentation, digestion, and mixing by earthworms; hence waste breakdown is equally influenced by aerobic mesophilic microbial activity. Along with worm activity, microbial activity in the environment also increases, therefore, organic materials in the environment that have not yet been consumed by the worms are broken down, decomposed and in a way composted. In addition to worm excrements (vermicast), the total of organic materials that mature in the environment due to this process creates the vermicompost product.

ASSESSMENT PARAMETERS FOR VERMICOMPOSTS

Identifying the desired final state of vermicomposted wastes is crucial for determining the key characteristics that define vermicompost quality. It is imperative to specify methods for measuring these characteristics and to establish the potential quality criteria for vermicompost. Detailed knowledge of vermicomposting processes and their operation is essential. Whether open or closed, continuous reactors or domestic vermicompost systems, the technological features of a setup must be customized to the raw materials used. The difference in process type and the significant differences in raw materials significantly influence the final quality of the product and its usefulness in respective applications (39, 40).

All pretreatments, for example, extraction or composting, should be fully described. This description must consider the effects of various weed seeds on quality criteria, including contamination with human pathogens. Additionally, the role of inorganic material applications, such as fertilizers, lime, and sulfur, should be noted. These factors are as crucial as the certification of vermicompost as an organic material and its final chemical properties (41-44).

Other information required for the characterization of vermicomposts includes the average worm population density in the system, the time required for the process and the precise determination of the worm species used. All of these affect the quality, stability and maturity of the end product. The ratio of the material input expressed exactly to the total worm weight, the final product vermicompost and the records made during vermicomposting are very important for classification.

Physical Attributes

Significant critical physical properties of vermicompost include solids composition, bulk density, moisture content, and water retention. These properties are crucial to measuring the quality of compost in agricultural and horticultural applications that better the structure and water management of soil (Figure 1). The primary indicator of the physical composition of vermicomposts is their organic matter content, which is crucial for assessing the overall stability of the materials. It's important to note that not all organic material, which serves as a source of potentially mineralizable nutrients and an energy source for biological activity in the soil, is stabilized (such as biodegrading organic matter), and this characteristic is significant. The organic matter content in completed vermicompost should be 25 to 50%. The total quantity of ash in composts is the non-evaporative solid part in composts, except materials that are relatively inert to all chemical reagents except glass, metal, plastic, large clay aggregates, etc. Materials like these represent little or no hazard to good-quality vermicompost as long as the contamination percentage is within the low-risk range, usually less than 0.5-1% (10).

Figure 2. Key physical properties of vermicompost.

Volume weight is a critical physical other property of vermicompost, influencing factors such as porosity, aeration, and water holding capacity, all of which are vital for plant growth. The final volume weight of vermicompost is also impacted by compression during production, transport in pots, and other mixtures. It is necessary, therefore, to establish properties of such mixtures by standardized methods. Total pore volume entails another prime characteristic that influences aeration water relationships; for organic materials, ideal for potting media, the volume of the pores should be 70-80% of the total volume. In commercial culture environments, excessive porosity is often undesirable because it reduces air capacity and increases transportation costs.

However, a volume weight that is too low can negatively impact substrate aeration and the amount of water available. Therefore, determined the optimum physical properties for the ideal substrate for plant development in 1972; The total minimum porosity should be 85%, and in the case of pots, it should be between 55-75%, and the air gaps should be 20-30%.

Particle size of the vermicomposted product is also one of the important measurement criteria. High-quality vermicomposts usually have a small particle size, the upper limit of which is generally less than 0.2 mm in diameter. The fine texture of vermicomposts is caused by the grinding of organic matter inside the intestine of a worm and from the processes of digestion and mixing where substantial reduction occurs. Because the intestines of worms may be cleaned over several hours, the longest time that organic matter can pass through their digestive system during the vermicomposting process hence remains relatively short (45). The distribution of individual particles is within the soil mixtures or potting media, which is influenced by the material's total volume weight and the material continuity and size of the interparticle pores. In most applications with vermicompost, the composition needs to be determined from particle distribution across size classes to warrant effectiveness and standardization (46).

It is often said that the number of water-resistant aggregates available in dry vermicompost and their stability on becoming re-wetted, is essential. Large aggregates that crumble when wet indicate the quality of the mixture. Therefore, assessing the water resistance of aggregates might be necessary for certain uses of vermicompost and can be evaluated using standard methods (47-49).

The percentage of moisture content that a vermicompost should have is important. During the vermicomposting process, moisture content should be maintained between 75-90%, although this can vary significantly in the finished product. Wet materials are costly to transport due to their increased weight and are difficult to stack and manage. Moisture content also greatly influences the commercial value of vermicomposts sold by weight. Conversely, excessively dry materials can pose problems when rewetted and may alter the microbial community, slightly reducing the ability to suppress plant pests and affecting other beneficial properties related to soil nutrient chain composition and structure. Certain applications, such as specific soil or pot mixtures, may require precise moisture levels in vermicompost. Generally, an acceptable moisture content ranges from 30-50% (33, 50-52).

The moisture content of vermicomposts can be altered either through the addition of water or drying, and the capacity of a material to hold permanent moisture can also be more or less permanent. Defining both total and useful water holding capacity is particularly important for vermicomposts used as pot media content.

Moisture retention curves will allow the measurement of the water retention characteristics of different growth media or soil mixtures containing vermicompost or other components (42, 53, 54).

Chemical Attributes

Important chemical properties of vermicompost include pH, cation exchange capacity (CEC), and soluble salt concentration. It is fundamental in the chemical dynamics of vermicompost and highly related to soil fertility, which averages 35.6% dry mass (Figure 2). The amount and activity of hydrogen ions (H^+) are the primary determinants of soil pH directly related to acidity or alkalinity. The acidity of a prepared environment or soil affects productivity and plant growth in many ways. In plant growth environments, the acceptable level of acidity is between 5.5 and 8.0, but 6.0 and 7.0 may also be preferable; however, some plant species prefer the pH ranges to be much narrower. In the vermicomposting process, the pH value of the environment was determined as 7 or slightly above. The pH values of final vermicompost vary widely, depending on the organic matter from which they are made; It was determined that the pH of sheep manure vermicompost was 8.6, farm manure vermicompost was 6.0 and 6.7, pig manure vermicompost was 5.7 and 5.3, and waste sludge vermicompost was 7.2. More important than the pH of a vermicompost is its buffering capacity. The buffering capacity is necessary to make a precise definition of the environment (10). It is known that the vermicomposting process brings the pH value of the starting material closer to neutral.

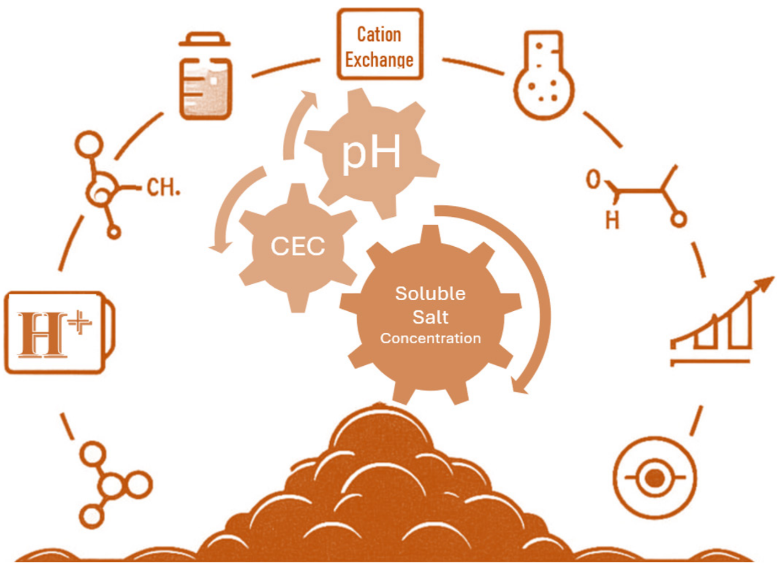

Figure 3. Interrelationship between key chemical properties of vermicompost: pH, cation exchange capacity (CEC), and soluble salt concentration.

Soil organic matter is another factor working in the soil such that it may hold nutrients due to its colloidal characteristics and can provide sustenance for plants. Like the clays, it has an elemental composition of wonderful water-suspending particles with an overwhelmingly sizeable negative surface charge. This increases the ability of the soil to attract and hold cationic nutrient ions. In the context of vermicomposts, which are organic fertilizers produced through the decomposition of organic matter by earthworms, the cation exchange capacity (CEC) is particularly important. The CEC measures how well soil or compost can retain and supply cations to plant roots. The CEC of the vermicomposts is within 50–100 meq/l, thus enabling it to maintain and release essential elements like potassium, calcium, and magnesium, resulting in soil fertility and robust plants .

Vermicompost, especially when obtained from animal manure, can inhibit plant growth or have a toxic effect on the plant if the total salt concentration is comparatively at high levels. High concentrations of soluble salts are a common problem in many composts and vermicomposts. Soluble salt concentrations (measured as electrical conductivity) in sature extracts of high-quality plant growth media should not exceed 2-3 dS/m for native plants and 1-2 dS/m for sensitive plants and seeds. What's more, Na concentration is an important factor for plants, especially with potentially harmful effects. In some cases, the harmful effects of high concentrations of soluble salts can be reduced if Ca is the dominant cation. Generally, vermicomposts have a low salt content because worm activity is inhibited above their concentration of 0.5%. Pig manure vermicompost has an electrical conductivity of 322 mS/m (3.22 dS/m) (28). When 5-10-25-50-100% pig manure vermicompost was mixed into Metromix360 as a plant growth medium, the electrical conductivity in the root environment increased due to the increase in vermicompost concentration (10).

Plant Nutrient Composition

The total carbon content in a vermicompost is more closely connected with the organic matter content. Organic material, when it decomposes through earthworms and other soil microorganisms under vermicomposting, undergoes an intensive biochemical metamorphosis. Changes in the composition of ingredients like total carbon, nitrogen, and other important nutrients act as very good indicators for their extent of stabilization through the vermicomposting process. It is essential to monitor these changes to assess the maturity and quality of the vermicompost (55). If a well-stabilized vermicompost has undergone thorough decomposition, it will end up with a meager ratio of carbon to nitrogen, and the fact that it means that most of the readily degradable organic materials have been mineralized. Such stabilization precludes the development of phytotoxic compounds and pathogens, making the vermicompost safe and beneficial for plant growth (56). A further reflection of the maturity of vermicompost itself is in its physicochemical and biological properties, showing a mature one with uniform texture, dark color, balanced pH, high cation exchange capacity, and appropriate carbon ratio. It should be teeming with beneficial microflora that improve soil health and plant growth biologically. Knowledge of overall carbon and changes in the ingredients provides valuable information regarding the efficiency during the vermicomposting process, hence the quality of vermicompost, which is helpful as a reliable and effective amendment to the soil.

The total nitrogen content of vermicomposts can range fairly widely (0.1%-2-4% or more) and is a significant criterion for determining the properties of vermicompost as a food source. However, this measure includes all nitrogen, not just what is directly available for plant growth. Therefore, it is important to determine the concentrations of inorganic forms of nitrogen (NH_4 and NO_3) as well as the organic nitrogen content to get a complete picture of the nitrogen available for plants (57, 58).

Saturation extracts of growth media should show total inorganic nitrogen concentrations over 100 mg^{-1}; however, the NH4-N concentrations do not have to be higher than 300 mg^{-1}, and NO3 concentrations have to be between 100-200 mg^{-1}. For vermicompost, NH_4-N should not exceed 10% of the total nitrogen, or the total nitrogen should be 0.04%. The basic form of inorganic nitrogen is NO_3 when the NO_3-N to NH_4-N ratio is ≥ 0.14 (10).

The carbon to nitrogen (C:N) ratio is one of the most widely used parameters in determining the stability of organic materials such as compost and vermicomposts. The C ratio for microorganisms is usually 15-25, while in humus, it is about 11-12. The C ratio of well-stabilized materials usually is lower than 20-22. If the C is higher than that, there remains some bioavailable carbon; the material is not fully stabilized. Changes in the C:N ratio of the raw material to the finished product can be as significant as the final value, so nitrogen-rich products may not need to be fully stabilized because the C:N ratio may already be too low (10).

The total extent of phosphorus (P) and potassium (K) forms should be determined in a completed vermicompost. In many countries, vermicompost must have a minimum content of N, P, and K on the label in order to be marketed as an organic fertilizer or soil conditioner. Generally, the P content should not be less than 0.5%. However, some plants are sensitive to high concentrations of P. The total amount of P in root environment for sensitive plants should not be less than 0.1% (5).

The determination of the total content of Ca, Mg, S, and B in vermicompost is essential in the evaluation of its nutritional value and effectiveness as an organic fertilizer. These elements contribute to several essential physiological and biochemical functions concerning plant growth and development. Calcium is essential in cell wall formation and stability, root development, and activation of a series of enzymes in plants. Magnesium forms part of chlorophyll, the molecule responsible for photosynthesis in plants. It acts as a cofactor in many enzyme reactions of plants. Sulfur is fundamental in amino acid, protein, and vitamin synthesis vital in plant metabolism and stress differentiation. Boron is involved in cell wall synthesis, membrane integrity, seed and fruit development, and regulation of carbohydrate metabolism. Nutrient-rich vermicompost, therefore, enhances the value of the compost and ensures that plants obtain a balanced supply of those critical elements for healthier growth and higher yield. The complete content analysis used for Ca, Mg, S, and B will be very relevant for the monitoring and changing of the nutritional profile in the efforts to make vermicompost more effective and reliable as an organic fertilizer. The holistic approach of nutrient management at this moment highlights the role of these elements in maintaining soil fertility that is consistent with sustainable agricultural practices (59, 60).

Many organic applications added to soils or growth media contain significant concentrations of microelements. Most microelements are in a suitable amount within vermicomposts. Humic substances from vermicomposts can be an active chelating agent that increases the reactivity of some microelements. However, the allometric amounts of certain microelements are harmful to the plant, and this thus calls for caution in monitoring and management of these elements concerning the application of vermicompost.

Nutrient Release Dynamics

Although total and extractable nutrient concentrations provide important information about the overall nutrient status of vermicomposts, the availability of nutrients useful to the plant is a dynamic process. Understanding the ability of a vermicompost to provide all nutrients on the basis of the nutrient-release characteristics of vermicomposts is more important than the total or ingestible amounts of nutrients. With many methods, the nutrient release characteristics of organic material in plant growth media or soil mixtures can be determined. Most of these require the incubation of materials over the course of weeks or months, and the management of this process is quite costly. However, the material to be used can be selected by comparing the oscillation characteristics of different mixtures. However, in general, a pot environment mix should contain enough nutrients for the first week or two. Environments that are recommended as soil conditioners, such as vermicomposts, should be able to provide minor nutrients for a period of development (61-63).

Heavy Metal Contamination

The microelements content of the raw material of the organic material in the vermicomposting process is important. Especially if a material contaminated with microelements is used, the vermicomposting process will be affected due to the content of the starting material. Microelements are also heavy metals whose toxicity limit range values are very close to each other. This situation reveals phytotoxicity. The bioaccumulation abilities of earthworms are known. If the levels are not at levels that will affect the vital functions of the worms but can also cause phytotoxicity, the worms store these toxic levels of heavy metals in their bodies. Heavy metal levels in earthworm feces fall below toxic level limit values.

Many vermicomposts made from food, paper waste, animal manure or plant residues are impossible to contaminate with heavy metals. The most common metals found in vermicomposts are usually lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), molybdenum (Mo) and zinc (Zn) if the source of raw materials is sewage sludge and biosolids. Heavy metal limitations in the application of compost used by our country: Pb<750, Hg<10, Cd<10, Cr<1000, Mo=0, Zn<2500 (mg/kg). Even though it can reduce the concentration of several elements, studies of vermicompost have shown that they still have appreciably higher levels of mixed contaminants in them compared with the starting feed. Consequent to this, proper management of sources of raw material and its monitoring at regular intervals is crucial so that the end product, vermicompost, is fit for use in agriculture and safe concerning the presence of heavy metals (57, 64-68).

Biological Attributes

Pathogen Reduction

In the vermicomposting process, unlike thermophilic composting, sufficient temperatures cannot be reached for pathogenic microorganisms to die. However, although the exact mechanisms are not known, there are increasing studies showing that the vermicomposting process provides sanitization of the material used and reduces human pathogenic microorganisms. Obviously, the type of raw material is an important factor in assessing the risks of a vermicompost contaminated with human pathogens. Some "clean" materials that are not contaminated with human pathogens, such as paper or food processing waste, have been studied less than animal waste and solid waste with a high contaminated load (37, 69-71).

As the organic material passes through the worm intestinal tract, it undergoes a rapid process of humification, detoxification, and sanitization. Vermicompost, which is the final product, is rapidly humified by beneficial microorganisms and enzymes they secrete in the system, organic matter is transformed and enriched with organic compounds, beneficial plant nutrients and plant growth regulators. The worm packs its feces with polysaccharides in its intestine and defecates. However, it prevents the development and infection of plant pathogens with the secretions of microorganisms with antagonistic effect living in the worm intestine and suppressing plant harvests. Some groups of microorganisms in the worm are fed with other pathogens (human/animal pathogens) and in this very rapid sanitization process, the final product is purified from pathogens by undergoing a sanitization process. This situation is in response to the disposal of the pathogenic load of barn manure by thermophilic composting under high temperature conditions, while in the mesophilic process of vermicomposting, both pathogenic microorganisms are destroyed and microbial activity, enzymes and proteins are protected from deterioration at thermophilic temperatures. Re-heat treatment of material that has undergone the sanitization process in the worm intestine is unnecessary and destructive to the enzymes, hormones and proteins that add value to the product. However, manufacturers must be careful to maintain sanitization and avoid contamination from the outside (with tools, equipment, etc.) (12, 72, 73).

Vermicomposts must adhere to the same health standards for human pathogens as thermophilic composts. Sometimes, conventional thermophilic composting of raw materials for up to 14 days is necessary to ensure pathogen levels are appropriate. Alternatively, end treatments like steam sterilization, fumigation, and other methods can eradicate pathogens entirely. However, such treatments also kill beneficial microorganisms, reducing the final product's value, and are therefore not recommended. Until more experimental and scientific data is available to effectively reduce pathogens during the vermicomposting process, and since "best management practices" as per EPA (U.S. Environmental Protection Agency) regulations for vermicomposting have not been established, frequent pathogen testing of vermicomposts is advised. This approach is preferred over heat treatments to comply with acceptable EPA health standards. The vermicomposting process itself sanitizes the raw material, producing a sanitary final product. However, if sanitation protocols are not adhered to during the process, or there is potential for contamination from external sources, routine testing becomes essential. Studies conducted by other researchers have shown that pathogen levels become so low during the process that they were non-detectable in the final vermicompost product, even with starting materials with an extremely high pathogen load that could not be pre-heat treated. To be classified as EPA class A, the material must be free of coliform, *E. coli*, salmonella, intestinal viruses, and pinworm eggs (37, 69, 74, 75).

Pathogen Reduction Mechanisms

The main reason why vermicomposting as an alternative to general organic waste management is not widely accepted is that there is not enough scientific information about waste biosolids or even animal manure potentially reducing human pathogens during vermicomposting. In contrast to conventional composting, which is defined by the presence of high heat or thermophilic phase (50-70 °C), vermicomposting is a mesophilic process, so the substrate temperature is below 35 °C. Otherwise, the worms will not be able to survive. The overall advantage of the high temperature generated during traditional composting is that they potentially reduce or eliminate salmonella, E. coli, enterococci, human viruses. A significant body of the scientific literature has developed EPA standards for vermicomposting processes as requiring a temperature above 55 °C for 72 hours to eliminate the risk of human pathogen contamination of a completed compost (not vermicompost) so that they can be safely used as class A materials for land use (10, 74).

The effects of worms and vermicomposting processes in human pathogens are obviously quite complex. The mechanisms by which human pathogens are reduced or eliminated include direct effects of mechanical dispersal due to gastrointestinal activity, digestion, microbial inhibition by antimicrobial agents or microbial antagonists produced by the worms themselves, and damage to microorganisms by enzymatic degradation and assimilation. Its indirect effect includes the production of antimicrobial substances such as humic acid, competition with and without worms, antagonism and stimulation of endemic or other microbial species caused by phagocytic activity, or other mechanisms (76).

Enzyme Activities

Enzyme activities play a crucial role in various processes related to composting, soil fertility, plant growth, and disease resistance. Studies have shown that certain enzyme activities are resistant to total microbial activity, indicating their significance in assessing the overall microbial activity of composts. Dehydrogenase enzyme activity, commonly used as a parameter for microbial activity in composts, reflects the microbial processes occurring during composting. During vermicomposting, a process involving earthworms, specific enzyme activities such as invertase, urease, and alkaline phosphatase, which are of microbial origin, are selectively enriched, further emphasizing the importance of enzymes in this context (54).

Vermicomposts, which are products of vermicomposting, have been found to be rich in microbial activity, making them excellent soil amendments due to their high porosity, good aeration, drainage, and water-holding capacity (54). These properties contribute to the enhancement of soil biological properties, such as microbial biomass C, basal respiration, and enzymatic activities, which are essential for soil fertility and plant growth (77). Additionally, vermicomposts have been shown to stimulate soil microbial growth and activity, further underlining their positive impact on soil health (78).

The enzymatic activities present in vermicomposts are crucial indicators of their quality and effectiveness. Studies have demonstrated that vermicompost-derived microbes possess enzymes like cellulase and xylanase, which are capable of breaking down plant-based substrates, highlighting the role of enzymes in nutrient cycling and availability (79). Furthermore, the application of humic acids extracted from vermicompost has been shown to improve soil properties and enhance the quality of field crops, indicating the beneficial effects of vermicompost-derived compounds on soil health and plant growth (80).

The microbial biomass content and associated activity in vermicompost regulate nutrient dynamics during maturation, influencing nutrient immobilization or release, which in turn affects plant nutrient uptake when vermicomposts are applied to the soil (81). This interaction between microbial activity, enzyme dynamics, and nutrient availability underscores the intricate relationship between soil microbes, enzymes, and plant health. Moreover, the application of vermicomposts has been found to increase soil alkaline phosphatase activity, which is essential for phosphorus cycling and plant nutrient uptake (82).

In the context of soil restoration and management, vermicomposts have been identified as valuable resources for improving soil biological properties. The application of vermicomposts has been associated with increased vegetal cover, enhanced soil microbial activity, and improved soil structure, all of which contribute to sustainable soil management practices (78). Additionally, the use of vermicomposts in soil enrichment has been linked to changes in soil biochemical parameters, such as soil respiration and enzyme activities, which serve as early indicators of soil health and quality (83).

Overall, the research on enzyme activities in composting, vermicomposting, and soil management highlights the critical role of enzymes in mediating microbial processes, nutrient cycling, and plant growth. Enzymes serve as key indicators of microbial activity and soil health, making them essential components in assessing the efficacy of organic amendments like vermicomposts in promoting sustainable agriculture practices.

Microbial and Faunal Populations

Studies are carried out in the soil and compost test laboratory to provide information about the populations and activity of selected microorganisms in composts, vermicomposts or vermicompost applied soil samples. It is generally estimated that the total populations of microorganisms are larger in compost or vermicompost (81, 84-87). In properly prepared thermophilic compost, the high-temperature phase (thermophilic) leads to the demise of most microorganisms and decomposing fauna. Although many microorganisms can recolonize during the lower temperature phase, the rate of colonization can vary. This process usually happens unconsciously, except for certain species of microorganisms and a few compost products that have been intentionally inoculated. Additionally, this recolonization is known to suppress plant pathogens. In vermicompost, the mesophilic temperatures must be sustainable, otherwise the worms will be inactive, escaped or dead. Despite some sensitive populations of organisms, all microbial activity may be reduced or eliminated (88, 89). The microorganism population in the vermicomposting medium should be evaluated from 3 sources. The first is the initial microorganism load, as the initially beneficial microorganism population carried by the organic material is not harmed; the second is the increase in the number of existing microorganisms with the organic compounds added to the environment with the worm activity and casts; the third is the microorganism population, which increases, albeit relatively slowly, by utilizing the substrates released by the decomposition of organic material that has not yet been consumed by the worm. This total data represents the very high microbial activity of the vermicomposting medium. Therefore, vermicomposts are sometimes called biological fertilizers.

CONCLUSION

Several research on advanced features and assessment criteria of vermicompost have acknowledged the viability and effectiveness on the worthiness of vermicomposting as a sustainable technology for managing waste and enhancing soil. Vermicomposting comprises earthworms that break down organic waste; the physical, chemical, and biological state of organic waste far surpasses other methods' products, as produced by the traditional thermophilic process. One of the final products of vermicomposting is nutrient availability; mature compost is rich in such. The end product, vermicompost, is available nutrient-rich because it has many valuable available elements such as nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and boron. Such available nutrients play their essential roles in the proper, healthy development of plants by making the soil fertile and enhancing the quality of plant growth. The cation exchange capacity (CEC) of its vermicompost varies between 50 and 100, considered by many as fruitful in holding and releasing the needed nutrients for the plants.

These explain the physical properties of fine-length particle size, porosity, and the ability of vermicompost to retain more moisture compared to ordinary compost. It allows better aggregation of the soil, better aeration properties, and better water-holding capacity—prerequisites for plant growth. Furthermore, the minimum risk of contamination of toxic substances, for example, heavy metals, and low concentration of pathogens means vermicompost is safe and effective for use as a soil fertilizer. The beneficial biologically derived microorganisms in the vermicompost are helpful in the management of soil health and suppression of plant pathogens. Vermicomposting enhances microbial activity and diversity slightly through the action of earthworms, which is essential in maintaining soil health and agricultural sustainability. Moreover, these beneficial microorganisms help in decomposing organic material, stabilizing some nutrients, and suppressing soil-borne pathogens, making vermicompost a potential input for ensuring organic farming and sustainable agriculture.

The research line must further point toward continuous analysis and evaluation of quality as reflected in the output vermicompost. The maintenance of essential nutrient concentrations and contents such as pH, CEC, and moisture allows the producer to be in a position to manipulate the nutritional profile of vermicomposts to maximize them for efficiency among divergent agricultural uses. It could be assumed that the findings of the current research confirmed the adaptability and potential of vermicomposting to its uses in organic waste management and soil enrichment. Besides, it does not only help in the development of a prosperous and stable product from compost but also guarantees the sustainability of farming and land management through rapid soil conditioning for optimum growth of plants with the most minor ecological damage. With many consumers seeking sustainably grown and organic products, the transformation of vermicomposting waste into fertilizers could be an essential part of the answer to the global recycling issue for organic waste in support of healthy soils. Future work would consider fine-tuning the different processes and parameters of vermicomposting to consolidate its benefits and application in sustainable agriculture adapted to local conditions.

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Chapter 3

APPLICATIONS OF BLUE BIOTECHNOLOGY IN HUMAN HEALTH

Mustafa ÜSTÜNDAĞ[1]

INTRODUCTION

The world population is increasing. It is estimated that the world population will be approximately 9.6 billion in 2050 (1). Today, factors such as rapidly developing industrialization, urbanization, pressure on natural resources, climate change, disrupted ecological balance and changing lifestyles also bring health problems, difficulty in accessing safe food and dependence on energy. Today, many countries in the world are faced with important and unsolved issues such as epidemics, climate change, access to safe food, alternative and renewable energy sources.

In order to meet future demand and find solutions to global problems, one of the most sensitive issues is to reduce pressure on natural resources. According to the Millennium Ecosystem Assessment Project; It is estimated that 60% of the world's 24 major ecosystems that support human societies, including rivers and lakes, ocean fisheries, forests, air quality and crop systems, are "degraded or used unsustainably" (2). Considering the degraded ecosystems and destroyed habitats, safe food production becomes increasingly difficult (1). While the spreading epidemics and pandemics experienced in recent years directly threaten human health (3,4), climate change poses an important global problem in terms of sustainable development (5,6).

With the advances in biotechnology, it is possible to minimize the effects of important issues that await solutions on a global scale or to resolve these issues. Today, biotechnological methods can increase the supply of food and feed production, improve sustainability, increase water quality, provide renewable energy, improve the health of animals and people, and help protect biodiversity by detecting invasive species.

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BIOTECHNOLOGY

Biotechnology is a multidisciplinary field that has a great impact on our lives. Today, biotechnology is used in almost every field. It is called the "technology of hope" that affects human health, the well-being of other life forms and ecology (7).

There are many different definitions of biotechnology. These definitions have been created over the years and inspired by each other. In addition, developments in biotechnology have shaped the definition of biotechnology.

The term "biotechnology" was used in 1919 by Hungarian engineer Karl Ereky to refer to methods used to obtain products from raw materials with the help of living organisms (8). The most important and accepted definition of biotechnology is the definition made by the OECD (Organization for Economic Co-operation and Development). According to the OECD definition, "Biotechnology is an interdisciplinary branch of science and technology that deals with the transformation and creation of living and non-living substances using living organisms, their parts or products derived from them" (7).

Biotechnology is a developing branch of science that combines different technologies and applications in all areas of life. As can be understood from its definition, biotechnology essentially involves the use of living organisms or their components to develop new products and processes that are beneficial to human health, agriculture, the environment and many other areas (7). Color codes have been developed to distinguish the main areas of biotechnology, which is a branch of science that works in such different areas (Figure 1). Today, biotechnology is divided into colors such as white (industrial), green (agricultural), blue (marine and freshwater), red (medicine), brown (desert biotechnology), yellow (insect biotechnology and its applications) and purple (patents and inventions) (7,9,10).

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Blue biotechnology is also called marine or water biotechnology and is related to the discovery and use of the world's marine resources. It is known that there are more life forms in the oceans and seas because most of the world is water.

Oceans and seas, the majority of which have not yet been discovered and are thought to have great potential in many areas, have attracted the attention of researchers and have become new areas of study for many researchers. Oceans and seas, which cover 70% of the globe, are rich sources of biological diversity. Although approximately 300,000 species have been identified in the marine environment, it is estimated that this number covers a very small portion of the existing species. Although the potential of the biological resources in the marine environment is very high, it is predicted that it has not yet been fully evaluated (12). Considering the potential of blue biotechnology, it has four main goals. These goals are:

1. Sustainable food production to meet increasing food supply needs,
2. Protecting the marine ecosystem and obtaining information about geochemical processes occurring in the oceans, protecting the seas and oceans,
3. Biofuel production,
4. Identifying and isolating important compounds that can benefit human health and other areas of human use. (11).

The most striking and important area of study among these goals is the human applications of blue biotechnology.

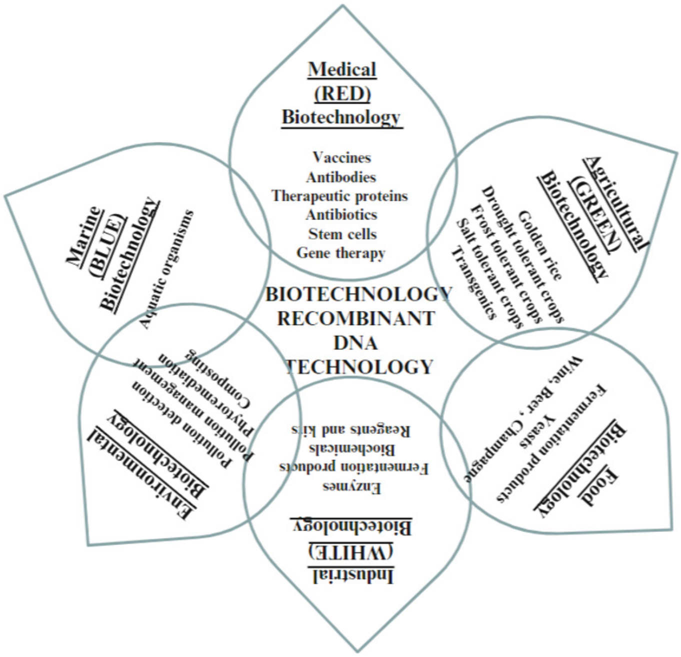

Figure 1. Major applications of biotechnology in different areas and some of their important products (11).

THE USE OF BLUE BIOTECHNOLOGY IN HUMAN HEALTH

Natural products obtained from seas and oceans have come to the forefront with research conducted in recent years. Although the vast majority of oceans and oceans have not yet been discovered, they offer a very rich biodiversity and opportunities. Obtaining bioactive substances and natural products to be used in the health sector from marine organisms is also one of the main purposes of biotechnology. These products generally cover the fields of medicine/pharmacy and functional food. Marine invertebrates (especially sponges) and algae are particularly prominent in this field (14).

THE USE OF BLUE BIOTECHNOLOGY IN MEDICINE/PHARMACY

In studies conducted in the past years, products used in medicine/pharmacy sciences were generally obtained from plants or terrestrial organisms. However, similar products were obtained in studies conducted on certain organisms after a certain period of time. In order to find a solution to the similarity in the products, researchers turned to different areas and aimed to obtain new products from less studied living spaces (15,16). In this sense, seas and oceans are areas of study with great potential. Biochemical compounds widely used in the pharmaceutical industry today are bioactive substances. Bioactive natural products are generally secondary metabolites produced by producer organisms to protect themselves and provide superiority in the natural environment they live in. These compounds can have effects in a wide range of fields, from therapeutic activity against human and animal diseases to neutralizing insects that harm agricultural production (16). Bioactive substances obtained from marine organisms and having therapeutic/pharmacological importance cover a wide range of areas such as anti-microbial, anti-tuberculosis, anti-viral, anti-parasitic, anti-protozoal, anti-inflammatory, anti-platelet, anti-diabetic and anti-cancer (17,18).

The first marine product used for therapeutic purposes was polysaccharide alginate (sodium alginate), which was used for gastroesophageal reflux disease and discovered from seaweed in 1881. Subsequent studies on sponges and marine fungi led to significant developments in the medical world (19). These studies have gained momentum in recent years. As of 2015, more than 8000 products have been examined as natural marine products. More than 1000 of these products are in the preclinical stage, 23 products are in the clinical stage and 7 products have received FDA (Food and Drug Administration) or EMA (European Medicines Agency) approval, while the number of drugs approved by FDA or EMA in 2021 is 13 (14,19). Although the approved drugs include drugs for chronic pain, hypertriglyceridemia and viral infections, most of these drugs are anti-cancer agents (ten out of thirteen) (19).

Research on the use of sea sponges in pharmacy began in the 1950s. "Sponogothymidine" and "Sponogouridine" isolated from sea sponges (*Cryptotheca crypta*) helped develop drugs called Ara-C, used to combat leukemia, and Ara-A, used against viral infections (FDA-1967/1976 approved) (13,20). When looking at the drugs in Table 1, in recent years, two natural marine products called "Ziconotide" (Prialt®) isolated from sea snails and "Trabectedin" (Yondelis®) obtained from another marine creature, Tunicates, have been commercialized by two different European pharmaceutical companies after receiving the necessary approvals (Table 1) (19,21). As seen in Table 2, there are some marine compounds and therapeutic agents in which phase studies are ongoing (22).

In addition, some marine compounds can be effective not only in cancer but also in different areas. It has been determined that 171 marine compounds published and structurally characterized by researchers in 42 countries between

2019 and 2021 have new pharmacological effects. While 49 of these compounds have antibacterial, antifungal, antiprotozoal, antituberculosis and antiviral effects, 87 have been determined to have antidiabetic and anti-inflammatory effects that also affect the immune and nervous systems (23).

In summary, many studies have shown that compounds and therapeutic agents obtained from aquatic organisms can be used beneficially for human health by affecting different areas of health.

Table 1. Marketed drugs by the EMA and/or the FDA (19).

Generic Name	Brand Name/s	Date of Marketting Authorisation	Natural Source	Clinical Use
Cytarabine	Cytosar-U Aracytin C.-Hospira	1969 (FDA)	Sponge	Leukemia
Vidarabine	Vira-A	1976 (FDA)	Sponge	Antiviral
Fludarabine	Fludara	1992 (FDA) 1994 (EMA)	Sponge	Leukemia
Ziconotide	Prialt	2004 (FDA) 2005 (EMA)	Mollusk	Chronic pain
Omega-3 acidethyl esters	Lovaza (US) Eskim (EU) and others	2004 (FDA) 2005 (EMA)	Fish	Hypertriglyceridemia
Nelarabine	Arranon (US) Atriance (EU)	2005 (FDA) 2007 (EMA)	Sponge	Leukemia
Trabectedin	Yondelis	2007 (EMA) 2015 (FDA)	Tunicate	Ovarian cancer, soft tissue sarcoma
Eribulin	Halaven	2010 (FDA) 2011 (EMA)	Sponge	Breast cancer
Brentuximabvedotin	Adcetris	2011 (FDA) 2012 (EMA)	Mollusk/ *Cyanobacterium*	Lymphomas
Lurbinectedin	Zepzelca	2020 (FDA)	Tunicate	Ovarian cancer
Polatuzumabvedotin	Polivy	2019 (FDA) 2020 (EMA)	Mollusk/ *Cyanobacterium*	Breast cancer
Enfortumavedotin	Padcev	2019 (FDA) 2021 (EMA)	Mollusk/ *Cyanobacterium*	Urothelial cancer
Belantamab mafodotin	Blenrep	2020 (FDA) 2020 (EMA)	Mollusk/ *Cyanobacterium*	Multiple myeloma

FDA: Food and Drug Administration; EMA: European Medicines Agency.

Table 2. ADCs containing MMAE or MMAF as payloads in clinical trials (22)				
Clinical	**Compound**	**Payload**	**Marine Organism**	**Therapeutic Use**
Phase I	ALT-P7	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase I	RC88	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase I	SGN-CD228A	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase II	CX-2029	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors lymphomas
Phase II	Disitamab vedotin	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase II	Enapotamabvedotin	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase II	Ladiratuzumab vedotin	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase II	Telisotuzumab vedotin	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase II	Tisotumab vedotin	MMAE	Mollusk/ *Cyanobacteria*	Solid tumors
Phase I	FS-1502	MMAF	Mollusk/ *Cyanobacteria*	Solid tumors
Phase II	AGS	MMAF	Mollusk/ *Cyanobacteria*	Solid tumors
Phase III	Depatuxizumabmafodotin	MMAF	Mollusk/ *Cyanobacteria*	Solid tumors

USE OF BLUE BIOTECHNOLOGY IN FUNCTIONAL FOODS

Due to their positive effects on human health, interest in functional foods has been increasing in recent years. Functional foods contain vitamins, phytochemicals, enzymes, antioxidants and essential oils, as well as drug-like effects. The positive effects of using some compounds obtained from aquatic organisms in functional foods have been observed and their use in this area has gradually increased. Especially chitin/chitosan and some compounds obtained from blue green algae and fish are very valuable in this area.

Chitin-chitosan is the second most used biopolymer in the world after cellulose. It has many uses in the medical field (24). Omega-3 fatty acids are very important for the development of children. They are one of the most important substances in the recovery of children's brains (25). Astaxanthin, produced from blue green algae, has a very high antioxidant value (26). In addition, some fish proteins have been determined to have anti-diabetic effects and are also useful in controlling obesity. It is known that taurine obtained from fish such as cod, salmon and mackerel is used in the treatment of Alzheimer's disease (27).

In addition to these, another product that is not consumed in our country but has a market in the Far East is jellyfish. It is used for medical purposes and as food in Far Eastern countries. It can be marketed fresh/cooled, salted or dried. In addition to its use in the pharmaceutical industry, another marine organism group that is important as a functional food is Sea Cucumbers (*Holothrurioidea spp.*). Its largest market is Far Eastern countries and it is sold under the name "Beche-de-mer" (12).

THE USE OF BLUE BIOTECHNOLOGY AS BIO-BASED MATERIALS

According to the European Standards Committee, bio-based materials are products obtained completely or partially from biomass such as plants, trees or animals (28). In recent years, many different enzymes, bio-polymers and other bio-based materials have been isolated or produced from marine organisms (29).

Bio-polymers are frequently used in the food industry, textiles and the production of plastic materials. With the studies conducted in recent years, bio-based materials are used in many areas related to the skeletal system (treatment of joints, thin metal sheets used to fix broken bone ends, bone filling material, treatment of bone deformities, artificial tendons and ligaments etc.), in many areas related to the circulatory system (cardiovascular system, blood vessel prostheses, heart valves etc.), as dental materials and in the treatment of sensory organs (30). However, marine bio-material science is still a new field and promises greater opportunities as the knowledge of marine living resources increases.

Marine-based polysaccharides are suitable for many chemical modifications. Polysaccharides that can be used in different areas are produced from macroalgae. The most important marine polysaccharides are Alginate from *Laminaria sp.* and agar-agar produced from *Gracilaria sp.* (31,32). Today, alginates;

- In the food industry (ice cream production, soft drinks, puddings, jams, gels, frozen fish and meat production technology, soups, mayonnaise production, soft cheese production, etc.)

- In detergent, soap and cosmetic product ingredients,
- In dental technology,
- In medicine, maintaining the consistency of liquid drugs (in coating drugs, as a carrier and binding agent in making pastilles, ensuring easy dissolution of tablets, preparation of ointments, etc.)
- In the paint industry, (Especially in water-based paints)
- In the textile industry,
- They are widely used in the production of leather, rubber, ceramics, sculpture and porcelain, adhesives and putties, foam sponges, paper, carbon, masks, mummies, pencils and colored pencils (33–36).

Agar agar, just like alginates, is widely used in many sectors. The areas of use of agar agar are as follows:

- In the food industry (as a stabilizer, thickener-gelling agent, clarifier, pectin substitute, transparency enhancer, shelf life extender)
- As a microbiological culture agent,
- In dental technology
- It is used in the production of laxatives (37,38).

Like agar-agar and alginate, chitin is one of the most common biopolymers used in different sectors. It is the main component of shellfish such as crabs and shrimps, and is also found in the skeleton of insects and the structure of the cell walls of fungi. Although there are many derivatives of chitin, the most important of these is chitosan (24,39). Chitin / Chitosan;

- In water treatment,
- In agriculture (plant additives, antimicrobial substances, etc.),
- In biotechnology (enzyme immobilization and chromatographic methods),
- In the food industry (thickener, additive, etc.),
- In cosmetics,
- It is used in the medical field (40,41).

USE OF BLUE BIOTECHNOLOGY IN COSMETICS

The use of marine products in cosmetics is becoming increasingly widespread. Due to the beneficial substances (fatty acids, antioxidants, photochemicals and enzymes) contained in marine products, many companies have started to use these products (42). Europe is the largest market in the world for cosmetic products. COLIPA (European Cosmetics Association) announced that the best-selling cosmetic products are skin care (anti-aging in particular) products

(23.7%). According to COLIPA, the new trend in terms of market/consumer is towards products obtained from plants and aquatic organisms (43). Today, many compounds obtained from marine organisms have become indispensable main products of the cosmetics sector.

Many nitrogenous compounds, tocopherols, polysaccharides, carotenoids and amino acids obtained from aquatic bacteria, micro and macro-algae, arthropods (such as krill, shrimp and crab), bivalves (scallops/oysters) and fish are either used in the cosmetics industry or their use is being investigated (44,45).

In addition to micro and macro algae, another aquatic creature used in cosmetic products is the “Leech”. Our country is the largest leech exporter in the world. Leeches have been used in the treatment of some diseases since ancient times. There are several species of medical leeches, and it is known that H. medicinalis and H. verbana live in Turkey. Currently, Turkey is one of the most important leech exporting countries in the world. The salivary gland secretions of leeches contain over 100 different bioactive substances. These secretions have vasodilators, bacteriostatics, analgesics, anti-inflammatories and edema solvents, prevent microcirculation disorders, reduce organ and blood pressure, increase immunity, relieve pain events and increase the bioenergetic status of the organism. Turkey is the luckiest country in terms of medical leeches. It is very important to evaluate this valuable product better and to use hirudotherapy more effectively as a supportive treatment in modern medical practices (46,47).

CONCLUSION

Natural marine-derived drugs are promising in the treatment of many diseases, especially cancer, Alzheimer’s, and schizophrenia. In addition, with the use of bio-based materials in health sciences, many treatment methods have been developed and significant progress has been made in the treatment of diseases. On the other hand, chemicals obtained from natural marine products are also used in cosmetic products. Therefore, oceans and seas have great potential. The evaluation of this potential is very important for the scientific world, the pharmaceutical industry and human health. In order for oceans and seas to be highlighted as a new research area in health sciences and to reach the potential envisaged in this field; more studies are needed with growth, education and R&D policies.

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Bölüm 4

THE ROLE OF BIOSTIMULANTS IN ENHANCING YIELD, QUALITY, AND STRESS TOLERANCE IN SUSTAINABLE VEGETABLE PRODUCTION

Suat SENSOY[1]

INTRODUCTION

Vegetables, herbaceous horticultural crops integral to human nutrition, are consumed directly or after minimal processing, either raw or cooked, fresh or preserved. These crops encompass a wide array of plant parts, including roots, tubers, stems, leaves, flowers, fruits, seeds, and more. As an essential part of the human diet, vegetables are generally low in fat and carbohydrates while being rich in vitamins, minerals, and dietary fiber. Major vegetable-producing nations include China, India, the United States, Türkiye, Iran, Egypt, and Italy (FAOSTAT, 2022).

Sustainable vegetable production involves adopting environmentally and human-friendly practices that ensure long-term agricultural viability. Biostimulants have gained a critical role in this context, offering substantial benefits for enhancing vegetable yield, improving quality, and increasing resilience against both biotic and abiotic stressors. These biostimulants comprise a diverse group of substances or microorganisms known to positively influence plant growth, yield, and biochemical composition, while also bolstering the plant's capacity to tolerate stress (Shahrajabian et al., 2021a; Yılmaz & Gazioglu Sensoy, 2021).

Biostimulants, often referred to as bioactivators, include a wide array of compounds, containing humic substances, protein hydrolysates, amino acids, nitrogenous compounds, seaweed extracts, polymers, inorganic compounds, beneficial fungi, and bacteria, organic wastes, vermicompost, and various plant-derived exudates (Shahrajabian et al., 2021a; Yılmaz & Gazioglu Sensoy, 2021). These substances can be applied to leaves, soil, or seeds, and are known to enhance vegetable growth, improve nutrient uptake, increase yields, and elevate product

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quality; Moreover, biostimulants contribute greatly to improving plant resilience against environmental stressors like drought, salinity, and pathogen attacks, while also promoting soil health and structure (Figure 1).

In the field of sustainable agriculture, biostimulants contribute significantly to the preservation of natural resources, like soil and water and are instrumental in soil erosion control, biodiversity enhancement, and integrated pest management. The application of biostimulants aligns with contemporary agricultural goals of improving soil fertility, achieving ecological balance, and safeguarding the health of humans and other organisms. As the demand for sustainable food production grows, the health and well-being of future generations drive scientists and producers toward environmentally friendly practices like the use of biostimulants.

This chapter will examine the mechanisms and impacts of biostimulants on soil health, crop yields, and product quality, drawing from both academic research and recent literature to provide a comprehensive understanding of their role in sustainable vegetable production.

MECHANISMS OF ACTION: HOW BIOSTIMULANTS IMPROVE PLANT PERFORMANCE

Biostimulants exert a wide array of beneficial effects on crops, significantly enhancing nutrient uptake, enhancing plant growth and development while increasing overall crop yield and quality These compounds stimulate physiological processes that contribute to better root architecture, more efficient water use, and increased resilience to both biotic and abiotic stressors. Through mechanisms such as enhanced nutrient solubilization, improved metabolic activity, and modulation of hormonal pathways, biostimulants enable plants to optimize their growth even under suboptimal environmental conditions. Additionally, biostimulants improve plant tolerance to stresses like drought, salinity, and pathogen attacks by strengthening the plant's natural defense systems and stress-response mechanisms. This multifaceted action not only boosts individual plant performance but also supports more sustainable and resilient agricultural practices (Shahrajabian et al., 2021a; Yılmaz & Gazioglu Sensoy, 2021). These synergistic effects make biostimulants critical tools for enhancing crop production in an era of increasing environmental challenges.

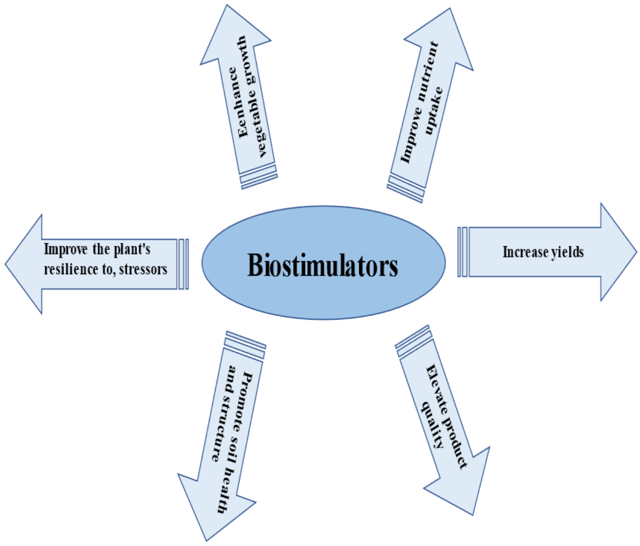

Figure 1. Effects of biostimulants in sustainable agriculture.

Beneficial fungi and beneficial bacteria

Microbial inoculants, including arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and Trichoderma-based products, are among the most widely recognized biostimulants. These PGPR and symbiotic microorganisms exert their effects through multiple mechanisms, including the modulation of plant hormone levels, enhancing nutrient availability, biosynthesis of volatile organic chemicals, and enhancing tolerance to abiotic stresses by the stimulation of systemic resistance (Vessey, 2003; Yang et al., 2009). However, there is significant variability in the results obtained from different studies. The mechanisms of action of these biostimulants remain only partially understood, and soil physicochemical parameters play a critical role in their efficacy. Therefore, it is crucial to analyze soil characteristics carefully before applying specific PGPR strains that are best suited to the prevailing conditions (Çakmakçı et al., 2007; Wu et al., 2005).

PGPR can be categorized into two main groups: symbiotic bacteria (*Rhizobium*, *Bradyrhizobium etc.*) and non-symbiotic bacteria (*Pseudomonas*, *Bacillus*, *Azotobacter etc.*). These bacteria affect plant development with direct mechanisms like nitrogen fixation, solubilization of phosphorus and potassium, and synthesis of phytohormones, and through indirect mechanisms, including stress management, disease resistance, and the synthesis of protective enzymes and volatile organic compounds (Saharan and Nehra, 2011). Notably, *Azospirillum spp.* is one of the most studied PGPR due to its role in biological nitrogen fixation via nitrogenase enzymes, regulated by *nif* genes (Seymen et al., 2021).

Endophytic bacteria, a subset of rhizobacteria, have been identified as potent growth promoters, contributing to both generative and vegetative growth in plants (Nadeem et al., 2014). These bacteria can improve plant resilience under abiotic and biotic stress conditions by enhancing nutrient uptake and reducing the accumulation of phosphates and nitrates in the soil, thus lowering the dependence on fertilizers (Yang et al., 2009). Given the growing challenges related to water stress, sustainable alternatives are needed. Research indicates that endophytic bacteria not only enhance plant tolerance to abiotic stress but also improve drought tolerance and provide protection against certain diseases (Dobbelaere et al., 2001).

Furthermore, PGPRs have been shown to solubilize phosphate, fix nitrogen, produce phytohormones, increase enzyme activity, lower ethylene production under stress conditions, and mitigate the adverse impacts of abiotic stressors (Çakmakçı et al., 2005; Çakmakçı et al., 2007; Çakmakçı, 2014). The ability of PGPR to alleviate the adverse impacts of abiotic and biotic stressors on plant development makes them a vital tool in sustainable agriculture (Wu et al., 2005). Recent focus has been placed on endophytic bacteria (EB), which reside within the plant's internal tissues without causing harm, for at least part of their life cycle (Rosenblueth et al., 2006; Hardoim et al., 2008; Akköprü et al., 2018). These bacteria, whether PGPR or EB, can enhance plant growth and development both directly and indirectly, providing new opportunities in sustainable production (Saharan and Nehra, 2011).

Among biostimulants, AMF are notable effective symbiotic microorganisms found in the rhizosphere, where they supply substantial benefits to agricultural crops through improved resistance and growth (Demir et al., 2015). AMF form a symbiotic relationship with plant roots by extending their hyphae, which are finer and longer than root hairs, greatly increasing the soil volume accessible for nutrient uptake. This extended network is particularly effective in absorbing immobile mineral matters including phosphorus (P), zinc (Zn), and copper (Cu), which are then exchanged for carbon derived from the plant's photosynthesis. This mutualistic association both boosts and improves the plant's tolerance to environmental stressors including drought and salinity, while influencing its phytochemical composition and metabolic processes. Additionally, AMF colonization has a vital role in mineral matter cycling, organic material decomposition, and enhances plant gas exchange (Seymen et al., 2021).

AMF are the commonest root symbionts, forming associations with nearly all higher plants. AMF ameliorate soil structure (Miller and Jastrow, 2000) and significantly improve plant development and production by facilitating mineral matter absorption, particularly for essential but less mobile elements, highlights their importance in plant development (Smith and Read, 2008). Beyond nutrient uptake, AMF help plants better withstand both biotic and abiotic stresses. They have been shown to contribute to plant disease resistance (Sensoy et al., 2013b; Demir et al., 2015), lessen mineral matter deficits, enhance drought and salinity tolerance (Türkmen et al., 2005, 2008; Sensoy et al., 2007, 2011; Çakmakcı et al., 2017). These multifaceted benefits position AMF as integral components in promoting sustainable plant growth and resilience in challenging environmental conditions.

Trichoderma species are also well-known as potent biostimulants in agriculture because of their capacity to promote plant development, improve mineral matter uptake, and increase crop resilience to stress. Acting as plant biostimulant, *Trichoderma* can significantly boost seedling vigor, root development, and overall crop yield without negatively affecting fruit quality. This makes them valuable tools in sustainable agriculture, particularly in high-value crops like vegetables. Studies have demonstrated that *Trichoderma* isolates can increase productivity in melon cultivation, highlighting their potential to improve both plant growth and fruit quality under nursery and greenhouse conditions (Fernando et al., 2018) and Fusarium wilt control in lettuce (Bellini et al., 2023).

Humic substances

Humic substances, such as humic and fulvic acids, are crucial in promoting plant growth and improving nutrient absorption, a phenomenon that has been extensively documented over the years (Pujola et al., 1992; Aleshin et al., 1994; Wang et al., 1995; Adani et al., 1998; Turkmen et al., 2005; Sensoy et al., 2013b; Demir et al., 2015; Ekincialp et al., 2016; Tahir et al., 2022). One of the key mechanisms through which humic acids (HA) exert their beneficial effects is their ability to chelate heavy metals, thereby mitigating their uptake by plants. Specifically, HA reduces the absorption of heavy metals through its chelating properties (Pujola et al., 1992). Yonebayashi et al. (1994) further elucidated that the chelating capacity of humic substances is notably enhanced at higher pH levels, leading to the transformation of heavy metals into forms that are less bioavailable for plant uptake under alkaline conditions. The underlying mechanisms of action appear to result from synergistic interactions among the various bioactive compounds present in raw materials; however, these effects can differ based on factors such as crop type, soil composition, and the microbial community present in the rhizosphere. Additionally, humic substances may stimulate plant growth through hormone-like activities, contributing further to their role in sustainable agriculture (Karakurt et al., 2017).

Seaweed extracts, and macro- and microalgae

Seaweed extracts, predominantly extracted from brown seaweeds like *Ascophyllum nodosum*, *Ecklonia maxima*, *Macrocystis pyrifera*, and various other macro- and microalgae, are recognized for their rich content of plant growth-promoting hormones and essential mineral matters (Sadak and Sensoy, 2022; Barot et al., 2023; Al-Ramamneh, 2024). Seaweed extract is an organic fertilizer derived from various types of seaweed, such as kelp, and is gaining popularity in agriculture for its multiple benefits. It is abundant in essential mineral matters (nitrogen, potassium, phosphorus, iron, zinc, magnesium, etc.), which are crucial for plant growth and health. Additionally, it includes natural growth hormones (auxins, cytokinins, gibberellins) that promote plant growth by enhancing cell division and root development. Seaweed extract also improves plants' stress resistance to environmental stressors including drought, salinity, and temperature fluctuations. Its compounds enhance nutrient uptake efficiency and promote microbial activity, improving soil structure and fertility. Furthermore, seaweed extract has potential antifungal and antibacterial properties, contributing to disease resistance in plants. Recognized as a natural biostimulant, it enhances seed germination, root development, and overall plant health while aligning with sustainable agricultural practices. Its compatibility with organic farming makes it a valuable input in many organic certification programs (Barot et al., 2023).

Seaweed extracts play a vital role in vegetable production by serving as natural fertilizers and soil amendments. They enrich the soil with essential nutrients, including nitrogen, phosphorus, potassium, and various growth-promoting compounds, which are readily absorbed by plants, thereby enhancing growth, development, and yield. The mechanisms underlying the beneficial effects of seaweed extracts are still being explored; however, the mechanism behind these benefits includes increased photosynthetic efficiency, enhanced water and nutrient absorption, along with the presence of plant hormones like auxins and cytokinins. Additionally, seaweed improves soil structure and moisture retention, particularly in sandy soils, while also enhancing plants' resilience to environmental stresses like drought and salinity. Its biostimulant properties promote seed germination, root development, and flowering, contributing to overall plant health. Furthermore, seaweed extracts may possess disease-suppressive qualities, helping to bolster the natural defense mechanisms of vegetable crops against pathogens. The use of seaweed in agriculture is aligned with sustainable practices, reducing reliance on synthetic fertilizers and minimizing environmental impacts (Barot et al., 2023).

In spite of scientific proof demonstrating the hormonal effects of seaweed extracts, various challenges impede a thorough understanding of their mechanisms of action. These challenges encompass inconsistencies in experimental designs, the wide variety of seaweed-based products available commercially, species-specific responses, limited evidence on the analytical formulation of these commodities, and the changing structure of raw materials thru different seasons. Consequently, additional research is required to clarify the exact mechanisms by which seaweed extracts influence plant growth and development.

Biopolymers such as Chitin and chitosan-like polymers

Chitosan, a natural biopolymer originating from chitin, is primarily sourced from the exoskeletons of crustaceans like shrimp, crabs, and lobsters. It is produced through the deacetylation of chitin, resulting in a positively charged polysaccharide. Its agricultural significance lies in its biocompatibility, biodegradability, non-toxicity, and antimicrobial characteristics (İkiz et al., 2024). Chitosan's multifunctionality allows it to act as a biopesticide, biofertilizer, and biostimulant, supporting plant growth, boosting crop yields, and offering protection against abiotic stressors and diseases. Moreover, chitosan strengthens plant defense mechanisms against various biological and environmental stressors, promotes growth by increasing stomatal conductance and reducing transpiration, and can be used as a seed coating material. It also fosters the growth of chitinolytic microorganisms, prolongs the storage life of produce through post-harvest treatments, and enhances nutrient delivery by minimizing leaching and facilitating the slow release of fertilizers. Additionally, chitin promotes plant growth, improves nutritional content, and enhances resistance to abiotic and biotic stressors. The review by Shahrajabian et al. (2021b) offers a comprehensive overview of the effects of chitin, chitosan, and their derivatives on horticultural crops, highlighting their essential role in sustainable crop production while discussing the limitations and future prospects of this category of biostimulants.

Protein hydrolysates (PHs), amino acids (AAs), and other nitrogenous compounds

Protein hydrolysates (PHs), amino acids (AAs), and other nitrogenous compounds are essential elements of biostimulants, originating from various natural sources such as aquatic and terrestrial ecosystems, along with microbial and plant metabolites. These components have a significant function in improving plant development and resilience (Santiago et al., 2021; El-Nakhel et al., 2023; İkiz et al., 2024). These eco-friendly substances support sustainable agriculture by utilizing by-products like hydrolyzates and extracts. Their production involves various technologies such as fermentation and hydrolysis, resulting in diverse mono- or multi-component products. Biostimulants enhance horticultural crop performance through bioactive compounds that improve primary and secondary metabolism, nutrient uptake, and phytochemical synthesis. They can increase pigment levels and antioxidant capacity in leafy vegetables, optimize leaf color, and reduce nitrate content, addressing health concerns associated with high nitrate intake. Moreover, applying protein hydrolysates through foliar or root treatments promotes root development, enhances carbon and nitrogen assimilation, and improves nutrient uptake by modulating metabolic processes through auxin-like signaling pathways. This treatment also strengthens plant defenses against abiotic stress and enhances quality traits in fruit and leafy vegetables. Nonetheless, the efficacy of biostimulants is subject to variables such as environmental conditions, application rates, timing, and the specific plant species or cultivars involved.

Inorganic compounds

Several inorganic compounds, such as phosphite, silicon, and nanoparticles, have demonstrated potential as effective biostimulants. Phosphite, an emerging biostimulant, not only serves as a phosphate source that promotes plant development and production but also acts like a biocide against plant diseases and alleviates abiotic stressors (Bellini et al., 2023). Silicon (Si) exhibits diverse biostimulant properties, especially in supporting crop growth under abiotic stress. Its beneficial effects include reducing oxidative stress, enhancing water relations, boosting photosynthesis, improving ion uptake, and regulating hormonal activity. These actions are primarily mediated through silica deposition in plant tissues, which strengthens mechanical structure (Santiago et al., 2021).

Nanofertilizers represent another innovative approach to nutrient management in plants, as they effectively regulate nutrient availability through their unique release mechanisms (Chen and Wei, 2018). Their application requires smaller quantities compared to conventional fertilizers, leading to reduced transportation and application costs (Fan, 2014). Moreover, because they are applied in lower doses, nanofertilizers help prevent soil salinization caused by the over-application of traditional fertilizers, both in the short and long term (León-Silva et al., 2018). Additionally, nanofertilizers can be customized to meet the specific nutritional needs of the crops being produced (Kah et al., 2018).

Organic biostimulants such as vermicompost and biochar, exudates and extracts of plants

Organic biostimulants, such as vermicompost and biochar, along with plant exudates and extracts, play significant roles in promoting plant growth and health. Vermicompost leachates exhibit hormonal activity due to their content of trace elements and phytohormones, including cytokinins, indole-3-acetic acid, gibberellins, and brassinosteroids. Phytohormones - cytokinins, auxins, and gibberellins - have a crucial function in promoting plant development by accelerating development, increasing leaf formation per plant, and improving overall yield. Additionally, vermicompost enhances phytoremediation by accumulating heavy metals and promoting higher levels of chlorophyll, carotenoids, and proteins (Uluğ, 2018; Kabay et al., 2019; Alp and Sensoy, 2023; İkiz et al., 2024).

Biochar, derived from waste and by-products, acts as a biostimulant by improving soil structure, enhancing nutrient retention, promoting beneficial microbial activity, adjusting soil pH, and contributing to carbon sequestration, all of which are crucial for sustainable agriculture (Chan et al., 2007; Çakmakcı et al., 2021). Research shows that amending soil with biochar can enhance soil organic materials; this, consequently, improves soil fertility (Xu et al., 2012) and increases fertilizer efficiency (Asai et al., 2009), ultimately resulting in higher plant yields (Chan et al., 2007). In addition, biochar has been shown to decrease the toxic mineral matter uptake to plants (Glaser et al., 2002; Namgay et al., 2010). Its use modifies soil structure, improves aeration, and enhances water retention capacity (Madiba et al., 2016), leading to improved water use efficiency and greater plant resilience under drought or water-limited conditions.

COMPARATIVE EFFECTS OF DIFFERENT BIOSTIMULANTS ON VEGETABLE CROPS

In addition to the effects observed with single biostimulant products, numerous studies have reported that the combination of different biostimulants can lead to significantly enhanced outcomes in horticultural crops. These combined effects often surpass the benefits provided by individual biostimulants, indicating a synergistic interaction that promotes plant growth and resilience. Research has shown that utilizing a mixture of biostimulants can improve various parameters, such as crop yield, quality, and stress tolerance. Notable studies supporting these findings include those conducted by Turkmen et al. (2005), Sensoy et al. (2013b), Demir et al. (2015), Ekincialp et al. (2016), Uluğ (2018), Santiago et al. (2021), Alp and Sensoy (2023), Bellini et al. (2023), and El-Nakhel et al. (2023). These studies collectively emphasize the potential of biostimulant combinations in enhancing the performance of vegetable crops, thereby contributing to more sustainable agricultural practices.

CASE STUDIES: EFFECTIVE USE OF BIOSTIMULANTS IN VEGETABLE CULTIVATION

The successful application of biostimulants in vegetable cultivation has been demonstrated through various case studies, highlighting their potential to enhance crop growth, yield, and quality. By improving nutrient uptake, increasing stress tolerance, and promoting beneficial microbial activity in the soil, biostimulants have emerged as an essential tool for sustainable agriculture. The following paragraphs present case studies that illustrate the versatility of biostimulants across different vegetable crops and growing conditions, offering practical insights into their benefits and their potential for broader adoption in commercial farming systems.

In a controlled growth chamber study conducted by Sensoy et al. (2007), eight distinct pepper genotypes were treated with two AMF, *Glomus intraradices* (Gi) and *Gigaspora margarita* (Gm), under standardized seedling growth conditions. The findings revealed that AMF inoculation significantly enhanced the dry weights than controls. Notably, five out of the eight genotypes displayed positive growth responses to AMF inoculation, while three exhibited negative responses. There was considerable variation in the dependency on mycorrhizal colonization among the pepper genotypes; the N52 genotype had the highest relative mycorrhizal dependency (RMD) and the Karaisali genotype demonstrated the lowest. Additionally, an inverse correlation was found between RMD and the dry weight of the pepper genotypes, suggesting that genotypes with lower dry weights tended to be more reliant on mycorrhizal colonization.

A study by Turkmen et al. (2008) examined the influence of two AMF species (*Glomus intraradices* and *Gigaspora margarita*) on the development and mineral matter content of pepper seedlings subjected to moderate salt stress. Although the saline conditions adversely affected seedling growth, both AMF species notably improved salt tolerance. This enhancement was reflected in various growth traits such as shoot height, stem diameter, root length, and overall biomass. Furthermore, AMF inoculation facilitated increased absorption of important mineral matters like phosphorus, potassium, calcium, and sodium. While *G. intraradices* emerged to be more efficient in promoting seedling growth compared to *G. margarita*, the difference in their performance was not statistically significant.

In a study by Ciftci et al. (2010), the effects of AMF species - *Glomus mosseae*, *Glomus intraradices*, and *Glomus fasciculatum* - on the development and mineral matter content of four common bean cultivars under salt stress were examined. The results showed that AMF inoculation significantly enhanced plant growth and development. Additionally, AMF inoculation increased the uptake of important mineral matters. Among the AMF species, *G. mosseae* was found to provide the most significant benefits for plant growth and nutrient absorption.

In a growth chamber study by Sensoy et al. (2011), four hybrids of *Cucurbita pepo* were treated with AMF species - *Glomus intraradices*, *Glomus etunicatum*, and *Gigaspora margarita* - to assess their effects on seedling development. The results demonstrated significant variability in RMD among the hybrids, with inoculations of *G. margarita* yielding notably higher positive RMD values compared to the lower, sometimes negative, values associated with *G. intraradices*. The Focus F1 hybrid inoculated with *G. margarita* exhibited the highest RMD. Furthermore, AMF-inoculated seedlings displayed wider cotyledons and thicker stems, along with substantial enhancements in most nutrient content analyzed. These findings highlight the beneficial role of AMF as biostimulants in improving the growth and nutritional status of *Cucurbita pepo* seedlings.

Tüfenkçi et al. (2012) examined the effectiveness of various AMF species on four hybrid cucumber cultivars, focusing on colonization, nutrient uptake, mycorrhizal dependency, and seedling traits. The growth chamber experiment included three AMF species - *Glomus intraradices*, *Glomus etunicatum*, and *Gigaspora margarita* - alongside a non-inoculated control group. The findings revealed that AMF-inoculated cucumber seedlings had shorter hypocotyls and exhibited wider and longer cotyledons compared to non-inoculated seedlings. However, seedlings inoculated with *G. margarita* displayed the narrowest stem diameters and the fewest leaves. AMF inoculation led to shorter shoots and longer roots across all treatments, with a significant increase in iron content in the shoots and a notable mycorrhizal colonization rate in the roots of AMF-treated plants. Additionally, considerable variability in relative mycorrhizal dependency (RMD) was observed among the hybrid cultivars, indicating that selecting cultivars with high RMD may enhance cucumber seedling production in future endeavors.

In the study conducted by Sensoy et al. (2013a), four hybrid melon cultivars were inoculated with three AMF species - *Gigaspora margarita*, *Glomus intraradices*, and *Glomus etunicatum* - to assess their effects on seedling growth. The results indicated variability in mycorrhizal colonization rates among the melon cultivars, ranging from moderate to high percentages. Nutrient uptake, including nitrogen, phosphorus, potassium, magnesium, and manganese, was influenced by the specific cultivars and the combinations of AMF species used. Relative mycorrhizal dependency (RMD) also varied significantly across the cultivars, with only half of the AMF combinations demonstrating positive dependencies. Notably, inoculations with *G. intraradices* resulted in higher positive RMDs, while those with *G. margarita* produced lower negative RMDs. These findings highlight the varying effectiveness of AMF in promoting growth and nutrient acquisition in melon seedlings.

In a growth chamber study by Turkmen et al. (2005), the effects of combined HA application and inoculation with AMF *Glomus intraradices* on the pepper cv. Demre were investigated. The results indicated that nearly all seedling growth parameters were favourably influenced by both HA application and AMF inoculation. Additionally, the interaction between HA and *G. intraradices* suggested that HA not only initiated but also amplified the beneficial effects of AMF inoculation. These findings indicate that the combined use of HA and *G. intraradices* could significantly enhance the growth of pepper seedlings under saline conditions.

Karakurt et al. (2027) showed that the application of humic acid (HA) through foliar and soil methods significantly enhanced both hydrophilic and lipophilic antioxidant activities in cucumber fruit. Furthermore, HA application notably increased the levels of total carotenes, xanthophylls, beta-carotene, lycopene, and chlorogenic acid. These results indicate that HA could serve as an effective strategy for improving the quality of cucumber fruit by elevating its antioxidant compound content.

In the study by Sensoy et al. (2013b), the influences of AMF, specifically *Gigaspora margarita*, along with whey and HA, on seedling traits and the incidence of Fusarium wilt caused by *Fusarium oxysporum* sp. *niveum* in the hybrid *Cucurbita pepo* L. cultivar Focus F1 were examined. The results demonstrated significant enhancements in seedling growth and nutrient content across the different treatments, with the single application of AMF achieving the highest level of Fusarium wilt suppression, followed by the combination of AMF, HA, and whey. These findings highlight the potential of AMF as an effective biostimulant for improving plant resilience and growth in *Cucurbita pepo* L. under disease pressure.

The research by Demir et al. (2015) investigated the impacts of AMF, HA, and whey applications on Verticillium wilt (*V. dahliae* Kleb.) in three solanaceous crops. The study demonstrated that the application of either *Glomus mosseae* or *Glomus intraradices* AMF inocula, in combination with HA and W, significantly enhanced the plant development and mineral matter status of these crops while effectively reducing the severity of wilt disease. Notably, the combination of these treatments reduced the number of *V. dahliae* microsclerotia. Moreover, the use of whey and HA stimulated AMF growth, resulting in increased levels of AMF colonization and spore density in comparison with controls. These findings highlight the potential of these biostimulants in managing disease and enhancing the overall health of solanaceous crops.

Ekincialp et al. (2016) examined the effects of two AMF species—*Gigaspora margarita* and *Glomus intraradices*—along with HA and whey applications on various traits of melon, watermelon, and summer squash cultivated in open field conditions. The study found that the application of these biostimulants significantly enhanced yield and branch length in melons, as well as yield, branch length, and fruit pedicle length in watermelons. Improvements were also noted in summer squash regarding yield, total soluble solids content, and fresh leaf weight. Importantly, the combination of AMF, whey, and humic acid produced the highest average yield across all three cucurbit species. These findings highlight the considerable potential of these biostimulants for improving the growth and productivity of cucurbits in field settings.

Çakmakcı et al. (2017) examined the function of AMF as biostimulants in melon seedlings subjected to deficit irrigation stress. The study found that the application of two mycorrhizal species, *Glomus intraradices* and *Glomus spp.*, significantly enhanced various physiological and photosynthetic parameters compared to non-AMF plants, especially under different levels of water deficit. The results indicated that AMF not only increased chlorophyll content but also enhanced the plants' drought tolerance. This research highlights the potential of mycorrhizal symbiosis to mitigate the negative impacts of water scarcity on melon growth.

Erdinc et al. (2017) investigated the impacts of AMF as biostimulants on the seedling traits of 21 common bean genotypes inoculated with four AMF species - *Glomus intraradices*, *Glomus mosseae*, *Gigaspora margarita*, and a commercial AMF - under controlled growth conditions. The data showed that inoculated plants generally displayed improved seedling characteristics in comparison with non-inoculated ones. Additionally, the phosphorus content in the shoots was significantly higher in the inoculated plants. The study also revealed considerable variation in mycorrhizal colonization and relative mycorrhizal dependency (RMD) among the genotypes, indicating complex interactions between AMF inoculation and the traits of the bean genotypes. Positive correlations were established between RMD and various seedling features, as well as with phosphorus content and mycorrhizal colonization, suggesting that AMF inoculation can enhance the growth and nutrient uptake of common beans.

Fernando et al. (2018) explored the biostimulant effects of two isolates of *Trichoderma saturnisporum* (T1 and T2) on the melon (*Cucumis melo*) production. The study aimed to assess how these isolates influenced various growth parameters and overall fruit quality, highlighting the potential benefits of *T. saturnisporum* as a biostimulant in melon cultivation. Germination trials and experiments were conducted in nursery and greenhouse settings over two crop cycles. The results demonstrated that both *T. saturnisporum* isolates significantly enhanced seedling vigor, root length, and overall plant quality in both conventional and large plant systems. Furthermore, treatments with *T. saturnisporum* resulted in notable increases in melon productivity and average fruit weight, without negatively impacting fruit quality. This research supports the application of *T. saturnisporum* as an effective biostimulant in melon cultivation, showing its potential to enhance crop performance while maintaining high fruit quality.

Bellini et al. (2023) investigated IPM strategies to address Fusarium wilt (*Fusarium oxysporum* f. sp. *lactucae*) in lettuce. The study emphasized the limitations of chemical control methods, particularly due to the restricted availability of fungicides and the growing consumer preference for organic products. Over two years, three IPM strategies were tested in two separate fields: (i) compost supplemented with *Trichoderma*, (ii) a mixture of *T. gamsii*, *T. asperellum*, *Bacillus amyloliquefaciens*, and potassium phosphite, and (iii) a blend of *T. polysporum* and *T. atroviride*. The results demonstrated that these IPM approaches significantly diminished disease severity and improved crop yields compared to untreated controls. Although these treatments did not substantially alter the overall composition of the rhizosphere microbiota, significant variations were noted between the two locations and across the years, demonstrating a microbial buffering effect influenced by soil conditions.

Tunçtürk et al. (2019) investigated the influences of nine plant growth-promoting rhizobacteria (PGPR) isolates on the Gina bean variety under biotic fungal stress induced by *Xanthomonas axonopodis* pv. *phaseoli*. The PGPR isolates included various strains of *Pseudomonas* and *Bacillus subtilis*. The results indicated that these PGPR isolates significantly reduced disease severity and positively influenced several growth traits. Additionally, the isolates enhanced carotenoid and chlorophyll levels, as well as the nutrient content in plant and root tissues. These findings highlight the potential of these PGPR isolates as effective biostimulants for mitigating the adverse impacts of pathogenic infections on bean plants.

Bilge et al. (2019) explored the influences of PGPR as biostimulants on seedling growth parameters and nutrient content of bean plants subjected to salt stress. The study found that increasing salinity levels negatively impacted the development of the bean plants. However, the application of four different bacterial isolates resulted in varying enhancements in growth parameters, with one isolate demonstrating superior performance in shoot height compared to the others and the control. While salinity adversely affected the intake of macro and micronutrients, certain PGPR isolates showed significant positive effects on nutrient uptake, particularly for magnesium, zinc, copper, and manganese. Furthermore, the application of PGPR generally increased chlorophyll content, highlighting their potential to diminish the adverse effects of salt stress on common bean.

Sadak et al. (2021b) revealed that the use of PGPR endophytic bacteria, specifically *Ochrobactrum sp.* and *Bacillus sp.*, as biostimulants significantly improved various plant growth parameters in pepper seedlings. The findings highlight the potential of these biostimulants to diminish the adverse effects of water stress and promote overall plant development.

In the study by Can et al. (2022), the use of microalgae (*Chlorella vulgaris*) as a biostimulant significantly enhanced plant growth parameters in spinach. Additionally, this treatment reduced the requirement for mineral fertilizers. These findings suggest that combining microalgae with reduced fertilizer doses can promote a more environmentally friendly and sustainable approach to spinach production.

Turhan et al. (2022) revealed that the application of microalgae positively influenced plant growth in rocket salad, as evidenced by improved growth parameters in comparison with the control group. The most favorable results were observed in treatments that combined NPK fertilizer with microalgae, indicating the potential of this combination to enhance growth performance.

The study by Sadak and Sensoy (2022) demonstrated that applications of microalgae (*Chlorella vulgaris*) significantly enhanced various plant growth parameters in garden cress, including shoot height, shoot fresh weight, total soluble solids (TSS), and chlorophyll content. Additionally, the application improved the uptake of crucial mineral matters. These findings indicate that microalgae biostimulants can effectively promote plant development and nutrient absorption, particularly when combined with reduced doses of mineral fertilizers.

The use of seaweed extracts, particularly *Ascophyllum nodosum* and *Spirulina platensis*, had a significant impact on the levels of endogenous IAA hormones in cucumber leaves. Moreover, these biostimulants influenced the composition of main nematode taxa in the cucumber rhizosphere, including the proportions of free-living nematodes and bacterivores and fungivores. These findings suggest promising possibilities for developing sustainable organic production strategies for cucumbers that utilize safe biostimulants as alternatives to conventional chemical inputs (Al-Ramanneh, 2024).

The study by Çakmakcı et al. (2022) demonstrated that silver nanoparticles (AgNPs), particularly at higher concentrations (80 ppm), significantly improved root growth parameters, including length, diameter, fresh weight, and dry weight, in radish plants, especially under deficit irrigation conditions. However, the effect on leaf number was limited, highlighting the potential of AgNPs as an effective biostimulant for enhancing nutrient and water use efficiency in water-stressed environments.

In contrast, Tahir et al. (2022) found that in hot climatic conditions, the application of humic acid (HA) through soil drenching and foliar feeding of potassium fertilizer did not significantly enhance vegetative or yield traits when compared to the control group. This indicates that the effectiveness of humic acid as a biostimulant may be constrained by climatic factors.

Kabay et al. (2019) investigated the effects of vermicompost as a biostimulant on lettuce production in a pot experiment conducted under greenhouse conditions. The results demonstrated that vermicompost application positively influenced various growth parameters, chlorophyll content, total phenolic content, total antioxidant values, and the activity of antioxidant enzymes. Notably, plants grown in a substrate consisting of a 1:3 volume mixture of peat, perlite, and vermicompost, irrigated solely with tap water, showed significant growth improvements compared to control plants cultivated in a peat and perlite medium fertilized with a Hoagland nutrient solution. This highlights the potential of vermicompost to enhance lettuce growth in organic cultivation systems.

In another study exploring the influences of vermicompost and mycorrhiza on the development and production of beans and onions, it was found that the application of vermicompost significantly enhanced pod length and width in beans compared to other treatments (Uluğ, 2018).

Çakmakcı et al. (2021) examined the use of biochar derived from rose plant pruning waste as a biostimulant under deficit irrigation conditions, focusing on various growth parameters of pepper plants. While deficit irrigation significantly reduced plant height, stem diameter, and fruit length, the application of biochar mitigated these negative effects, leading to notable increases in plant height and stem diameter, although fruit length remained statistically unchanged. The study also highlighted variations in leaf color parameters, with the control treatment (without biochar) exhibiting the highest values of L, a, b, and C. Conversely, the highest hue (h°) value was recorded in pots treated with the highest concentration of biochar (3% w/w), indicating that incorporating biochar can significantly enhance pepper plant development, even with restricted water availability.

Santiago et al. (2021) evaluated the biostimulant Codasil®, which contains oligo/polypeptides, amino acids, silicon, and potassium, for enhancing lettuce growth and tolerance under water stress conditions. Conducted at 75% field capacity, the research analyzed various parameters related to growth, oxidative stress, photosynthesis, pigment concentrations, and proline metabolism. The results showed that Codasil® significantly improved plant growth while reducing lipid peroxidation and hydrogen peroxide levels, thus protecting photosynthetic performance. Treated plants also exhibited increased silicon accumulation and lower proline levels, suggesting enhanced stress response mechanisms. Overall, the study confirms that Codasil® successfully alleviates the adverse impacts of water scarcity on lettuce, fostering improved growth and physiological resilience.

Alp and Sensoy (2023) found that different fertilizer applications, including vermicompost, resulted in significant differences in various morphological traits of fresh beans. Organomineral and vermicompost treatments often outperformed chemical fertilizers, indicating that organic fertilizers like vermicompost could serve as sustainable alternatives to chemical fertilizers in bean cultivation.

El-Nakhel et al. (2023) carried out a protected cultivation trial to study four biostimulants: (i) two derived from enzymatic hydrolysates of Fabaceae species, (ii) one formulated with betaine, alginic acid, and caidrin, and (iii) another based on alfalfa extract, algae, and molasses rich in low-molecular-weight amino acids. The study aimed to assess their effectiveness in reducing nitrate accumulation in wild rocket leaves while enhancing yield and both quantitative and qualitative traits over successive harvests.

İkiz et al. (2024) conducted a greenhouse study on sustainable hydroponic lettuce cultivation under saline water stress, demonstrating that biostimulant applications helped mitigate the negative effects of salt on plant weight, height, leaf number, and leaf area. Notable yield increases were observed with the use of various treatments under 50 mM NaCl, including significant improvements attributed to vermicompost, PGPR, fulvic acid, amino acid, and chitosan. Additionally, these applications enhanced stomatal conductance, chlorophyll content, nutrient uptake, and water status, while also reducing malondialdehyde (MDA) levels. The findings indicate that PGPR, vermicompost, and fulvic acid were particularly effective in promoting growth, yield, phenolic compounds, and mineral content, while lowering nitrate levels in saline conditions.

Numerous scientific studies have been conducted in the field of biostimulants, further highlighting their potential benefits in enhancing plant growth, yield, and resilience under various environmental stresses.

REGULATORY FRAMEWORK AND MARKET TRENDS FOR BIOSTIMULANT PRODUCTS

In the comparative analysis by Pujari (2023) on regulatory frameworks for biopesticides and biostimulants across the EU, US, India, and Japan, distinct approaches are highlighted. The EU employs rigorous assessment processes under its Plant Protection Products Regulation, promoting sustainable agricultural practices. In the US, the EPA facilitates a more streamlined registration for biochemical pesticides, offering cost and time advantages. India's framework under the Insecticides Act emphasizes various biopesticide categories and recent regulatory developments to support organic farming. Japan's approach combines historical usage with a tiered assessment for biopesticides, while biostimulants are regulated under existing fertilizer laws. This analysis highlights the diverse regulatory landscapes that influence the adoption and development of biopesticides and biostimulants in these regions.

Okumuş and Alçıkaya (2019) reviewed that in Türkiye, the regulatory framework for biofertilizers and biopesticides was primarily governed by the Ministry of Agriculture and Forestry. The registration process for biofertilizers involves the identification, formulation, and field testing of soil microorganisms, followed by an application to the relevant ministry unit. Companies must submit several documents, including an Organic Fertilizer Production Permit Application Form, production processes, capacity reports, and health-related permits. Additionally, for microbial fertilizers, specific trials must demonstrate their effectiveness on local soil conditions and crop yields. For biopesticides, registration is guided by the 2008 regulation on the licensing, import, production, and use of biological control agents. This includes conducting biological efficacy trials to validate the effectiveness of imported beneficial organisms under local conditions. Both biofertilizers and biopesticides must adhere to strict guidelines, including labeling requirements detailing the product's composition, usage instructions, and safety information.

FUTURE PERSPECTIVES ON BIOSTIMULANTS IN VEGETABLE CROP IMPROVEMENT

Biostimulants represent an innovative and sustainable strategy for enhancing vegetable production, particularly in the context of increasing biotic and abiotic stressors. Their efficacy is linked to enhanced photosynthetic activity and improved tolerance to stress factors primarily through the increased activity of antioxidant enzymes. Additionally, biostimulants exhibit auxin-like effects that facilitate nitrogen uptake, regulate the nutrient ratios, and promote osmoprotectants accumulation, thereby acting as against stressors.

Application methods for biostimulants consist of foliage application, soil amendment, or seed coating. However, the effectiveness of these biostimulants varies significantly based on the crop type, soil characteristics, and the native microbial communities within the rhizosphere. This variability highlights the necessity for future research to evaluate biostimulant performance across diverse environmental conditions.

Crop genotype significantly influences responses to biostimulants, especially under stress conditions. Therefore, exploring optimal combinations of biostimulants with specific crop species and cultivars is essential. Notably, the use of microalgae in conjunction with mineral fertilizers has demonstrated positive effects on nutrient uptake and plant growth, yielding significant increases in productivity while reducing reliance on chemical fertilizers by 25-50%. Future studies should investigate the synergistic interactions between microalgae and beneficial microbes like AMF and PGPR to further boost vegetables development.

Numerous studies have shown that biostimulants can produce vegetable yields and quality that are on par with, or even surpass, those achieved using chemical fertilizers. This highlights their potential as effective alternatives in sustainable vegetable cultivation. Continued research is warranted to explore optimal combinations and ratios of biostimulants to maximize their benefits while providing eco-friendly solutions for crop enhancement.

Moreover, while abiotic stresses like drought adversely affect plant growth, biostimulants - particularly endophytic bacteria - can enhance resilience by promoting growth parameters and mitigating stress impacts. This suggests a promising avenue for using biostimulants to address the challenges posed by environmental stresses, emphasizing the need for further exploration of plant-microbe interactions to develop stress-tolerant crops and improve agricultural sustainability.

Given the increasing global population and the urgent need for food production amongst environmental challenges stemming from excessive chemical fertilizer use, this study emphasizes the advantageous role of microalgae as biostimulants in promoting plant growth and nutrient uptake. Incorporating microalgae into agricultural practices may help reduce dependency on synthetic fertilizers, warranting additional research on their application to foster sustainable farming practices and enhance crop production while minimizing environmental impacts.

In light of escalating challenges related to climate change and water scarcity, biochar has emerged as a promising biostimulant, enhancing soil fertility, improving water retention, and promoting plant resilience against abiotic stressors such as deficit irrigation. Its incorporation significantly enhances key growth parameters, ultimately leading to increased agricultural productivity and quality.

The combined application of various biostimulants demonstrates significant potential for sustainable vegetable production. By improving plant growth, yield, and stress tolerance, biostimulants enhance nutrient use efficiency and soil nutrient availability, which are crucial for sustainable agricultural productivity and crop quality. Additionally, biostimulants contribute positively to soil health by supporting microbial communities and enhancing soil fertility, thus ensuring long-term agricultural sustainability. Humic substances are particularly important in enhancing soil's physical and chemical characteristics, which in turn boost nutrient availability and improve water retention.

Integrating biostimulants into modern vegetable production systems presents a promising strategy for achieving higher yields while maintaining or improving soil health. Their multifaceted benefits position biostimulants as essential tools for addressing the challenges posed by climate change and resource depletion, particularly in degraded ecosystems or semi-arid regions. Future research should focus on optimizing biostimulant application techniques and understanding their interactions with soil microbiomes to maximize their potential in enhancing crop productivity and environmental sustainability.

Moving forward, research on biostimulants should target several key areas to advance their application in sustainable agriculture. This includes the exploration of novel biostimulants with superior growth-promoting properties, investigating synergistic effects with other biostimulants such as AMF and PGPR, and assessing various application methods and timings. Assessing the sustainable influnces of biostimulants on soil health and microbial diversity will offer crucial insights. Future research should also examine how biostimulants improve plant resilience to environmental stress, their influence on nutrient use efficiency, and the biochemical mechanisms driving these interactions. Transitioning to field trials will be essential for assessing the practical benefits of biostimulants, while studies addressing their impact on crop quality and consumer acceptance will facilitate their integration into mainstream agricultural practices.

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Chapter 5

AGRICULTURE 4.0 AND VITICULTURE: THE CURRENT STATUS OF DIGITAL TECHNOLOGIES AND FUTURE PERSPECTIVES

Ruhan Ilknur GAZIOGLU SENSOY[1]

INTRODUCTION

Agriculture 4.0 refers to the integration of advanced technologies such as digitization, automation, and data analytics into the agricultural sector. Viticulture represents an important area in the transition from traditional agricultural practices to digitalization. Various innovations, including sensor technologies, the use of drones, AI-supported decision-making systems, and automation applications, are among these technologies. In addition to analyzing the current status, the potential benefits of these technologies in viticulture are also examined. These benefits include increased productivity, more efficient use of resources, improvements in quality control, and strengthening sustainability measures.

The concept of Industry 4.0, known as the Fourth Industrial Revolution, was introduced in Germany in 2011. It can be described as the replacement of muscle power by machines and human management by automation, because of technological advancements (Kilic and Alkan, 2018). The agricultural sector has been continuously influenced by these technological innovations. Following and implementing these innovations in the agricultural sector has become increasingly essential. According to the FAO's report titled "How to Feed the World in 2050," the global population is projected to increase by 34% and reach 9.1 billion by 2050. This growth is expected to make food security a more pressing issue, and FAO recommends that countries invest in agricultural R&D and prioritize policies in this area. As one of the top 10 agricultural economies in the world and the largest agricultural producer in Europe, it is crucial for Türkiye to focus on agricultural R&D and work to increase production in line with these recommendations (Pakdemirli et al., 2021).

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The agricultural sector is undergoing significant transformation through digitalization. Agriculture 4.0, as part of this transformation, encompasses technology and data-driven approaches. The digital transformation process, known as Agriculture 4.0, has played a critical role in the modernization of agriculture. Agriculture 4.0 offers an approach aimed at increasing productivity, sustainability, and efficiency by integrating traditional agricultural practices with digital technologies (Ercan et al., 2019; Sevli, 2023).

Countries such as the United States, the Netherlands, Australia, Brazil, and New Zealand are among the global leaders in Agriculture 4.0 applications (Saygili et al., 2018; Anonymous, 2021). Digital agriculture and technologies have significant potential in the agricultural sector. These technologies can help improve productivity while preserving biodiversity, maintaining soil health, enhancing food security, and combating climate change. Technologies such as the Internet of Things (IoT), wireless sensor networks (WSN), remote sensing (RS), unmanned aerial vehicles (UAVs), big data analytics, machine learning (ML), deep learning (DL), and artificial intelligence (AI) are critical for the long-term sustainability of agriculture (Cakmakci and Cakmakci 2023). Digital agriculture facilitates data-driven, intelligent decision-making, supporting safer, more sustainable, and highly productive food production. Technological innovations are essential in overcoming the economic, social, and environmental challenges faced by agriculture. Technologies such as IoT, big data analytics, AI, UAVs, unmanned ground vehicles (UGVs), and robotics can make agricultural processes more efficient. National and international policies and strategies should focus on applications such as smart spraying, monitoring of plant diseases, and tracking of crops and soil (Sahin, 2022). In an article titled "Digital Agriculture, Agriculture 4.0, Smart Farming, Robotic Applications, and Autonomous Systems," the applicability and potential of Agriculture 4.0 technologies in the viticulture sector are examined.

In a study evaluating the applicability of Agriculture 4.0 in Türkiye, survey results from three different companies working on Agriculture 4.0 were analyzed. These data, evaluated using a SWOT analysis, highlighted the strengths and weaknesses of Agriculture 4.0 in Türkiye. The existence of organizations offering smart agricultural technologies and the growing awareness among farmers were identified as strengths. However, the lack of IT literacy, the high average age of producers, and insufficient infrastructure were considered weaknesses. As a result, it was emphasized that modern practices, cooperativism, and educated farmers are necessary for the successful implementation of Agriculture 4.0 in Türkiye (Ercan et al., 2019).

While Agriculture 4.0, which refers to the use of digital technologies in agriculture, has contributed significantly to the global transformation of agriculture, a further step toward Agriculture 5.0 has emerged in recent years. Agriculture 5.0 involves integrating technology-based agricultural practices with human-machine collaboration. This concept was introduced by Japanese Prime Minister Shinzo Abe during the CEBIT fair in Hannover, Germany, in 2017, where the Society 5.0 philosophy began to gain wider recognition (CEBIT, 2020). Agriculture 5.0 aims to develop more flexible, personalized, and sustainable production methods by placing the human factor at the center of the process, while still leveraging technology-driven production processes. In Agriculture 5.0, which utilizes advanced robotics and AI technologies, systems that enhance human-machine interaction, as well as biotechnology and genetic engineering applications tailored to farmers' needs, play a key role. Agriculture 5.0 focuses on social, environmental, and ethical values. In this context, Agriculture 5.0 is seen as a perspective concerned with developing more sustainable and ecosystem-friendly agricultural practices. While Agriculture 4.0 can be described as a period that focuses on productivity through the use of technology, Agriculture 5.0 represents a transition to a more personalized and sustainable production model that emphasizes human-machine collaboration. It places greater importance not only on technology but also on social and environmental values. Agriculture 5.0, seen as the next step beyond Agriculture 4.0, aims to develop solutions that are in harmony with both people and nature in the future of agriculture (Cam, 2023).

CURRENT AND POTENTIAL HIGH-TECH APPLICATIONS IN VITICULTURE

Technology is causing significant changes in the agricultural sector, as in all other sectors. Viticulture stands out as one of the first sectors to develop innovative techniques to simplify fieldwork. Systems such as GIS (Geographic Information Systems) for positioning rows, supports, and plants, precision viticulture applications, decision support systems (DSS) for pathogen monitoring, and innovative leaf analysis systems are increasingly used in viticulture. For example, before planting, existing digital systems enable highly accurate analysis of vineyard soils. By integrating geographic information systems with real-time and systematic soil analysis, innovative analytical systems now allow for the creation of very specific maps by integrating planimetric data with soil information such as aspect, elevation, and orography (Anonymous, 2014; Akin et al, 2015; Karaman, et al, 2022).

A digital field diary, enriched with information and data recorded by sensors and weather stations arranged in the field, allows for the gathering of all vineyard-related information in a single program. The digitization of data reduces workload. Drones, robots, and advanced sensor technology can be used to collect data in the field, which is then transmitted to the main software. This data is processed and analyzed under the guidance of Agriculture 4.0 experts and automated systems. In wineries, precision viticulture systems are also used, enabling the monitoring of processes such as temperature, humidity, and micro-oxygenation in the cellar via remote control systems. Agriculture 4.0 aims to minimize human intervention and thereby improve management and product quality (Anonymous, 2022). When it comes to winemaking, interconnected systems can be instructed to make adjustments, such as correcting the temperature or humidity in the cellar. Traceability in the Agriculture 4.0 world is one of the most advanced areas and is one of the most widely adopted solutions by companies. By placing a QR code or NFC tag on the label, it is possible to trace back a wide range of information about the wine. The VRA (Variable Rate Application) systems applied to harvesting machines, especially for monitoring the fertilization process, are among the main technologies implemented during this period (Saygili et al., 2018; Anonymous, 2022).

Agri 4.0 consists of 1, Smart Sensors and Monitoring Systems; 2. Use of Biotechnology; 3. Data Analytics and Artificial Intelligence; 4. Image Processing Technologies; 5Software and Hardware; and 6. Communication Systems (Figure 1.).

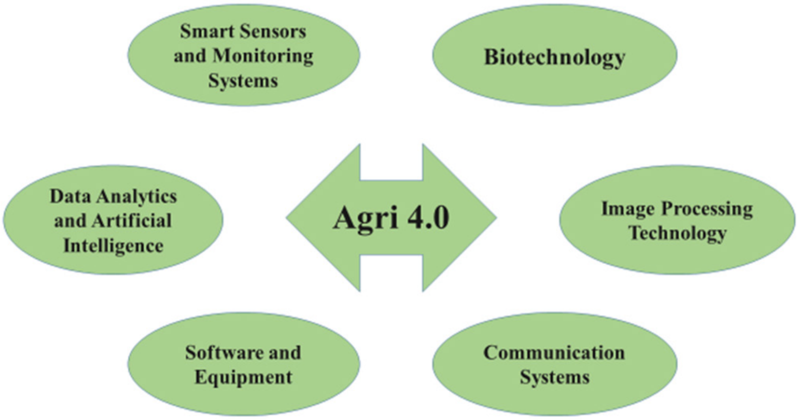

Figure 1. Agriculture 4.0 Practices

SMART SENSORS AND MONITORING SYSTEMS

In viticulture, sensor technologies such as soil moisture, humidity, light, air temperature, CO_2, and solar energy sensors continuously collect data for smart agriculture. These sensors can monitor a wide range of parameters, from weather conditions and soil quality to crop phenology, employee performance, and equipment efficiency (Cesco et al., 2021).

Weather Sensors: These are used to monitor weather conditions in vineyards. By collecting data on temperature, humidity, wind speed, and sunlight, they help optimize viticultural operations.

Soil Sensors: These measure soil moisture, pH levels, and nutrient content, providing valuable information for irrigation and fertilization strategies.

Plant Sensors: Used to monitor plant health by measuring photosynthesis rate, leaf water potential, and signs of disease, which are critical in vineyard management (Ammoniaci et al., 2021; Oreški et al., 2021).

Data Analytics and Artificial Intelligence: The data collected from sensors are processed through analytical algorithms and artificial intelligence, making viticulture processes more efficient. One such application, the Metaverse, allows multiple users to interact in a virtual environment in ways not possible in the real world (Anonymous, 2022; Ozguven et al., 2022).

Remote Monitoring and Automation: Vineyards can be monitored remotely through sensors, and automated systems can manage irrigation, spraying, and harvesting with greater precision and efficiency (Ercan et al., 2019, Sevli, 2023). In Türkiye's eastern Erciş district and surrounding villages, a study was conducted to identify potential areas for grape cultivation by analyzing climate, soil, and topography. Using Geographic Information Systems (GIS), areas suitable for grape varieties with different ripening times were identified. The study examined parameters such as total effective temperature, frost-free days, minimum winter temperatures, slope, aspect, soil depth, drainage, and land use capability. Results showed that the Erciş region is suitable for cultivating early- and mid-season grape varieties (Dogan and Guzel, 2020).

In a study evaluating the effects of canopy management techniques in small-scale viticulture operations, field research and unmanned aerial vehicle (UAV) remote sensing were used. Results from Italian local grape varieties showed that canopy management can enhance productivity and improve environmental sustainability. Remote sensing provided real-time vegetation indices (VIs) that can be utilized to increase quality and sustainability in viticulture operations (Brunori et al., 2022).

Using GIS techniques, a study in the Erciş district identified potential areas for cultivating cold-resistant Amur grapes by analyzing climate, soil, and topographical factors. The study produced suitability maps based on various parameters and found that 83,297 hectares of the region were suitable for Amur grape cultivation. This study serves as a valuable resource for agricultural planning and support (Guzel and Dogan, 2020).

Pagliai et al. (2022) evaluated canopy size parameters such as thickness, height, and volume in grapevines using digital tools like a mobile application, mobile laser scanner, and UAV. Using Pix4Dmapper Pro software, 3D point clouds of vineyard rows were created and processed in MATLAB. Canopy parameters were calculated through spatial manipulations considering local soil gradients, with the tools showing good correlation.

USE OF BIOTECHNOLOGY

Biotechnology is a key component of Agriculture 4.0 and is used to enhance agricultural production, develop plants resistant to diseases and pests, and optimize water and energy usage. In grape cultivation, biotechnology offers rapid solutions to challenges such as climate change, nutrient deficiencies, salinization, and diseases, improving yield, resilience, and quality (Kazancıoglu et al., 2024).

Technologies and applications of biotechnology in Agriculture 4.0 include

Genetic Engineering: Modifying the genetic structure of plants to develop desired traits. This can lead to more productive, disease-resistant, or climate-adaptable plants (Ford-Lloyd and Jackson, 1991).

Plant Breeding: Traditional plant breeding methods are enhanced with modern biotechnology techniques, speeding up the process and improving agricultural productivity (Caglar and Sensoy, 2021; Atak, 2024).

Biological Control: The use of biological methods to combat harmful organisms instead of chemical pesticides, representing an environmentally friendly approach (Compant and Mathieu, 2016; Tanyolac et al., 2010).

Plant Biotechnology: The manipulation of plant cells, tissues, or genetic material in laboratory settings, improving traits like disease resistance, productivity, and quality. Molecular markers are widely used in grape plants for genetic distance and relationship studies, as well as for developing stress-resistant varieties (Gazioglu Sensoy and Balta, 2018; Durna Dastan, 2023).

Agricultural Biotechnological Products: Products developed using biotechnology, such as genetically modified organisms (GMOs), can enhance yield, improve resistance to pests, or boost nutritional content (Turgut, 2020; Gungor and Demiryurek, 2021).

Rapid Selection: The use of fast and accurate techniques for selecting genetic material in plant or animal breeding, enabling quicker development of organisms with desired traits (Acun, 2024; Borghi et al., 2024).

Biotechnology, as part of Agriculture 4.0, contributes to making agriculture more efficient, sustainable, and environmentally friendly. However, ethical and environmental concerns surrounding these technologies must be considered (Kılavuz and Erdem, 2019).

DATA ANALYTICS AND ARTIFICIAL INTELLIGENCE:

Artificial intelligence (AI) develops technologies that simulate human brain functions such as thinking, learning, and problem-solving (Cakmakci and Cakmakci, 2023). AI is used in viticulture to optimize decision-making processes. Machine learning algorithms analyze collected data to predict diseases and pests, optimize fertilization programs, and determine harvest timing. In the context of Viticulture 4.0, a study conducted at a winery in Abruzzo evaluated the use of digital technologies, finding that they made agricultural production processes more efficient and supported decision-making. Creating a digital data archive has helped optimize processes by supporting future decisions based on past production data (Romualdi, 2019).

In a thesis evaluating a fermenter-connected system as a sustainable innovation for traditional farms, the evolution of Agriculture 4.0 was examined, with technologies like robotics and AI being explored. It was concluded that this system provided winemakers with real-time data analysis and control through a sensor network and mobile application (Quarato, 2018). Another study focused on the use of a real-time control system and sensors in large-scale vineyards for digital farm management. The smart software helped predict vineyard diseases based on previous data, allowing timely interventions, while an alarm system sent warnings and advice to farmers' smartphones for early detection (Bento et al., 2019).

A study aimed at developing low-cost IoT hyperspectral devices for Viticulture 4.0 reported the testing of a prototype designed to reduce costs compared to commercial models. This solution was developed for evaluating the water and phytosanitary conditions of vineyards, offering real-time decision-making capabilities (Tugnolo et al., 2022).

In another study, vis/NIR spectroscopy was tested for its ability to rapidly assess grape polyphenol content in winemaking. A process spectrometer with a spectral range of 400-1650 nm was used for non-contact analysis. The results indicated that the system could provide useful information on polyphenol content, contributing to better management of the winemaking process (Pampuri et al., 2022).

Despite some challenges, studies on smart and digital agriculture indicate that the technology supports and facilitates human decision-making (Mazzon, 2019).

IMAGE PROCESSING TECHNOLOGIES:

As a part of Agriculture 4.0, Image Processing Technologies refer to image analysis and computer vision techniques utilized in the agricultural sector. These technologies are applied across various agricultural domains to enhance efficiency, optimize resource use, and improve the sustainability of farming (Agin and Malaslı, 2016).

Plant Recognition and Disease Diagnosis: Image processing technologies are employed in agricultural fields for plant recognition and disease diagnosis. High-resolution images obtained through drones or sensors provide insights into plant health. These images can be analyzed to identify plant diseases, pests, or stress symptoms.

Harvest Prediction and Yield Analysis: These technologies can also be used to predict harvest times and perform yield analyses. Factors such as plant growth rates, fruit maturation processes, and harvest potential can be evaluated through image analysis.

Field Mapping and Management: Image processing technologies assist in mapping and managing agricultural fields. Factors like topography, plant density, and irrigation needs can be determined via image analysis, providing farmers with valuable insights for field management.

Pest Control: These technologies can identify and control pests or weeds by recognizing specific features (e.g., particular colors or patterns) in images, helping detect pests and inform appropriate intervention strategies.

Irrigation Management: Image processing technologies can improve irrigation management by analyzing factors such as leaf color and plant growth status to determine irrigation needs and help automate irrigation systems.

As a component of Agriculture 4.0, image processing technologies contribute to making agriculture smarter, more efficient, and sustainable. They provide farmers with critical data to make informed decisions and optimize agricultural production processes (Sanchez et al., 2011; Reis et al., 2012).

SOFTWARE AND HARDWARE:

Software and hardware, integral to Agriculture 4.0, refer to the technologies used to digitize agricultural processes, enhancing productivity, resource efficiency, and sustainability.

Agricultural IoT (Internet of Things): Sensors, devices, and equipment in agriculture are integrated to collect and communicate data on soil moisture levels, weather conditions, water consumption, and plant growth rates, allowing continuous monitoring and analysis.

Smart Farming Machines: Tractors, harvesters, and irrigation systems are equipped with sensors and automated control systems as part of Agriculture 4.0, automating agricultural operations and increasing efficiency while reducing labor costs.

Cloud-Based Agricultural Software: Cloud-based agricultural software is utilized for storing, managing, and analyzing farm data. It helps farmers with tasks like field mapping, crop management, harvest forecasting, and irrigation planning. The software also provides real-time data access for improved decision-making.

Robotic Agricultural Systems: The use of robotic systems in agriculture is increasing. These systems are employed in tasks such as automatic harvesters, weed control robots, and greenhouse automation systems. Robotics reduces labor costs, enhances efficiency, and minimizes risks faced by human workers.

UAVs (Unmanned Aerial Vehicles) and Drones: UAVs and drones are used for aerial surveillance of agricultural fields, monitoring plant health, field mapping, and irrigation management. These devices facilitate rapid data collection and analysis, saving farmers time and resources. Software and hardware are key elements of Agriculture 4.0, accelerating the digital transformation in the agricultural sector and fostering the adoption of more sustainable and efficient farming practices. Drones play a significant role in agricultural and viticultural applications, especially in monitoring and surveillance over large areas. They can be used to evaluate plant health, detect pests, and increase harvest efficiency. Drones (unmanned aerial vehicles) are crucial in the agricultural field. In a study examining traditional and alternative viticulture systems, including organic, biodynamic, and adaptive landscape systems, the importance of precision agriculture technology for enhancing grape yield was emphasized. The Agrofly TF1A high-precision agricultural drone-sprayer device has been reported to significantly enhance the phytopathological resistance of each vine by combating major diseases prevalent in the subtropical regions of southern Russia. Supplying local viticulture with these agricultural drone-sprayer devices is expected to greatly increase grapevine yield and provide the wine industry with high-quality raw materials (Dorofeeva et al., 2021).

Monitoring and Surveillance: Drones can observe agricultural fields from the air, monitoring plant health and detecting issues like diseases, pests, or water shortages. In a study aimed at disease-pest control, drones equipped with RGB and multispectral cameras were used to detect downy mildew in vineyards. A method based on the combination of infrared images was employed to generate disease maps (Kerkech et al., 2020). Altas et al. (2018) successfully detected Cercospora leaf spot in sugar beet fields using drone-captured images and a developed image processing algorithm. In another study, drones were used to detect pests in fruit orchards, with the collected images transmitted to an NVIDIA Jetson TX2 embedded system for further analysis of pest development stages and locations (Chen et al., 2021).

Data Collection: Drones can gather soil, plant, and atmospheric data through sensors and cameras, aiding agricultural management. In a study using drone, Remote Sensing, and Geographic Information Systems (GIS) technologies, the aim was to investigate floods in a specific area to minimize damage in agriculture and other sectors through early warning systems (Dolo, 2018).

Precision Agriculture: Drones are used in precision farming applications, such as applying the right amount of fertilizers or pesticides to specific areas. Alkan and Ertugrul (2022) emphasized the significance of widespread and conscious use of agricultural drones in pesticide applications for reducing fuel and chemical costs, minimizing environmental impacts, and engaging the younger generation in agriculture (Alkan and Ozgünaltay, 2022). Mattivi et al. (2021) used a low-cost commercial drone to detect weeds in corn fields, achieving 99.38% to 99.55% accuracy through three different methods (Maximum Likelihood Classifier, Artificial Neural Network model from the OpenCV library, and Object-Based

Image Analysis). Prescription maps for site-specific weed management were generated using these results (Mattivi et al., 2021).

Mapping and Route Planning: Drones can map agricultural fields and plan routes based on data. This helps tractors operate more efficiently. Bohler et al. (2020) combined UAV and APEX datasets to distinguish between different plants using a random forest-based method, achieving 92% accuracy with the use of additional NIR-RGB texture features.

Harvest Monitoring: Drones can monitor crops during harvest to increase efficiency. In a study using deep learning techniques, drone-acquired images were used to automatically detect, count, and estimate the size of citrus fruits on trees with the Faster R-CNN model, showing more accuracy than predictions made by expert technicians (Apolo-Apolo et al., 2020). In a study aimed at monitoring the ripening parameters of Chardonnay grapes, a cost-effective visible/near-infrared optical prototype was tested. Based on a combination of spectroscopic data and predictive models, the prototype quantified qualitative parameters. The results showed that the prototype could assist operators in estimating ripening stages in Chardonnay grapes, supporting Viticulture 4.0 with a sustainable approach (Pampuri et al., 2022).

Water Management: Drones can measure soil moisture to determine irrigation needs and help optimize water use. A study investigating water stress in vineyards obtained various reflection indices such as NDVI, TCARI/OSAVI, and PRInorm using drone data. These indices showed positive correlations with water stress indicators, and researchers reported that thermal images could be used to detect water stress (Gago et al., 2015; Turker et al., 2020; Cakır and İslek, 2021; Ozguven et al., 2022; Bal and Bal, 2023).

COMMUNICATION SYSTEMS

Within the framework of Agriculture 4.0, communication systems refer to the technologies that facilitate information exchange, data transmission, and collaboration within the agricultural sector. These systems enable farmers, agricultural enterprises, research institutions, and other stakeholders to communicate, share information, and coordinate effectively.

Mobile Communication Tools: Mobile communication devices are used to enhance communication among stakeholders in the agricultural sector. Cell phones, smartphones, tablets, and other portable devices allow farmers and agricultural experts to exchange information and stay connected (Dogancukuru, 2009; Pakdemirli et al., 2021).

Internet Access and Email: Internet connectivity is a critical component for increasing communication and information sharing in agriculture. Farmers, agricultural specialists, and other stakeholders can communicate and exchange information through digital tools such as email, online forums, and social media platforms (Arklan, 2008; Altin, 2021).

Cloud-Based Collaboration Platforms: Cloud-based collaboration platforms in agriculture enable farmers, agricultural experts, and other stakeholders to share data and collaborate on joint projects. These platforms support functions like document sharing, project management, task assignment, and tracking (Unal and Topakci, 2013).

Remote Education and Consulting: As part of Agriculture 4.0, remote education and consulting services can be offered. Farmers can receive training on agricultural techniques, new technologies, and best practices. Additionally, experts can provide remote consulting to help farmers solve problems and make informed decisions (Gulcubuk and Aluftekin, 2006; Sevli, 2023).

IoT (Internet of Things) and Sensor Networks: IoT and sensor networks facilitate data collection and communication in the agricultural sector. Sensors placed in agricultural fields continuously collect data and wirelessly transmit it to provide farmers with real-time information. The wine industry, blending tradition with innovation, uses IoT as a key tool in viticulture to address climate change and support decisions in production, storage, and sales, marking its integration into modern viticulture (Bicakci, 2019; Giordano and Verrastro, 2020).

Communication systems are a vital component of Agriculture 4.0, contributing to more efficient and sustainable agricultural practices by enhancing information exchange, collaboration, and coordination across the agricultural sector.

Autonomous Agricultural Vehicles: Automation is another key Agriculture 4.0 technology used to optimize viticulture operations and save labor. Applications like automated irrigation systems, mechanical harvesters, and automatic fertilization systems can enhance the efficiency of vineyard operations. The grape drying process can also be adapted to automated systems (Koken, 2019). Machine learning methods have been employed to automate traditionally difficult and time-consuming tasks, such as grape detection, harvesting, spraying, and yield estimation. Tests have shown that the proposed method operates with high accuracy and precision (Al-Saffar, 2019; Sahin, 2022). One study focusing on smart agriculture developed a rover - a quadruped robot - that uses multispectral and hyperspectral cameras to monitor vineyard stress and perform rational fertilization. The project was implemented using low-cost electronics like Raspberry Pi and open-source platforms. This approach highlights the importance of ICT skills and digital literacy in industrial design education while encouraging students to work creatively in robotics (Faoro et al., 2022).

There are some advantageous and disadvantageous aspects of Agriculture 4.0 Applications in Viticulture as mentioned below:

Advantages of Agriculture 4.0 Applications in Viticulture

Increased Efficiency: Agriculture 4.0 enhances the efficiency of agricultural operations through the use of advanced technologies such as automation, sensor technologies, and data analytics. With technologies like sensors, drones, and image analytics, vineyard management becomes more effective, resulting in higher efficiency and improved product quality (Kılavuz and Erdem, 2019; Abbasi et al., 2022; Aydinbas, 2024).

Optimizing Resource Use: Digital agriculture technologies allow for more efficient use of resources such as water, fertilizer, and energy. Sensors and smart irrigation systems ensure that water is used in the right amount at the right time, preventing waste (Aldag and Eker, 2018; Cengiz and Das, 2022).

Early Detection of Diseases and Pests: Sensors, imaging systems, and AI-supported analysis can detect diseases and pests in vineyards early on. This enables timely intervention, preventing crop loss and increasing productivity (Arslan et al., 2018; Turker et al., 2020; Demir et al., 2021; Aydin, 2022).

Water and Fertilizer Management: With smart irrigation systems and sensors, water and fertilizer usage can be optimized. This leads to water conservation and prevents over-fertilization of the soil, reducing environmental impact (Kılavuz and Erdem, 2019; Agizan et al., 2022; Cakmakci and Cakmakci, 2023).

Safety and Labor Efficiency: Technologies such as automated tractors, robots, and automated harvesters make vineyard work safer and more efficient while reducing labor costs (Dogru and Mecik, 2018; Baran and Karacuha, 2021).

Environmental Sustainability: Digital agriculture can enhance environmental sustainability by promoting more efficient use of resources, reducing chemical fertilizer and pesticide use, and implementing erosion control measures (Ertas, 2020; Senol, 2021; Korkmaz, 2023; Govez, 2023).

Data Analytics and Decision Support Systems: Data collected from vineyards can be analyzed to assist farmers in making better decisions. For example, weather forecasts and soil data analysis can help determine pruning and harvesting times (Terribile et al., 2017; Zhai et al., 2020; Mehedi et al., 2024).

Innovation and Development: Agriculture 4.0 promotes continuous innovation and development in the agricultural sector. The application of technologies like AI, machine learning, and IoT leads to the development of more effective farming practices (Sarri et al., 2020; Ferro and Catania, 2023; Araujo et al., 2023).

Market Access and Trade Facilitation: Digital platforms and e-commerce solutions enable vineyard owners to market their products to wider audiences, providing better marketing opportunities and increased revenue.

Agriculture 4.0 aims to create a more sustainable, efficient, and innovative agricultural system, offering significant advantages. Applications of Agriculture 4.0 in viticulture enhance sustainability, efficiency, and profitability for farmers, increasing the competitiveness of the viticulture sector (Cokuysal, 2021; Bilgin and Medeni, 2023).

Potential Disadvantages of Agriculture 4.0 Applications in Viticulture

High Costs: Agriculture 4.0 technologies require significant investments in equipment, training, and maintenance. Tools such as sensors, robots, drones, and other smart farming equipment come with high initial costs, which may impose financial burdens on farmers (Sassu et al., 2021; Tziolas et al., 2023).

Requirement for Technical Skills: The implementation of advanced technologies necessitates a certain level of technical knowledge and skills from farmers. Those who are not proficient in these technologies may struggle to utilize them to their full potential or may misuse them (Cengiz and Demirel, 2019; Yarım and Celik, 2020).

Data Security and Privacy Concerns: Agriculture 4.0 involves the collection and processing of vast amounts of data. Ensuring the security and privacy of this data is a major concern for farmers. Data breaches or misuse could erode their trust in these technologies (Elciyar, 2018; Duman and Sen, 2019; Aksoy, 2024).

Digital Divide: Access to Agriculture 4.0 technologies may not be equally distributed, especially between developed and rural areas. This can exacerbate the digital divide, limiting access to technology for certain farmers and reducing their competitiveness (Akman, 2023; Gucenmez, 2023).

Environmental Impact: Some Agriculture 4.0 applications may have environmental drawbacks. For instance, the energy demands of large data centers and the disposal of electronic waste can increase environmental harm (Cokuysal, 2021; Baran et al., 2023).

Unemployment Risk: Automation and robotics in farming can lead to a reduction in traditional agricultural jobs, raising the risk of unemployment, particularly in developing countries where Agriculture 4.0 becomes more prevalent (Sarac, 2022; Sheikh, 2022; Onder, 2023).

These disadvantages must be considered to ensure the successful implementation of Agriculture 4.0, and appropriate policies and regulations should be put in place to mitigate them (Dressler and Paunovic, 2021; Sassu et al., 2021; Barrile et al., 2022; Kadagan Gurbuz, 2022; Ferro and Catania, 2023).

CONCLUSION AND RECOMMENDATIONS

This study explores the feasibility and potential of Agriculture 4.0 technologies in the viticulture sector, assessing both the current situation and future perspectives. Agriculture 4.0 offers a range of technologies such as sensors, drones, and AI-supported decision-making systems, bringing significant transformation and innovation to viticulture.

The research delves into how these technologies are being utilized in viticulture. Innovations like Geographic Information Systems (GIS), sensor technologies, drone applications, and AI-enhanced decision-making tools optimize viticulture processes and improve efficiency. Precision farming practices and data-driven management systems, in particular, increase the success of grape cultivation and promote sustainability.

Additionally, the availability of these technologies in the viticulture sector has helped to identify the strengths and weaknesses of Agriculture 4.0. While weaknesses such as the lack of digital literacy among farmers were highlighted, strong points, like the presence of organizations offering smart farming technologies, were also underscored.

In conclusion, Agriculture 4.0 technologies have substantial potential to enhance efficiency, optimize resource use, and promote sustainable production in viticulture. Through the application of these technologies, vineyards can be managed more effectively, product quality can be improved, and environmental impacts can be minimized. However, challenges such as access to technology, training, and infrastructure remain, and various measures should be taken to overcome them.

In summary, Agriculture 4.0 is characterized by the digitalization and automation of farming processes, focusing on improving efficiency through technological innovation. However, with the shift toward Agriculture 5.0—a model that emphasizes human-machine collaboration, personalized production, and sustainability—the disadvantages identified within Agriculture 4.0 can be minimized. Agriculture 5.0, which values societal and environmental concerns, can be seen as the next step beyond Agriculture 4.0, enabling solutions that are more aligned with human and environmental harmony in the future of farming.

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Bölüm 6

FAUNISTIC AND FLORISTIC DIVERSITY AROUND THE MARBLE QUARRIES IN THE VICINITY OF LAKE YARIŞLI (BURDUR, TÜRKİYE)

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INTRODUCTION

Türkiye is located at the crossroads of three floristic regions and continents, and has an undulated topography enabling different microclimatic features, hence relatively high biological diversity and richness levels are recorded for various organism groups.

Within the Mediterranean ecoregion borders, Burdur Province lies in the southwestern corner of the country within Lakes Region (named after the lakes occupying tectonic depressions to the north of Taurus Mountains) and exhibits a transient climatic character between Mediterranean and continental climates (9). Due to climatic factors and anthropogenic pressure, almost all lakes in Burdur area are in a drying up process (18-19), some of which like Lake Akgöl have become seasonal lakes. Surface levels of the spring fed alkaline lake Yarışlı varies greatly though the year due to changes in the evaporation-rainfall balance (2). Being among the smallest natural lakes of Burdur (1), it is hence more vulnerable to anthropogenic factors like pollution, land erosion and habitat destruction.

Marble mining activity in Burdur province has developed significantly since early 90s, bringing Burdur the top rank nationwide in marble and travertine production (21). Currently, more than half of the licensed quarries are concentrated in small area between lakes Burdur and Yarışlı, two different marble types produced around the latter is commercially favored due to appearence and quality (20). Therefore, Lake Yarışlı is amongst the most suitable locations to monitor biotic and other environmental impacts of marble mining.

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Marble mining industry causes a variety of environmental problems such as pollution, erosion, habitat fragmention and biodiversity loss (5). Open mining practices widely applied as well in Burdur, depending on the scale and size of the area, involve clearing top soil and plant cover (5). Furthermore, increased noise pollution and human traffic levels cause spatiotemporal avoidance and behavioral shifts among wildlife.

Biodiversity loss has now become a global problem, with the rise in the global population and destruction of natural or protected areas in quality and quantity. To understand and minimize the effects of various activities on the biodiversity, monitoring studies and inventories should be performed around protected areas. Following study aims to present findings of a brief faunal and floral survey of the close proximity of a marble mining area.

STUDY AREA

Vertebrate fauna and flora of a mining area to the south of Lake Yarışlı (Yeşilova, Burdur) were investigated in 2013. Lake Yarışlı is an alkaline lake situated in a tectonic depression forming an endorrheic (closed) basin, fed by karstic springs and Gençali stream (only flowing in winters) (22) and having greaty changing lake levels within a year (1,21-22), from a full extent during winters to almost the drying point in some summers.

To the east and south, there is a steep valley divides between the site and rocky mountain slopes, while to west there are low hills covered with dense maquis shrubland and in the north and npprtheast direction lies the transformed agricultural land below the site.

METHODOLOGY

To sample from as different habitat pieces as possible, 8 collection spots were selected on the way to a random mining area and peripherial areas. The plant specimens lacking flower, fruit, or leaves bearing characters that can be used for identification due to grazing or season, also the plants growing outside the periphery of mining area or the road are not taken into consideration.

The faunal inventory is based on interviews with local people, direct observations, and additional material (feathers, traces, sound recording, scat analysis etc.) from likewise immediate surroundings of the study area.

VEGETATION AND FLORA

Limestone rocks (Triassic Dutdere formation) covering much of the area provide a variety of habitats with rich flora. A homogenous secondary forest dominated by a single species kermes oak (*Quercus coccifera* L.) characterize the study area. Even though grazing risk poses a lesser threat in the active mining areas, effects of overgrazing below the study area and pastures in the forest clearings around it are clearly seen.

Less elevated areas surrounding Lake Yarışlı have been transformed into cultivated land. Forest is the dominant vegetation type in the elevated terrain where the marble quarries are located, formed mainly by the kermes oak (*Quercus coccifera* L.), along with sparse groups of juniper (*Juniperus excelsa* Bieb.) and interspersed cade (*Juniperus oxycedrus* L. subsp. *oxycedrus*).

Clearings along the road to the site from the main road junction has a bushy maquis type vegetation mixed with the kermes oak. Western Anatolian endemic *Crocus biflorus* subsp. *crewei* found in this habitat is a new record for the region.

Slopes to the southern and eastern directions is covered with a sparse *Juniperus excelsa* forest. The two juniper species can be encountered individually amongst the degraded shrubland even close to the mining area. The mining area itself is devoid of any vegetation growth, while leaves and stems of surrounding vegetation is observed to be covered with a layer of dust.

In pastures and clearings formed within maquis, several shrub or bush species exemplified by *Berberis crataegina*, *Verbascum* sp., *Alhagi pseudalhagi* and seldom *Picnomon acarna* can be seen.

Rock vegetation is common and represented by species like *Astragalus angustifolius* subsp. *pungens*, *Cyclamen coum*, and *Sedum album*.

According to the findings of our excursions, 34 seed plant taxa and 1 fern species (*Asplenium ceterach* L.) have been determined, none of which are threatened according to Red Data Book of Turkish Plants (8).

Table 1. The seed plant taxa determined from the study area.

	Latin name
1	*Juniperus oxycedrus* L. subsp. *oxycedrus*
2	*Juniperus excelsa* Bieb.
3	*Berberis crataegina* DC.
4	*Alyssum linifolium* Steph. ex Willd. var. *linifolium* Steph. ex Willd.
5	*Linum tenuifolium* L.
6	*Erodium cicutarium* (L.) L Herit.
7	*Alhagi pseudalhagi* (M. Bieb.) Desv.
8	*Astragalus angustifolius* Lam. subsp. *pungens* (Willd.) Hayek
9	*Sedum album* L.
10	*Sanguisorba minor* Scop. subsp. *minor*
11	*Scabiosa argentea* L.
12	*Scorzonera cana* (C.A. Mey.) Hoffm. var. *jacquiniana* (W. Koch) Chamb.
13	*Crupina vulgaris* Cass.
14	*Cichorium intybus* L.
15	*Picnomon acarna* (L.) Cass.
16	*Cyclamen coum* Mill. var. *coum*
17	*Verbascum* sp.
18	*Salvia viridis* L.
19	*Teucrium polium* L.
20	*Phlomis bourgaei* Boiss.
21	*Sideritis libanotica* Labill. subsp. *linearis* (Bentham) Bornm
22	*Lamium amplexicaule* L.
23	*Acantholimon acerosum* (Willd.) Boiss. Var. *acerosum*
24	*Origanum onites* L.
25	*Euphorbia rigida* Bieb.
26	*Quercus coccifera* L.
27	*Ornithogalum comosum* L.
28	*Crocus biflorus* Miller subsp. *crewei* (Hooker Fil.) Mathew
29	*Brachypodium pinnatum* (L.) P. Beauv.
30	*Bromus hordeaceus* L. subsp. *hordeaceus*
31	*Cynodon dactylon* (L.) Pers.
32	*Festuca arundinacea* Schreber subsp. *arundinacea*
33	*Catapodium rigidum* (L.) C. E. Hubbard
34	*Poa annua* L.

A flora listing of 350 plant taxa from 72 families has been compiled from the lake basin proper (2), which gives *Verbascum dudleyanum*, *Bolanthus minuartioides*, *Hedysarum pestalozzae*, *Ballota nigra* subsp. *anatolica*, *Micromeria cristata* subsp. *cristata* as the endemic plant taxa encountered in the lake environs. Southern shores of the lake are characterized with a halophytic vegetatition dominated by Juncus *heldreichianus* subsp. *orientalis* (2). Aquatic flora was reported as rich by Özçelik et al. (14). Common reed (*Phragmites australis*) and southern cattail (*Typha domingensis*) are found in groups on the shores of the lake (14).

There is no previous direct flora study of marble mining areas in Lake Yarışlı and other similar areas. Our relatively smaller list is devoid of aquatic and mesic species, and restricted to a much smaller area. Even though it has been reported that marble mines threaten local plants especially narrow endemic species (14), to understand direct or indirect effects on the flora which shows some consistency in the area according to our observation future studies are needed.

OVERVIEW OF THE VERTEBRATE FAUNA

Rather uniform rocky and shrubby character of study site provides a limited habitat diversity. Constant noise during the day time radiated from densely spaced quarries, network of busy roads surrounding them, and the limited food availability are the limiting factors to the foraging behavior and daily activity of animals as direct observations could only be made at a distance to quarry site.

According to our findings 1 amphibian, 6 reptile, 28 bird and 5 mammal (sub) species inhabit the study area. The species numbers are highly likely to increase with inclusion of more sites, increasing the radius of study or number of visits to the site.

AMPHIBIANS AND REPTILES

The only amphibian species in the fully terrestrial habitat is the common variable toad. Unlike birds and mammals, avoiding the study site during day time, reptiles can commonly be found among the bush cover and on rocks, or under stones near the site. The blotched snake record is based on an anectodal evidence. One lizard species, *Anatololacerta ibrahimi* is endemic to Taurus range and Burdur sets the western limit of its distribution (3,10).

Table 2. The amphiban and reptile species determined from the study area.		
	Vernacular name	Latin name
1	Variable toad	*Bufotes sitibundus* (Pallas, 1771)
2	Greek tortoise	*Testudo graeca ibera* Pallas, 1814
3	Snake-eyed lizard	*Ophisops elegans macrodactylus* Berthold, 1842
4	Baran's rock lizard	*Anatololacerta ibrahimi* (Eiselt and Schmidtler, 1986)*
5	European snake-eyed skink	*Ablepharus kitaibelii* Bibron & Bory St. Vincent, 1833
6	Caspian snake	*Dolichophis caspius* (Gmelin, 1789)
7	Blotched snake	*Elaphe sauromates* (Pallas, 1811)

*Endemic

In a previous study on herpetofauna of Lakes Region (7), in addition to Baran's rock lizard and snake-eyed lizard, two species restricted to the springs connected to the lake (Beyşehir frog *Pelophylax caralitanus* Arikan, 1988 and European pond turtle *Emys orbicularis* (Linnaeus, 1758) and common species of the region (*Trachylepis aurata* (Linnaeus, 1758), *Stellagama stellio* (Linnaeus, 1758) and *Eirenis modestus* (Martin, 1838)) were found in three localities surounding the lake. In another inventory study, along with the Beyşehir frog and European pond turtle, the halotolerant dice snake (*Natrix tessellata*) was determined occur in the lake area.

In a thesis study on the ornithofauna of the lake, Dut (6) reported Beyşehir frog (as marsh frog) and European pond turtle (as Caspian turtle), dice snake, variable toad, starred agama, snake-eyed lizard, and greek tortoise to occur in the lake area as well.

Although threatened by organic wastes (2,14), impacts of marble mining to springs feeding the lake are largely unknown. Along with the endangered Beyşehir frog and European pond turtle, two Burdur endemic fish species *Anatolichthys fontinalis* (Akşiray, 1948) and *Pseudophoxinus ninae* Freyhof and Özuluğ, 2006 inhabit these springs as well (2). As some of the newer quarries are in closer proximity of the lake, this issue should be investigated in the future.

BIRDS

The steep cliffs and agricultured lands surrounding the study site possess particular ornthologcal importance, serving as nesting and foraging grounds. Ravens and possibly ruddy shelducks nest in the distant rocks above the site. Even though diurnal passage migrants and summer visitors preferring open habitats could use the site temporarily, this seems unlikely due to poor habitat quality parameters and the noise. Most species were observed by the agricultural lands along way to the site. The doubtful record of the golden eagle, previously unrecorded from the lake area, is based on anectodal evidence of former occurrence and nesting on high cliffs above the study site.

Table 3. The bird species determined from the study area.

	Vernacular name	Latin name
1	Ruddy shelduck	*Tadorna ferruginea* (Pallas, 1764)
2	White stork	*Ciconia ciconia* (Linnaeus, 1758)
3	Golden eagle	*Aquila chrysaetos* (Linnaeus, 1758)
4	Eurasian sparrowhawk	*Accipiter nisus* (Linnaeus, 1758)
5	Long-legged buzzard	*Buteo rufinus* (Cretzschmar, 1827)
6	Eurasian kestrel	*Falco tinnunculus* Linnaeus, 1758
7	Rock dove	*Columba livia* Gmelin, 1789
8	Eurasian collared dove	*Streptopelia decaocto* (Frivaldszky, 1838)
9	Eurasian scops owl	*Otus scops* (Linnaeus, 1758)
10	Little owl	*Athene noctua* (Scopoli, 1769)
11	Eurasian hoopoe	*Upupa epops* Linnaeus, 1758
12	Red-backed shrike	*Lanius collurio* Linnaeus, 1758
13	Eurasian jay	*Garrulus glandarius* (Linnaeus, 1758)
14	Hooded crow	*Corvus cornix* Linnaeus, 1758
15	Common raven	*Corvus corax* Linnaeus, 1758
16	Coal tit	*Periparus ater* (Linnaeus, 1758)
17	Great tit	*Parus major* Linnaeus, 1758
18	Crested lark	*Galerida cristata* (Linnaeus, 1758)
19	Eurasian crag martin	*Ptyonoprogne rupestris* (Scopoli, 1769)
20	Barn swallow	*Hirundo rustica* Linnaeus, 1758
21	Lesser whitethroat	*Sylvia curruca* (Linnaeus, 1758)
22	Eastern olivaceous warbler	*Iduna pallida* (Hemprich & Ehrenberg, 1833)
23	Western rock nuthatch	*Sitta neumayer* Michahelles, 1830
24	Common blackbird	*Turdus merula* Linnaeus, 1758
25	Eurasian chaffinch	*Fringilla coelebs* Linnaeus, 1758
26	European goldfinch	*Carduelis carduelis* (Linnaeus, 1758)
27	Common linnet	*Linaria cannabina* (Linnaeus, 1758)
28	Corn bunting	*Emberiza calandra* Linnaeus, 1758

Previous studies on ornithofauna of the lake (6,16) or the lake basin (2) give varying numbers of species counts: Dut (6) reports 98 species, Öztürk and Tavuç (16) mentions 34 species, while in an inventory study combining all available literature data, a record of 141 species in total were given for the whole basin (2).

Lake Yarışlı holds important numbers of bird species like flamingo, lapwing and avocet in diffrent periods of the year. Rocky islets and cliffs to the east of the lake serve as breeding grounds for avian taxa. 141 species determined from the endorrheic lake basin, including 50 waders and 9 diurnal raptors, are composed of 52 resident, 46 wintering, 34 summer visitor ve 9 passage migrant species, 104 of which are protected under Bern convention. Wintering white headed duck (*Oxyura leucocephala* (Scopoli, 1769)) and passage migrant Egyptian vulture (*Neophron percnopterus* (Linnaeus, 1758)) are the two threatened bird species of the lake area listed as endangered (EN) according to risk categories of IUCN. The ruddy shelduck (*Tadorna ferruginea*), common shelduck (*Tadorna tadorna* (Linnaeus, 1758)) and mallard (*Anas platyrhynchos* Linnaeus, 1758) are the most frequently observed birds during the winter season. Iconic non-breeding visitor flamingo (*Phoenicopterus roseus* Pallas, 1811) can be seen across the year in varying numbers (2).

MAMMALS

It is notable to mention that the fauna list consists of highly adaptable small to moderate sized species (17,21). Traces, scat samples and other indirect evidence were obtained from agricultural land below the study site. Presence of the fox and weasel is based on anectodal evidence.

Tablo 4

	Vernacular name	Latin name
1	European hare	*Lepus europaeus* Pallas, 1778
2	East European vole	*Microtus mystacinus* (de Filippi, 1865)
3	Anatolian blind mole-rat	*Nannospalax xanthodon* (Nordmann, 1840)
4	Least weasel	*Mustela nivalis* Linnaeus, 1766
5	Red fox	*Vulpes vulpes* (Linnaeus, 1758)

In a thesis study on the ornithofauna of the lake, Dut (6) reported red fox, Anatolian squirrel (*Sciurus anomalus anomalus* (Gmelin, 1778)) (as red squirrel) and southern white-breasted hedhehog (*Erinaceus concolor* Martin, 1838) (as European hedhehog) to occur in the lake area.

A previous study based on the phototrap method records 12 mammal species around marble sites near lakes Burdur and Yarışlı (21). Among these, six species (European hare, wild boar, Eurasian lynx, gray wolf, red fox and golden jackal) were selected as target species. European hare is noticeable with the highest detection rate and extensive use of marble sites due to lack of hunting pressure. Presence of potential prey (hare) seems to attract carnivores to marble sites which even though restrict their activity due to human activity, preferring nocturnal or crepuscular activity around the marble sites, showing lesser activity and population size in marble sites as compared to the control area. However, contrastingly, adaptive carnivore golden jackal show a markedly higher activity in human dominated areas, where it is known to adopt a scavenging lifestyle. Despite decreased hunting pressure, excessive grazing pressure observed in the marble sites, responded by lesser habitat use of marble sites by the wild boar. Habitat fragmentation and destruction seem to be the most significant impacts of marble quaaries on the mammal populations, implicated as well by virtual absence of the lynx around Lake Yarışlı even though it exists around Lake Burdur sites having a higher habitat integrity in small numbers (21).

CONCLUSION

In Burdur area, aside from the environmental aspect, human-centered adverse effects like overuse of ground water supplies, loss of aesthetic and recreational value, as well as problems concerning public and crop health due to dust cloud have been reported (11). Loss of the forest cover as an indirect effect of open mining also causes land erosion, also problems involving access to clean water, pollution, and flood control (15). However, to develop mitigation strategies and gain public support for possible restrictions, detailed documentation of impacts marble industry is required necesssarily.

Lakes in Burdur area and surrounding vegetation provide many ecosytem services, and bear cultural sgnificance for the local people (12). Under climate change and the increasing demand for natural resources, sustainable use and protection of water sources especially has become essential, yet regulations and legislations fall behind to handle the problem or fail to balance the economixal growth and sustainability.

Due to feasibility of potential quarries are understudied, the rate of leftover mining sites are fairly high in the area (20). Such areas are not replanted nor restored, as opposed to legislations (13), therefore renewability of ecosystems is not possible. Avoidance of regulations would also trigger several after-effects like land erosion and pollution.

Public perception of the lakes in Burdur has been negatively affected by the drying process (4), and seasonal drying of the lake is often viewed as complete drying, which in turn is misconceived and associated wit uselessness. Therefore, to protect the lake biodiversity, education of Burdur people is of critical importance.

We expect our findings will contribute to the future biodiversity and habitat management studies in the Burdur area.

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