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Legal Responsibility

From The Editor;

Dear Readers and Authors,

As "International Journal of Science Letters (IJSL)", we are pleased and honored to present the first issue of 2021. IJSL, is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, we are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in this issue of International Journal of Science Letters.

24.02.2021

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Research Article

Investigation of the dyeing properties of wool fabrics with *Alkanna tinctoria* root extract

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Abstract

In this work, dyeing properties of wool samples with *Alkanna tinctorum* root extract was investigated. For this purpose, two different dyestuff extracts, red and blue are gained from its roots with acetone and ethanol solvents, as well. Wool fabrics (194 pieces) dyed with $AIK(SO_4)\cdot 12H_2O$, $FeSO_4\cdot 7H_2O$, $CuSO_4\cdot 5H_2O$, $AgNO_3$ and $CoCI_2\cdot 6H_2O$ mordants at pH 2, 4, 6 and 8, respectively. Unmordanting, pre-mordanting, meta-mordanting, and post-mordanting methods were used for the dyeing experiments. In addition, wool fabrics were pretreated with artificial animal urine system (AAUS) including NH_3 (3%, v/v), CaC_2O_4 (3%, m/v) and urea (3%, m/v) before dyeing processes in order to see the improvement in the fastness properties of the dyed samples with meta-mordanting method. The color codes were determined with Pantone Color Guide, and also washing-, rubbing fastness levels were evaluated using gray scale. According to the evaluations, it is observed that AAUS mordant system helps to improve the light fastness values in general. Good results were obtained both for the dyeings with red and blue components, as well. Optimum dyeing parameters were also evaluated for wool fabric in terms of usage in textile industry.

Article History

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Alkanna tinctorum,
Artificial animal urine system,
Dyeing,
Fastness,
Mordant,
Wool

1. Introduction

Dyes have a wide range of usage including textile, paper, cosmetic, food, pharmaceutical and leather industry (Kamat and Alat, 1990; Samanta and Agarwal, 2009). The discharge of non-biodegradable colored effluents of textile-dyeing causes water pollution which is regarded as one of the main environmental problems today (Glover, 1995). Because of the synthetic effluents that threaten human life, many researchers start to look for eco-friendly products. Hence, the importance and usage of natural dyes are increasing day by day around the World (Smith and Wagner, 1991). Using of mordant is important in the dyeing of fabric. It

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provides an affinity between the dye and the fabric (Tawfik et al., 2007; Oztav, 2009). Although natural dyes have some limitations they offer more advantages such as renewable sources, minimal health hazards, mild reaction conditions, no disposal problems and harmonization with nature (Singhee, 2020). Moreover, they are non-toxic, non-allergic to skin and non-carcinogenic (Esfahani et al., 2012; Tappenier et al., 2014).

It is known that *A. tinctoria* contains alkannin and shikonin compounds in its roots which are responsible for coloration and biological activities, as well (Kourounakis et al., 2002; Rekaby et al., 2009). Shabbir et al. (2019) studied the dyeing potential of *A. tinctoria* root extract on wool yarns. Another study was carried out by Shabbir et al. (2020) including determination of color characteristics and antibacterial properties of *A. tinctoria* and other plant on woolen yarns. The dyeing performances of *A. tinctoria*, *Acacia catechu*, and *Curcuma longa* combinations were investigated on cotton and silk fabric without any additives (salt, mordant, etc.) for eco-friendly colorant resources (Jose et al., 2017). The dyeing of cotton fabric was performed by pad-steam dyeing technique using various mordants (Khattak et al., 2015).

The dyestuffs of *A. tinctoria* -alkannin and shikonin- had been detected in the root of the plant (Papageorgiou et al., 1999). Shikonin is a light red naphtoquinone pigments of *Lithospermum erythrorhizon* plant cells. Alkannin is the first pigment material to be isolated from the root of the aviary plant and is considered to be an essential element of dark red pigments. It is used in food and cosmetic industry for coloration, as well (Khattak et al., 2015).

In 1935, the extracts of shikonin and alkannin were found to be the enantiomer of each other. Alkannin and shikonin and their derivatives have been known to have antitumor activity over the past 25 years, which has prevented tumor formation in some skin cancers in cancer treatment (Zhang et al., 2018).

A. tinctorum is used in the pharmaceutical industry, in cosmetics, and in the dyeing of liquors. Furthermore, extracts prepared from *A. tinctorum* with fennel, cinnamon, rosemary, cumin, black pepper and olive oil are used in rheumatic diseases (Baytop, 1984). Its roots are used in vegetable dyeing (Kayabasi et al., 2000; Dogan et al., 2003) and also has been used in the dyeing of wool carpet yarn with some mordants (Onal, 1996).

In this research, we aimed to investigate the dyeing potential of *A. tinctoria* extract on wool fabrics under different conditions. Besides, we targeted to determine the optimum dyeing conditions for wool fabrics dyed with *A. tinctoria* root extract using both some metal salts and Article Animal Urine System (AAUS), which was developed in our research group before.

2. Materials and Methods

2.1. Reagents and Equipment

All chemicals used in this work were purchased from Merck. Distilled water was used for all steps. $FeSO_4 \cdot 7H_2O$, $AlK(SO_4)_2 \cdot 12H_2O$ and $CuSO_4 \cdot 5H_2O$ were purchased from Merck. Extraction was performed by soxhlet apparatus. The mordanting and the dyeing procedures were carried out in a Termal HT 610NHT model dyeing machine. Color codes were determined by Pantone Color Guide. The washing, rubbing (wet, dry) and light-fastness tests of all dyed samples were established according to ISO 105-C06 and to CIS, respectively, and fastness values were determined by Atlas Weather-ometer, a Launder-ometer and a 255 model crock-meter, respectively (Eser and Onal, 2015).

2.2. Dyestuff Extraction from A. tinctoria Root

Ethanol and acetone were determined as the extraction solvents for the extraction of the blue and red components from *A. tinctoria* roots according to TLC spots. For this purpose, 80 g sample was extracted with ethanol (1 L x 3) for 30 minutes, at room temperature. Same procedure was repeated with acetone, as well. Extraction of the blue component was achieved with ethanol extraction and the red component was gained in the presence of acetone. After the evaporation of the solvents, the yield of crude extracts (dye) were found as 3.6% and 2.6% for ethanol and acetone extraction, respectively. The dye bath was prepared at a 30:1 liquor ratio containing 0.5% of dye.

2.3. Dyeing Procedures

The 7x7 cm^2 of wool fabrics were dyed with the blue solution (ethanol extract) and the red solution (acetone extract), respectively. The dyeing procedures were carried out using the pre-mordanting, meta-mordanting, and post-mordanting methods with $AlK(SO_4)_2 \cdot 12H_2O$, $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $K_2Cr_2O_7$, $AgNO_3$, $CoCl_2 \cdot 6H_2O$ mordants at various pH values (pH:2, 4, 6, and 8). The effect of AAUS mordant at different pH values (pH: 2, 4, 6 and 8) was investigated in terms of high fastness values on wool fabrics.

2.3.1. Dyeing of fiber material by unmordanting method

For unmordanting dyeing of fiber material, 1 g of wool fabric sample was put into the 100 mL of dye solution. The dyeing of the wool fabric was performed at its boiling point for 1 hour. After the end of the process, the fabric was cooled, rinsed with distilled water and air-dried.

2.3.2. Dyeing of fiber material by pre-mordanting method

1 g of fiber material (wool fabric) was treated with 0.1 M, 100 mL of mordant solution for 20 minutes and then filtered. The mordanted fabric was dyed with the dye solution for 1 hour at pH 2. Then it was cooled, filtered and rinsed. The same process was repeated in the same way at pH: 4, 6, 8, respectively.

2.3.3. Dyeing of fiber material by meta-mordanting method

Both mordant (in solid state which is equivalent to 0.1 N 100 mL mordant solution) and dyestuff solution poured into a flask and the pH was adjusted. The fabric was placed into the mixture and it was dyed at its boiling point for 1 h. Finally, it was cooled, filtered and rinsed.

2.3.4. Dyeing of fiber material by post mordanting method

Plant extract (100 mL) was taken into an Erlenmeyer and pH was adjusted. The dyeing of the fabric was carried out at its boiling temperature for 1 hour. After the end of the process, the fabric was rinsed with distilled water and mordanted with mordant solution

(0.1 M 100 mL) for 20 minutes, at its boiling point. Then, it was cooled, filtered, and rinsed.

2.3.5. Dyeing of fiber material with AAUS mordant

Fiber sample (wool fabric, 1 g) kept in AAUS for 24 hours. For this purpose, meta-mordanting method was used for its time-saving and economic properties.

3. Results and Discussion

3.1. Dyeing Results with Red Component

Experimental results of 97 pieces of wool samples dyed with red component using $AlK(SO_4)_2 \cdot 12H_2O$, $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $K_2Cr_2O_7$, $AgNO_3$, $CoCl_2 \cdot 6H_2O$ and AAUS mordants according to the pre-mordanting, meta-mordanting, and post mordanting methods are given in Table 1. Generally, good fastness values were obtained in the dyeing of wool fabrics with the red component. The washing fastness was found to be moderate to outstanding (3-5) in all mordanting methods, mordants, and pH values except the dyeing with $CoCl_2 \cdot 6H_2O$, using pre-mordanting method at pH 2. It is known that washing fastness value of the dyed sample depends on the diffusion rate of the dye and concentration of the dye within the fiber (Jothi, 2008). Among the samples, the dyeings with copper mordant ($CuSO_4 \cdot 5H_2O$) using post-mordanting method and the dyeings with iron mordant ($FeSO_4 \cdot 7H_2O$) using meta-mordanting method gave the best results at all pH values. This can be explained by the coordination tendency of the dyed wool fabrics (Hou et al., 2013) whereas copper (II) and iron (II) can form complexes with the wool fiber on one site and with phenolic colorants of the extract on the other site. According to the data obtained, all the dyed wool samples showed considerable rubbing fastness degrees in both dry and wet forms while the values of the rubbing fastness ranged from 3 to 5, i.e., moderate to outstanding (Table 1). Satisfactory rubbing fastness results can be observed with iron mordant using post-mordanting method, AAUS mordant, and alum mordant ($AlK(SO_4)_2 \cdot 12H_2O$) with meta-mordanting method, as well. It is difficult to obtain high light fastness values in natural dyeing. Generally, good results were observed in the dyeing of wool fibers with the red component of *A. tincroria*. The lowest grade (3) was recorded with alum mordant. This may be due to formation of a complex with the metal salts which protects the chromophore to

minimum photolytic degradation (Ali et al., 2009). Alkannin and shikonin contain –OH groups in their chemical structures. Previous studies revealed that, two hydroxyl groups give good fastness values due to the increase or decrease of electron density through the substituent may accelerate oxidation (Ali et al., 2009; Kanchana et al., 2013). Excellent light fastness values (7) were also obtained especially with iron mordant using AAUS and post mordanting methods at acidic pH levels. Good light fastness could be easier to transfer the excitation energy from the dye molecule to the macromolecular fiber chains due to the strong dye-fiber bond. The dye–fiber bond thus provides a bridge for transferring the energy of excitation between the two components of the dye molecule and the fiber-ambient system. If this bond facilitates energy transfer, the light fastness increases (Shabbir et al., 2017). It is observed that, AAUS mordant system especially improves light fastness values. The results of the study revealed that pH of the dyeing solution changes the fastness values, but it is not a determinative factor as well (Table 1).

Table 1. Fastness values of wool samples dyed with red component

		Pre-mordanting				Meta-mordanting				Post-mordanting				AAUS			
	pH	Washing	Rubbing (dry)	Rubbing (wet)	Light	Washing	Rubbing (dry)	Rubbing (wet)	Light	Washing	Rubbing (dry)	Rubbing (wet)	Light	Washing	Rubbing (dry)	Rubbing (wet)	Light
$CuSO_4 \cdot 5H_2O$	**2**	3	4-5	3-4	4	4	5	4–5	4	5	5	5	4	4–5	5	4–5	4
	4	3	5	4	4	5	5	4–5	4	5	4–5	4–5	7	5	5	4–5	5
	6	3–4	3–4	3	5	5	5	4–5	4	5	5	4–5	4	5	5	5	4
	8	5	5	3–4	5	5	5	5	6	5	5	5	3	5	5	5	4
$FeSO_4 \cdot 7H_2O$	**2**	4–5	4–5	3–4	6	5	5	4	6	5	5	4–5	7	5	5	5	7
	4	3	5	4	4	5	5	4–5	4	5	5	4–5	7	4–5	5	5	7
	6	3–4	3–4	3	5	5	5	4–5	3	4	5	4–5	5	5	5	5	4
	8	4	5	3–4	5	5	5	4–5	5	4	5	4–5	3	5	5	5	4
$K_2Cr_2O_7$	**2**	3	5	5	4	3	5	5	4	3	5	5	4	4–5	5	5	4
	4	3	5	5	5	3	5	4–5	4	4	5	4–5	3	4	5	4–5	3
	6	5	5	4–5	3	4	4–5	4–5	3	3	5	5	3	3–4	5	3–4	3
	8	4	5	4–5	3	3	5	5	5	3–4	5	5	3	3–4	5	5	7
$AlK(SO_4)_2 \cdot 12H_2O$	**2**	3	5	4–5	3	3	5	5	3	4	5	4–5	3	3–4	5	4–5	3
	4	3–4	5	4–5	3	3	5	5	3	3-4	4–5	4	3	3–4	5	4–5	3
	6	4	5	4–5	4	4	5	5	3	4	4–5	4–5	3	3–4	5	5	3
	8	4	5	4–5	3	3	5	5	3	4	5	5	3	3–4	5	4	3
$AgNO_3$	**2**	3–4	5	4	5	3–4	4–5	4	3	4	5	4–5	6	3	4–5	4	5
	4	3	5	4	7	3–4	4–5	4	4	4	5	4–5	6	3–4	4	4	5
	6	3–4	5	4–5	5	3–4	4–5	4–5	5	3–4	4–5	4–5	5	3–4	4	3–4	5
	8	4	5	4	5	3–4	5	4	4	4	4–5	4–5	6	4–5	4–5	4	7
$CoCl_2 \cdot 6H_2O$	**2**	2–3	5	4–5	6	3	5	4–5	3	5	5	4–5	3	4	4–5	4–5	4
	4	3	5	4	5	5	5	5	3	5	5	5	3	5	5	4–5	3
	6	3–4	5	4	3	5	5	5	3	4	5	4–5	3	5	5	4–5	3
	8	5	4–5	4	5	5	5	5	4	5	5	4–5	3	4–5	5	5	5

3.2. Dyeing Results with Red Component

Wool samples (97 pieces) were dyed with blue component under similar dyeing conditions for the red component and high fastness values were gained. The fastness values of the wool samples with the blue component using the pre-mordanting, meta-mordanting, and post-mordanting methods are given in Table 2. Although, pH of the medium effects the fastness values, type of mordant is more effective than pH value on the fastness values of the dyed samples. AAUS mordant system changes fastness quality, but it is not the basic criteria especially for the dyeing with blue component (Table 2). Generally, considerable fastness values were obtained with *A. tinctoria* extracts. Washing fastness degree ranges between 3 and 5, i.e., moderate to outstanding. $K_2Cr_2O_7$ mordant gave the best washing fastness results along with meta-mordanting, post-mordanting, and AAUS mordanting systems, as well. The rubbing fastness values range between 4 and 5, i.e., very good to outstanding. Outstanding rubbing fastness values were obtained with alum (meta- and post-mordanting), copper (pre- and post-mordanting), iron (meta-mordanting), and $K_2Cr_2O_7$ (post-mordanting) mordants at all pH values. For the light fastness, fading was taken as a criterion for the determination of the light fastness values of all the dyed samples. The light fastness ranges between 3 and 7, i.e., fair to excellent. Outstanding light fastness values were gained with AAUS mordanting system with $CoCl_2 \cdot 6H_2O$ mordant.

Generally, color tones of purple and blue were obtained in the dyeing of wool fibers with the red component while blue and cream tones were gained with the blue component (Table 3). The results show that the pH values of the dye bath have considerable effects on the dyeing of wool fabrics with all extracts. The effect of the dye bath can be attributed to the relationship between the natural dye structures and the structure of the wool fibers (Mansour et al., 2020). The dyeing with $AgNO_3$ mordant gave generally brown tones. It is concluded that, mordants, mordanting methods, and pH values are effective in the color hue, as well. When all the parameters were evaluated, the most suitable dyeing conditions for wool fabric samples dyed with blue component seems the dyeing with $FeSO_4 \cdot 7H_2O$ mordant using the post-mordanting method at pH = 2, and the dyeing by using the $FeSO_4 \cdot 7H_2O$ and $K_2Cr_2O_7$ mordants with meta-mordanting method at pH = 4, as well. When the relationship between fastness and pH for wool samples were examined, it was seen that the fastness increases when the pH decrease. The optimum pH value for wool samples was determined as 2 and 4, respectively. This is due to the fact that the wool fiber is protein. Protein fibers exhibit an

amphguoteric feature. Due to its chemical structure (because it contains free amino and carboxyl groups), wool has high affinity for both mordant and dyestuff. Under acidic conditions, all the cationic groups in wool are potential sites for the attraction of negatively charged, anionic acid dyes to wool. The negative charge on wool under alkaline conditions (above pH 8) makes the fiber substantive to dyes that carry a cationic charge (basic dyes). Since natural dyes used are sparingly soluble in water, containing OH groups, they interact ionically with the protonated terminal amino groups of wool fabric at acidic pH via ion exchange reaction because of the acidic character of the OH groups (Mansour et al., 2019). Oxo chrome groups in natural dyes can be directly bound to wool. This binding is caused by electrostatic pulling of the opposite loads in wool and dyestuffs. The ionic amino groups are also released as the decomposition of carboxyl groups decreases below of the isoionic pH. These amino acids combine with acid anions to form salt.

In this salt quickly decomposed and dyestuff anion passes to instead of the acid anion. The dyestuff anions form more resistant salts with wool molecules than their acid roots. This is explained by the binding of dyes to the wool molecule not only by electrostatic gravitational forces, but also by intermolecular bonds such as hydrogen bond, dipole-dipole interaction. The formation of salt bridge bonds between the ammonium and carboxyl groups at the isoionic point enhances the staining of the wool fibers at this pH. Therefore, the wool is more suitable to be stained in acidic environment at pH below isoionic point. Our experimental work has supported this information. Dyestuffs are not preferred in the basic environment because the dye decomposition of the dyestuffs will decrease and the dyes cannot be attached to the wool.

Table 2. Fastness results of wool samples dyed with blue component

		Pre-mordanting				Meta-mordanting				Post-mordanting				AAUS			
	pH	**Washing**	**Rubbing (dry)**	**Rubbing (wet)**	**Light**	**Washing**	**Rubbing (dry)**	**Rubbing (wet)**	**Light**	**Washing**	**Rubbing (dry)**	**Rubbing (wet)**	**Light**	**Washing**	**Rubbing (dry)**	**Rubbing (wet)**	**Light**
$CuSO_4 \cdot 5H_2O$	**2**	3–4	5	5	4	5	5	5	4	5	5	5	5	4–5	5	4–5	4
	4	3	5	5	4	4–5	5	5	4	5	5	5	5	5	5	5	4
	6	5	5	5	4	5	5	5	3	5	5	5	5	5	5	5	6
	8	3–4	5	5	4	5	5	4–5	4	5	5	5	5	5	5	4–5	3
$FeSO_4 \cdot 7H_2O$	**2**	3–4	5	5	5	5	5	5	6	5	5	5	7	4–5	5	5	5
	4	4	5	4–5	6	5	5	5	7	5	5	4–5	7	5	5	4–5	6
	6	4	5	5	5	4–5	5	5	6	5	5	4–5	7	4–5	5	4–5	6
	8	5	5	5	3	5	5	5	5	4–5	5	4–5	5	5	5	4–5	7
$K_2Cr_2O_7$	**2**	3	5	5	5	5	5	5	6	5	5	5	6	5	5	5	6
	4	3–4	5	4–5	4	5	5	5	7	5	5	5	5	5	5	5	6
	6	4	5	5	4	5	5	4–5	6	5	5	5	5	5	5	4–5	4
	8	5	5	5	4	5	5	5	4	5	5	5	6	5	4–5	4	4
$AlK(SO_4)_2 \cdot 12H_2O$	**2**	3	5	4–5	3	3–4	5	5	6	4–5	5	5	5	5	5	5	3
	4	4	5	4–5	3	3–4	5	5	6	3–4	5	5	4	4	5	4–5	3
	6	4	5	5	3	3–4	5	5	4	5	5	5	4	5	5	4–5	3
	8	5	5	5	3	3–4	5	5	4	5	5	5	4	5	5	4–5	3
$AgNO_3$	**2**	3–4	5	5	5	3–4	5	4	4	3–4	4–5	4–5	4	3–4	4–5	3–4	5
	4	3–4	5	5	6	4	4	4	3	4–5	5	4	4	3–4	4	4	6
	6	3	5	4–5	5	3–4	4	4	5	3	4	4	6	3–4	4	4	7
	8	5	5	4	5	4	4–5	4–5	4	3–4	4-5	4	4	3–4	4	3–4	6
$CoCl_2 \cdot 6H_2O$	**2**	3	5	5	5	5	5	5	4	4–5	5	5	5	5–4	5	4–5	7
	4	3–4	5	5	6	5	5	4–5	4	4–5	5	4–5	4	4	5	5	7
	6	3–4	5	5	5	4	5	5	5	4	5	4–5	4	4	5	4–5	7
	8	3–4	5	5	4	3–4	5	4–5	6	4–5	5	4–5	3	5	5	4–5	7

Table 3. Colors of the dyed samples

unmordaned									
	pH	Pre-mordanting		Meta-mordanting		Post-mordanting		AAUS	
		Red comp.	Blue comp.	Red comp.	Blue comp.	Red comp.	Blue comp.	Red comp.	Blue comp.
$CuSO_4 \cdot 5H_2O$	2								
	4								
	6								
	8								
$FeSO_4 \cdot 7H_2O$	2								
	4								
	6								
	8								
$K_2Cr_2O_7$	2								
	4								
	6								

	8								
$AlK(SO_4)_2 \cdot 12H_2O$	**2**								
	4								
	6								
	8								
$AgNO_3$	**2**								
	4								
	6								
	8								
$CoCl_26H2O$	**2**								
	4								
	6								
	8								

3.3. Dyeing Mechanism

The mechanism of wool dyeing by pre-mordanting can be explained as follows. The pre-mordanting method consists of 2 steps. Firstly, the cation in the metal salt used as mordant forms a complex compound by binding on the free amino and carboxyl groups in the wool with a coordinative bond. The metal cation, which is then bonded to the wool by the coordinative bond with oxygen and nitrogen atoms, stabilizes the dye to the fibers by making a coordinative bond with the binding groups such as -OH in the dye molecule and the basic color of the wool is released after this step (Onal, 2000).

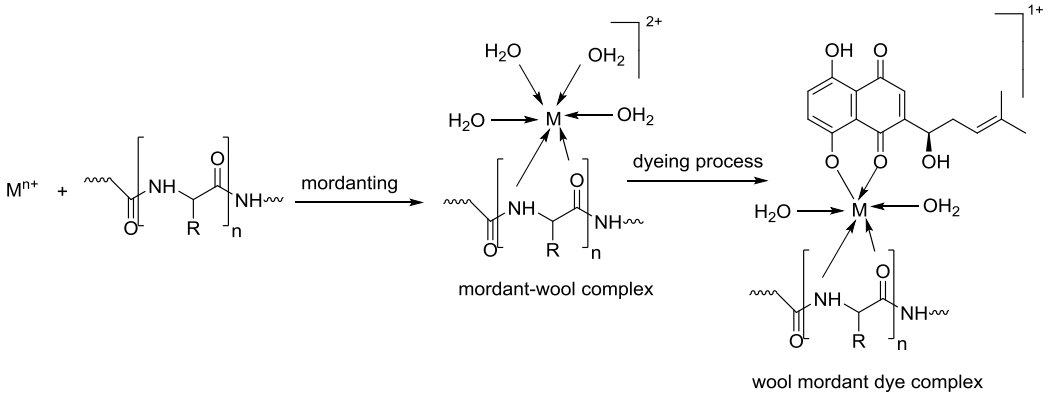

Figure 1. Dyeing mechanism of wool samples with pre-mordanting method [M: metal salt (iron mordant)]

The dyeing mechanism of dyed wool samples with pre- mordanting method is seen in Figure 2.

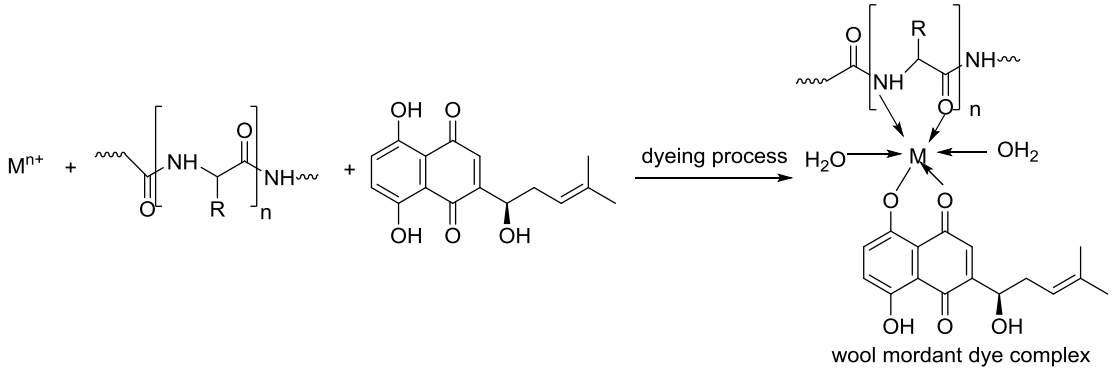

Figure 2. Dyeing mechanism of the wool samples with meta-mordanting method [M: metal salt (iron mordant)]

The dyeing of wool samples occurs one step for together mordanting method is seen in Figure 3.

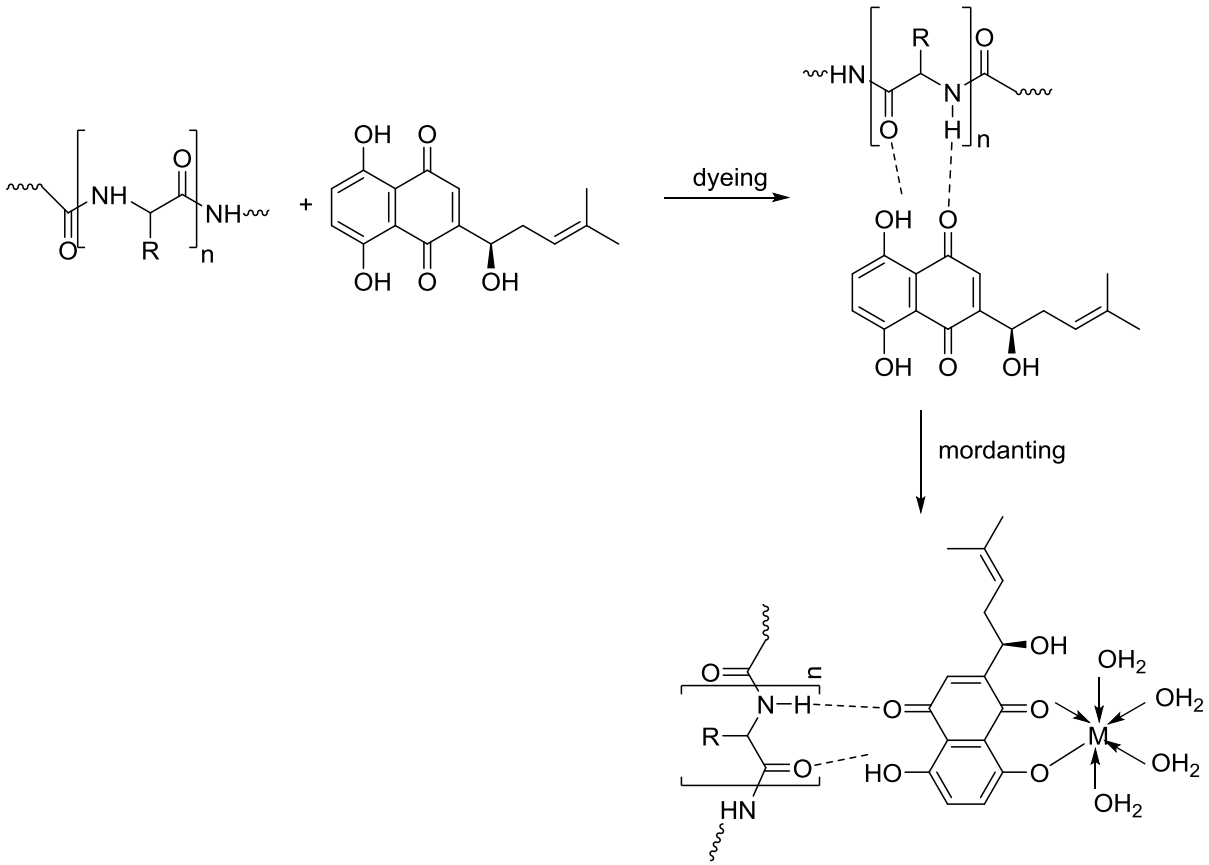

Figure 3. The dyeing mechanism for wool samples with post-mordanting method [M: metal salt (iron mordant)]

The dyeing mechanism of the wool samples with last mordanting method is carried out in two steps. The wool is first dyed with dyestuff, then is mordanted with the metal cation used as mordant. The main color of the wool (Onal, 2000) is revealed by the binding in the last step, it is seen in Figure 3.

The complexes formed by the cation in the metal salt used as mordant by binding to the amino group in the protein fiber can be bound to the dye molecule as seen in the mechanisms, as well as from the O atom in the lower -OH and carbonyl groups, but sterically it is more difficult to bind and more energy requires. Therefore, when forming mechanisms, it was assumed that the dyestuff is bound at the top of the molecule by O atoms in the OH and carbonyl groups, where the steric barrier is the least.

The ideal dyeing temperature for wool is 80 °C while the period is 45-60 minutes. In case of high temperatures for wool, the structure of keratin will be damaged, it causes wrinkle and felting in wool fibers. In this study, fiber samples were dyed in the ideal temperature range in the ideal time period as seen in the tables.

Blue, navy blue, magenta, green, brown, gray and lead color and color shades are obtained with red compound; purple, blue, navy blue, brown, pink, beige color and color tones are obtained with blue compound. The fastness analyses of dyed fabrics are high and the colors obtained are bright and vivid.

Different colors and color tones were obtained in the dyeing of wool fabric with AAUS mordant system, and fastness values were higher than others. This situation can be explained as follows. In this mordant system, ammonia opens the fiber micelle and facilitates the penetration of the dye into the fiber and oxalate stabilizes the complex formed in the environment. Urea increases the solubility of the dyestuff, causing swelling of the fiber , decreases the degree of aggregation (clustering of ions in the environment) and facilitates diffusion.

The rate of diffusion is the transfer rate to the interior of the fiber. The high diffusion ratio allows for quick set-up of the balance and rapid removal of irregularities in the adsorption of the dye. Thus, a system resistant to external influences is formed with AAUS mordant and fastness is high. In addition to this, fiber samples stained with $FeSO_4 \cdot 7H_2O$ mordant were found to have high fastness values. This can be explained by the fact that the iron metal is less than the coordination number and the iron has two different oxidation steps in its compounds.

4. Conclusion

In this study, the *Alkanna tinctoria* roots were used for the dyeing of wool fabrics. Natural dye solution was extracted and applied to the selected fabrics using pre-, meta, and post-mordanting techniques. Blue, navy blue, magenta, green, brown, gray and lead color and color shades are obtained with red compound; purple, blue, navy blue, brown, pink, beige color and color tones are generally obtained with blue compound. The fastness analyses of dyed fabrics are found high and colors are bright and vivid. Fastness of color obtained using artifical

animal urine system (AAUS) was higher than other dyed samples which are unpretreated with AAUS. We can say that the pre-treatment of fabrics with AAUS increases the fastness. Here, amonia opens the micelles of the fabric, urea inreases the solubility of dye and oxalate improves the stability of metal complex. The dyeing results of the study showed that *Alkanna tictoria* roots can be used as a natural dyestuff source in the dyeing of wool fabrics with suitable mordant.

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Research Article

Purification and characterization of protease from *Bacillus thuringiensis* isolated from soil

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Abstract

Proteases are one of the most important groups of industrial enzymes. These enzymes are used in detergent, protein, meat, leather, dairy, pharmaceutical and food industry. In this study, protease enzymes produced from *Bacillus thuringiensis* which was isolated from soil was purified. The optimum conditions of this purified enzyme were investigated. The effect of the different production environment, different pH, different temperature degrees and different metal ions of the enzyme produced from *Bacillus thuringiensis* were observed. It was seen that the highest protease activity was at 55°C and at pH-7.

Article History

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Keywords

Bacillus thuringiensis,
Protease,
Thermophilic enzyme

1. Introduction

Proteases are enzymes with a molecular weight of 18-90 kDa and have a wide range of applications, especially in the food, detergent, textile, leather, pharmaceutical and chemical industries. Approximately 60% of the industrial protease used in the world is obtained by bacteria (Kobata et al., 1972; Ferrero et al., 1996). Proteases are usually classified according to the optimum pHs which are acidic, neutral and alkaline. A lot of studies were carried on neutral protease. Proteases are not only industrial but also the elucidation of mechanisms involved in thermostability of enzymes (Imanaka et al., 1986; Helmann, 1995). These enzymes are divided into 3 groups as intracellular protease (in cell), periplasmic (with cell wall) and extracellular protease (in medium) (Kohlmann et al., 1991; El-Safey and Abdul-Rauf, 2004; Nascimento et al., 2004). While intracellular proteases are vital in cellular and

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metabolic activities, extracellular proteases are important for obtaining hydrolytic products of proteins in commercial processes (Kalisz, 1988; Kumar and Tagaki, 1999; Gupta et al., 2002). Recently, both extracellular and intracellular protease enzymes have been investigated for industries (Brar et al., 2007).

The protease enzyme derived from *Bacillus thuringiensis*, which is considered to be an excellent source of protease enzymes, is vital in a wide variety of biological processes such as cell cycle regulation, sporulation, and protoxin activation of biopesticides (Suresh Kumar and Venkateswerlu, 1998; Reddy and Venkateswerlu, 2002). Proteases have appealed the interests of many researchers for a long time and been extensively studied for production from different sources for their cellular role, downstream processing and characterization (Rao et al., 1998; Gupta et al., 2002). However, *Bacillus thuringiensis* have been studied in literature focused for only their role insecticidal activity. In this study, protease enzyme was purified from *Bacillus thuringiensis* isolated from soil and characterized. Then, the studies were carried out to figure out the best research conditions of the enzyme in different mediums, at optimum temperatures, at different pH and in the effect of different metal ions and inhibitors.

2. Materials and Methods

2.1. Isolation of Microorganisms

The soil samples taken from different fruit gardens in Amasya were inoculated in nutrient broth. Inoculated samples were cultivated at 37^{o}C by shaking at 120 rpm for 24 h. The cultures were diluted with physiological saline water and transferred into nutrient agar. Single colony was transferred into skim milk agar for screening protease activity. Protease positive single colony was inoculated into LB medium, which contains peptone from casein 10 g/L, yeast extract 5 g/L and sodium chloride 10 g/L and incubated at 25^{o}C, 130 rpm for 24 h. After the incubation, concentration of bacteria was adjusted OD_{600}:0.3 in %15 glycerol stock solution and storaged at -20^{o}C. 16S rRNA sequence analysis were performed with 27F and 1492R primer set.

2.2. Growth Conditions and Enzyme Sources

Different growth mediums were used for the cultivation of microorganisms. These mediums are Schaeffer's medium (SG) (containing nutrient broth (difco), 16 g per liter; $MgSO_4.7H_2O$ 0.5 g per liter; KCl 2.0 g per liter; 10^{-3} M $Ca(NO_3)_2$, 10^{-4} M $MnCl_2$, 10^{-6} M $FeSO_4$ and glucose 0.1%), 2X SG medium, nutrient broth and tryptose broth (pepton 10 g per liter; NaCl 5 g per liter). Then, inoculated culture (1%) was carried into the 1000 ml shaking flask containing 500 ml medium at 25°C and 150 rpm for 12, 24, 36, 48, 60 hours on Innova R 40 shaking incubator. The culture was centrifuged at 14.000 rpm for 7 min at 4°C. The culture supernatant was used as an extracellular enzyme source. For intracellular enzyme source pellet was washed three times with 50 mM Tris-HCl buffer (pH-8) and then pellet was grinded with alümina in porcelain muller. Extract was dissolved in 25 ml of 50 mM Tris-HCl buffer (pH-8) and was centrifuged at 14.000 rpm for 7 min at 4°C. Supernatant was used for intracellular enzyme source.

2.3. Protease Activity

Protease activity was measured by hydrolysis of casein (1% w/v) pH-8 at 37°C in shaking water bath during 10 min. Reaction was stopped with TCA 5%. After centrifugation 14.000 rpm for 7 min. at 4°C, supernatant was filtered by 0,45 μm filter (Johnvesly and Naik, 2001). Absorbance of supernatant was measured at 280 nm.

2.4. Determination of Protein Content

The protein content of samples was estimated by the methods (Lowry et al., 1951) using bovine serum albumin as the standart.

2.5. Purification of Protease

2.5.1. Ammonium sulphate precipitation and dialysis

Proteins were precipitated between 0-20%, 20-45% and 45-70% with ammonium sulphate saturation. Then protein fractions were dissolved in 2.5 ml 50 mM Tris-HCl buffer (pH-8) and dialyzed against same buffer.

2.5.2. DEAE cellulose column chromatography

The dialysate was put on a jacket column (1.5x20 cm) of DEAE-cellulose pre-equilibrated with Tris-HCl buffer (pH-8). Column was washed with 80 ml Tris-HCl buffer (pH-8) and then protein fractions were pooled by elution buffer (80 ml) containing 0.05 M, 0.1 M, 0.2 M and 0.4 M NaCl ($1ml^{-1}$).

2.5.3. Sephadex G-100 gel filtration

The active fractions pooled in DEAE cellulose column were selected and put on a jacket column (1x45 cm) of Sephadex G-100 pre-equilibrated with 50 mM Tris-HCl buffer (pH-8) ($0.5\ ml^{-1}$).

2.6. Characterization of Protease

Molecular weight of purified protein was determined with SDS PAGE (Figure 3) (Laemmli, 1970). Zymogram Analysis was executed in PAGE and the staining was done with Commassie brilliant blue-R250 (Figure 4). For thermostability testing, the purified protein was incubated at 25, 35, 45, 55, 65 and 75°C for 30 min in 50mM Tris-HCl buffer (pH-8) and 1% casein. For determination of optimum pH, the purified protein was incubated at 6 and pH-7 sodium phosphate buffer, 8 and pH-9 Tris-HCl buffer, 10 and pH-11 in glycine NaOH buffer and 1% casein for 30 min. The effects of different ions on protease thermostability were studied by incubating purified protein at 37°C in 50mM Tris-HCl buffer (pH-8) containing 5 mM ion concentrations for 30 min. For determining the effect of inhibitors on protease activity, the enzymes solution was pre-incubated with 1 $mmol^{-1}$ of inhibitor such as EGTA, EDTA, PMSF, 1-10 Phenontrolin, SDS, CTAB, Triton, Tween for 30 min at 37°C in Tris buffer and then caseinolytic activity was assayed. Afterwards, the residual activities were assayed with 1% casein as the substrate at 37°C for 10 min.

3. Results

In our study, the microorganism isolated from soil samples was examined in terms of potential protease production. In addition, optimization of the environment for the maximum protease enzyme production of the bacteria and partial purification of the enzyme were carried

out. The sequence analysis of the 16S rRNA gene region of the strain was confirmed as *Bacillus thuringiensis*.

In the carried out study, the purification results of the protease from *Bacillus thuringiensis* are summarized on Table 2. After the final purification step, the protease was purified (Table 1). After grinding with alumina pellet resuspansed with 2.5 ml Tris-HCl buffer pH-8, ammonium sulphate precipitation, dialysis was performed. Then, ion exchange chromatography was done and one peak was observed (Figure 1).

Table 1. Intracellular protease activity of *Bacillus thuringiensis*

Incubation Time (h)	1. Result (U/ml)	2. Result (U/ml)	3. Result (U/ml)
12	124	177	118
24	387	400	409
36	361	343	359
48	208	211	221
60	168	160	176
72	143	157	165

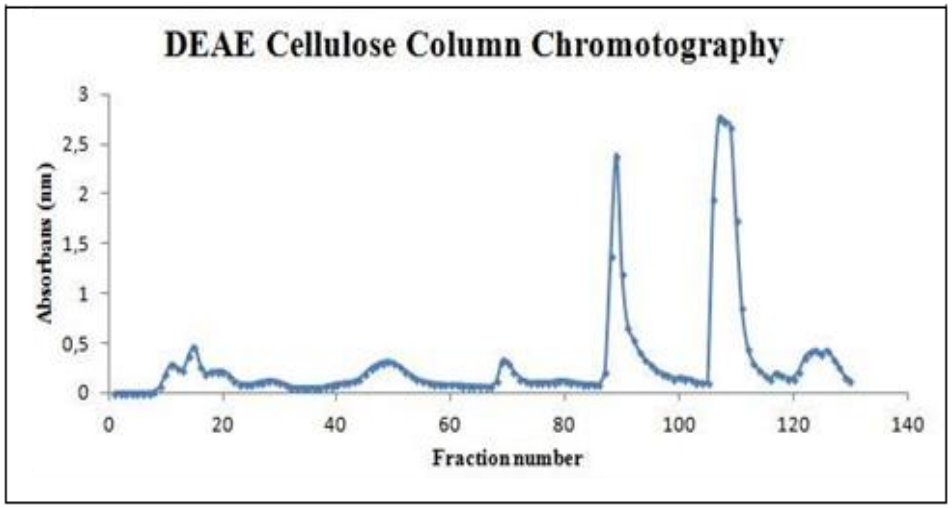

Figure 1. DEAE cellulose column chromatography

108th fraction tube was chosen for gel filtration chromatography (Figure 2) and one type notrale intracellular protease was purified from *Bacillus thuringiensis*. Specific activity and purification fold values of fractions were given in Table 2.

Table 2. Protease purification results

	Activity Absorbance (nm)	**Activity**	**Protein Content**	**Specific Activity**	**Purification Fold**
Crude	0,711	547	13,42	40,6	1
Ammonium Sulphate (0-20%)	0,463	356	5,39	66,09	1,62
Ammonium Sulphate (20-45%)	0,71	546	17,39	31,41	0,77
Ammonium Sulphate (45-70%)	2,14	1646	27,5	59,86	1,47
Dialysis	1,06	815	15,75	51,74	1,27
Ion Exchange (89^{th} fraction)	0,059	45,4	1,49	30,47	0,75
Ion Exchange (107^{th} fraction)	0,44	338,5	0,9	376,11	9,26
Gel Filtration(6^{th} fraction)	0,095	73,08	0,09	812	20

The characterization study was done on the partially purified product obtained after the ammonium sulphate step. The purified enzyme was screened with SDS PAGE (Figure 3) and zymograme analysis was done (Figure 4). Zymography was performed in conjunction with SDS PAGE. Finally, the gel attained with Coomassie Brillant Blue R-250 for zymogram analysis. The development of clear zones on the blue background indicated the presence of the activity.

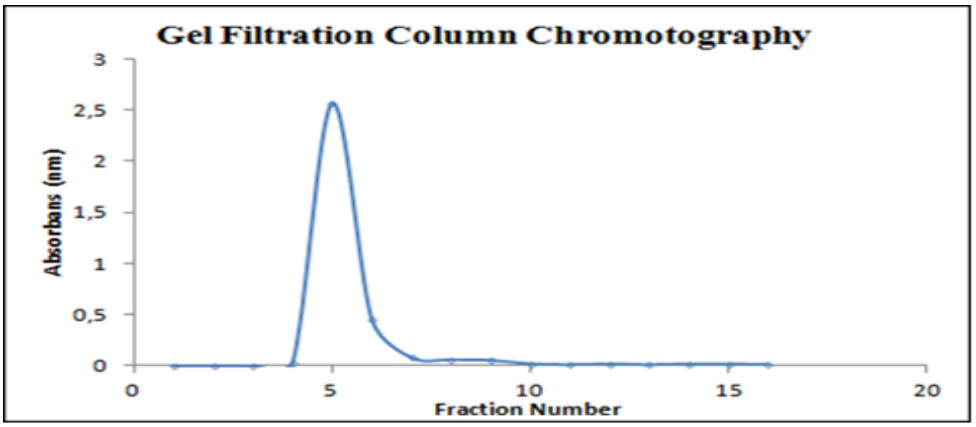

Figure 2. Gel filtration column chromatography

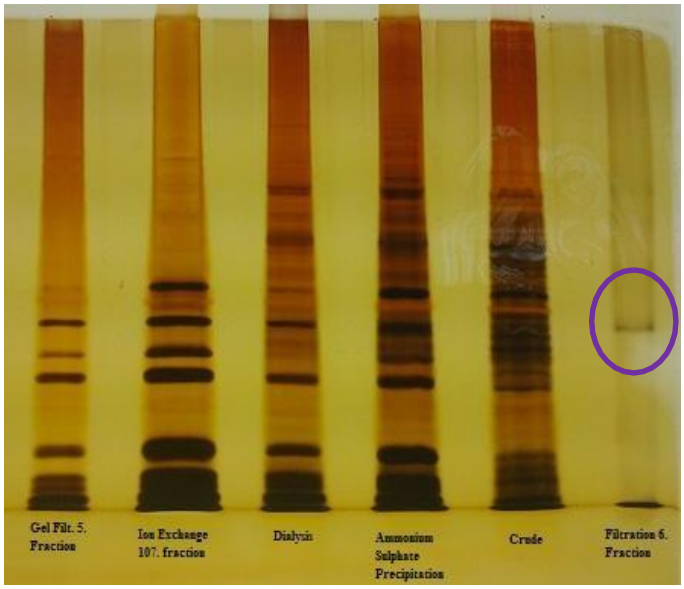

Figure 3. SDS PAGE purification steps

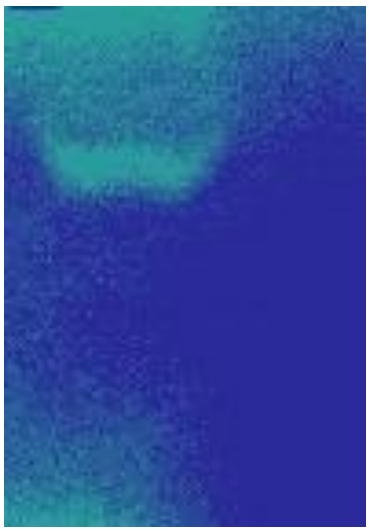

Figure 4. The enzyme zymogram image

The effect of temperature on the activity of B.thuringiensis protease was examined. It is found out that the maximum activities of the enzyme was at 55°C when its activity was controlled and the enzyme was incubated between 25-75°C in 30 minutes (Figure 5). Thus, it can be classified as thermophilic protease.

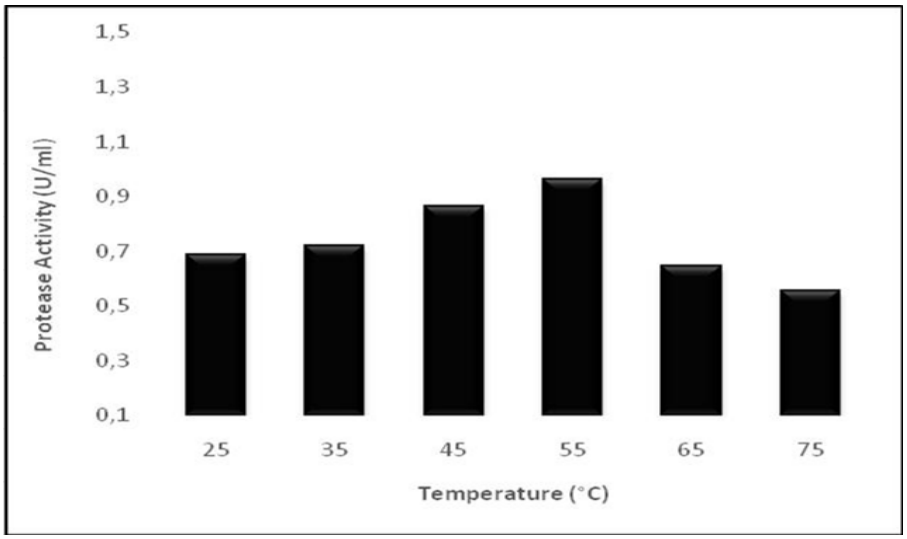

Figure 5. Effect of temperature on protease activity

The effect of pH on protease activity of the purified enzyme was studied at various pH values (pH 5-11). Purified enzyme was showed maximum activity at pH 7 (Figure 6).

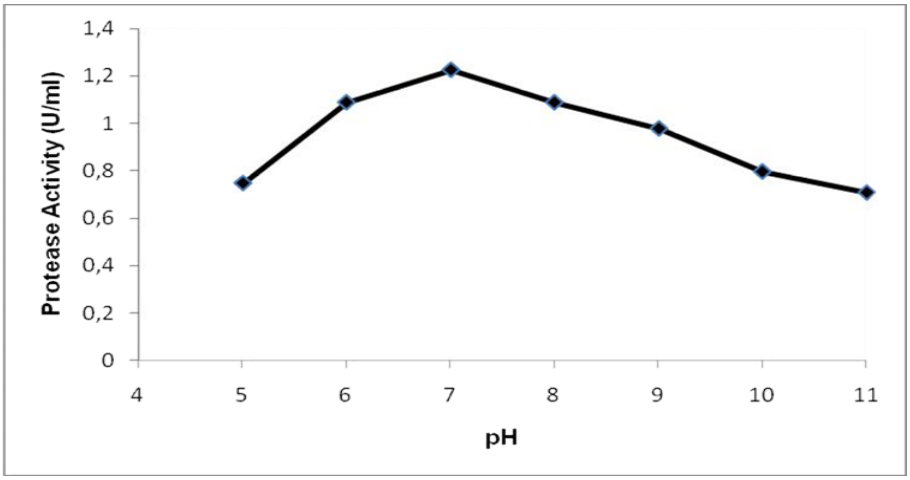

Figure 6. Effect of pH on protease activity

The effect of various enzyme inhibitors were also investigated (Table 3). It was seen that 1-10 phenanthroline, Tween and SDS were effective inhibitors on the enzyme activity.

Table 3. Effect of inhibitors on protease activities

Additives (5mM)	**Relativeactivity (%)**
Distile water (Control)	100
Methanol (Control)	100
CTAB	105,7411
EDTA	94,88518
1-10 Phenontrolin	79,18455
PMSF	97,2103
Triton X-100	104,6973
Tween	85,38622
SDS	82,3

The effects of various metal ions were observed on the activity of protease at a concentration of 5 mM (Table 4). Most of the metal ions did not effect the activity or decreased the enzyme activity slightly while Mg^{2+} inhibited the activity at the ratio of 21%. Five different isolates were obtained from soil samples. Protease activity was observed only in a single isolate onto skim milk agar. In this part of this study, four different growth mediums were used. The results showed that maximum protease activity was observed in SG medium at 37°C for 48 and 60 h (Figure 7).

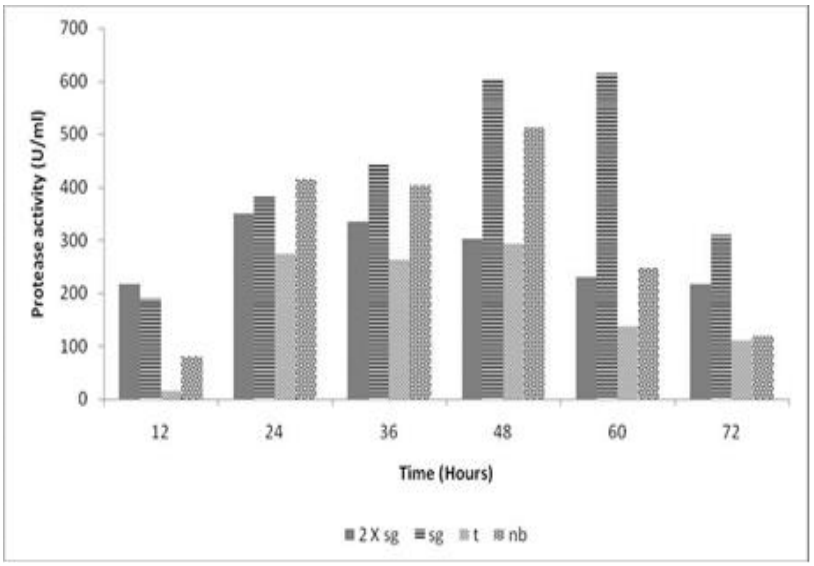

Figure 7. Protease activity in different broths

The effect of different metal ions were also investigated (Table 4). It was seen that Mg^{2+} effective inhibitors on the enzyme activity.

Table 4. The effect of metal ions on *Bacillus thuringiensis* protease activities

Metal ions (5mM)	**Relative activity (%)**
Cu^{2+}	95
Mn^{2+}	87
K^{+}	86
Hg^{2+}	89
Fe^{3+}	101
Na^{+}	95
Mg^{2+}	79
Ca^{2+}	97
Zn^{2+}	94
Control	100

4. Discussion

Metabolic enzymes that have vital tasks in the cell are molecules in the protein structure that catalyses biochemical reactions and even they enter into our daily and economic life for various purposes. Enzymes used in almost all areas of the industry are generally derived from microorganisms. Enzymes produced from microorganisms have some advantages such as they have high catalytic activities, not to produce undesired side products, more stable and cheaper and they can be produced more comparing to the enzymes produced from plants and animals. These microorganisms are chosen not only for their ability to produce the enzyme but also according not to be non-toxic and pathogenic microorganisms (Eren et al., 2006). Time course for the production of protease by *B. thuringiensis* was studied at 24 h. Similar results reported (Haddar et al., 2009; Shivanand and Jayaraman, 2009; Bekler et al., 2015; Ahmetoglu et al., 2015) the optimum protease production at 24 h. Other study on the optimization of pH and temperature for production of maximum enzymes as well as thermostability, pH stability, effect of activators and inhibitors on the proteases (Kaur and Pandy, 2009). Our study also includes optimization factors such as pH, temperature and also effect of inhibitors and activators on the activity of protease enzyme. In Figure 7, the optimum pH was 7.0. Similarly reported the optimum pH to be 7.0 for protease activity. In the same study done on enzyme activity, it is determined that different fungus displayed optimum activity on pH-7 (Muthulakshmi et al., 2011; Jellouli et al., 2011; Asker et al., 2013; Bekler et al., 2015).

As shown in Figure 8, protease was significantly activated by Fe^{3+} and Ca^{2+}. It was known that Ca^{2+} acts as an activator for proteases (Hmidet et al., 2009; Annamalai et al., 2014). An increase in the activity in the presence of Ca^{2+} can may be due to stabilisation of enzyme (Divakar et al., 2010). Similarly the protease activity was accelerated by Zn^{2+} and it was inhibited Mg^{2+} and Ca^{2+} (Samal et al., 1991).

In Figure 9, it was shown that effect of inhibitors on protease activities. Protease strongly inhibited by EDTA, SDS, Tween and 1-10 Phenontrolin (Ahmetoglu et al., 2015). In previous studies researchers found that Ca^{2+} activated and EDTA inhibited the protease activity (Wang et al., 2009; Wu et al., 2011). Enzyme was stable in the presence of triton X-100 (Wang et al., 2009). The inhibitory effect of SDS on the protease activity was confirmed the other study (Sellami-Kamoun et al., 2008). Higher enzyme production was found to be at 50°C. Earlier

studies report that different species of *Bacillus cereus* (Kebabci and Cihangir, 2011) and *Bacillus licheniformis* (Bekler et al., 2015).

As a result, intracellular protease was purified from *B.thuringiensis* that was isolated from soil. After the purification process, the best research conditions of the enzyme were determined. It was found out that protease displayed the best activity in pH-7 and at 55°C.

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Research Article

Determination of Auchenorrhyncha species distributed in apple orchards in Amasya, Turkey with a new record for Turkish fauna

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Abstract

Apple is the economically most important agricultural product in Amasya. So, identifiying the potentially pest species in apple orchards is very important for agriculture and economy. Auchenorrhyncha species, known as plant pests, adversely affect the growth of plants directly or indirectly and as a result cause abnormal development, damage and even death of plant tissues and cells. In this study, it was aimed to determine the Auchenorrhyncha species which are potentially pests in apple orchards in Amasya, Turkey. 38 species belonging to the Auchenorrhyncha were determined in apple orchards in Amasya and Trypetimorpha occidentalis is recorded for the first time for the Turkish Auchenorrhyncha fauna.

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1. Introduction

Auchenorrhyncha is one of the sub-order belonging to the order Hemiptera (Insecta), which has great number of families. The functional significance of the insects belonging to this group is little known. Since they are in high numbers in tree, shrub and herb layers, the biomass of them are high in vegetation. Because of this, they are significant components of terrestrial food chain. Insects belonging to this group move by jumping. There are mottling and patterning in different colors and shapes on the body and wings. At the rest position, the wings rest on the abdomen to form a roof.

By means of their piercing-absorbent mouth structure, they feed by sucking plant sap from the intracellular, intercellular and transmission bundles of the plants. Therefore, species belonging to this group known as plant pests and affect the development of plants directly or

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indirectly. In addition, they secrete toxic substances while sucking sap from the plant, which cause abnormal development, damage, or even death of plant tissues and cells. The species belonging to the Auchenorrhyncha group are damage plants directly by feeding and laying eggs, and indirectly by blocking the conduction tissues during feeding and carrying and spreading viral and mycoplasmal diseases (Ossiannilsson, 1978). The diagnosis of Auchenorrhyncha species is usually based on morphological characters. Some of these characters are visible from the outside, and some are visible as a result of dissection (Holzinger et al., 2003). In the part after the seventh abdominal segment removed from the body, genital structures bearing important taxonomically reliable characters are aedeagus, stylus, pygofer, genital plate, pregenital sternitis in men, and the seventh pregenital sternitis and ovipositor in female. In order to clash against Auchenorrhyncha species, which are very important in economic terms, it is necessary to know the species existing in nature, the density of their population and their distributions. The detection of these species is also important in terms of Turkey's biodiversity and to demonstrate Auchenorrhyncha fauna.

The first study on Auchenorrhyncha suborder in Turkey was carried out by Fahringer (1922). Fahringer (1922) listed some species from Turkey and then Dlabola (1957, 1971a, 1971b, 1974, 1979, 1985, 1987), Lodos and Kalkandelen (1985a, 1985b, 1985c, 1986a, 1986b, 1986c, 1987a, 1987b, 1987c, 1987d, 1988), Kartal and Zeybekoğlu (1991, 1992, 1994a, 1994b, 1996, 1997) studied on Auchenorrhyncha fauna. But, there are no studies on Auchenorrhyncha species in economically important apple orchards.

In the literature, only one study was found on Auchenorrhyncha species in apple orchards in Turkey. Ayaz and Yücel (2010) reported five species belonging to Auchenorrhyncha in apple orchards in Elazığ. In addition, Bleicher, Markó and Orosz (2006) determined 109 species in two apple orchards in Hungary. Bleicher, Orosz, Cross and Markó (2010) determined 77 species in three apple orchards in England.

In this study, it was aimed to determine the Auchenorrhyncha species which are potentially pests in apple orchards in Amasya, Turkey. Apple is the economically most important agricultural product in Amasya. So, identifiying the potentially pest species in apple orchards is very important for agriculture and economy.

2. Materials and Methods

The Auchenorrhyncha species used as research materials were collected from the apple orchards selected from different localities in Amasya. Samples were collected with sweep net by hitting the trunk, branch and leaf parts of the apple trees and weeds in the orchards. Insect samples were prepared according to standart methods. In Auchenorrhyncha specimens, species diagnosis is made according to the shape and structural features of the genital organs. In the male and female insect, the end part after the seventh segment of the abdomen where the genital organs are located, was detached from the body with the help of a dissection needle in a stereo microscope. The shapes and structures of these genital parts are taxonomically reliable characters for identifiying. Each sample was examined in detail in terms of body shape, structure, genital structures, color, patterning characters under the stereomicroscope, and identified compared with the definitions given in the literature for the relevant taxa and the previously diagnosed samples.

3. Results and Discussion

As a result of the research, 38 species belonging to 8 families of the suborder Auchenorrhyncha were detemined in apple orchards of Amasya Province. These species are;

Cixiidae

Pentastira rorida (Fieber, 1876)

Material examined: 1♀, 28.07.2016, Yıkılgan Village, Amasya

Distrubioun in Turkey: Balıkesir, Edirne, Erzincan, İzmir, Manisa, Mardin, Niğde, Tokat (Önder et al., 2011).

Zoogeographical distribution: Southeast of Europe, Eastern Mediterranean and Southeast of Central Europe (Holzinger et al., 2003)

Reptalus cuspidatus (Fieber, 1876)

Material examined: 1♂1♀, 18.07.2016, Merzifon; 1♀, 08.06.2016, Ayrancı Village, Suluova; 28.06.2016, Ziyaret, Amasya.

Zoogeographical distribution: Europe and Central Asia (Holzinger et al., 2003)

Delphacidae

***Asiraca clavicornis* (Fabricius, 1794)**

Material examined: 1♂, 06.08.2016, Yenimahalle, Suluova; 1♂, 07.09.2106, Korkut, Hamamözü, Amasya.

Distrubution in Turkey: Ankara, Antalya, Aydın, Çorum, Denizli, Erzurum, İzmir, İstanbul, Konya, Kütahya, Muğla, Samsun, Sinop, Tokat, Yozgat (Lodos and Kalkandelen, 1980; Asche, 1982; Güçlü, 1996; Karavin, 2012).

Zoogeographical Distribution: Afghanistan, Albania, Armenia, Austria, Azerbaijan, Belgium, Bulgaria, China, Cyprus, Czech Republic, England, France, Georgia, Germany, Hungary, Ireland, Israel, Italy, Kazakhstan, Kyrgyzstan, Latvia, Moldavia, Morocco, Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Switzerland, Tajikistan, Tunisia, Turkmenistan, Ukraine, Uzbekistan, Yugoslavia (Nast, 1972).

***Kelisia sabulicola* Wagner, 1952**

Material examined: 1♂, 06.08.2016, Yenimahalle, Suluova, Amasya.

Distrubution in Turkey: Amasya, Çorum, Samsun, Sinop, Tokat (Karavin, 2012).

Zoogeographical Distribution: Austria, France, Germany, Greece, Hungary, Italy (Holzinger et al., 2003).

***Javesella dubia* (Kirschbaum, 1868)**

Material examined: 1♂, 06.08.2016, Ayrancı Village, Suluova, Amasya.

Distrubution in Turkey: Amasya, Ankara, Çorum, Erzurum, Ordu, Samsun, Tokat (Lodos and Kalkandelen, 1980; Asche, 1982; Güçlü, 1996; Karavin, 2012).

Zoogeographical Distribution: Austria, Azores Islands, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, England, Estonia, Finland, France, Germany, Ireland, Italy, Kazakhstan, Latvia, Moldavia, Morocco, Netherlands, Norway, Poland, Romania, Slovakia, Sweden, UkraineUzbekistan (Nast, 1972).

Laodelphax striatellus (Fallén, 1826)

Material examined: 10♂♂16♀♀, 27.06.2016, Yenimahalle, Suluova; 16♂♂24♀♀, 28.06.2016, Çekmece Orchards, Harmanağılı Village, Suluova; 9♂♂12♀♀, 28.06.2016, Ziyaret; 1♂4♀♀, 24.07.2016, Çiğdemlik Village, Merkez; 55♂♂41♀♀, 28.07.2016, Yıkılgan Village; 20♂♂26♀♀, 06.08.2016, Ayrancı Village, Suluova; 5♂♂1♀, 06.08.2016, Yenimahalle, Suluova; 3♂♂2♀♀, 10.08.2016, Dutlupınar, Gümüşhacıköy; 7♂♂2♀♀, 18.08.2016, İpekköy; 16♂♂10♀♀, 01.09.2016, Kurnaz, Suluova; 4♂♂1♀, 07.09.2016, Alıcık-Merzifon, Amasya.

Distrubution in Turkey: Adana, Adıyaman, Amasya, Ankara, Antalya, Bilecik, Bitlis, Çorum, Diyarbakır, Elazığ, Erzincan, Erzurum, Eskişehir, Iğdır, İçel, İzmir, Kahramanmaraş, Kars, Malatya, Mersin, Muğla, Niğde, Ordu, Rize, Samsun, Siirt, Sinop, Şırnak, Tokat (Dlabola, 1957; Lodos and Kalkandelen, 1980; Asche, 1982; Güçlü, 1996; Karavin, 2012; Karavin and Özgen, 2017; Gözüaçık and Özgen, 2018).

Zoogeographical Distribution: Afghanistan, Albania, Algeria, Armenia, Austria, Azerbaijan, Bulgaria, Canary Islands, China, Czech Republic, England, Estonia, Finland, France, Georgia, Germany, Hungary, Iran, Iraq, Israel, Italy, Japan, Kazakhstan, Korea, Kyrgyzstan, Latvia, Lebanon, Moldavia, Mongolia, Netherlands, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Tajikistan, Tunisia, Turkey, Ukraine, Uzbekistan, Yugoslavia (Nast, 1972).

Ribautodelphax albostriata (Fieber, 1866)

Material examined: 3♂♂1♀, 06.08.2016, Yenimahalle, Suluova; 15♂♂20♀♀, 07.09.2016, Korkut, Hamamözü, Amasya.

Distrubution in Turkey: Ankara, Erzincan, Tokat (Lodos and Kalkandelen, 1988; Karavin, 2012).

Zoogeographical Distribution: Austria, Belgium, Caucasus, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Kazakhstan, Kyrgyzstan, Moldavia, Mongolia, Netherlands, Norway, Poland, Portugal, Romania, Russia, Siberia, Slovakia, Spain, Sweden, Switzerland, Tunisia, Ukraine, Yugoslavia (Nast, 1972).

Toya propinqua (Fieber, 1866)

Material examined: 4♂♂2♀♀, 27.06.2016, Yenimahalle, Suluova; 2♀♀, 28.07.2016, Yıkılgan Village; 1♂3♀♀, 18.08.2016, İpekköy; 1♀, 07.09.2016, Alıcık-Merzifon, Amasya.

Distrubution in Turkey: Adana, Afyon, Amasya, Ankara, Antalya, Aydın, Çanakkale, Çorum, Denizli, Diyarbakır, Erzurum, Gaziantep, Hatay, Isparta, İçel, İskenderun, Kastamonu, Mardin, Muğla, Ordu, Samsun, Siirt, Sinop, Şırnak (Silopi),Tokat (Dlabola, 1957; Lodos and Kalkandelen, 1980; Asche, 1982; Güçlü, 1996; Karavin, 2012; Karavin and Özgen, 2017).

Zoogeographical Distribution: Afghanistan, Albania, Algeria, Armenia, Austria, Bulgaria, Canary Islands, Cyprus, Czech Republic, Egypt, France, Georgia, Germany, Hungary, Iran, Iraq, Israel, Italy, Japan, Jordan, Kazakhstan, Kyrgyzstan, Libya, Moldavia, Morocco, Poland, Portugal, Romania, Russia, Slovakia, Spain, Tajikistan, Tunisia, Turkey, Uzbekistan, Yugoslavia (Nast, 1972).

Dictyopharidae

Dictyophara europaea (Linné, 1767)

Material examined: 1♂2♀♀, 24.07.2016, Çiğdemlik Village, Merkez; 2♂♂2♀♀, 06.08.2016, Ayrancı Village, Suluova; 2♂♂2♀♀, 06.08.2016, Yenimahalle, Suluova, Amasya.

Distrubution in Turkey: Ankara, Denizli, Diyarbakır, Elazığ, Eskişehir, Isparta, İstanbul, İzmir, Kars, Kastamonu, Manisa, Mardin, Muğla, Muş, Ordu, Samsun, Siirt, Van (Özgen et al., 2009; Önder et al., 2011).

Zoogeographical Distribution: Afghanistan, Albania, Algeria, Armenia, Austria, Azerbaijan, Belgium, Bulgaria, China, Czech Republic, France, Georgia, Germany, Greece, Hungary, Iraq, Italy, Kazakhstan, Kyrgyzstan, Moldavia, Poland, Portugal, Romania, Russia, Slovakia, Spain, Switzerland, Tunisia, Ukraine, Uzbekistan, Yugoslavia (Nast, 1972).

Tropiduchidae

Trypetimorpha occidentalis Huang & Bourgoin, 1993

Material examined: 1♂, 28.06.2016, Çekmece Orchards, Harmanağılı Village, Suluova, Amasya.

Distrubution in Turkey: New record for the Turkish Auchenorrhyncha Fauna.

Zoogeographical Distribution: Armenia, Austria, Bulgaria, Cyprus, Czech Republic, France, Hungary, Israel, Italy, Kyrgyzstan, Moldavia, Romania, Russia, Slovakia, Ukraine (Nast, 1972).

Issidae

Agalmatium flavescens (Olivier, 1791)

Material examined: 4♂♂2♀♀, 27.06.2016, Gökçebağ Village, Merzifon, Amasya.

Distribution in Turkey: Ankara, Bursa, Çorum, İstanbul, Edirne, Kastamonu, Tekirdağ, Van, Zonguldak (Önder et al., 2011).

Zoogeographical Distribution: Albania, Algeria, Austria, Bulgaria, Cyprus, Czech Republic, France, Greece, Hungary, Italy, Jordan, Morocco, Portugal, Romania, Slovakia, Spain, Switzerland, the Canary Islands, Turkey (Nast, 1972; Önder et al., 2011).

Agalmatium bilobum (Fieber, 1877)

Material examined: 1♂, 27.06.2016, Yenimahalle, Suluova, Amasya.

Distribution in Turkey: Adıyaman, Afyonkarahisar, Amasya, Ankara, Aydın, Balıkesir, Bilecik, Burdur, Bursa, Çanakkale, Çorum, Denizli, Eskişehir, Gaziantep, Gümüşhane, İzmir, Kütahya, Malatya, Manisa, Muğla, Tekirdağ, Tokat, Uşak (Önder et al., 2011).

Zoogeographical Distribution: France, Greece, Israel, Italy, Russia, Spain, Syria, Tunisia, Turkey (Lodos and Kalkandelen, 1981).

Aphrophoridae

Aphrophora alni (Fallen, 1805)

Material examined: 1♂, 07.09.2016, Korkut, Hamamözü, Amasya.

Distribution in Turkey: Adana, Afyonkarahisar, Ankara, Artvin, Aydın, Balıkesir, Bitlis, Bolu, Çanakkale, Çorum, Diyarbakır, Erzincan, Erzurum, Giresun, İstanbul, İzmir, Kayseri, Kırklareli, Konya, Kütahya, Mardin, Manisa, Muğla, Ordu, Rize, Samsun, Sinop, Tekirdağ, Trabzon, Yozgat (Önder et al., 2011).

Zoogeographical Distribution: Albania, Algeria, Armenia, Austria, Azerbaijan, Belgium, Bulgaria, China, Czech Republic, Denmark, England, Estonia, Finland, Georgia, Germany,

Greece, Hungary, Ireland, Italy, Japan, Kazakhstan, Moldova, Mongolia, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Turkmenistan, Ukraine (Nast, 1972; Önder et al., 2011).

Lepyronia coleoptrata (Linné, 1758)

Material examined: 1♂, 27.06.2016, Yenimahalle, Suluova; 1♂, 18.07.2016, Merzifon; 1♂1♀, 10.08.2016, Dutlupınar, Gümüşhacıköy, Amasya.

Distribution in Turkey: Adana, Afyonkarahisar, Ankara, Artvin, Aydın, Bilecik, Bursa, Çanakkale, Çankırı, Çorum, Diyarbakır, Edirne, Elazığ, Erzincan, Gümüşhane, İzmir, Kahramanmaraş, Kars, Kütahya, Manisa, Mardin, Muğla, Muş, Sakarya, Siirt, Tokat (Önder et al., 2011; Özgen et al., 2018).

Zoogeographical Distribution: Afghanistan, Albania, Algeria, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iraq, Italy, Mongolia, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Syria, Turkey (Önder et al., 2011).

Philaenus spumarius (Linne, 1758)

Material examined: 1♂1♀, 27.06.2016, Gökçebağ Village, Merzifon; 1♂, 28.06.2016, Ziyaret, Amasya.

Distribution in Turkey: Ağrı, Amasya, Ankara, Artvin, Aydın, Balıkesir, Bilecik, Bitlis, Bolu, Bursa, Çanakkale, Elazığ, Erzincan, Erzurum, Eskişehir, Giresun, Gümüşhane, Hakkari, İstanbul, İzmir, Kars, Kırklareli, Kütahya, Kocaeli, Malatya, Manisa, Mardin, Muğla, Ordu, Rize, Samsun, Siirt, Sinop, Tekirdağ, Trabzon, Tokat, Van (Önder et al., 2011; Özgen et al., 2018).

Zoogeographical Distribution: Afghanistan, Albania, Algeria, Austria, Azores, Balearic Islands, Belgium, Bulgaria, China, Cyprus, Czech Republic, Denmark, England, Finland, France, Germany, Greece, Hungary, Iraq, Ireland, Italy, Japan, Mongolia, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Sweden, Switzerland, Syria, Tunisia, Turkey (Önder et al., 2011).

Membracidae

***Stictocephala bisonia* Kopp & Yonke, 1977**

Material examined: 1♂, 28.06.2016, Ziyaret; 1♂, 24.07.2016, Çiğdemlik Village; 1♂1♀, 28.07.2016, Yıkılgan Village; 1♂1♀, 06.08.2016, Yenimahalle, Suluova; 1♀, 18.08.2016, İpekköy, Amasya.

Zoogeographical Distribution: North America, Europe, Central Asia (Holzinger et al., 2003).

Cicadellidae

***Anaceratagallia laevis* (Ribaut, 1935)**

Material examined: 6♂♂6♀♀, 27.06.2016, Gökçebağ Village, Merzifon; 2♂♂, 27.06.2017, Yenimahalle, Suluova; 1♂, 28.06.2016, Çekmece Orchards, Harmanağılı Village, Suluova; 5♂♂2♀♀, 28.06.2016, Ziyaret; 11♂♂5♀♀, 24.07.2016, Çiğdemlik Village; 1♂, 28.07.2016, Yıkılgan Village; 4♂♂2♀♀, 18.08.2016, İpekköy, Amasya.

Distribution in Turkey: Adana, Ağrı, Ankara, Bilecik, Erzurum, Eskişehir, İçel, İstanbul, İzmir, Konya, Malatya, Nevşehir (Önder et al., 2011).

Zoogeographical Distribution: Afghanistan, Albania, Bulgaria, Cyprus, Egypt, France, Greece, Hungary, Iran, Iraq, Israel, Italy, Jordan, Morocco, Portugal, Romania, Russia, the Canary Islands, Turkey (Nast, 1972; Önder et al., 2011).

***Anaceratagallia ribauti* (Ossiannilsson, 1938)**

Material examined: 3♂♂, 27.06.2016, Yenimahalle, Suluova; 4♂♂, 06.08.2016, Ayrancı Village, Suluova; 4♂♂2♀♀, 10.08.2016, Merzifon; 1♂1♀, 07.09.2016, Korkut, Hamamözü, Amasya.

Distribution in Turkey: Adana, Ankara, Balıkesir, Çankırı, Elazığ, Hatay, İçel, İzmir, Malatya, Mardin, Samsun (Önder et al., 2011; Kaplan and Yücel, 2014).

Zoogeographical Distribution: England, Europe, Iran, Russia, Turkey (Önder et al., 2011).

Eupelix cuspidata (Fabricius 1775)

Material examined: 1♀, 27.06.2016, Yenimahalle, Suluova; 3♂♂2♀♀, 18.07.2016, Merzifon, Amasya.

Distribution in Turkey: Adana, Adıyaman, Afyonkarahisar, Ankara, Artvin, Çanakkale, Diyarbakır, İçel, Kahramanmaraş, Konya, Malatya, Mardin, Muğla, Nevşehir, Niğde, Şanlıurfa (Önder et al., 2011; Tolga and Yoldaş, 2019).

Zoogeographical Distribution: Algeria, Canary Islands, Cyprus, Europe, Iran, Iraq, Ireland, Israel, Mongolia, Morocco, Russia, Syria, Tunisia, Turkey (Nast, 1972; Önder et al., 2011).

Aphrodes albifrons (Linnaeus 1758)

Material examined: 1♂, 27.06.2016, Gökçebağ Village, Merzifon, Amasya.

Distribution in Turkey: İzmir, Manisa (Önder et al., 2011).

Zoogeographical Distribution: Israel, Spain, Turkey (Önder et al., 2011).

Aphrodes bicinctus (Zachvatkin 1948)

Material examined: 1♀, 27.06.2016, Yenimahalle, Suluova, Amasya.

Distribution in Turkey: All over the Turkey (Önder et al., 2011).

Zoogeographical Distribution: Afghanistan, Algeria, Britain, Cyprus, Europe, Iran, Ireland, Lebanon, Madeira Island, Mongolia, Morocco, North America, Russia, Syria, Tunisia, Turkey (Nast, 1972; Önder et al., 2011).

Cicadella viridis (Linnaeus, 1758)

Material examined: 8♂♂6♀♀, 06.08.2016, Ayrancı Village, Suluova; 7♂♂6♀♀, 18.08.2016, İpekköy, Amasya.

Distribution in Turkey: Amasya, Artvin, Balıkesir, Bursa, Çanakkale, Diyarbakır, Edirne, Erzincan, Erzurum, İzmir, Kars, Kırklareli, Manisa, Mardin, Muğla, Samsun, Tekirdağ (Önder et al., 2011).

Zoogeographical Distribution: China, Europe, Iran, Ireland, Japan, Korea, Mongolia, Russia, Turkey (Önder et al., 2011).

Empoasca decipiens **Paoli, 1930**

Material examined: 1♀, 24.07.2016, Çiğdemlik Village Köyü; 2♂♂12♀♀, 28.07.2016, Yıkılgan Village; 3♂♂11♀♀, 10.08.2016, Dutlupınar, Gümüşhacıköy, Amasya.

Distribution in Turkey: All over the Turkey (Önder et al., 2011).

Zoogeographical Distribution: Cyprus, Czech Republic, Egypt, Iran, Iraq, Italy, Jordan, Libya, Pakistan, Russia, Sardinia and Sicily Islands, Turkey (Nast, 1972; Önder et al., 2011).

Zyginidia pullula **(Boheman, 1845)**

Material examined: 4♂♂17♀♀, 28.06.2016, Çekmece Orchards, Harmanağılı Village, Suluova, Amasya.

Distribution in Turkey: Amasya, Ankara, Aydın, Bolu, Çankırı, Çorum, İzmir, İzmit, Kayseri, Manisa, Nevşehir, Sinop (Önder et al., 2011).

Zoogeographical Distribution: Europe, Iran, Kazakhstan, Moldova, Mongolia, the island of Sardinia, the North Caucasus, Turkey, Ukraine (Nast, 1972; Önder et al., 2011).

Zygina flammigera **(Fourcroy, 1785)**

Material examined: 1♂1♀, 27.06.2016, Yenimahalle, Suluova, Amasya.

Distribution in Turkey: Amasya.

Zoogeographical Distribution: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, England, Finland, France, Germany, Hungary, Ireland, Italy, Kazakhstan, Kyrgyzstan, Latvia, Moldova, Netherlands, Norway, Poland, Romania, Russia, Scotland, Slovakia, Sweden, Switzerland, Ukraine, Uzbekista (Nast, 1972).

Arboridia versuta **(Melichar, 1897)**

Material examined: 1♂4♀♀, 27.06.2016, Yenimahalle, Suluova; 2♂♂16♀♀, 24.07.2016, Çiğdemlik Village; 4♂♂58♀♀, 28.07.2016, Yıkılgan Village; 17♂♂33♀♀, 06.08.2017, Ayrancı Village, Suluova; 18♂♂35♀♀, 06.08.2016, Yenimahalle, Suluova; 30♂♂78♀♀, 10.08.2016, Dutlupınar, Gümüşhacıköy; 9♂♂14♀♀, 18.08.2016, İpekköy; 24♂♂46♀♀, 01.09.2016, Kurnaz, Suluova; 86♂♂114♀♀, Korkut, Hamamözü; 28♂♂46♀♀, 07.09.2016, Alıcık-Merzifon, Amasya.

Distribution in Turkey: Bitlis, Kars (Önder et al., 2011).

Zoogeographical Distribution: France, Germany, Italy, Russia, Siberia, Switzerland, Turkey, Ukraine (Önder et al., 2011).

Balclutha punctata (Fabricius, 1775)

Material examined: 1♂, 27.06.2016, Yenimahalle, Suluova; 1♂2♀♀, 24.07.2016, Çiğdemlik Village; 5♂♂9♀♀, 18.08.2016, İpekköy; 4♂♂10♀♀, 01.09.2016, Kurnaz, Suluova; 5♂♂6♀♀, 07.09.2016, Korkut, Hamamözü; 20♂♂24♀♀, 17.09.2016, Alıcık-Merzifon, Amasya.

Distribution in Turkey: Adana, Adıyaman, Amasya, Ankara, Bitlis, Bolu, Diyarbakır, Erzincan, Erzurum, Hakkari, Hatay, Iğdır, Isparta, İçel, Kars, Ordu, Samsun, Sinop (Özgen and Karsavuran, 2009; Önder et al., 2011).

Zoogeographical Distribution: Afghanistan, Algeria, Austria, Azerbaijan, Bulgaria, China, Denmark, Cyprus, Czech Republic, England, Estonia, Europe, Finland, France, Germany, GreeceHungary, Iran, Ireland, Italy, Japan, Kazakhstan, Korea, Kyrgyzstan, Moldova, Mongolia, Netherlands, Norway, Poland, Portugal, Russia, Sardinia, Sicily, Slovakia, Tunisia, Turkey (Nast, 1972; Önder et al., 2011).

Recilia schmidtgeni (Wagner, 1939)

Material examined: 3♂♂, 18.07.2016, Merzifon; 1♂, 10.08.2016, Dutlupınar, Gümüşhacıköy, Amasya.

Distribution in Turkey: Adana, Ankara, Diyarbakır, Erzincan, İçel, İzmir, Kırıkkale, Nevşehir, Samsun, Sinop, Şanlıurfa, Tokat (Önder et al., 2011).

Zoogeographical Distribution: Albania, Bulgaria, Crete, France, Iran, Italy, Jordan, Manchuria, Morocco, Romania, Russia, the Czech Republic, Turkey (Önder et al., 2011).

Doratura homophyla (Flor, 1861)

Material examined: 1♂1♀ 18.07.2016, Merzifon, Amasya.

Distribution in Turkey: Adana, Ankara, Bolu, Bursa, Diyarbakır, Eskişehir, Gaziantep, İzmir, Manisa, Van (Önder et al., 2011).

Zoogeographical Distribution: Albania, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Hungary, Iran, Iraq, Israel, Italy, Mongolia, Netherlands, Poland, Romania, Russia, Sweden, Turkey (Nast, 1972; Önder et al., 2011).

Doratura stylata (Boheman, 1847)

Material examined: 2♂♂, 28.07.2016, Yıkılgan Village, Amasya.

Distribution in Turkey: Orta ve Doğu Anadolu, Ankara, Bayburt, Gümüşhane, Kars, Samsun, Van (Önder et al., 2011).

Zoogeographical Distribution: Algeria, England, Europe, Russia, Tunisia, Turkey (Önder et al., 2011).

Platymetopius undatus (De Geer, 1773)

Material examined: 1♀, 10.08.2016, Dutlupınar, Gümüşhacıköy, Amasya.

Distribution in Turkey: Adana, Ankara, Diyarbakır, İzmit, Mardin (Önder et al., 2011).

Zoogeographical Distribution: England, Europe, Israel, Korea, Russia, Sicily, Tunisia, Turkey (Önder et al., 2011).

Phlepsius intricatus (Herrich-Schäffer 1838)

Material examined: 2♂♂1♀, 27.06.2016, Gökçebağ Village, Merzifon; 1♂1♀, 27.06.2016, Yenimahalle, Suluova; 1♂, 18.07.2016, Merzifon; 3♂♂1♀, 18.08.2016, İpekköy; 1♂, 01.09.2016, Kurnaz, Suluova; 1♂, 17.09.2016; Alıcık-Merzifon, Amasya.

Distribution in Turkey: Amasya, Bolu, Çanakkale, Diyarbakır, Elazığ, Isparta, İzmir, Kars, Kırşehir, Malatya, Mardin, Muğla, Nevşehir, Samsun (Önder et al., 2011).

Zoogeographical Distribution: Afghanistan, Albania, Austria, the Balearic Island, Bulgaria, Algeria, Czech Republic, Morocco, Palestine, France, Iraq, Iran, Spain, Italy, Cyprus, Hungary, Portugal, Romania, Russia, the island of Sardinia, Turkey, Jordan, Greece (Nast, 1972; Önder et al., 2011).

Mocydia crocea (Herrich-Schäffer, 1837)

Material examined: 1♂3♀♀, 06.08.2016, Yenimahalle, Suluova; 1♂, 10.08.2016, Dutlupınar, Gümüşhacıköy; 14♂♂15♀♀, 01.09.2016, Kurnaz, Suluova, Amasya.

Distribution in Turkey: Amasya, Ankara, Bolu, Bursa, Denizli, Giresun, İçel, Konya, Manisa, Nevşehir, Ordu, Sakarya, Samsun, Sinop, Tokat, Trabzon (Önder et al., 2011).

Zoogeographical Distribution: Albania, Algeria, Austria, Belgium, Bulgaria, Cyprus, Czech Republic, England, France, Germany, Hungary, Ireland, Italy, Jordan, Poland, Portugal, Romania, Russia, Switzerland, Turkey (Önder et al., 2011).

Euscelis lineolatus (Brullé, 1832)

Material examined: 73♂♂100♀♀, 27.06.2016, Gökçebağ Village, Merzifon; 3♂♂4♀♀, 27.06.2016, Yenimahalle, Suluova; 1♀, 28.06.2016, Ziyaret; 30♂♂60♀♀, 18.07.2016, Merzifon; 1♀, 24.07.2016, Çiğdemlik Village; 6♂♂1♀,10.08.2016, Dutlupınar, Gümüşhacıköy, Amasya.

Distribution in Turkey: Amasya, Ankara, Artvin, Balıkesir, Bursa, Denizli, İstanbul, İzmir, Kırıkkale, Kırklareli, Kırşehir, Konya, Malatya, Manisa, Niğde, Ordu, Samsun, Sinop, Tokat, Trabzon, Uşak (Zeybekoğlu, 1991; Önder et al., 2011).

Zoogeographical Distribution: Albania, Algeria, Azerbaijan, Bulgaria, France, Greece, Hungary, Iran, Ireland, Italy, Jordan, Morocco, Portugal, Sardinia, Sicily, Spain, Switzerland, the Canary Islands, the Netherlands, Tunisia, Turkey (Nast, 1972; Önder et al., 2011).

Arocephalus longiceps (Kirschbaum, 1868)

Material examined: 33♂♂28♀♀, 07.09.2016, Korkut, Hamamözü, Amasya.

Distribution in Turkey: Amasya, Ankara, Bolu, Erzincan, Erzurum, Hakkari, İzmir, Konya, Nevşehir, Samsun, Sinop (Önder et al., 2011).

Zoogeographical Distribution: Austria, Belgium, Bulgaria, Czech Republic, Denmark, France, Germany, GreeceHungary, Italy, Mongolia, Netherlands, Poland, Romania, Russia, Spain, Switzerland, Turkey (Önder et al., 2011).

Psammotettix confinis (Dahlbom, 1850)

Material examined: 3♂♂2♀♀, 17.09.2016, Alıcık-Merzifon, Amasya.

Distribution in Turkey: Adana, Ağrı, Ankara, Balıkesir, Bitlis, Edirne, Erzincan, Erzurum, Giresun, Hakkari, Isparta, Kars, Konya, Ordu, Samsun, Siirt, Sivas, Van (Önder et al., 2011).

Zoogeographical Distribution: Austria, Bulgaria, Corsica, Czech Republic, Denmark, England, Estonia, Finland, France, Germany, Greece, Holland, Hungary, Ireland, Italy, Kazakhstan, Kyrgyzstan, Norway, Poland, Romania, Russia, Slovakia, Sweden, Switzerland, Turkey, Ukraine, Uzbekistan (Nast, 1972; Önder et al., 2011).

Psammotettix provincialis (Ribaut, 1925)

Material examined: 2♂♂3♀♀, 27.06.2016, Gökçedağ Village, Merzifon; 45♂♂56♀♀, 27.06.2016, Yenimahalle, Suluova; 8♂♂20♀♀, 28.06.2016, Çekmece Orchards, Harmanağılı Village, Suluova; 9♂♂10♀♀, 28.06.2016, Ziyaretz; 22♂♂54♀♀, 18.07.2016, Merzifon; 3♂♂2♀♀, 24.07.2016, Çiğdemlik Village; 15♂♂35♀♀, 28.07.2016, Yıkılgan Village; 4♂♂7♀♀, 06.08.2016, Ayrancı Village, Suluova; 4♂♂9♀♀, 06.08.2016, Yenimahalle, Suluova; 23♂♂25♀♀, 10.08.2016, Dutlupınar, Gümüşhacıköy; 13♂♂29♀♀, 18.08.2016, İpekköy; 4♂♂5♀♀, Kurnaz, Suluova, Amasya.

Distribution in Turkey: Adana, Ankara, Antalya, Aydın, Bolu, Bursa, Çankırı, Diyarbakır, Edirne, Erzurum, Hatay, İçel, İstanbul, İzmir, Kayseri, Konya, Manisa, Mardin, Nevşehir, Sakarya, Samsun, Van (Önder et al., 2011).

Zoogeographical Distribution: Afghanistan, Bulgaria, China, Cyprus, Czech Republic, France, Greece, Hungary, Iran, Italy, Mongolia, Romania, Russia, Sardinia, Sicily, Turkey (Nast, 1972; Önder et al., 2011).

Artianus manderstjernii (Kirschbaum, 1868)

Material examined: 1♂, 27.06.2016, Gökçebağ Village, Merzifon; 1♂, 27.06.2016 Yenimahalle, Suluova; 1♂3♀♀, 18.07.2016, Merzifon, Amasya.

Distribution in Turkey: Ağrı, Ankara, Balıkesir, Çankırı, Diyarbakır, Edirne, Gaziantep, İzmir, Kırklareli, Şanlıurfa,Van, Zonguldak (Önder et al., 2011).

Zoogeographical Distribution: Bulgaria, France, Hungary, Italy, Lebanon, Romania, Russia, the Czech Republic, Turkey (Önder et al., 2011).

By this study, the species belonging to the Auchenorrhyncha which distributed in the apple orchards in Amasya were determined. The taxonomical characters of the species were similar with definitions of the related taxa in the litereture.

These are potentially pests and economically important, because they prevent the normal development of plants by absorbing the plant sap and cause the spread of plant diseases in a short time due to their dense population and the large number of species. This study is a preliminary information for agriculture. The density, damage patterns and economic importance of the species can be better demonstrated by detailed studies to be carried out in other type of agricultural fields. It is hoped that this study will contribute to future studies on Auchenorrhyncha.

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Review Article

Food biotechnology and food safety

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Abstract

Food security has a multidimensional nature that has an important and strategic priority in protecting consumers of today and citizens of countries. The application of modern biotechnological methods in food ingredients and food products is evaluated in terms of research and development studies and legal regulations in terms of food consumption and human health. On the other hand, safe food production is important for the detection, management and control of physical, chemical and biological risks that may arise in food. Today, modern biotechnological studies are carried out on transgenic plants, animals and microorganisms for health, safety, economic, cultural and ethical reasons and national, regional and international security in some developed and developing countries. Each country has started to discuss the legal regulations related to the application of modern biotechnology according to its own conditions, especially due to biosecurity concerns. The production of genetically modified foods and control of legal arrangements in Turkey carried out effectively and are continuing to work on this issue.

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Biotechnology,
Food,
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1. Introduction

Biotechnology is a modern discipline that has revolutionized many fields since the late 20th century, primarily the health, agriculture, and environment (Gultekin, 2005). Research and education studies in the field of biotechnology are of great importance in developed and developing countries and are supported by many national and international organizations, and some countries take on world leading roles by conducting large investment campaigns in

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these areas (Hall, 2005). Biotechnology has various fields of implementation including industry and science.

Biotechnology is the most important application areas with the production of new and specific products together with technological developments and has a long tradition in the food industry. Biologic systems are used without making any modifications. Traditional biotechnological applications are used in the production process of bread, alcohol, some alcoholic beverages, vinegar, yogurt, etc. On the other hand, Modern Biotechnology is a field where the biological systems are changed using genetic engineering methods to produce useful products such as genetically modified food, enzymes, human hormones, insulin, and biotech vaccines. Both modern and traditional variants are in use today (Pinstrup-Andersen and, 2000; WHO, 2005; Jauhar, 2006). Nevertheless, the rapid increase of biotechnology applications have a strong influence on the need new legal regulations of suitable for the countries themselves. In this way, It is aimed to discuss and research that biotechnology addressed the potential effects on health, safety, economics, culture and ethics in both national and international platforms while at the same time to benefit from the opportunities offered by biotechnology. (Comert, 2011). The progress of biotechnology in food and agriculture created a tendency to share knowledge and cooperate among the industries (Baskaya et al., 2009).

2. Role of Biotechnology in Food Processing

Biotechnology can be used in the all ring of this chain in order to improve the safety of the food supply and nutritional quality, as well. The food industry is the lifeline connection between the farmer and the supermarket. Many agricultural products are processed after leaving the farm, except the vegetables and fruits generally eaten raw. Biotechnology can be used in every ring of this chain to improve the safety of the food supply and nutritional quality (Haroon and Ghazanfar, 2016). Thus, the aim of the study is to evaluted the food safety and how food biotechnology can be used to improve food processing following the product from the farm to the table.

2.1. Genetic Development of Food Fermentation Microorganisms

Given the fact that humankind is already benefiting from living systems to produce, process, and serve food for centuries, it could be noted that biotechnology is not something new for the food industry. Mutation and selection methods were used to enhance the bacteria

and yeast types used to produce fermented products such as cheese, sausage, bread, and wine. The processed foods including vitamins, stabilizers, enzymes, flavor enhancers, and preservatives are produced by the bacteria. Bacteria, yeast, and fungi have been used for centuries in fermented food production (Harlander, 1990; Fraiture et al., 2020). In traditional biotechnology methods, classical specimen enhancing based on the selection of bacteria and mutations are not under control, although it is not certain. Also, it is impossible to identify from all possible mutations and the screening process is time consuming and laborious (Ross et al., 2002). Modern biotechnology with genetic engineering provides a mechanism that can overcome such boundaries with the method providing selection and transfer of single, well-defined characteristics of many live organisms in a controllable and certain manner (Demain, 2000; Mosier and Ladisch, 2009). Effects of genetically enhanced microorganisms used in food fermentation on production are presented in Table 1.

Table 1. Effects of genetically enhanced microorganisms used in food fermentation on production (Harlander, 1990).

Food Product	Effects	Product Improvement Effects
Cheese	Bacteriophage (virus) resistance	Eliminate economic losses from loss of beneficial bacterial cultures
Wine	Insertion of malolactic gene in industrial yeast strain	Reducing the acidity level of the wine Prevented economic losses
Sausage	Bacteriocin production	Inhibition of pathogens
Beer	α- amylase producion	Low calorie beer
Bread	Higher levels of maltose	Improved leavening

2.2. Food Microorganisms

Food microorganisms are critically important in flavor-enhancing, preservation, and creating the aroma and texture of fermented foods. Genetic engineering is one of the new applications of the biotechnologies, providing successful results industrially (Koffas and Marienhagen, 2014) by developing genome calibration tools enhancing values or upgrading the microbial beta-galactosidase enzymes produced by Lb. delbrueckii subsp. bulgaricus and *Streptococcus thermophilus* (Markakiou et al., 2020), which are used in yogurt production. Serious studies are conducted on genome engineering for acquiring high yield products in lactic acid bacteria (Rothstein et al., 2020).

2.3. Probiotics

Probiotics are capable of implanting and competing many bacteria strains into human and animal gastrointestinal channels (Kerry et al., 2018). These organisms are widely named "probiotics" because they are beneficially helping the host (Gorbach, 2002) and probiotic microorganisms are shown in Table 2. Strains competitively inhibiting pathogenic intestinal organisms potentially have much useful utilization in agriculture and the food industry. Lactic acid bacteria can be found in multiple ecosystems, including food, animal feed, plants, and animals. Considering the importance of Lactic acid bacteria in biotechnology and medicine, this organism group is an important economical factor (Wuyts et al., 2020). Many methods and techniques are being investigated such as DNA and RNA specific sequence region analyses and microdroplet scanning use to describe probiotic isolates effectively and in a cost-efficient manner. Microdroplets can be used to describe isolates that can possibly affect the probiotic activity and are advanced products of short-chain fatty acids (Mekonnen et al., 2020). Detailed analysis of the effects of the probiotic product on intestinal bacteria populations can be made by molecular methods and PCR usage. These techniques being in the development phase today will become the most frequently used methods in the near future (Fuller, 1989).

2.4. Microbial Food Ingredients

Microorganisms produce an array of metabolites used as components in already processed food products (Neidleman, 1990). There are some regulators such as acetic acid, benzoic acid, lactic acid (for example, acetic acid is substantially produced by *Bifidobacteria*), aromas like diacetyl, pyrazines, and lactone (for example, ethyl acetate is produced by *E. coli* ATF1) (Lee and Trinth, 2019; 2020), flavor enhancers (for example, glutamic acid is produced by *Corynebacterium glutamicum*) (Nakayama et al., 2019), pigments (for example, the monascus-nata color agent is produced by *Monascus purpureus*) (Sheu et al., 2000) stabilizers and thickeners (for example, dextrans are synthesized by *Lactobacillus acidophilus*) (Gibbon and Banghart, 1967), nutritional additives(vitamins, amino acids) (Revuelta et al., 2016), sweeteners (aspartame) (Erbeldinger et al., 2000) and preservatives (nisin) (Joshi et al., 2009). Using these components in food production make the product more functional, enhance nutritional values, improve food quality, extend shelf life, and provide safety. Most of the ingredients mentioned above are produced by microorganisms with a long history of safe use

in the food industry. Moreover, many microorganisms can be found in nature which produces interesting components that can be used in processed foods. For example, many bacteria produce extracellular biopolymers such as stabilizing agents, viscosifiers, surfactants, flavor encapsulating agents, which can be used as non-caloric gelling agents or sources of soluble fiber in regimens. Genes coding the production of these biopolymers are under great interest to transfer them to food-grade microorganisms (Torino et al., 2015; Revuelta et al., 2016).

Table 2. Probiotic microorganisms (Holzapfel et al., 2001; Mercan, 2020)

Lactobacillus species	*L. bulgaricus, L. cellebiosus, L. delbrueckii, L. lactis, L. acidophilus, L. reuteri, L. brevis, L. casei, L. curvatus, L. fermentum, L. plantarum, L. johnsonii, L. rhamnosus, L. helveticus L. salivarius, L. gasseri, L. amilovorus, L. crispatus, L. gallinarum*
Bifidobacterium species	*B. adolescentis, B. bifidum B. breve, B. infantis B. longum, B. thermophilum, B. animalis, B. lactis*
Bacillus species	*B. subtilis, B. pumilus, B. lentus B. licheniformis, B. coagulans*
Pediococcus species	*P.scerevisiae, P. acidilactici, P. pentosaceu*
Streptococcus species	*S. faecalis, S. cremoris, S. lactics, S.intermedius, S. thermophilus*
Bacteriodes species	*B. capillus, B. suis B. ruminicola, B. amylophilus*
Propionibacterium species	*P. shermanii* ssp. *freudenreichii*
Leuconostoc species	*L. mesenteroides*
Yeasts	*S. cerevisiae, C. torulopsis, Saccharomyces boulardi*
New generation probiotic bacteria (NGP)	*Bacteroides xylanisolvens* DSM 23694, *Bacteroides ovatus* D-6 , *Bacteroides ovatus* V975, *Bacteroides ovatus* V975, *Bacteroides dorei* D8, *Bacteroides fragilis* ZY-312, *Bacteroides acidifaciens* JCM 10556(T), *Clostridium butyricum* MIYAIRI 588, *Faecalibacterium prausnitzii*, *Lactococcus lactis::trefoil factor 1* or IL-10

2.5. Enzymes

Enzymes are widely used as process helping agents in the food industry to control and enhance processed food texture, flavor, and nutritional values (Niedleman, 1986). In the last couple of years, food processing companies are using enzymes produced by genetically modified organisms. Enzyme groups comprising proteases and carbohydrates are cloned to have them produced with higher yield in a shorter time. These enzymes are used in the food industry to produce food substances such as sweeteners, cheese, and curd cheese. Rennin and

α-amylase are such enzymes (Haroon and Ghazanfar, 2016). The first recombinant enzyme (rennin) to be used directly in food is acknowledged by the Food and Drug Administration (FDA) as "generally recognized as safe" (GRAS) status, and it is considered as the first in food biotechnology. It is important to note that the recombinant product comprises more active enzymes per protein unit and it is microbiologically safer than the traditional equivalent extracted from the front stomach of calves (Flamm, 1991).

Table 3. Enzyme samples obtained from genetically modified microorganisms (Zhang et al., 2019)

Enzyme	Application Area	Microorganism	Improvement
α- amylase	Breaking down maltose and dextrin Stain remover Fortification of flour Glucose syrup	*Bacillus licheniformis* *Rhizopus oryzae*	Sensitive to acid Thermostable
β-glucosidase	Food, animal feed, textile, fuel and chemical industry	*Aspergillus aculeatus* *Thermotoga maritima*	Hydrolytic has improved efficiency
Proteases	Bakery, detergent industry	*Aspergillus* ve *Bacillus* species	Enhanced Glycine Releasing Activity
Lipase	Breaking down animal and vegetable oils and paper, cosmetics, pharmaceutical and detergent industry and agricultural chemistry	*B. subtilis*	Thermostability

Some techniques of genetic engineering (region-specific mutagenesis: specifically changing enzyme primary amino acid sequences) to enhance enzymes' functionality in food systems. Some genetically modified enzyme examples are presented in Table 3 (Zhang et al., 2019).

3. Safety of Products and Foods Produced with the Help of Modern Biotechnology

Food safety is the term defining the rules and measures to be followed in the production, processing, preservation, transportation, and distribution of food safely, and it generally emphasizes topics of safe, healthy, and preserved food (Artık et al., 2021). Food-related risks are considered separately in every aspect of food processing, from the initial production to the final result of consumption (Koç and Uzmay, 2015). In another explanation, food safety is the

capacity of all social groups and individuals to acquire the amount and quality of the food which will satisfy their nutritional needs, with the whole process also being sustainable. To this extent, the system that provides food safety needs to meet the basic conditions given below:

a) Availability: Capacity of producing, storing, and importing the necessary amount of food to satisfy the needs of all groups.

b) Accessibility: To ensure the impacts of international and political imposition are at a minimum and to physically and economically ensure all can acquire food.

c) *Sufficiency:* An environment of trust which can get through seasonal and periodical threats to food acquirement and the food production being nutritional, safe, and environmentally sustainable.

d) *Acceptability:* Food supply being suitable to cultural habits, not hampering human rights and honor.

e) *Individual and Institutional Factors:* The institutions which are the policymakers and manage the whole process, with the responsibility of food safety (Cankaya and Sancar, 2009; Koc and Uzmay, 2015).

In short, food safety is the consumed food not being harmful to health. However, in the 21st century, genetically modified (GM) products are proposed as the only option of providing food safety (Cankaya and Sancar, 2009). Modern biotechnology has various areas of use in medicine and the agricultural- food industry. One of those is the usage of GM in the food production chain. Thanks to the production of genetically modified products in the laboratory environment by humans, the resistance to herbicides, insects and disease factors (bacteria, fungi, virus) has increased and the product efficiency has been increased, the tolerance of the plants to salt, cold and drought has been improved, the nutritional values and shelf life of the products have been increased, It has been made more attractive in terms of color, shape and size, and products that are free or reduced from natural toxic substances and allergens are obtained (Gultekin, 2005). Studies of Codex Commission concentrated on the principles and guidelines of evaluation of food safety of products of modern biotechnology, and the food products of modern biotechnology are evaluated under three main topics as a result of this study (WHO, 2009).

I. Food safety evaluation and management of food products derived from Recombinant-DNA plants

II. Food safety evaluation and management of food products derived from Recombinant-DNA microorganisms
III. Food safety evaluation and management of food products derived from Recombinant-DNA animals.

Generally accepted food safety level by the public for new foods reflects the history of safe consumption by people. In many instances, it is accepted that the knowledge required to manage the risks related to food comes from long histories of use. These foods are generally considered safe. Risk analysis of food produced by the means of modern biotechnological methods provides a platform to designate general principles. The main goal of these principles is to set the ground for the risk analysis of foods produced with modern biotechnology concerning food nutrition and safety. These principles and the Codex Alimentarius Commission report do not address the environmental, ethical, moral, and socio-economical aspects of research, development, producing and marketing of biotechnologically produced foods. Risk evaluation comprises of a safety evaluation designed to identify whether there are any threats, nutritional or any other harms and if any, gather information on the nature and severity of the possible threat. Safety evaluation should focus on the determination of similarities and differences, comparing the modern biotechnologically produced food and the traditional equivalent. If any new or changed threat or any other safety issue is detected in safety evaluation, the related risk should be characterized to identify the relativity of the threat to human health. A safety evaluation is characterized by the comparison of the food or a component and the generic equivalent :

A. Considering both desired and undesirable effects,
B. Determining new threats or threats changed with the product,
C. Determining the changes in nutritional values related to human health

Accordingly, the safety of all new food derived from DNA plants, animals, and the products of these should be evaluated with a generic equivalent with a safe history of use in comparison as a principle considering expected and adverse effects (WHO, 2009; Amin et al., 2011; Kramkowska et al., 2013). The priority is to identify the new or changed threats compared to the traditional equivalent, not trying to identify all the threats of a given food. Safety evaluation of a food product derived from a recombinant DNA plant, animal or microorganism comprises of relative factors given below.

- Evaluation of toxicity
- Assessment of allergy status (proteins)
- Composition analysis of key components
- Evaluation of metabolites
- Food processing
- Nutritional modification
- Potential accumulation of substances important for human health
- Use of antibiotic resistance marker genes

The genetically modified food certification procedure and the conditions of genetically modified products that can be traded and are regulated by legal procedures and rules. Many characteristics are being examined, not only the features of the parent organism, but source of genes also used for the modifications and statement products (WHO, 2009).

4. Legal Regulations in Biosafety

Biotechnology products or (GMO) became commercially available 20 years ago. During this time, the effects of biotechnology products on health and food safety remained a controversial topic (Dincoglu, 2016). It is important to regulate some basic points with laws and topple these concerns and controversies for the good of the public. The Europen Union established a lawful frame by various regulations to provide the ground for the safe development of biotechnology and especially GMOs. These topics of regulations cover scientific subjects, measure rules, technological evaluations, trade, and the environment. The European policy and legislations are prepared and acted upon by considering the international statutes. Accordingly, European laws and their applications are in compliance with the following documents.

- Regulations of the World Trade Organization (WTO),

- Provisions of Cartagena Biosafety Protocol, which had been prepared and acknowledged as an additional protocol of the UN Biodiversity Agreement,

- Codex Alimentarius and studies of International Interim Workgroup on Codex of Food Biotechnology

Conditions on which the GMO products or food products derived from GMOs can be developed, used, or marketed are defined by the European Parliament and Council Charter,

which had been prepared according to these regulations. All GMO products and products derived from GMOs should also comply with the labeling and traceability regulations. In 2010, Biosafety Law was published in Turkey to establish a biosafety system, to ensure biodiversity is preserved, and to prevent any possible threats of GMOs and related products. Within the light of these evaluations, procedures and principles were determined regarding the tracing, auditing, and indoor activities of GMOs. Biosafety Committee was found to provide necessary scientific evaluations. To make sure the institution is independent, it was established as an autonomous board, never taking any orders from institutions, individuals, or companies. The mission of this Biosafety Committee is: To evaluate applications concerning the indoor use of GMOs, the marketing of GMO and related products as food, feed, and processing agents, and GMO and other related products being decontrolled for experimental purposes; and to provide experts and committees for these evaluations. According to the 5th Article of the Biosafety Law, below actions are prohibited:

a) Releasing GMO and related products to the market without obtaining the approval.

b) Using GMO and related products by the owner or third parties against the decisions of the Committee.

c) Production of genetically modified plants and animals.

d) Using GMO and related products except for the set purposes and goals before releasing to the market by the Committee.

e) Using GMO and related products in infant food, baby formulas, follow-on baby food, follow-on baby formulas, and baby and children supplementary food products (Ministry of Agriculture and Forestry, 2021).

A national inspection of food-related GMOs is the responsibility of the Ministry of Agriculture and Forestry. Controls of imported food and feed samples are made by Provincial Food Control Laboratories and other food analysis laboratories affiliated under the ministry, at a frequency specified by the Ministry according to the risk conditions (Başkaya et al., 2009).

5. Conclusion

In conclusion, transgenic plants are not allowed to have free trade and imported to our country. Transgenic plant elements and seeds coming for registry purposes are field-tested by the Ministry of Agriculture and Forestry. Turkish studies on legislations concerning biosafety

are greatly affected by the Cartagena Biosafety Protocols, where Turkish government is also a party.

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Review Article

Medicinal values of a horticultural plant - *Coleus hadiensis* (Forssk.) A. J. Paton

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Abstract

Coleus hadiensis (Forssk.) A.J.Paton is a horticultural herb that goes to the *Lamiaceae* family. This plant species has been using to treat diarrhea, skin and digestive disorders, diabetes, and carcinoma in ethnomedicines. This minireview work purposes to analyze, summarize, and document the reported bioactivities of *C. hadiensis.* Suitable published works were obtained employing the Web of Science, Scopus, PubMed, Semantic Scholar, and ScienceDirect databases from 1900 to December 2020. Hitherto, *in vitro* level of scientific evidence is the highest level of scientific evidence available for the bioactivities of this plant species. Various parts of *C. hadiensis* exhibited antioxidant, antibacterial, anti-inflammatory, anticancer, and antimalarial activities in a range of assays. To date, eight bioactive (antimalarial and antioxidant) compounds have been isolated from *C. hadiensis.* This minireview analyzed, summarized, and documented the reported bioactivities of *C. hadiensis.* In addition, this minireview provides a basis for further bioactivities researches using *C. hadiensis* in future.

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Coleus hadiensis,
Lamiaceae,
Plectranthus hadiensis,
Plectranthus zatarhendi,
Siddha medicine,
Sri Lanka

1. Introduction

Coleus hadiensis (Forssk.) A.J.Paton [synonyms: *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger; *Plectranthus zatarhendi* var. tomentosus (Benth.) Codd] is a horticultural herb that goes to the *Lamiaceae* family (Figure 1). This plant species grows up to

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1.5 m high and 1 m broad. Its stems are semi-succulent and red at the lowest part. Leaves are alternately organized and they are oval to round shape, from 35 to 100 mm broad, soft, gentle, furry, occasionally multicolored. The blossoming is 50 cm lengthy and contains lateral divisions. The flowers are white or violet and exist 1 to 3 cm at a distance. The conservation status of *C. hadiensis* is Least Concern and it is usually grown in forest, and grassland. The grow soil is well drained rich soil (Kew Science, 2020). It is native to Asia (Sri Lanka, Maldives, and Yemen) and Africa (Angola, Mozambique, Malawi, South Africa, Ethiopia, Eritrea, Kenya, Chad, Egypt, Djibouti, Swaziland, Rwanda, Zimbabwe, Somalia, Zaïre, Tanzania, and Uganda) (Kew Science, 2020). *C. hadiensis* has the name Iruveli in Tamil. This plant species has been using to treat diarrhea, skin and digestive disorders, inflammations, coughs, diabetes, and carcinoma in ethnomedicines (Lukhoba et al., 2006; Van Zyl et al., 2008; Rice et al., 2011; Menon et al., 2013; Sathasivampillai et al., 2015; 2017; 2018; Sripathi et al., 2018). Phytochemicals including rosmarinic acid, chrysosplenol D, desacetyl plectranthone, quercetin, casticin, ayanin, (+)-plectranthone, piperitone oxide, L-fenchone, β-farnesene, copaene, 2,3-dimethyl hydroquinone, α-caryophyllene, 1,8-naphthalenedione, limonene, copaene, 8,11,15-eicosatrienoic acid, β-cubebene, β-farnesene, α-caryophyllene, 2-isopropenyl-5-methylhex-4-enal, germacrene D, piperitone oxide, δ-cadinene, disophenol, p-cymen-8-ol, isolongifolan-8-ol, δ-cadinol, α-hydroxymyristic acid, p-cymen-3-ol, octern-3-ol, linalool, nerol, z-citral, geraniol, neryl acetate, α-copaene, geranyl acetate, α-cadinene, α-cadinol, β-cubebene, and valencene have been discovered in this plant species (Menon and Gopalakrishnan, 2015a; Sripathi and Ravi, 2017; Sripathi et al., 2018; Ji et al., 2019).

As there is no systematic review regarding the bioactivities of *C. hadiensis,* this minireview work purposes to analyze, summarize, and document the reported bioactivities of *C. hadiensis.* This minireview will be an advantageous for future bioactivities and phytochemistry related researches of *C. hadiensis.*

Suitable published works were obtained employing the Web of Science, Scopus, PubMed, Semantic Scholar, and ScienceDirect databases from 1900 to December 2020. "*Coleus hadiensis"*, "*Plectranthus hadiensis"*, and "*Plectranthus zatarhendi* var. tomentosus" were used as search terms and the subjects were narrowed down to Biology, Chemistry, Medicine, Agriculture, Pharmacology, Pharmaceutics, Toxicology, Biochemistry, Genetics, Molecular Biology, and Multidisciplinary.

Figure 1. *Coleus hadiensis* (Forssk.) A. J. Paton (http://pza.sanbi.org/coleus-hadiensis)

2. Reported Bioactivities of C. hadiensis

Reported bioactivities of *C. hadiensis* have been listed on Table 1. Hitherto, *in vitro* level of scientific evidence is the highest level of scientific evidence available for the bioactivities of this plant species. Various parts of *C. hadiensis* exhibited antioxidant, antibacterial, anti-inflammatory, anticancer, and antimalarial activities in a range of assays (Van Zyl et al., 2008; Mothana et al., 2010; Menon et al., 2010; 2011; 2012; 2014; Menon and Gopalakrishnan, 2015; Sripathi and Ravi, 2017; Rijo et al., 2018; Sripathi et al., 2018; Ji et al., 2019). A larger number of studies reported the antioxidant activities of this plant species and the aerial part and ethanol extracts were used in the majority of the investigations. To date, eight bioactive compounds (ayanin, casticin, chrysosplenol D, luteolin 7-O-glucuronide, quercetin 3, 7-dimethyl ether, rosmarinic acid, abietane diterpene 1, and 2) have been isolated from *C. hadiensis.* Reported anti-inflammatory, anticancer, and antibacterial activities provide scientific evidence for the ethnomedicinal uses such skin disorders, inflammations, coughs, and carcinoma. Anyway, ethnomedicinal uses to treat illnesses like diarrhea, digestive disorders, and diabetes have no scientific evidence. Noteworthy investigations (based on the minor concentrations used) are only deliberated beneath.

Table 1. Reported *in vitro* bioactivities of *C. hadiensis*

Bioactivity	Part used	Extract compound	Assay	Dose concentration	Reference
Antibacterial	Aerial	Essential oil	*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*	32 mg/dl (MIC)	Sripathi et al., 2018
Antibacterial	Leaf	Aqueous, Methanol	*Bacillus subtilis*, *Micrococus flavus*, *Staphylococcus aureus*, *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis*	4 mg	Mothana et al., 2010
	Root	Aqueous	*Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*	4 mg	
	Root	Methanol	*Bacillus subtilis*, *Micrococus flavus*, *Staphylococcus aureus*, *Staphylococcus aureus (ATCC 6538)*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*	4 mg	
Antibacterial	Seed	Essential oil	*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	NS	Sripathi and Ravi, 2017
Antibacterial	Whole plant	Acetone	*Bacillus subtilis*	1 mg/ml	Rijo et al., 2018
Anticancer	Stem	Methanol	Cervical cancer (HeLa) cell line	141.3 µg/ml (IC_{50})	Menon et al., 2011
Anticancer	Aerial	NS	Human colon cancer cell (HCT-15)	17.27 µg/ml	Menon and Gopalakrishnan, 2015
Anti-inflammatory	Stem	Methanol	Bovine Serum Albumin denaturation inhibitory, Human red blood cell membrane stabilization, Platelet aggregation inhibitory	1 mg/ml	Menon et al., 2011
Anti-inflammatory	Aerial	Ethanol (80%)	Bovine Serum Albumin denaturation inhibitory	56.18 µg/ml (IC_{50})	Menon et al., 2014
	Aerial	Ethanol (80%)	Human red blood cell membrane stabilization	250 µg/ml	
	Aerial	Ethanol (80%)	NO radical scavenging	79.84 µg/ml (IC_{50})	
	Aerial	Ethanol (80%)	Platelet aggregation inhibitory	54.26 µg/ml (IC_{50})	
	Aerial	Ethanol (80%)	Potassium ferricyanide reduction	50 µg/ml	
Antimalarial	Leaf	NS (Abietane diterpene 1)	*Plasmodium falciparum*	4.6 µM (IC_{50})	Van Zyl et al., 2008
	Leaf	NS (Abietane diterpene 2)	*Plasmodium falciparum*	29.2 µM (IC_{50})	
Antioxidant	Leaf, Root	Aqueous	DPPH radical scavenging	> 1000 µg/ml (IC_{50})	Mothana et al., 2010
	Leaf	Methanol	DPPH radical scavenging	150 µg/ml (IC_{50})	
	Root	Methanol	DPPH radical scavenging	> 1000 µg/ml (IC_{50})	

Bioactivity	Part used	Extract compound	Assay	Dose concentration	Reference
Antioxidant	Aerial	Ethanol (80%)	DPPH radical scavenging	22.76 µg/ml (IC_{50})	Menon et al., 2014
Antioxidant	Aerial	Ayanin, Casticin	DPPH radical scavenging	> 100 µM (EC_{50})	Ji et al., 2019
	Aerial	Ayanin	TBARS	53.7 µM (IC_{50})	
	Aerial	Casticin	TBARS	22.8 µM (IC_{50})	
	Aerial	Chrysosplenol D	DPPH radical scavenging	48.3 µM (EC_{50})	
	Aerial	Chrysosplenol D	TBARS	2.5 µM (IC_{50})	
	Aerial	Ethanol (95%)	DPPH radical scavenging, TBARS	20 µg/ml	
	Aerial	Luteolin 7-O-glucuronide	DPPH radical scavenging	26.2 µM (EC_{50})	
	Aerial	Luteolin 7-O-glucuronide	TBARS	2.8 µM (IC_{50})	
	Aerial	Quercetin 3, 7-dimethyl ether	DPPH radical scavenging	31.2 µM (EC_{50})	
	Aerial	Quercetin 3, 7-dimethyl ether	TBARS	3.8 µM (IC_{50})	
	Aerial	Rosmarinic acid	DPPH radical scavenging	19 µM (EC_{50})	
	Aerial	Rosmarinic acid	TBARS	1.5 µM (IC_{50})	
	Leaf	Ethanol (30%, 50%, 70%, 95%)	DPPH radical scavenging	100 µg/ml	
	Leaf	Ethanol (50%, 70%, 95%)	TBARS	5 µg/ml	
	Stem	Ethanol (30%, 50%, 70%, 95%)	DPPH radical scavenging	100 µg/ml	
	Stem	Ethanol (50%, 70%, 95%)	TBARS	5 µg/ml	
Antioxidant	Aerial	Methanol	DPPH radical scavenging, FRAP	100 µg/ml	Menon et al., 2012
	Aerial	Methanol	NO radical scavenging	1 mg/ml	
Antioxidant	Whole plant	Acetone, Aqueous	TLC-DPPH bleaching	1 mg/ml	Rijo et al., 2018

DPPH: 2,2-diphenyl-1-picrylhydrazyl; NS: Not stated; TBARS: Thiobarbituric acid reactive substance; IC_{50}: Half maximal inhibitory concentration; EC_{50}: Half maximal effective concentration; MIC: Minimum Inhibitory Concentration; NO: Nitric oxide; FRAP: Ferric reducing antioxidant power; TLC: Thin-Layer Chromatography

2.1. Antibacterial activity

Acetone extract prepared used the whole of showed antibacterial activity at 1 mg/ml concentration in Bacillus subtilis assay (Rijo et al., 2018).

2.2. Anticancer activity

Menon and Gopalakrishnan (2015) reported that aerial extract (17.27 µg/ml) showed anticancer activity human colon cancer cell (HCT-15) line (Menon and Gopalakrishnan, 2015). Anyway, the authors did not mention the solvent used to prepare the extract.

2.3. Anti-inflammatory Activity

Aerial ethanol (80%) extract showed anti-inflammatory effects in potassium ferricyanide reduction assay at a concentration of 50 µg/ml (Menon et al., 2014).

2.4. Antimalarial Activity

So far, two antimalarial compounds have been identified from leaves this plant species. Abietane diterpene 1 and 2 revealed antimalarial properties in Plasmodium falciparum assay at IC_{50} of 4.6 µM and 29.2 µM respectively (Van Zyl et al., 2008).

2.5. Antioxidant activity

Antioxidant compounds including ayanin, casticin, rosmarinic acid, chrysosplenol D, luteolin 7-O-glucuronide, and quercetin 3,7-dimethyl ether have been discovered in C. hadiensis (Ji et al., 2019). Among these compounds, rosmarinic acid isolated from aerial part unveiled antioxidant effects in thiobarbituric acid reactive substance assay at IC_{50} 1.5 µM.

3. Conclusion

Published bioactivities related articles involving *C. hadiensis* parts provide some scientific evidence for its ethnomedicinal uses. Still, some other ethnomedicinal uses have no scientific evidence. Thus, more bioactivities related studies should be conducted and active compounds

should be isolated. Then these compounds should be studied further in *in vitro, in vivo*, and clinical trial studies. Further, toxicity studies of various extracts and isolated bioactive compounds should be conducted for safety and efficacy determinations. As this plant species exhibited several bioactivities in various *in vitro* assays only, these bioactivities should be further studied in suitable *in vivo* models. These studies would provide new drugs for killer diseases like cancer in the future. This minireview analyzed, summarized, and documented the reported bioactivities of *C. hadiensis.* In addition, this minireview provides a basis for further bioactivities researches using *C. hadiensis* in future.

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Research Article

Proline accumulation in three closely related *Salsola* L. taxa

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Abstract

Halophytes, gypsicoles and serpentinicoles are adapted to specialized edaphic conditions at arid and semi-arid regions. These arid and semi-arid areas possess physical and chemical stress factors for all plants. For these plants, one of the most important parameter that provides stress tolerance is the proline accumulation. Proline is a water-soluble amino acid generally accumulated under stress and behave like an indicator for adaptation of plants against extreme conditions. In this study, the amounts of proline accumulation in three different taxa, *Salsola boissieri* subsp. *serpentinicola*, *Salsola boissieri* subsp. *boissieri* and *Salsola turcica*, were determined. These taxa are phylogenetically close to each other but adapted to different soil types. The highest proline accumulation measured in leaves of *S. turcica* with the value of 2.510 ± 0.020 µmol g^{-1} FW and the lowest accumulation measured in leaves of *S. boissieri* subsp. *serpentinicola* with the value of 0.996 ± 0.024 µmol g^{-1} FW. As a result of these proline accumulations, it can be concluded that the high amount of proline accumulation in halophytic *S. turcica* is a response against stress conditions but the low proline accumulation of *S. boissieri* subsp. *serpentinicola* means this species may have other adaptations against the stress factors that caused by serpentinicolous soils. Both of the studied taxa accumulate proline and the findings show that proline accumulation can be a marker in the assessment of stress tolerance of *Salsola* species.

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1. Introduction

Environmental conditions like aridity, salinity, extreme changes in temperatures, extreme precipitations etc. cause abiotic stress factors and influence the growth and propagation of

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plants (Taiz and Zeiger, 2010). Under abiotic stress conditions, plants made some physiological and metabolic changes to adapt themselves against any harm at growth and development (Kalefetoğlu and Ekmekçi, 2005).

Proline, a water-soluble amino acid, is one of the most important parameter in determining the tolerance of plants to stress in saline and arid habitats and used as an indicator for plants adaptation to these harsh conditions (Bian et al., 1988). Proline accumulation is important for balancing osmotic pressure in plants (Iba, 2002; Ashraf and Harris, 2004), protecting the cell membrane by reducing lipid peroxidation in arid conditions (Molinari et al., 2007), adjusting cytosolic pH and regulating hydroxyl radicals (Matysik et al., 2002), photosynthetic activity and preservation of mitochondrial functions, chlorophyll molecules and enzyme activities (Rai et al., 2011).

Like halophytic plants, both gypsicole and serpantinicole plants generally distributed at arid and semi-arid areas and these areas contain physical and chemical stress factors in many aspects. Serpentine soils restrict plant growth and propagation because they have high Fe and Mg and low Ca concentrations, they are rich in heavy metals like Ni, Cr and Co, and also they are poor soils especially for some nutrients like N, P and K (Gordon and Lipman, 1926; Vlamis and Jenny, 1948; Walker, 1954; Proctor and Woodell, 1975; Brooks, 1987; Avcı, 2005). Plants on serpentine soils are also exposed to secondary water stress because of the presence of heavy metals. They have to adapt heavy metal toxicity, water stress and also lack of nutrients. Gypsaceous soils are also poor in organic matter and increase in gypsum cause low cation exchange capacity. Ca concentration of gypsaceus soils is high and decrease Mg and K availability, also cause increase in Ca:Mg ratio in plant tissues (FAO, 1990). Increase in gypsum also causes decrease in water availability so plants face with water stress (Llinares et al., 2015).

Salsola L. genus from Chenopodiaceae/Amaranthaceae family has a distribution area at arid regions of Central and Southeastern Asia, northern Africa and Mediterranean region (Guma et al., 2010). In Turkey, genus *Salsola* L. represented with 18 species and totally 23 taxa (Yaprak, 2012). Species of genus *Salsola* L. generally have adaptations to live on saline or semi-saline areas (Aellen, 1967). Even though *Salsola turcica* Yıldırımlı, *Salsola boissieri* Botsch. subsp. *boissieri* and *Salsola boissieri* Botsch. subsp. *serpentinicola* (Freitag & Özhatay) Freitag & Uotila are phylogenetically closely related, they are adapted on different

edaphic conditions and became habitat specialists which make them more vulnerable against habitat loss and degradation (Pueyo et al., 2008).

Salsola turcica Yıldırımlı is member of steppe vegetation with gypsaceous and saline soils at 950-1000 m (Yıldırımlı, 2010). It is endemic for Turkey and halophytic and gypsicole ecotypes were studied. *Salsola boissieri* Botsch. subsp. *boissieri* prefers rocky and stony slopes at 900-2500 m around Sivas and Kahramanmaraş provinces (Aellen, 1967). *Salsola boissieri* Botsch. subsp. *serpentinicola* (Freitag & Özhatay) Freitag & Uotila geographically separated from the other subspecies and prefers serpentine rocks and rock cracks between 1600 and 2000 m around Burdur and Muğla provinces, it is also endemic for Turkey (Güner et al., 2000). According to the literature surveys there is not any record about the proline accumulation of studied taxa. In this study, the authors aimed to determine the proline accumulation of leaf tissues of the aforementioned *Salsola* taxa and whether these conditions cause stress over the plants or they are adapted to these harsh environmental conditions.

2. Materials and Methods

2.1. Collection of Plant Specimens

The distribution and sampling areas of three taxa were given at Table 1. From each locality leaf samples were taken from 5 different individuals in 2016 and preserved at -20°C at the laboratory.

Table 1. Taxa, collection number of specimens and localities

Taxon	Collection number of specimen (IBÇınar)	Locality
S. turcica (gypsicole ecotype)	1055	Ankara, Beypazarı, about 14 km west of Beypazarı, on the left side of Beypazarı-Nallıhan highway
	1074	Ankara, Beypazarı, about 14 km west of Beypazarı, on the right side of Beypazarı-Nallıhan highway
	1075	Eskişehir, Sivrihisar, Aşağıkepen village
S. turcica (halophytic ecotype)	1088	Ankara, Şereflikoçhisar south of Akin village, Tuz Lake
	1096	Konya, Cihanbeyli, around Yavşan Saltpan, saline steppe
	1105	Konya, Cihanbeyli, Bolluk Lake, saline alkaline areas
S. boissieri subsp. *serpentinicola* (serpentinicole subspecies)	1110	Muğla, Fethiye, Beyağaç, Köyceğiz, Sandras Mountain
	1111	Burdur, Altınyayla, Dirmil pasture, serpentine soils
S. boissieri subsp. *boissieri* (glycophyte subspecies)	1115	Sivas, Yıldızeli, Yusufoğlan village
	1131	Kahramanmaraş, Ahır Mountain, southern slopes

2.2. Determination of Free Proline Accumulation

Proline accumulation was determined by ninhydrin assay according to the method of Bates et al. (1973). At a wavelength of 520 nm, the absorbance of the proline extract in toluene was determined by Perkin Elmer 1420 Multilable Counter VICTOR3V. The accumulation of proline was calculated by comparison with a standard calibration curve previously made by using different concentrations of proline.

2.3. Statistical Analysis

Analysis results were calculated as mean values for each *Salsola* taxa. Measured proline values were analysed by repeated measures analysis of variance (One way ANOVA). Variance analysis was conducted by using SPSS 20 software. Based on the variance analyses conducted for proline, the difference was found to be statistically significant ($p<0.05$). Data

used in the statistical analysis are mean values ± standard error measures for three replicates (n = 3).

3. Results and Discussion

Plants generally have two main mechanisms of tolerance against stress factors like salinity and extreme temperatures. First one is the avoidance from the stress factor by making some morphological and/or chemical modifications. The second mechanism is the resistance against stress factors by making cellular and tissue level modifications (Avcıoğlu et al., 2003). Against salinity stress plants accumulate some organic molecules like proline, glycine, betain and sucrose to protect osmotic potential of cytoplasm (Taban et al., 1999). Proline accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008). It is a common physiological response in many plants in response to a wide range of biotic and abiotic stresses (Choudhary et al., 2005; Munns, 2005; HongBo et al., 2006; Ashraf and Foolad, 2007; Saed-Moucheshi et al., 2013; Sharma et al., 2014; Hunt et al., 2017; De Freitas et al., 2019; Trovato et al., 2019).

In this study, specimen with collection number IBÇınar1096 was used as a control group because most of the taxa of genus *Salsola* are halophytic. In the present work, proline amounts in gypsicoles (1055, 1074, 1075) and halophytic species (1088, 1105) were found to be higher than subspecies of *S. boissieri* (1110, 1111 and 1131) ($p<0.05$) (Table 2). In *S. boissieri* subsp. *boissieri* (1115), it was found that there was an increase in the amount of proline compared to the control group ($p<0.05$) (Table 2). According to the results the highest proline accumulation was found in *S. turcica* (1105) with the value of 2.510 ± 0.020 μmol g^{-1} and the lowest was found in *S. boissieri* subsp. *serpentinicola* (1111) as 0.996 ± 0.024 μmol g^{-1} ($p<0.05$) (Table 2). In *S. turcica* (1105) species, compared with control group, the increase in proline level were approximately 136.8 % ($p<0.05$).

Table 2. Proline amounts in Salsola species (Values are means ± SE, n = 3)

Taxa	Collection Number	Free Proline
		µmol g^{-1} FW
S. turcica (Gypsicole ecotype)	1055	1.826 ± 0.035
S. turcica (Gypsicole ecotype)	1074	1.890 ± 0.135
S. turcica (Gypsicole ecotype)	1075	1.713 ± 0.023
S. turcica (Halophytic ecotype)	1088	1.893 ± 0.066
S. turcica (Halophytic ecotype)	1096	1.060 ± 0.025
S. turcica (Halophytic ecotype)	1105	2.510 ± 0.020
S. boissieri subsp. *serpentinicola*	1110	1.336 ± 0.018
S. boissieri subsp. *serpentinicola*	1111	0.996 ± 0.024
S. boissieri subsp. *boissieri*	1115	2.000 ± 0.046
S. boissieri subsp. *boissieri*	1131	1.316 ± 0.016

All plants including glycophytes synthesize proline as a response to abiotic and biotic stress and it is also true for halophytes (Flowers and Hall, 1978; Tipirdamaz et al., 2006; Grigore et al., 2011). In this study, the high proline accumulation of gypsicole and halophytic species may be related to the osmotic stress caused by the aridity and salinity of the soil. The osmotic stress caused by gypsum depending on salinity was seen as a stimulating factor for proline biosynthesis in plants (Boscaiu et al., 2013). Osmotic stress caused by salt stress activates proline synthesis may have had. Increased proline under salt stress conditions causes an increase in cellular osmotic pressure and may have helped osmotic adjustment in the cytoplasm, which may help increase tolerance. Moreover, the ability to retain water under saline conditions with proline accumulation can increase salt tolerance by eliminating an excess ion concentration by a dilution effect. Saradhi et al. (1995) and Hare and Cress (1997) reported that proline amino acid, as one of the most common osmolite, was accumulated in cytosol as a response to abiotic stress factors like salinity, aridity, extreme temperatures, nutrient deficiencies etc. According to Boscaiu et al. (2013) the main trigger of proline synthesis is the lack of water at gypsaceous and saline soils as in arid and semi-arid areas. The lowest value measured at *S. boissieri* subsp. *serpentinicola* may be the result of adaptation of this taxon to serpentine soils. Proline accumulation as an osmoregulator occurs due to water stress in plants growing in serpentine soils with high metal content (Avcı, 2005). However, the amount of proline can vary between species. Also, these different reactions can be associated with different genotypes.

4. Conclusion

In nature, there are so many stressful factors and their influences change from species to species, and many plants have developed some adaptation mechanisms through their evolutionary processes and do not show any sign of stress under unfavourable conditions. By having these adaptation mechanisms, plants can invade extreme habitats. According to the proline levels it can be said that *S. boissieri* subsp. *serpentinicola* successfully adapted to its habitat or have some other adaptation mechanisms as well as proline synthesis. But the soil and climatic conditions cause stress over especially both ecotypes of *S. turcica* and *S. boissieri* subsp. *boissieri* (1115) according to their proline accumulation and the presence of proline help them to survive at these unfavourable conditions.

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