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JOURNAL OF AGRICULTURAL PRODUCTION

RESEARCH ARTICLE

Evaluation of Early Spring Grazing on Meadow in Erzurum, Turkey

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ABSTRACT

This study was conducted in irrigated meadow with deep water table level in 2014 in Erzurum, Narman, Demirdag, and aimed to evaluate the effects of early spring grazing on meadows. Soil properties and dry matter yield and some quality parameters such as dry hay yield, crude protein, ADF, NDF and crude ash rates were assessed in meadow. Average dry matter yield was lover in spring grazed site than that of the ungrazed meadow site. Crude protein content was determined as 8.25%, 8.35% in grazed and ungrazed meadow sites, respectively. In spring grazed site ADF and NDF ratio were lower than that of the ungrazed one (36.95%, 36.72%; 55.98%, 56.82%). In grazed meadow site digestible dry matter ratio (60.29%) was lower than that of the ungrazed site (61.40%). Based on the results of the study, it is important to prevent of spring grazing in meadows for increasing of dry matter yield and forage quality.

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Taşçı, M. and Altunok, Z. (2020). Evaluation of Early Spring Grazing on Meadow in Erzurum, Turkey. *Journal of Agricultural Production*, 1(1): 1-4.

Introduction

Animal production is an important agricultural activity in Turkey and in addition to rangelands forage crop production areas meadows is one of the main food sources for livestock in winter periods because the high quality roughage need of livestock in long winter periods is mainly obtained from natural meadows. Our country has 1 449 313 ha of meadow area and the dry forage production is approximately 4 347 939 ton per year from this area (Topcu and Ozkan, 2017).

Meadows are mainly managed to produce dry hay for livestock needed the winter period by individual owners. Also, meadows are grazed in early spring or late summer after harvesting. Although, this practice is providing food for animals, grazing of meadows in early spring periods leads to decrease in yield and forage quality. Unlike the spring grazing, it is expressed that there is no negative effect of late fall grazing on yield and forage quality of meadows after harvest (Gokkus, 1989). In Eastern region due to the vegetation period is pretty short and forage plants dries early, meadows can be an important feed sources for animal in the fall period (Altin et al., 2005).

The aims of this study to evaluate the effects of spring grazing on yield and forage quality of irrigated meadows.

Materials and Methods

This study was carry out in irrigated meadow with deep water table level in 2014 in Erzurum, Narman, Demirdag. The enclosed meadow area was fenced in the early spring of the year 2014 to protect animal grazing. Meadow irrigated three times along the year and fertilized with animal manure at doses 1 ton ha^{-1}. The experiment was designed in a randomized complete block design, replicated three times. The size of grazing plots was 1 da for grazing treatments, 300 m^2 for enclosure treatment.

Grazing treatment plots freely grazed by cattle from the early spring, middle of April to the first week of May. It was about 15-20 days period, traditionally practiced in meadows of the region.

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Figure 1. A meadow in Eastern Region of Turkey

Figure 2. Fencing of meadow to protect animal grazing in early spring

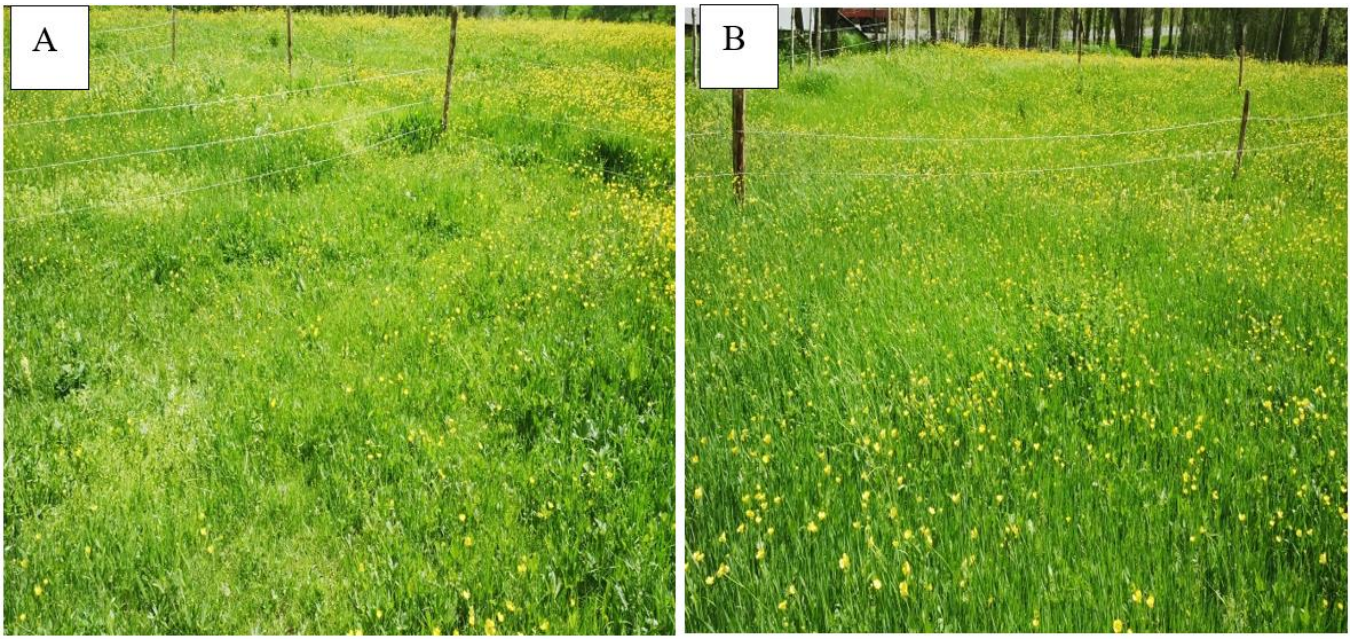

Figure 3. Grazed meadow site in spring time (A) and ungrazed meadow site (B)

Dominant plant species were Buttercup (*Ranunculus sp*), meadow fescue (*Festuca pratensis*) and Timothy (*Phleum sp*). Also, orchard grass (*Dactylis glomerata*), Kentucky bluegrass (*Poa pratensis*), Clower (*Trifolium sp*) and Docks (*Rumex sp*) were determined common plant species in the vegation of meadow.

Long term average temperature was 5.6 °C, total annual precipitation was 403.3 mm, and in study year average temperature was 3.24 °C, and total annual precipitation was 362.5 mm.

Three composite soil samples were collected from the surface layer of each plots and analyzed for physical and chemical properties. The soil texture was determined by a Bouyoucos hydrometer (Gee and Bauder, 1986) as sandy clay loam, the soil pH, determined by a pH meter (McLean, 1982) with glass electrode (1:2.5 soil-water suspension) for meadow as 7.25. Soil organic matter content was determined by SmithWeldon method (Nelson and Sommers, 1982) as 2.75%; available K was determined by a flame photometry (Thomas, 1982) as 130.2 and Olsen P content was determined by moliybdophosphoric blue color method (Olsen and Sommers, 1982) as 4.21. $CaCO_3$ content was determined by a Scheibler calcimeter (Nelson, 1982) as 5.24%.

Dry matter yield was determined by harvested three quadrats (1 m^2) area of central part of each plots when dominant species at flowering period and weighing after oven dried at 70 °C for 24 h. Total N content was determined by the Kjeldahl method and multiplied by 6.25 to give crude protein content (Jones, 2001).

Acid detergent fiber and Neutral detergent fiber analyzes were determined by (Anon, 1995). Total digestible dry matter was determined by (Moore and Undersander, 2002; Schroeder, 2004) equations [TDN%=88.9-(0.779 x ADF %)].

All data were subjected to analysis of variance using the SPSS (SPSS for Windows). Means were separated using the t test.

Results and Discussion

Average dry matter yield was determined 302.7 kg da^{-1}, in meadow area and dry matter yield significantly different in two meadow sites; it was higher in ungrazed site than grazed site. Average crude protein (%), ADF (%), NDF (%) and DDM (%) were 8.32, 36.83, 54.40, and 60.84, respectively. The results showed that in grazed site crude protein content (%) was higher than ungrazed site; on the other hand ADF, NDF, DDM ratios were higher in ungrazed site than grazed site. But there were no significant difference between meadow sites based on crude protein content, ADF, NDF and DDM ratios.

Table 1. Dry matter yield in early spring grazed and ungrazed meadow sites (kg da^{-1})

	Grazed Site	Ungrazed site	Average
Dry Matter Yield (kg da^{-1})	253.0 **B**	352.3 **A**	302.7
Crude Protein Ratio (%)	8.35	8.29	8.32
ADF (%)	36.72	36.95	36.83
NDF (%)	56.82	55.98	54.40
DDM (%)	60.29	61.40	60.84

The higher dry matter yield in grazed site than ungrazed site is most probably resulted from decreasing effects of early spring animal grazing on yield in grazed site. Some study results supported our results stated that early spring animal grazing decreases dry matter yield in meadows (Gökkuş, 1989; Wenick et al., 2007).

Crude protein content may change depend on plant species, plant growth stage, leaf/stem ratio, and some other environmental conditions (Ball et al., 2001). In grazed site, legumes with high protein content selectively grazed and on the other hand effects of grazing the vegetation may be greener than ungrazed site. This two-way effect may be revealed any significant difference between grazed and ungrazed sites based on crude protein content. Most probably, the same effects of grazing may be caused to near values ADF, NDF and DDM in both sites.

Conclusion

According to one year results of this study indicated that early grazing may effects on dry matter yield of meadows but there were no significant difference between early spring grazing treatment and enclosure treatment. Also, the study should be continued more than one year for more stubble results.

Acknowledgements

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RESEARCH ARTICLE

Investigation of Antibacterial Activity of Two Different Medicinal Plants Extracts Against Fish Pathogens

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ABSTRACT

Diseases are one of the leading factors affecting the sustainability of aquaculture industry. Due to the undesirable effects of the methods used in the prevention or treatment of diseases, the usability of herbal products has been investigated recently. Thus, in the present study, we aimed to investigate the effects of aqueous methanolic extracts of two different medicinal plants (*Laurus nobilis* and *Brassica nigra*) against *Vibrio anguillarium*, *Yersinia ruckeri*, *Pseudomonas putida*, and *Aeromonas hydrophila* by using minimum inhibitory concentration (MIC). The MIC values of leaf aqueous methanolic extract of *Laurus nobilis* for Aeromonas *hydrophila* and leaf aqueous methanolic extract of *Brassica nigra* for *Vibrio anguillarium* were determined as 3.125 µg ml^{-1} and 100 µg ml^{-1}, respectively. The results showed that *Laurus nobilis* could be used against *Aeromonas hydrophila* and *Brassica nigra* against *Vibrio anguillarium*. Further *in vivo* studies should be conducted to evaluate the usability of these plants.

Please cite this paper as follows:

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Introduction

Aquaculture industry is one of the most fast growing food industry in the world (Fisheries, 2018). Increased technology could give opportunities to the fish farmers to increase their production rate (Bilen et al., 2013). However, diseases seem to be most limiting factor in the industry. Increasing stoking density and the different technology usage may trigger stress conditions and this can favour occurrence and the spread of the bacterial diseases (Fazio et al., 2013).

Among the bacterial pathogens *Vibrio anguillarium*, *Yersinia ruckeri*, *Pseudomonas putida* and *Aeromonas hydrophila* are opportunistic and ubiquitous, and most commonly infect not only freshwater fish species but also marine fishes. Antibiotic have been mostly used to prevent or treat fish from those pathogens (Corum et al., 2020; Terzi et al., 2020). In some cases, vaccine usage also gives an opportunity to overcome the problem. However, antibiotics have some adverse effect on animal and the environment (Capkin et al., 2015), and vaccines are used for specific pathogens.

Medicinal plants extracts already discovered to cure the fish against diseases (Bilen and Elbeshti, 2019; Bilen et al., 2019) or protect them from many different fish diseases (Bilen et al., 2020a; Bilen et al., 2020b; Bilen et al., 2014) and even in some cases as reproductive promoter (Sonmez et al., 2019). Also, medicinal plants have growth performance and immune system activation in fishes (Amhamed et al., 2018; Bilen et al., 2020c; Elbesthi et al., 2020; Mohamed et al., 2018). Laurel (*Laurus nobilis)* is a tree and has been used for its astringent, healing and diuretic properties (Nayak et al., 2006). Laurel has also antimicrobial and antibacterial effects (Digrak et al., 2001). Black mustard (*Brassica nigra*), native to the southern Mediterranean region of Europe, which has been cultivated for thousands of years. In a previous study, addition of a medicinal plant, *Brassica nigra*, to *Oreochromis niloticus* food improves both the immune and biotransformation systems after

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exposure to a polycyclic aromatic hydrocarbon, BaP (Abbas et al., 2016).

In the present study, we performed to demonstrate that the effects of aqueous methanolic extract of *Laurus nobilis* and *Brassica nigra* against fish pathogen such as *Vibrio anguillarium*, *Yersinia ruckeri*, *Pseudomonas putida* and *Aeromonas hydrophila*.

Materials and Methods

Plant and Preparation of the Extracts

The plants were purchased from herbalist in Kastamonu province. Aqueous methanolic extraction of the plants were performed as previously described (Bilen et al., 2016).

Table 1. The list of the plants used in the study

Scientific Name	Family	Vernacular Name
Laurus nobilis	Lauraceae	Laurel
Brassica nigra	Cruciferae	Black Mustard

Bacterial Strains

The plant extracts were tested against Gram negative bacteria, *Vibrio anguillarium* (SBVA1), *Yersinia ruckeri* (SBYR1), *Pseudomonas putida* (SBPP1) and *Aeromonas hydrophila* (SBAh1) which are isolated from fish and identified using conventional and molecular methods.

Minimum Inhibitory Concentration (MIC) Determination with Broth Microdilution Method

The antibacterial activity of the plants aqueous methanolic extracts were determined using sterile 300 µl 96-well plates as previously described (Wiegand et al., 2008) with small modifications. Briefly, all plant extracts were diluted up to 3200 µg ml^{-1} starting from 1.5625 µg ml^{-1} (3200 µg ml^{-1}, 1600 µg ml^{-1}, 800 µg ml^{-1}, 400 µg ml^{-1}, 200 µg ml^{-1}, 100 µg ml^{-1}, 50 µg ml^{-1}, 25 µg ml^{-1}, 12.5 µg ml^{-1}, 6.25 µg ml^{-1}, 3.125 µg ml^{-1} and 1.5625 µg ml^{-1}). 150 µl plant extract and same amount of the bacterial suspension each contains $1x10^8$ CFU was mixed by pipetting. For control, only bacterial suspension and the only methanolic extraction of the plant were prepared and added to 96-well plates. The plates were then placed in the incubator and kept at 25 ℃ for 48 hours. Each bacteria and all concentrations were studied in triplicate.

Results

The results of the study were given in Table 2.

Table 2. Antimicrobial activity of aqueous methanolic extracts of the medicinal plants used in the study

Plant Species	Bacterial Strains			
	VA	YR	PP	AH
Laurus nobilis (µg ml^{-1})	1600	3200	--------	3.125
Brassica nigra (µg ml^{-1})	100	3200	--------	800

VA: *Vibrio anguillarium*; YR: *Yersinia ruckeri*; PP: *Pseudomonas putida*; AH: *Aeromonas hydrophila*.

The MIC value of laurel aqueous methanolic extract showed the strongest activity against *Aeromonas hydrophila*. Black mustard aqueous methanolic extract showed the strongest activity against *Vibrio anguillarium*. Both of the plant extracts exhibit no activity against *Pseudomonas putida*. Also the activity of the plants against *Yersinia ruckeri* was very weak (3200 µg ml^{-1}).

Discussion

Medicinal herbs have many different chemical complex and substance and novel mechanism of the plans haven't been explained yet. In the present study effectiveness of the laurel and black mustard were demonstrated against *A. hydrophila* and *V. anguillarium*.

Kamaraj et al. (2012) have reported that the methanolic extract of the *A. indica* showed strong activity against *Klebsiella pneumonia*. Also, strong antimicrobial activity of *Cotinus coggyria* was determined against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Salmonella typhimurium*, *Salmonella typhi*, *Hanseniaspora guilliermondii*, *Rhodotorula rubra*, *Kluyveromyces fragilis*, *Kluyveromyces marxianus* and *Debaryomyces hansenii* (Dulger et al., 2009). Dulger et al. (2005) showed that several extracts and fractions of some Hypericum species have antimicrobial activity against bacterial pathogens.

In conclusion, this study highlights *Laurus nobilis* has antimicrobial activity against *Aeromonas hydrophila* and *Brassica nigra* has similar activity against *Vibrio anguillarium*. *In vivo* studies should be conducted using these plants extracts for the fish.

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RESEARCH ARTICLE

In vitro Effect of Bacterial Biocontrol Organisms against *Pectobacterium carotovorum* on Potato

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ABSTRACT

Soft rot is a disease that cause substantial economic losses in potato production. *Pectobacterium carotovorum* is known as one of the most common soft rot causative agents and the disease has not effective control methods being developed yet. In this study the effect of 3 different bacterial isolates of *Bacillus megaterium* (B60d, TV6D, TV91C), 2 of *Paenibacillus polymyxa* (Ç9, TV12E), 2 of *Bacillus subtilis* (TV6F, TV17C), 1 of *Pantoea agglomerans* (B79), 1 of *Agrobacterium radiobacter* (A16), 1 of *Bacillus megaterium* gc. subgroup A (FDG161), 1 of *Bacillus atrophaeus* (FD1), 1 of *Pseudomonas fluorescens* biotype F (FDG37) and 1 of *Bacillus pumilus* (TV3D) were tested against 5 pathogenic bacterial strains of *P. carotovorum* subsp. *carotovorum* (F37, F680, F741, F331, F742), using dual culture method in *in vitro* conditions. The most effective bacterial isolate against strains *Pectobacterium* were *P. carotovorum* F37 + *Bacillus megaterium* B60d (55,00 mm) isolate, followed by *P. carotovorum* F680 + *Bacillus megaterium* B60d (44,00 mm) and *P. carotovorum* F742 + *Bacillus megaterium* B60d (39,33 mm) isolates. Therefore, *Bacillus megaterium* has a promising biocontrol activity against tested plant pathogenic bacteria under *in vitro* conditions.

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Introduction

In terms of global production, potato (*Solanum tuberosum L.*) is the fourth most important food crop after corn, rice and wheat. This crop is grown throughout the world. According to FAO (Food and Agriculture Organization), in 2016 the world production was about 376 million tons potato. Asia and Europe are the world's major potato producing regions, accounting for more than 80% of world production (FAO, 2008).

Potato production globally is constrained by factors which cause substantial economic losses. These can be in the form of biotic and abiotic factors. The greatest losses are due to diseases and some of the important bacterial diseases are tuber soft rot, blackleg and aerial stem rot in the field (Ngadze, 2012).

For the soft rot, its characterized symptoms like sprouts of infected tubers that causes failure to emerge from the soil following planting and the emerged sprouts may show curled upper leaves, compact foliage, stunting, and fading from green to yellow-green. Infected plants later assume a distinct yellow color and gradually die as the lower stem rots away. When pulled, affected plants will have slimy, rotted, dark or inky black, mushy stems (Seebold, 2014). This disease mainly is caused by *Pectobacterium* and *Dickeya spp.*

Pectobacterium carotovorum (previously belonged to the genus *Erwinia*) is a Gram-negative plant-specific pathogen, causes soft rot diseases in monocot and dicot host plants in at least 35% of angiosperms (Marquez-Villavicencio et al., 2011). In potato it causes the disease by degradation of the plant cell wall (Aizawa, 2014).

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The effective control methods against the disease has not been developed, yet (Jeong-A et al., 2013). Various strategies such as chemical antibiotics and copper have been developed and used for many years (Cooksey, 1990). However, copper resistance has been reported in many bacterial pathogens and few effective bactericides have been developed (Cooksey, 1990; Bender et al., 1990). Chemicals are not effective in controlling soft rot pathogens; control strategies rely on the use of resistant cultivars, good agronomic practices such as planting certified disease-free seed, planting in well-drained soil and good sanitation (Ngazde, 2012; Rahman et al., 2017). Thus, novel strategies for the control of bacterial diseases are required and currently particular attention has been paid to biologically based strategies, such as bacteriocins or bacteriophages (Jeong-A et al., 2013).

In this context, the focus of biological control studies reflects the desire of several sectors to develop sustainable methods for plant disease control. However, efficient antagonists must be obtained for biological control to become a reality (Mota et al., 2016). Development of *in vivo* biocontrol agent selection is not a simple task due to the diversity of agents and interactions with the host plant, and therefore, efficient search methods are required. Therefore, it is necessary to develop efficient selection strategies to reduce costs and increase the possibility of selecting organisms that can be produced in a large scale at low cost and that maintain their viability and efficiency for long periods (Mota et al., 2016; Schisler and Slininger, 1997).

Materials and Methods

Material

Pathogen bacteria and biological control agent bacteria

Pathogen isolates used in this study were identified by using microbial identification system (MIS) and BIOLOG system. 5 pathogenic bacterial isolates were obtained from the culture collection in the Department of Plant Protection, Faculty of Agriculture at Ataturk University, Erzurum, Turkey. Pathogenic bacteria isolates information are given in Table 1.

Table 1. Pathogenic bacteria isolates (Dadaşoğlu, 2013)

Bacteria No	Identification Results
F- 37	*P. carotovorum* subsp. *carotovorum*
F- 331	*P. carotovorum* subsp. *carotovorum*
F- 680	*P. carotovorum* subsp. *carotovorum*
F-741	*P. carotovorum* subsp. *carotovorum*
F-742	*P. carotovorum* subsp. *carotovorum*

Bacterial biocontrol isolates were obtained from the Cultural Collection of the Department of Plant Protection, Faculty of Agriculture, Ataturk University. Bioagent bacteria isolates were grown on nutrient agar (NA) for routine use, and maintained in Luria Bertani (LB) Broth with 30% glycerol at –80°C for long-term storage. These isolates have been determined to be used as a plant growth agent and biosystems against pests and bacterial and fungal plant pathogens. In this study, 13 bacterial isolates were used among the numerous bacterial isolates considering the results of the previously conducted studies (Table 2).

Table 2. Bioagent bacteria isolates

Bacteria No	Identification Results
B60d	*Bacillus megaterium*
B79	*Pantoea agglomerans*
B16	*Agrobacterium radiobacter*
Ç9	*Paenibacillus polymyxa*
FDG161	*Bacillus megaterium* **gc subproup** *A*
FD1	*Bacillus atrophaeus*
FDG37	*Pseudomonas fluorescens* biotype F
TV6F	*Bacillus subtilis*
TV12E	*Paenibacillus polymyxa*
TV17C	*Bacillus subtilis*
TV6D	*Bacillus megaterium*
TV3D	*Bacillus pumilus*
TV91C	*Bacillus megaterium*

Method

Bioagent bacterial isolates test in petri against pathogens

Frozen pathogens and potential bioagent bacterial cultures were placed in petri plates containing NA and left to incubation at 25-27°C, 24 hours later fresh culture were obtained for *in vitro* dual culture method. Fresh pathogens developed cultures were taken and spread on the surface of the nutrient agar and the bacterial potential bioagent was applied in the middle of the petri plates (diameter 6 mm). Petri dishes were wrapped with parafilm and allowed to incubate for 48 hours at 27 ° C. Finally, for the evaluation of the inhibition zones or the hyperparasite effects, the propagation of the colonies of bacterial bioagent on the surface of the Petri dishes was measured.

Pectolytic activity tests

Fresh and healthy potato tubers were sterilized in 5% sodium hypochlorite for 10 minutes. Sterilized tubers were sliced 5 mm diameter and placed onto petri dishes containing sterilized wet papers. Bacterial broth cultures of 24 hours incubation were injected into potato slices in 1 ml volume and these slices were incubated at 28°C. After 24-72 h observed softness was assessed as positive result. As a control solution, sdH_2O was used (Figures 1 and 2).

The data analyses

The data obtained were subjected to transformation with arcsin, then one-way variance analysis was applied and the differences between the means were compared to LSMeans Student test at P <0.01 significance level. Data analysis in the present study were processed using statistical software JUMP IN (SAS Institute, cary, NC,% .0 PC version).

Figure 1. Pectolytic activity

Figure 2. Control

Results and Discussion

Pectobacterium sp. has a large list of hosts, including many species of agricultural and scientifically important plants. Pathogen produces pectolytic enzymes which hydrolyze pectin between individual plant cells. This causes the detachment of cells and soft rot. In this study, the objective was control diseases without using chemicals. 23 bacterial isolates were used as bioagents. In this study, 13 different bacterial species were applied in vitro against the pathogen. Table 2 shows the 13 bacterial biocontrol isolates tested for activity against *P. carotovorum* subsp. *carotovorum* F37, F680, F741, F331, F742.

According to MIS, bacterial isolates were identified as 3 *Bacillus megaterium* (B60d, TV6D, TV91C), 2 *Paenibacillus polymyxa* (Ç9, TV12E), 2 *Bacillus subtilis* (TV6F, TV17C), 1 *Pantoea agglomerans* (B79), 1 *Agrobacterium radiobacter* (A16), 1 *Bacillus megaterium* gc. subgroup A (FDG161), 1 *Bacillus atrophaeus* (FD1), 1 *Pseudomonas fluorescens biotype* F (FDG37) and 1 *Bacillus pumilus* (TV3D).

The best results against *P. carotovorum* subsp. *carotovorum* (F37, F680, F741, F331, F742) are shown in Figures 3, 4, 5 and Table 3. Inoculated zone values with bacterial bioagent were found to vary between 55.00-11.67 mm. The highest activity was observed between F37 + B60d (55.00 mm) isolate, followed by F680 + B60d (44.00 mm) and F742 + B60d (39.33) isolates. The lowest activity were obtained between F37 + Ç9 (11.67 mm), F741 + Ç9 (13.33 mm) and F331 + Ç9 (16.67 mm) isolates.

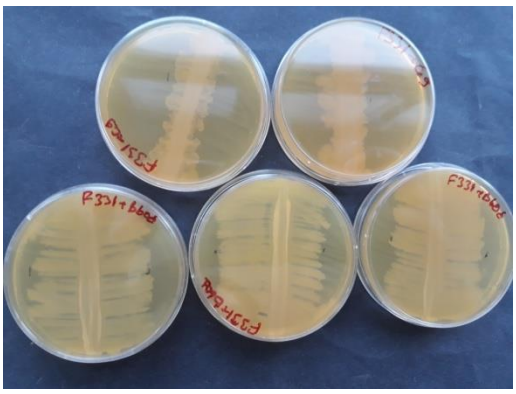

Figure 3. Hyperparasitic activity results (1)

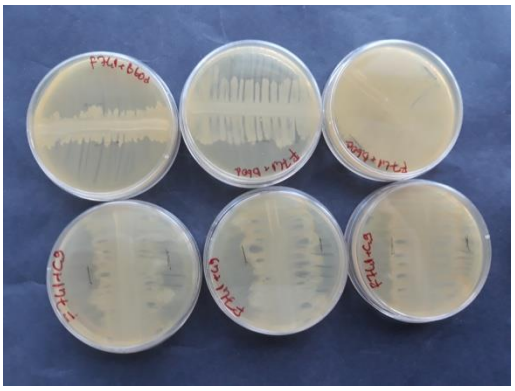

Figure 4. Hyperparasitic activity results (2)

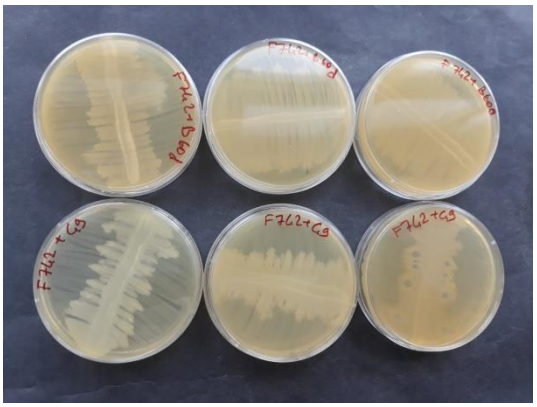

Figure 5. Hyperparasitic activity results (3)

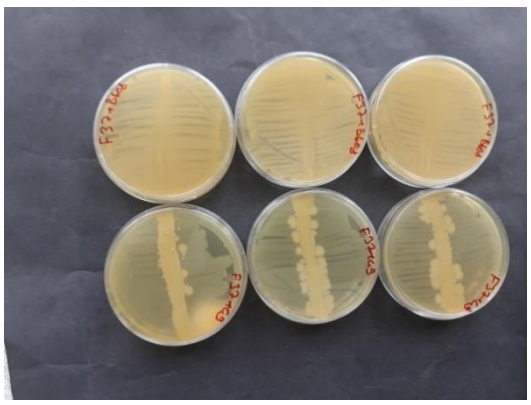

Figure 6. Hyperparasitic activity results (4)

Table 3. Inhibition rates of *P. carotovorum* subsp. *carotovorum* isolates in the dual culture tests of bacterial biocontrol isolates

No. Pathogen and bioagent bacterial isolate	ZONE (mm)	
F37 and B60d	55.00	A
F680 and B60d	44.00	B
F742 and B60d	40.00	B
F331 and B60d	39.33	BC
F741 and B60d	29.33	CD
F742 and Ç9	26.33	DE
F680 and Ç9	20.00	DEF
F331 and Ç9	16.67	EF
F741 and Ç9	13.33	F
F37 and Ç9	11.67	F
Control B60d	0.00	G
Control and Ç9	0.00	G
LSD	10.01	
CV	24.01	

Bacillus megaterium was the bacterial biocontrol isolates that had the greatest growth zone and prevented the pathogen development.

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RESEARCH ARTICLE

Phylogeny, Characterisation and Identification of Creatine Kinase Genes (*ckma* and *ckmb*) in Zebrafish (*Danio rerio*)

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Bioinformatics

ABSTRACT

Creatine kinase genes (*ckma* and *ckmb*) in zebrafish (*Danio rerio*), an aquatic model organism, have been characterized and identified. However, the gene structure is designed using exons, introns, amino acids produced by the exons. TATA boxex, poly A tails and 5' UTR and 3' UTR regions of zebrafish *ckma* and *ckma* genes are showed at the gene structure. In addition, chromosomal regions of *ckma* and *ckmb* genes were determined. The other genes which are placed in the same region with *ckma* and *ckmb* genes were found in medaka and human which are the orthologs of zebrafish, and conserved gene syntheny was designed manually according to these regions. In addition, phylogenetic relationship was determined between zebrafish and it's some orthologs using *ckma* and *ckmb* gene sequences. Genetic affinity between zebrafish and its orthologs was calculated as similarity-identity % rate and given as a table. For all these studies, bioinformatics databases (NCBI database, Ensembl genomic database, Expasy, Reverse Complementary) and programs (MEGA6 program, BLOSUM62 matrix program and BioEdit software) were used. In this study, characterization and identification of *ckma* and *ckmb* genes in zebrafish (*D. rerio*) was completed using bioinformatics tools and some data to be used in the future studies on molecular stress response were presented.

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Introduction

Zebrafish (*Danio rerio*) is a tropical freshwater fish that has its natural habitat in rivers of Northern Pakistan, Bhutan and Nepal, as well as rivers of South Asia and Northeast India (Carpio and Estrada, 2006). Zebrafish belonging to the Cyprinidae family in the ray-finned fish (Actinopterygii) class is a teleost fish (Carpio and Estrada, 2006). It has many advantages, such as being a rapidly developing creature such as being completed to a large extent afterwards (Gilmour et al., 2002). It has been one of the most researched model organisms due to its existence (Carpio and Estrada, 2006). In addition, zebrafish embryos are an aquatic model organism that is powerful enough for experimental-mental manipulations such as microinjection and cell transplantation experiments, and therefore highly preferred in genetic studies (Gilmour et al., 2002). Due to the transparency of the embryo, it has been possible to directly observe its internal development and the embryos can be genetically and embryologically manipulated through microinjection, making zebrafish an excellent complementary research model for human disease and development (Ma, 2004; Lieschke and Currie, 2007).

Analysis of fish muscle protein levels indicates that creatine kinase is one of the most highly expressed proteins in fish muscle (McLean et al., 2007). It has both cytosolic and mitochondrial forms involved in the regulation of energy production (mitochondria) and utilization (cytosol) through actions related to ATP. There is a chemical cycle in the alive fish muscle. These chemical events provide energy to the muscle while the fish swim, providing the substances necessary for growth and regeneration of dead tissue. The substances that create and control chemical reactions in living muscle are

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enzymes, and the energy source required for this application is ATP, which converts chemical energy into mechanical energy. While ATP consumption and re-formation and contraction-relaxation events in living tissue are continuous, the amount of ATP decreases rapidly as a result of the interruption of blood circulation and oxygen supply in the post-mortem tissue, and contraction and relaxation events continue to be limited during this decrease. The energy required for the contraction of the muscle in living fish is provided by the ATP formed during glycolysis. ATP is broken down into adenosine diphosphate (ADP) and inorganic phosphate (P) by the ATPase enzyme, and the energy released at this time is used for the contraction of the muscle. ADP and creatine are catalyzed by the keratin kinase enzyme to regenerate ATP from phosphate (Stryer, 1995).

Although it is known that there is genetic similarity between species in organisms, the thesis that studies on an organism can be used as a data source for other species (Collins et al., 1998) increased the importance of model organisms in scientific studies. Therefore, in this study, bioinformatics analysis of *ckma* and *ckmb* genes in zebrafish will provide pioneering data for molecular studies in other organisms. Creatine kinase one of the enzymes that maintains cellular energy homeostasis and high ATP/ADP and ATP/AMP ratios in vertebrates in order to meet the high energy demand for physiological responses in living organism (Wallimann et al., 1992). In order to provide higher energy, it is necessary to catalyze phosphocreatine and ADP of creatine kinase, creatine and ATP in ADP more effectively (Wu et al., 2011). Also, zebrafish cannot survive in water temperatures below 12 ^{o}C or cannot be fed when the temperature is below 16 ^{o}C (Chou et al., 2008). Therefore, this study is of great importance in providing basic information for studies on both zebrafish and other teleost fish.

Materials and Methods

To investigate whether the creatine kinase genes (*ckma* and *ckmb*) are functional or they are nonfunctional or pseudo-genes in zebrafish, the cDNA sequences of these genes were obtained from the ENSEMBL database and blasted (http://blast.ncbi.nlm.nih.gov) using the NCBI database described in our previous publication (Bayır et al., 2020), and it was confirmed that both *ckma* and *ckmb* genes are functional genes in zebrafish. Then, ENSEMBL data bank was used to characterize creatine kinase genes. The ensembl number of the zebrafish *ckma* gene was ENSDART00000032481.6 and the UNIPROT number was A2BHA3, while the ensembl number of the *ckmb* gene was ENSDART00000059366.7 and the UNIPROT number was Q7T306. It was also determined that the *ckma* gene encodes a protein of 381 amino acids, while the *ckmb* gene encodes a protein of 380 amino acids.

Conserved gene syntheny was designed manually by detecting conserved common genes and determining their locations to detect genes that are preserved in the same way as their orthologists. For this purpose, conserved genes in zebrafish (*Danio rerio*), human (*Homo sapiens*) and medaka (*Oryzias latipes*) used for the *ckma* gene while conserved genes in zebrafish, human (*Homo sapiens*) and spotted gar (*Lepisosteus oculatus*) was using for *ckmb* gene. First of all, it was determined in which chromosomes and in which regions the *ckma* and *ckmb* genes were found in zebrafish (*D. rerio*) and then the other genes on this chromosome were found and the locations of these genes were recorded. Later, a conserved gene syntheny was formed by detecting the chromosomes and locations of these genes, which were detected in medaka (*Oryzias latipes*), spotted gar *(Lepisosteus oculatus*) and human (*Homo sapiens*), which are orthologists of zebrafish (*Danio rerio*) (Figure 1). Using the CLUSTALW (Thompson et al., 1994) BioEdit program the proteins of these organisms were aligned and then using MEGA6 (Using the program Tamura et al., 2013) a phylogenetic tree (Kell et al., 2018) was created according to the maximum likelihood method (Figure 2). Medaka (*O. latipes*) glutathione reductase (*gsr*) gene was used as outgroup (Figure 2).

The gene structures consisting of the starting point (+1) of transcription, exon-intron organization, amino acids produced by the exons, the 5 'UTR regions of these two genes (with the TATA box in this region) and the 3'UTR region (showing the poly A tail located in this region), of zebrafish (*D. rerio*) *ckma* and *ckmb* genes are shown in Table 1 and Table 2. For the creation of these tables, ENSDART00000032481.6 transcript for the *ckma* gene and the cDNAs of the ENSDART00000059366.7 transcript for the *ckmb* gene were used.

Zebrafish (*Danio rerio*) with medaka (*Oryzias latipes*), platy fish (*Xiphophorus maculatus*), spiny (*Gasterosteus aculeatus*), blowfish (*Fugu rupripes*) human (*Homo sapiens*) *ckma / CKM* proteins and zebrafish (*Danio rerio*) with cave fish (*Astyanax mexicanus*), eel (*Electrophorus electricus*), Mexican tetra fish (*Astyanax mexicanus*), spotted gar (*Lepisosteus oculatus*) human (*Homo sapiens*) ckmb / CKM proteins by aligning the in the Bioedit program, using CLUSTALW. Identity rates were calculated (Thompson et al., 1994) (Table 3,4).

Results and Discussion

The big effect of industrial enterprises wastes, oxygen deficiency is also a major factor in the increase in creatine in fish (Arslan, 2015). The stress responses of vertebrates also include different interactions between physiological pathways that can be characterized in both acute and chronic situations. Creatine kinase (CK); is an important enzyme used in the determination of damage to tissues and organs such as Glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) enzymes.

These enzymes except from CK are liver enzymes and they are also used to understand liver-related problems. CK and GOT enzymes tend to increase in fish skin wounds, muscle tissue and brain disorders. In addition, the CK enzyme provides the renewal of ATP in the contraction or transport systems. Therefore, it is of great importance to complete a detailed bioinformatics study of *ckma* and *ckmb* genes, which are the stress markers in fish whose acute or chronic stress response varies with environmental differences (Iwama et al., 1999) in great importance as a model organism zebrafish (*D. rerio*).

Table 1. Nucleotide sequence of Zebrafish (*Danio rerio*) *ckma* gene

```
5'atggatatgggaaggaagggggcaccacccacagctgccacctcatctaggatgcct
ggggcctaaattgaagccttcttacactaaacagggcataagagaccagcgccagccaa
tcataattcagtgagctctaaaatgggccagccaatggctgcaggggctagaggtaTATA
                      +1
tatccaaatcaaactcttcttgCTTGGGTGACCCCTATTTCGGCTTGGTGAACAGGATCT
GATCCCAAGGACTGTTACCACTTTTGTTGTCTTTTGTGCAGgtaaa'N1538'atcagTG
TTAGAAACGCAATCATGCCTTTCGGAAACACCCACAACAACTTCAAGCTGAACTACTCAG
              -M--P--F--G--N--T--H--N--N--F--K--L--N--Y--S--
TTGATGAGGAGTATCCAGACCTTAGCAAGCACAACAACCACATGGCCAAGGTGCTGACTA
V--D--E--E--Y--P--D--L--S--K--H--N--N--H--M--A--K--V--L--T--
AGGAAATGTATGGCAAGCTTAGGGACAAGCAGACCTCCACTGGATTCACTGTGGATGATG
K--E--M--Y--G--K--L--R--D--K--Q--T--S--T--G--F--T--V--D--D--
TCATCCAGACCGGTGTTGACAATCCAGgtgag'N95'tccagGCCACCCCTTCATCATGA
V--I--Q--T--G--V--D--N--P--                 G--H--P--F--I--M--
CCGTCGGCTGTGTTGCTGGTGATGAGGAGTCCTACGAAGTGTTCAAGGATCTGTTCGACC
T--V--G--C--V--A--G--D--E--E--S--Y--E--V--F--K--D--L--F--D--
CCGTCATTTCCGACCGTCACGGTGGATACAAGGCAACTGACAAGCACAAGACCGACCTCA
P--V--I--S--D--R--H--G--G--Y--K--A--T--D--K--H--K--T--D--L--
ACTTTGAGAACCTGAAGgtaca'N783'tgtagGGTGGTGATGACCTGGACCCCAACTAC
N--F--E--N--L--K-                  -G--G--D--D--L--D--P--N--Y-
GTCCTGAGCAGCCGTGTGCGTACCGGACGCAGCATCAAGGGATACGCCCTGCCCCCCCAC
-V--L--S--S--R--V--R--T--G--R--S--I--K--G--Y--A--L--P--P--H-
AACAGCCGTGGAGAGCGCAGAGCTGTGGAGAAGCTGTCTGTTGAAGgtctg'N971'tcc
-N--S--R--G--E--R--R--A--V--E--K--L--S--V--E--
agCTCTGAGCAGCTTGGATGGAGAGTTCAAGGGCAAGTACTACCCCCTGAAGTCCATGAC
  A--L--S--S--L--D--G--E--F--K--G--K--Y--Y--P--L--K--S--M--T
TGATGCCGAGCAGGAGCAGCTGATCGCTGACCACTTCCTCTTTGACAAACCCGTCTCCCC
--D--A--E--Q--E--Q--L--I--A--D--H--F--L--F--D--K--P--V--S--P
CCTGCTGCTGGCTGCTGGTATGGCCCGTGACTGGCCCGATGCCAGAGGCATTTGgtgag'
--L--L--L--A--A--G--M--A--R--D--W--P--D--A--R--G--I—W
N555'tatagGCACAATGAGAACAAGACCTTCCTGGTCTGGGTGAACGAGGAGGATCACC
          --H--N--E--N--K--T--F--L--V--W--V--N--E--E--D--H--
TGCGTGTCATTTCCATGCAGAAGGGTGGCAACATGAAGGAAGTGTTCAAGCGCTTCTGCG
L--R--V--I--S--M--Q--K--G--G--N--M--K--E--V--F--K--R--F--C--
TTGGTCTTCAGAGGgtatg'N79'gatagATTGAGGAAATTTTCAAGAAGCACAACCATG
V--G--L--Q--R-                -I--E--E--I--F--K--K--H--N--H--
GGTTCATGTGGAACGAGCATCTTGGTTTCGTCCTGACCTGCCCCTCCAACCTGGGCACAG
G--F--M--W--N--E--H--L--G--F--V--L--T--C--P--S--N--L--G--T--
GCCTGCGCGGTGGAGTCCACGTCAAGCTGCCCAAGCTCAGCACACATGCCAAGTTTGAGG
G--L--R--G--G--V--H--V--K--L--P--K--L--S--T--H--A--K--F--E--
AGATCCTGACCAGACTGCGCCTGCAGAAGCGTGGCACAGgtata'N93'ctcagGTGGTG
E--I--L--T--R--L--R--L--Q--K--R--G--T--              G--G--
TGGACACTGCCTCCGTTGGTGGAGTGTTTGACATTTCCAACGCTGACCGTATCGGCTCTT
V--D--T--A--S--V--G--G--V--F--D--I--S--N--A--D--R--I--G--S--
CAGAGGTTGAGCAGGTGCAGTGTGTGGTTGATGGTGTCAAGCTGATGGTGGAGATGGAGA
S--E--V--E--Q--V--Q--C--V--V--D--G--V--K--L--M--V--E--M--E--
AGAAGCTGGAGAAGGGCGAGTCCATCGACAGCATGATCCCTGCCCAGAAGTAAagcggga
K--K--L--E--K--G--E--S--I--D--S--M--I--P--A--Q--K--*-
gctcttccatttttttctcgtctttgtctgtttttttacagtccaacagcaatgcagagg
aaaactgctgctcaaaaagacagtctcacctttgcacctgtcttctttcctttttttcc
cttcttctctaatttccatgtcatttcgccatctttttttccactttgtttcctattaag
tcggtaacatcttgggatcagatacccggcgcaggagtgagtgcctgttgctgaggcttc
acctcaatttcagccttggttgtaaaaagtgaatcaatcaaagttgtatttcAATAAAAA
taccccataaaaca 3'
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* The exons of Zebrafish (*D. rerio*) *ckma* gene are shown in capital letters, starting point of transcription with +1, 5 'upstream sequence, 3' downstream sequence are shown in lowercase letters. The first five nucleotides and the last 5 nucleotides of the introns are shown in lowercase letters and in red, and the length of the intron other than these nucleotides is given. The TATA box and poly adenylation signal (AATAAAAA) are shown in capital letters and colored yellow. Stop codon (TAA) is indicated by asterisk.

Table 2. Nucleotide sequence of Zebrafish (*Danio rerio*) *ckmb* gene

```
5’gtgcctacagtttatttcataactgagcacagaggaataggttaccgtggggtgggga
cagctgtctccccttctcgggtgttgtgatgtagcttctttccttttagaacctgaaacc
cccatcaagaagtgcagctatccaatgaagttcaagcatgcaggagcgggacaaccaatg
                                                        +1
ggcagcaaacattgaagTATAtaaaccaaggtctgacgtcttgcaaaGTTGGGCAACCCC
TACATTGGCTTGGTGAACAGGATCTGATCCCAAGGACTGTTACCTTTTCTCCCATACAGg
taaa’N1073’ggtagATTGCCGGAAAAGTGCAATCATGACTAAGAACTGCAACAACGAT
                                          -M--T--K--N--C--N--N--D-
TACAAGATGAAGTTTGCTGTGGATGAGGAGTTTCCTGACCTCTCCCAGCACAACAACCAT
-Y--K--M--K--F--A--V--D--E--E--F--P--D--L--S--Q--H--N--N--H-
ATGTCCAAGGTCCTCACCAAGGACATCTACAACAAGCTCAGGAGCAAGTCAACCCCCAGT
-M--S--K--V--L--T--K--D--I--Y--N--K--L--R--S--K--S--T--P--S-
GGATTCACCCTTGATGACTGCATCCAGACTGGTGTGGACAACCCTGgtaag’N318’aca
-G--F--T--L--D--D--C--I--Q--T--G--V--D--N--P--
agGTCACCCCTTCATCATGACTGTCGGCTGTGTGGCTGGGGATGAGGAGTCCTATGAAGC
 G--H--P--F--I--M--T--V--G--C--V--A--G--D--E--E--S--Y--E--A
CTTCAAGGAGTTGTTCGACCCCGTCATTTCTGACCGTCATGGTGGCTATAAGCCCACCGA
--F--K--E--L--F--D--P--V--I--S--D--R--H--G--G--Y--K--P--T--D
CAAGCATCTTACTGATCTGAACTGGGAGAACCTGAAGgtatg’N57’cacagGGTGGTGA
--K--H--L--T--D--L--N--W--E--N--L--K-                -G--G--D
TGATCTTGACCCCAACTACGTTCTGAGCAGCCGCGTACGTACCGGCCGCAGCATCAAGGG
--D--L--D--P--N--Y--V--L--S--S--R--V--R--T--G--R--S--I--K--G
ATTCACCCTGCCTCCTCACAACAGCCGTGGTGAGCGCAGAGCTGTGGAGAAGCTGTCCAT
--F--T--L--P--P--H--N--S--R--G--E--R--R--A--V--E--K--L--S--I
TGAGGgtatg’N69’cacagCTCTGAACAGCCTGGATGGTGAGTTCAAGGGCAAGTACTA
--E--                A--L--N--S--L--D--G--E--F--K--G--K--Y--Y
CCCACTGAAGGACATGACTGACAAGGAGCAGGAGCAGCTCATTGCTGACCACTTCCTGTT
--P--L--K--D--M--T--D--K--E--Q--E--Q--L--I--A--D--H--F--L--F
TGACAAGCCTGTGTCCCCCCTGCTGTTGGCTGCTGGCATGGCCCGTGACTGGCCTGACGG
--D--K--P--V--S--P--L--L--L--A--A--G--M--A--R--D--W--P--D--G
TAGAGGTATCTGGCACAACGACAACAAGACCTTCCTTGTGTGGGTGAACGAGGAGGATCA
--R--G--I--W--H--N--D--N--K--T--F--L--V--W--V--N--E--E--D--H
CCTCCGTGTCATCTCTATGCAGAAGGGTGGCAACATGAAGGAGGTCTTCAAGAGGTTCTG
--L--R--V--I--S--M--Q--K--G--G--N--M--K--E--V--F--K--R--F--C
CGTTGGCCTGCAGAAGgtaaa’N77’tgcagATTGAGGATGTTTTCAAGAAGCACAACCA
--V--G--L--Q--K-                -I--E--D--V--F--K--K--H--N--H
CGGTTTCATGTGGAACGAGCATCTTGGTTTCATCTTGACCTGCCCCTCTAACTTGGGTAC
--G--F--M--W--N--E--H--L--G--F--I--L--T--C--P--S--N--L--G--T
CGGTCTGCGCGGTGGTGTCCACGTCAAGCTGCCCAAACTCAGCACACATGCCAAATTTGA
--G--L--R--G--G--V--H--V--K--L--P--K--L--S--T--H--A--K--F--E
GGAGATCCTGACCAGACTGCGTCTTCAGAAGCGTGGCACAGgtgag’N83’tccagGTGG
--E--I--L--T--R--L--R--L--Q--K--R--G--T--                G--G
TGTGGACACAGCCTCCGTCGGTGGTGTGTTCGACATCTCAAACGCTGACCGTCTGGGCTC
--V--D--T--A--S--V--G--G--V--F--D--I--S--N--A--D--R--L--G--S
CTCTGAGGTGCAGCAGGTGCAGCTGGTGGTCGATGGTGTGAAACTCATGGTTGAAATGGA
--S--E--V--Q--Q--V--Q--L--V--V--D--G--V--K--L--M--V--E--M--E
AAAGAAACTGGAGAAGGGCGAGTCCATCGATGACATGATCCCTGCCCAGAAGTAAactgc
--K--K--L--E--K--G--E--S--I--D--D--M--I--P--A--Q--K--*-
caaaagtgtttcttttttttatttgttcatgcagtcgtgccatcatgacttttccaaaag
acactccaccctgcttccttttgtcctactttttttttctccttcctttctttcctgtcg
tcttctttttccagcacttttttcttcttatcttgctgttcctcctgtaacatcctgggat
caaacatccacatagctagaaacccctagctgtgttgtattcacctaaacttttttggtt
gtacaaagtttAATAAAcggccccatgtaacaatcaa 3’
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* The exons of Zebrafish (*D. rerio*) *ckmb* gene are shown in capital letters, starting point of transcription with +1, 5 'upstream sequence, 3' downstream sequence are shown in lowercase letters. The first five nucleotides and the last 5 nucleotides of the introns are shown in lowercase letters and in red, and the length of the intron other than these nucleotides is given. The TATA box and poly adenylation signal (AATAAAAA) are shown in capital letters and colored yellow. Stop codon (TAA) is indicated by asterisk.

Since fish are aquatic organism, changes in the qualitative and quantitative properties of water can cause changes in the functional structure of the proteins of fish, therefore, from time to time, the protein folds can be opened and these proteins can combine with other proteins in the cell to form clumps.

As a result of this situation, proteins can lose their functions due to conformational deformation (Basu et al., 2000). Therefore, in this study, it was determined that *ckma* and *ckmb* genes are functional genes in zebrafish (*D. rerio*) with bioinformatics tools before the other bioinformatic studies such as determining the gene structure, creating a phylogenetic tree, constructing a preserved gene synthin and calculating the similarity-identity ratios with orthologists of zebrafish.

Bioinformatics studies should be completed before experimental studies in order to understand how the expression of genes changes with various stress factors in molecular studies. Therefore, this study will provide important bioinformatics data both for fish physiology studies and for studies on other vertebrates since zebrafish (*D. rerio*) is a model organism.

In this study, first of all, ENSEMBL, UNIPROT and NCBI databases and computerized algorithms such as BioEdit software, BLOSUM62 matrix program and MEGA6 program were used to reach and evaluate some data such as cDNAs, exons and introns of *ckma* and *ckmb* genes, amino acids produced by these genes, 5 'UTR and 3'UTR regions, chromosomes and locations where genes are located, protein sequences required for determining their phylogenetic affinity with other vertebrates. It was determined that zebrafish *ckma* gene has 8 exons and 7 introns while *ckmb* gene has 7 exons and 6 introns before the gene structures of these two genes were designed (Table 1, 2).

Sequence similarity was calculated to investigate the orthology of zebrafish *ckma* and *ckmb* genes with some other vertebrates. For this purpose, zebrafish (*D. rerio*) with protein sequences produced by *ckma* and *ckmb* genes, medaka (*Oryzias latipes*), platy fish (*Xiphophorus maculatus*), stickleback (*Gasterosteus aculeatus*), puffer fish (*Fugu rubripes*) cave fish (*Astyanax mexicanus*), eel (*Electrophorus electricus*), Mexican tetra fish (*Astyanax mexicanus*), spotted gar (*Lepisosteus oculatus*) and human *(Homo sapiens*) *ckma* and *ckmb*/*CKM* genes were sequenced using the Bioedit program in the BLOSUM62 matrix algorithm and the similarity of these organisms. Identity rates were calculated (Gromiha, 2010). Analysis results include zebrafish (*D. rerio*) with *ckma* gene, medaka (*O. latipes*) 93-97%, platy fish (*X. maculatus*) and stickleback (*G. aculeatus*) 90-96%, puffer fish (*F. rubripes*) and cave fish (*A. mexicanus*) 88-93%, human (*H. sapiens*) 88-93% and zebrafish (*D. rerio*) *ckmb* 88-94% (Table 3), zebrafish (*D. rerio*) with the *ckmb* gene medaka (*O. latipes*) 94-97%, eel (*E. electricus*) 93-97%, zebrafish (*D. rerio*) *ckma* gene 88-95%, human (*H. sapiens*) 84-92%, Mexican tetra fish (*A. mexicanus*) 82-85%, spotted gar (*L. oculatus*) showed 82-91% similarity-identity ratio (Table 4).

In order to determine the conserved genes of zebrafish (*D. rerio*) with medaka (*O. latipes*) and human (*H. sapiens*), it was first determined from the Ensembl genome database that the *ckma* and *ckmb* genes of this organism are on the 5th and 15th chromosomes and other genes located in these chromosomes were determined and their locations determined.

Table 3. Similarity-identity ratio of the zebrafish *ckma* gene with the other teleosts and human

```
Zf Ckma      1   MPFGNTHNNFKLNYSVDEEYPDLSKHNNHMAKVLTKEMYGKLRDKQTSTGFTVDDVIQTG
Me Ckma      1   ................D.F................L...M.....P....L.......
Pf Ckma      1   ..............K.E..F..............N.DI.A.......PS.Y.L.......
St Ckma      1   ..............K.ED.F................L...I..R..PS.Y.L.......
Fu Ckma      1   .AK-.C..DY.MKFA....F....Q............I.....G.S.PS..........
Hu Ckm       1   ........K.....KPE..................L.L.K.....E.PS..........
Zf Ckmb      1   .TK-.CN.DY.MKFA....F....Q.....S.....DI.N...S.S.PS...L..C....

Zf Ckma     61   VDNPGHPFIMTVGCVAGDEESYEVFKDLFDPVISDRHGGYKATDKHKTDLNFENLKGGDD
Me Ckma     61   I.........................L............P..................
Pf Ckma     61   ..........................E.L..I.........P..................
St Ckma     61   ..........................E.L............P.......M..........
Fu Ckma     60   ........................A....L............P..................
Hu Ckm      61   ..........................E....I.........P.........H........
Zf Ckmb     60   ........................A..E..............P....L....W........

Zf Ckma    121   LDPNYVLSSRVRTGRSIKGYALPPHNSRGERRAVEKLSVEALSSLDGEFKGKYYPLKSMT
Me Ckma    121   ................................I....I.....................
Pf Ckma    121   ....................T............I...A....T.................
St Ckma    121   ...................FT............I........T.................
Fu Ckma    120   ...................FT............I....I...A.............TG..
Hu Ckm     121   ....................T....C................N..T.............
Zf Ckmb    120   ...................FT.................I...N..............D..
```

Table 3 continued

```
Zf Ckma     181 DAEQEQLIADHFLFDKPVSPLLLAAGMARDWPDARGIWHNENKTFLVWVNEEDHLRVISM
Me Ckma     181 ........S.............TC.........G......D..................
Pf Ckma     181 ........S.............TC................DD..................
St Ckma     181 ........N.............TC.........G...M..D..................
Fu Ckma     180 ........................TC.........G......D..S..............
Hu Ckm      181 EK..Q...D...............S..............D..S..............
Zf Ckmb     180 .K..............................G......D..................

Zf Ckma     241 QKGGNMKEVFKRFCVGLQRIEEIFKKHNHGFMWNEHLGFVLTCPSNLGTGLRGGVHVKLP
Me Ckma     241 ......R...R.......K...................YI...................
Pf Ckma     241 ......R...R.......K...................YI...................
St Ckma     241 ......R...........K...................YI...................
Fu Ckma     240 ..........R.......K..A................YI...................
Hu Ckm      241 E.........R.......K.......AG.P....Q...Y...................A
Zf Ckmb     240 ..................K..DV................I...................

Zf Ckma     301 KLSTHAKFEEILTRLRLQKRGTG-GVDTASVGGVFDISNADRIGSSEVEQVQCVVDGVKL
Me Ckma     301 .....P.................-..................L.....A...L.......
Pf Ckma     301 .....P.................-..................L.....D...L.......
St Ckma     301 .....P..D.............S..................L.........L.......
Fu Ckma     300 ....QP.................-..................L.........L.......
Hu Ckm      301 H..K.P.................-.....A..S...V.....L.........L.......
Zf Ckmb     300 .......................-..................L.....Q...L.......

                                                 Similarity(%)     Identity (%)

Zf Ckma     360 MVEME CKMAKKLEKGESIDSMIPAQK      100               100
Me Ckma     360 ............A.........          93                 97
Pf Ckma     360 ............A..G......          90                 96
St Ckma     361 ...L........A...L.....          90                 96
Fu Ckma     359 ...............G......          88                 93
Hu Ckm      360 ...........Q...D......          88                 93
Zf Ckmb     359 ...............D......          88                 94
```

* Amino acid sequence alignment of Zebrafish (Zf) Ckma with medaka (Me) Ckma, eel (Ee) Ckma, Zebrafish Ckmb, Human (Hu) Ckm, Mexican tetra fish (Mt) Ckma, and spotted gar (Sg) Ckma. The dots represent same amino acids with the first line and tires represent amino acids that are not specified. The percent identity and similarity between the zebrafish Ckma and the other teleosts and human Ckma/Ckm proteins is shown at the end of each sequence.

Table 4. Similarity-identity ratio of the zebrafish *ckmb* gene with the other teleosts and human

```
Zf Ckmb   1   MTK-NCNNDYKMKFAVDEEFPDLSQHNNHMSKVLTKDIYNKLRSKSTPSGFTLDDCIQTG
Cf Ckmb   1   ...-..H.......SLE........L.....A............G.........V..V....
Ee Ckmb   1   ...-..H.......SLE..Y..........A.A...E..E..............I....
Zf Ckma   1   .PFG.TH.NF.LNYS....Y....K.....A.....EM.G...D.Q.ST...V..V....
Hu Ckmb   1   .PFG.TH.KF.LNYKPE..Y....K.....A....LEL.K...D.E......V..V....
Mt Ckmb   1   ...-..H.......SLE........L.....A............G.........V..V....
Sg Ckmb   1   .PFG.TH.N..LN.S.........TK.....A.A......A...D.Q......V..V....

Zf Ckmb   60  VDNPGHPFIMTVGCVAGDEESYEAFKELFDPVISDRHGGYKPTDKHLTDLNWENLKGGDD
Cf Ckmb   60  ........................V..D.L.................H.............
Ee Ckmb   60  ........................V..D.L.................N.............
Zf Ckma   61  ........................V..D..............A....K....F........
Hu Ckm    61  ........................V.......I..............K....H........
Mt Ckmb   60  ........................V..D.L.................H.............
Sg Ckmb   61  ......................DV..D......E...N.F......K....FG.......

Zf Ckmb   120 LDPNYVLSSRVRTGRSIKGFTLPPHNSRGERRAVEKLSIEALNSLDGEFKGKYYPLKDMT
Cf Ckmb   120 ................L.........................SV................
Ee Ckmb   120 ......Q............Y.....................MT................
Zf Ckma   121 ...................YA.................V...S.............S..
Hu Ckm    121 ...................Y.....C............V......T...........S..
Mt Ckmb   120 ................L.........................SV................
Sg Ckmb   121 ...................Y.....C.......I..M..D...T.E.............
```

Table 4 continued

```
Zf Ckmb   180 DKEQEQLIADHFLFDKPVSPLLLAAGMARDWPDGRGIWHNDNKTFLVWVNEEDHLRVISM
Cf Ckmb   180 ................................A.........................
Ee Ckmb   180 E...D..................S........A...Y.....................
Zf Ckma   181 .A.............................A......E...................
Hu Ckm    181 E...Q...D..............S........A.........S...............
Mt Ckmb   180 ................................A.........................
Sg Ckmb   181 .E..D...R..............S........A......ND.................

Zf Ckmb   240 QKGGNMKEVFKRFCVGLQKIEDVFKKHNHGFMWNEHLGFILTCPSNLGTGLRGGVHVKLP
Cf Ckmb   240 .L............T......ET.....................................
Ee Ckmb   240 ..............................................................
Zf Ckma   241 ..................R..EI................V.....................
Hu Ckm    241 E.........R.........EI...AG.P....Q...YV...................A
Mt Ckmb   240 .L............T......ET.....................................
Sg Ckmb   241 ..........R...........L....GRS...S....Y.....................

Zf Ckmb   300 KLSTHAKFEEILTRLRLQKRGTGGVDTASVGGVFDISNADRLGSSEVQQVQLVVDGVKLM
Cf Ckmb   300 ..............................................................
Ee Ckmb   300 .....P........................................................
Zf Ckma   301 ........................................I.....E...C........
Hu Ckm    301 H..K.P.....................A..S...V...........E............
Mt Ckmb   300 ..............................................................
Sg Ckmb   301 Q..K.P.....................AE.............F...E...M........

Zf Ckmb   360 VEMEKKLEKG-------ES----------IDDMIPAQK----------------------
Cf Ckmb   360 ..........-------..----------..N......----------------------
Ee Ckmb   360 I.........-------..----------.........----------------------
Zf Ckma   361 ..........-------..----------..S......----------------------
Hu Ckm    361 ..........-------Q.----------.........----------------------
Mt Ckmb   360 .........CSRTTFPRD.PPCSPSFFLF.LSLF.VAYNVFLLPSFLLSLSHSGHSGLRL
Sg Ckmb   361 I.........--AA---------------...IL--------------------------

                                  Similarity(%)     Identity(%)

Zf Ckmb   380 ----                    100               100
Cf Ckmb   380 ----                     94                97
Ee Ckmb   380 ----                     93                97
Zf Ckma   381 ----                     88                95
Hu Ckm    381 ----                     84                92
Mt Ckmb   420 SGHV                     82                85
Sg Ckmb   377 ----                     82                91
```

* Amino acid sequence alignment of zebrafish (Zf) Ckmb with cave fish (Cf) Ckmb, eel (Ee) Ckmb, Zebrafish (Zf ckma, human (Hu) Ckm, Mexican tetra fish (Mt) Ckmb and spotted gar (Sg) Ckmb. The dots represent same amino acids with the first line and tires represent amino acids that are not specified. The percent identity and similarity between the zebrafish Ckma and the other teleosts and human Ckma/Ckm proteins is shown at the end of each sequence.

The genes which are on chromosome 5 (*ckma, mark4a, kptn, crx, nfkbib, alkbh6, nova1, micu2, rhogc, nccrp1*) and chromosome 15 (*ckmb klc3, ercc2, zc3h4, sae1, bbc3, rad1, ift20, tmem97*) in zebrafish are found in the medaka, human and spotted gar in different location. However, these locations were identified and recorded before designed the conserved gene syntheny (Figure 1). As can be seen in the figure 1, the genes mentioned are conserved on the 5th, 11th, 13th, 14th, 17th, 19th chromosomes in humans, 14th and 21st chromosomes in medaka, and the 2nd and 22nd chromosomes in spotted gar. As it is known, teleost fish have evolutionarily conserved regions for the gene structure in the same gene family, and the designed conserved gene syntheny clearly demonstrates this. In addition, when the results are examined, it is thought that the zebrafish creatine kinase gene emerged as a result of the teleost genome duplication seen in teleost fish. Teleost fish can have two copies of genes found as a single copy in other living things as a result of whole genome duplication (Amores et al., 1998; Meyer and Schartl, 1999; Postlethwait et al., 2000; Braasch and Postlethwait, 2012; Çapan, 2019). When the Ensembl database is examined; zebrafish were found to have two copies of the creatine kinase gene, *ckma* and *ckmb*.

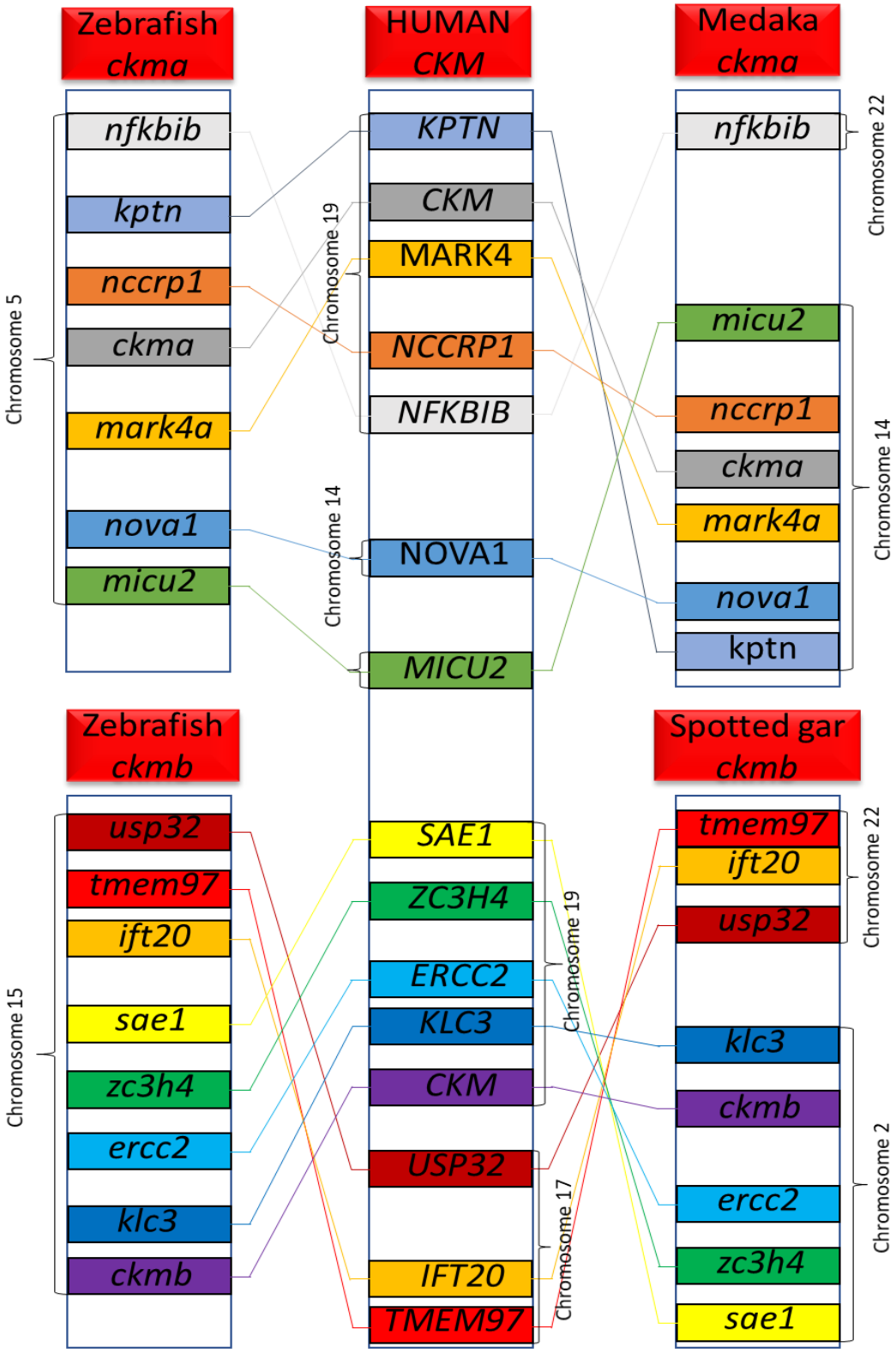

Figure 1. Conserved gene syntheny of Zebrafish (*Danio rerio*) *ckma* and *ckmb* genes

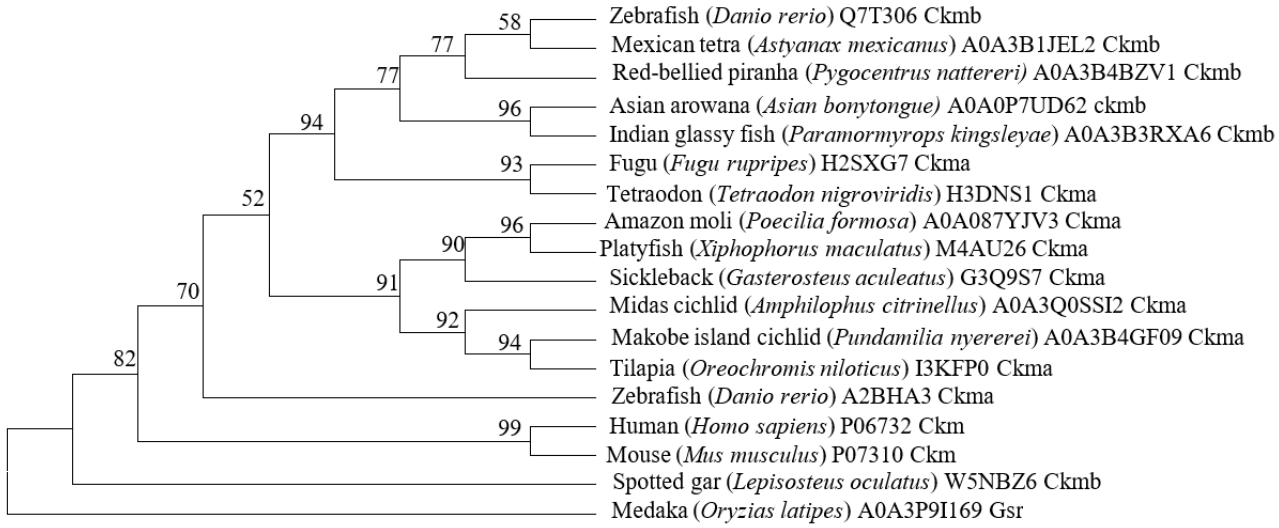

Figure 2. Phylogenetic tree of zebrafish (*Danio rerio*) *ckma* and *ckmb* genes

Phylogenetic tree was designed using protein sequences of Zebrafish Ckma A2BHA3 and Ckmb Q7T306 genes and the other vertebrates such as Amazon moli (*Poecilia formosa*) A0A087YJV3 Ckma, fugu (*Fugu rubripes*) H2SXG7 Ckma, human (*Homo sapiens*) P06732 *CKM*, Makobe island cichlid (*Pundamilia nyererei*) A0A3B4GF09 Ckma, Midas cichlid (*Amphilophus citrinellus*) A0A3Q0SSI2 Ckma, rat (*Mus musculus*) P07310 Ckm, platy fish (*Xiphophorus maculatus*) M4AU26 Ckma, stickleback (*Gasterosteus aculeatus*) G3Q9S7 Ckma, puffer fish (*Tetraodon nigroviridis*) H3DNS1 Ckma, spotted gar (*Lepisosteus oculatus*) W5NBZ6 Ckmb, Mexican tetra (*Astyanax mexicanus*) A0A3B1JEL2 Ckmb, Asian arovan (*Asian bonytongue*) A0A0P7UD62 Ckmb, Indian gladiolus (*Paramormyrops kingsleya*) A0A0P7UD62 Ckmb by obtaining from the UNIPROT genomic database, and the phylogenetic relationship between these genes was determined by Mega program and the maximum likelihood method (Felsenstein 1989). Medaka (*Oryzias latipes*) *gsr* gene (A0A3P9I169) was used as outgroup. In the phylogenetic tree, it was observed that *ckma* protein sequences clustered separately from *ckmb* protein sequences (Figure 2). The reliability of the tree was evaluated by a phylogenetic analysis with 1000 replicates (Felsenstein, 1989).

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RESEARCH ARTICLE

Properties and Importance of *Prometheum sempervivoides* (Fisch. Ex Bieb.) H. Ohba as Ornamental Plant Naturally Grown in Erzurum

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ABSTRACT

The purpose of this study is to reveal the usage possibilities in the landscape and to bring an alternative species to the ornamental plant sector by determining the form, length and habitat characteristics of *Prometheum sempervivoides* (Fisch. ex Bieb.) H. Ohba taxon, naturally grown in Erzurum province and its close environment. Within the scope of this research, Erzurum province and its surroundings were scanned and it is determined that there are large populations of this taxon in İspir and Aşkale districts. The plant vegetative characteristics of *P. sempervivoides* taxon in the regions where the plant was found were examined. At the same time, the altitude of the regions was determined and the soil structures were observed and the possibilities of use in landscape applications were evaluated. As a result of field trips, photographs of the plants were taken and their point coordinates were determined by GPS. The morphological characteristics of the specie (plant height, stem length, number of leaves, number of branches of the middle cluster, leaf width and length, flower diameter, stem diameter and branch diameter characteristics, habitat) were recorded. Plant height were varied between 9.0- 16.70 cm by taking the average of the data obtained from all locations. The highest plant height data were obtained from POS-4 coded genotypes from a location at an altitude of 1942 m in Aşkale district. Stem length varied between 3.60 and 9.30 cm, and the highest stem height was determined in the averages of POS-4 genotypes. While the maximum number of leaves was determined with an average of 19.00 in POS-10 genotype; POS-9, POS-8 and POS-2 genotypes were also included in the same statistical group. The highest value obtained for the number of branches of the middle cluster was determined in the POS-3 genotype. These values differed between 4.00 and 10.00 number/plant. As a result of the research, it has been revealed that the species can be used in landscape studies due to its efficacy with its flower color and star-shaped flowers, red showy leaves and ground cover properties. In addition, It can be recommended that they can be used in rock gardens, in collections of succulents etc., since they have adapted to different environments such as stony rocky habitats, steep slopes and arid soil conditions.

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Introduction

Natural plants, forming the flora of every region, have long withstood the long-term conditions of all kinds of environmental conditions. These natural plants are the best guides for evaluating the ecological sciences of that region, as they are organisms that have been resistant to environmental stresses for many years (Atashgahi et al., 2009). Therefore, regional floristic research is the most effective method of studying the geographical and floristic origins of each region in order to manage and conserve available genetic resources.

Turkey is among the richest countries in terms of floristic (Avcı, 2014). According to Turkey's Plants List; there are 11,707 plant taxa in our country and 3,649 of them are endemic (Güner et al. 2012). This number is almost equal to the 12,500 gymnosperm and angiosperm plant species in the whole European continent (Sezen et al., 2014).

Although Turkey has rich plant biodiversity, mostly imported plants are used in landscaping applications. This situation puts pressure on natural flora and threatens the ecological balance (Deniz and Şirin, 2005). The life form of each plant is a specific character based on environmental

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conditions and morphological adaptation (Muller-Dombois and Ellenberg, 1974). In other words, plant morphology is directly related to climatic factors. For this reason, the ease of adaptation of natural species to the ecological conditions of the region in which they are found can contribute to the restoration or reduction of degradation in flora (Tuttu et al., 2019). Low maintenance costs are among the benefits of using natural plants in landscaping due to their resistance to extreme climatic conditions, providing food and shelter for wildlife in urban areas, contributing to soil fertility and reducing erosion, and low fertilizer and irrigation needs (Özhatay, 2009). In addition, the use of natural plants in landscape applications will provide ecological, economic and aesthetic benefits, as well as ecological landscape applications will be realized and success will be higher (Var, 1992; Özyavuz, 2011; Tuttu et al., 2019).

Eastern Anatolia of Turkey Region has a rich flora due to the variable climate and different ecological zones (Özgökçe and Özçelik, 2004). This region has a high average altitude and shows a diverse structure with its high mountains and deep valleys. In the city of Erzurum, there are limited ornamental plant species in landscape applications due to the harsh winter conditions. In the 1970s, adaptation studies of existing ornamental plants and some cold-resistant plants were carried out in Erzurum and used in the Atatürk University campus (Tanrıverdi, 1973). 18 plant species grown in the region were taken to the experiment, it was determined that over 90% adaptation of 4 plant species was achieved (Güçlü, 1988). Yılmaz and Irmak (2004) reported that a total of 60 plant species, 36 trees and shrubs (13 species common) and 24 shrubs (5 species common) were used in Erzurum open-green areas. The city of Erzurum is one of the few places with high settlements in the world with an altitude of about 2000 m. In addition to altitude, the extreme climatic conditions also limit the development and diversity of the plant material to be used (Yılmaz and Irmak, 2004). Especially in the landscape of cities where such climatic conditions are extreme in order to gain more ecological and economic benefits, studies using natural plants should be carried out.

In the literature reviews; It has been determined that there are few studies (Güçlü, 1988; Yılmaz and Irmak, 2004; Yılmaz and Yılmaz, 2009) that could serve these purposes for the region. In addition, there is no study made for *Prometheum sempervivoides* (Fischer ex M.Bieb.) H.Ohba taxon naturally grown in stony rocky habitats, extreme altitudes, arid soil conditions, sunny-open areas and is effective with its ground cover features and eye-catching red stem and leaf beauty (Dilaver et al., 2020) was encountered.

Prometheum sempervivoides (Fischer ex M.Bieb.) H.Ohba taxon belongs to the Crassulaceae family. *Crassulaceae* family has about 1410 species in 34 genera containing three subfamilies, i.e., *Crassuloideae*, *Kalanchoideae* and *Sempervivoideae*. *Sedum* L., by far the largest genus of *Sempervivoideae* and indeed of *Crassulacea*e, contains ca.430 species (Thiede and Eggli, 2007). *Prometheum sempervivoides* is known with Turkish names which vary according to the region such as Horozlelesi (Anonymous, 2020a), Ömürotu; İkbal flower; Kader flower; Ömür flower. It has a succulent character. It grows on stony and rocky slopes (Tuttu et al., 2019; Dilaver et al., 2020). It has been stated that the taxon are synonyms of *Rosularia sempervivoides* (Fisch. ex M. Bieb.) Boriss., *Sedum kurdistanicum* Fröd., *Sedum sempervivoides* Fisch. ex M.Bieb., *Sedum sempervivum* Ledeb. ex Spreng. (Anonymous, 2020b, Anonymous, 2020c). The characteristic fiery-red flowers of this alpine *Rosularia* is quite unique in the family. Anthems and styles are also red or reddish. Distribution of the taxon is in Turkey (Anatolia), Georgia, Armenia, Caucasus, North Iran. *Rosularia* look similar to *Sempervivum* except that they have bell-shaped blooms instead of star-shaped (Eggli, 2003).

In this context, the aim of the study is determination of the qualities to be an ornamental plant (Dilaver, 2001; Erduran et al., 2010; Arslan, 2010; Gülbağ, 2016; Dilaver et al., 2020) such as habitat, plant height, stem length, number of leaves, number of branches of the middle cluster, leaf width and height, flower diameter, stem diameter and branch diameter of *P. sempervivoides*, naturally grown in the city of Erzurum and its close vicinity and rich in vegetative biodiversity. Thus, it was aimed to provide data for the cultivation and breeding studies and to suggest landscape using possibilities.

Materials and Methods

Study Area

Erzurum city center was established at an altitude of 1800-2000 m and surrounded by 3200 m high mountains. The topographical structure and geographical location of Erzurum creates a severe continental climate throughout the province. When the climate data of Erzurum city is examined, the average temperature for many years is 5.7 °C; The average lowest temperature was observed in January with -14.0 °C and the average highest temperature was observed in August with 27.2 °C. The annual average relative humidity in the region is 63.58%, and the total annual precipitation is 432 mm (Yağanoğlu, 2019). The temperature in the province, whose winter period covers more than 6 months, decreases in October and starts to increase in April. In the settlement, which is one of the coldest cities of our country, the temperature can drop below -25oC in winter. The period of snow cover extends from October to May. Also, in the city, which has a humid continental climate (Kottek et al., 2006), seasonal temperature differences are high (Yavaş and Yılmaz, 2019).

Field trips were organized to the province of Erzurum and its surroundings and large populations of this taxon were found in İspir and Aşkale districts. *P. sempervivoides* plants were collected from ten different locations (Table 2) in the designated regions. Vegetative measurements were taken and observations were made in the natural conditions of the plant (Figure 1, 5). Photographs of the plants were taken and collected and brought to the laboratory for further measurement.

Study Material

The main material of the study is the natural *P. sempervivoides* taxon taken from ten different locations of

İspir and Aşkale districts in Erzurum. The auxiliary material consists of literature data on the subject, photographs and land registration forms were obtained during field studies.

Method

This study was carried out by organizing field trips in Erzurum and its surroundings, three times a year, considering the seasonal life cycle of the plant between 2018-2020. The samples of *P. sempervivoides* were collected from ten different locations from 2 different districts (Table 2). Photographs of the plants were taken and their vegetative characteristics were measured (Table 3).

Plants were identified using the standard text "Flora of Turkey and the East Aegean Islands" (Davis, 1965-1985; Davis et al., 1988). After the name of the species, its locality, habitat, altitude, date of collection are given.

Figure 1. General view of the plant and the view from the part specified as the middle cluster

Ten plants with the best quality and aesthetics were selected from each of the determined locations. Plant height, stem length, number of leaves, number of branches of the middle cluster, leaf width and length, flower diameter, stem diameter and branch diameter characteristics were measured in the selected plants and recorded in the charts (Figure 1, 2). In addition, features such as life form (single, biennial, perennial) and habitats (slope, rocky) were noted in the field and plant photographs were taken (Figure 1, 3, 4, 5).

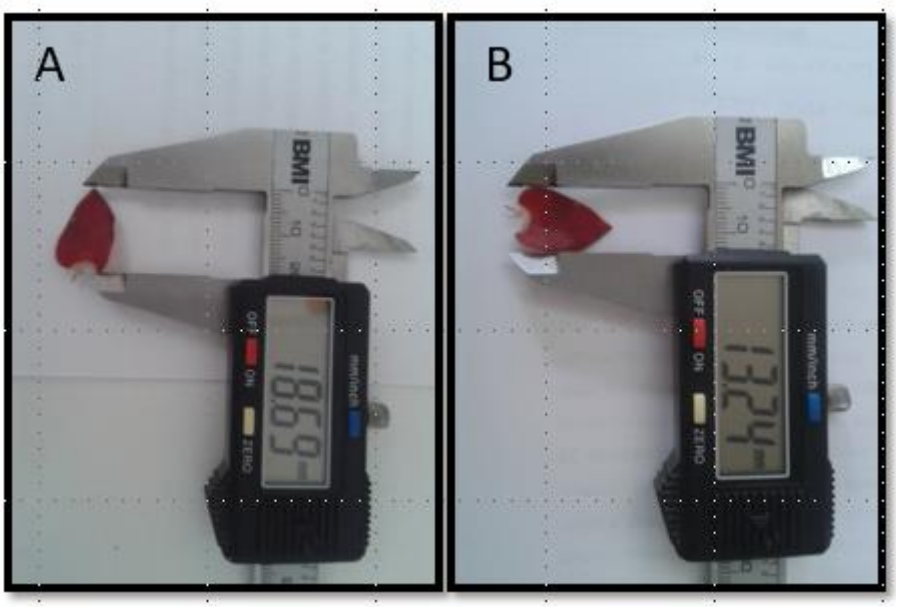

Figure 2. Image representing the plant's leaf height measurement (A); Image representing the leaf width measurement (B)

Data evaluation

In terms of the measured characteristics, the existence of differences between the plants collected from ten different locations of two different districts was determined by analysis of variance. Duncan test at 0.05 significance level was used to distinguish groups from each other in significant differences. SPSS (version 20.0) statistics package program was used in all statistical calculations.

Results

Plant Description

Prometheum sempervivoides is known with Turkish names which vary according to the region such as Horozlelesi (Anonymous, 2020a), Ömürotu; İkbal flower; Kader flower; Ömür flower. It blooms red in June-August. It is effective with its blood red flowers. It has a slightly short villus structure with an upright posture [regular-vertical form feature (Tuttu et al., 2019)]. The flower-bearing stem emerges from the leaves in the form of rosettes from the base and is about 7-20 cm tall. Each of the leaves is ovoid, flat and pointed and slightly purplish-green in color. The inflorescence, which consists of approximately 30-150 small flowers, is loose. Each flower has 5 pieces and a stem. The petal of each red flower is 6-8 mm long. It grows on open stony and rocky slopes (Tuttu et al., 2019; Dilaver et al., 2020; Messerschmid et al., 2020) (Table 1).

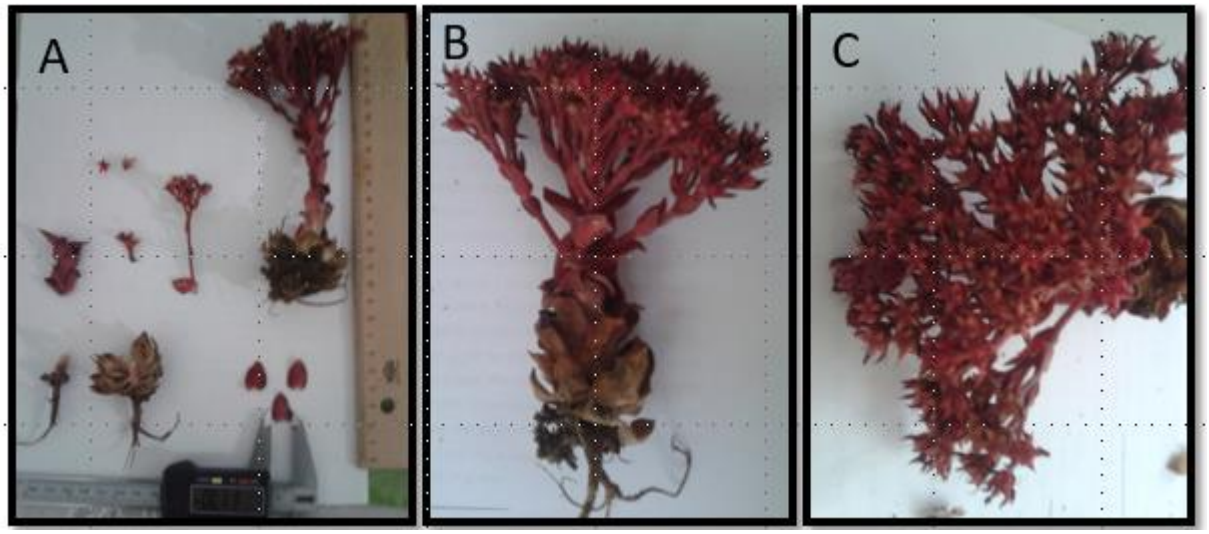

Figure 3. View of the plant's entire morphology (A, B) and view from top of flowers (C)

Table 1. Characters of *Prometheum sempervivoides* (Fischer ex M.Bieb.) H.Ohba defining by Eggli (2005) and Thiede & Eggli (2007) (Messerschmid et al., 2020).

Other Characters of *Prometheum sempervivoides* (Fischer ex M.Bieb.) H.Ohba	
Clade affiliation	Leucosedum
Inferred phylogeny	Monophyletic
Distribution	N Greece to N Iran, incl. Caucasus
Life form	Annual to perennial herbs
Phyllo taxis	Sessile rosettes, alternate on stems
Inflorescence insertion	Terminal or lateral
Flower merism	5
Petal fusion	Fused for ≤ 1/2 of Corolla length, never free
Petal colour	Yellow,cream, white,pink or red
Number of stamens	2x petals
Trichomes	Glandular
Rosette branching sympodial	No
Leaf apex	Rounded to mucronate
Filamentin sertion	n.s.
Seeds perfollicle	n.s.
Testa ornamentation	Costate

n.s. – not specified in the literatüre

Ten locations have been determined in two districts where the species grows in Erzurum, and the altitude range having the plants are found varied between 1447-1942 m. It has been determined that the habitat of the species is rocky places and its life form is biennial (Figure 5). It was determined that the flowering period was June-August (6-8) (Table 2) (Figure 4).

Figure 4. Views from the flowering period of *Prometheum sempervivoides* and the natural environment of the plant, taken during the field trip

Figure 5. Views of the plants that are in the drying stage and the natural environment of the plant, taken during the field trip on 15 August 2020

Table 2. Information on the locations where plant genotypes are collected

Genotip No	Province	District	Latitude	Longitude	Altitude (m)	Location
POS-1	Erzurum	Aşkale	39°59'18 "	40°32'17 "	1935	Askale province, Pırnakapan village
POS-2	Erzurum	Aşkale	39°59'25 "	40°32'33 "	1930	Askale province, Pırnakapan village
POS-3	Erzurum	Aşkale	39°59'32 "	40°32'23 "	1927	Askale province, Pırnakapan village
POS-4	Erzurum	Aşkale	39°59'45 "	40°32'29 "	1942	Askale province, Pırnakapan village
POS-5	Erzurum	Aşkale	39°59'23 "	40°32'36 "	1925	Askale province, Pırnakapan village
POS-6	Erzurum	İspir	40°26'34 "	40°59'52 "	1447	Ispir province, Korga Mountain
POS-7	Erzurum	İspir	40°27'42 "	40°62'55 "	1585	Ispir province, Korga Mountain
POS-8	Erzurum	İspir	40°26'79 "	40°57'98 "	1892	Ispir province, Korga Mountain
POS-9	Erzurum	İspir	40°25'38 "	40°53'58 "	1500	Ispir province, Korga Mountain
POS-10	Erzurum	İspir	40°28'12 "	40°51'63 "	1570	Ispir province, Korga Mountain

The Vegetative Characteristics

The vegetative characteristics of the natural *Prometheum sempervivoides* (Fischer ex M.Bieb.) H.Ohba taxon in the city of Erzurum and its surroundings are given in Table 3. Plant height were varied between 9.0-16.70 cm according to the table 3 created by taking the average of the data obtained from all locations. The highest plant height data were obtained from POS-4 coded genotypes from a location at an altitude of 1942 m in Aşkale district (Tables 2 and 3). Stem length varied between 3.60 and 9.30 cm, and the highest stem height was determined in the averages of POS-4 genotypes. While the maximum number of leaves was determined with an average of 19.00 in POS-10 genotype; POS-9, POS-8 and POS-2 genotypes were also included in the same statistical group. The middle cluster can be defined as the part that opens from the point where the flower stalks of the plant cluster to the tip of the plant (Figure 1). There is a relationship between the number of branches and flower diameter of the middle cluster. The highest value obtained for the number of branches of the middle cluster was determined in the POS-3 genotype. These values differed between 4.00 and 10.00 number/plant (Table 3).

Table 3. The vegetative characteristics of the natural *Prometheum sempervivoides* (Fischer ex M.Bieb.) H.Ohba taxon in the city of Erzurum and its immediate surroundings

Genotip	Plant height (cm)	Stem height (cm)	Number of leaves per plant	Number of branches of the middle cluster per plant	Leaf width (mm)	Leaf height (mm)
POS-1	9.50 f	3.60 e	13.00 d	6.00 c	13.50 ^{ns}	17.44 bc
POS-2	14.40 bc	7.80 b	17.00 ab	6.00 c	13.24	18.80 bc
POS-3	9.30 f	3.80 e	14.00 cd	10.00 a	13.41	21.92 a
POS-4	16.70 a	9.30 a	16.00 bc	4.00 e	13.25	19.23 b
POS-5	11.50 de	5.50 d	13.00 d	6.00 c	13.08	18.15 bc
POS-6	13.30 cb	6.70 c	14.00 cd	4.00 e	12.85	18.27 bc
POS-7	15.50 ab	8.60 ab	16.00 bc	6.00 c	11.73	19.39 b
POS-8	15.10 a-c	9.20 a	17.00 ab	5.00 d	12.10	16.48 c
POS-9	10.80 ef	6.00 cd	18.00 ab	4.00 e	13.27	10.82 d
POS-10	12.50 bc	6.50 cd	19.00 a	7.00 b	11.82	16.80 bc

Figure 6. View from the dried state of the plant (a) and its seeds (b)

In the light of the obtained data, the leaf width parameter was found to be statistically insignificant and it was determined that there were no significant differences in leaf width among genotypes. Leaf height values were found to be statistically significant and the values varied between 10.82 and 21.92 mm. The highest leaf size data were obtained from POS-3 genotypes (Table 3).

As a result of the study, the flowering and seed-tied samples taken from the locations were waited to dry (Figure 6) and germination was carried out to examine the seed properties. However, their germination has not been successful.

Discussion

Oudolf and Darke (2017) and Oudolf and Gerritsen (2019) stated that natural plants are a wealth and this wealth gains importance by being discovered and utilized in landscapes according to their usage areas. Researchers reported that natural plants do not require any maintenance because they are in their natural habitats. These plants are resistant to all natural phenomena around them because they are compatible with the conditions of their environment (Aydoğdu, 2018). Deniz and Şirin (2005) emphasize that in addition to nature protection, smooth transitions between natural vegetation in rural landscapes and urban landscapes can be achieved, and

thus the balance in the ecosystem and the continuity of this balance can be achieved. The success of achieving these smooth transitions depends on the use of many natural plant species in the urban landscape. Extending the natural plant species to be used in cities is only possible by knowing the design features of the plant species to be used. This can be possible by determining which feature of the plant has the potential to become an ornamental plant with previous studies.

Natural habitats (biotopes) of plants can be determined with these and similar studies, thus, the negative effects of vegetative designs intended to be made in urban open-green areas in applications can be minimized. In this context, according to the observations obtained as a result of our study, it was predicted that the natural *Prometheum sempervivoides* species found in the city of Erzurum and its immediate surroundings can find habitats in stony and rocky areas and at high altitudes such as 1942 m (Table 2) and can be used in this type of unfavorable soil and elevations in vegetal designs. Demircan et al. (2006) have been reported in their study that aimed to put forward tourism potential of Turkey's the succulent plant diversity, that *P. sempervivoides* grows in altitude 1200-2900 m, its habitat is rocky places and its the life form is biennial. The findings and observation results of our study were in parallel with this finding. In addition, the same researchers stated that the flowering period of *Prometheum sempervivoides* species was June-August (6-8). As a result of our field trip observations, its the flowering period was determined as the same months.

Ten locations were determined in two districts where the species grows in Erzurum, and the altitude where the plants are found has varied between 1447-1942 m. It has been determined that the habitat of the species is rocky places and its life form is biennial. Babacan et al. (2017) reported that *Prometheum sempervivoides* (Fischer ex M. Bieb.) H.Ohba identified in open places in an oak forest at an altitude of 1590 m in Tunceli province. It was stated that *Prometheum sempervivoides* species identified from the area of Soguksu National Park (Ankara-Kızılcahamam), grows naturally at an altitude of 1500-1800 m and in 0-20 degrees inclination (Dilaver et al. 2020). The altitude ranges that this species can grow in TÜBİVES records are specified as 1200-2900 m (Anonymous, 2020a).

Due to the global climate change and the intense drought expected to be experienced it is seen that practices are carried out to reduce water consumption all over the world. Drought threat is concerned as well as all over the world and our country. The amount of rainfall that has decreased in recent years is also reflected in the city of Erzurum. For the city of Erzurum, priority should be given to the use of natural species in landscape areas in terms of rational use of water. It will be possible to create, preserve and improve natural environments inside and outside the city and create areas with high aesthetic value by choosing of local species such as *Prometheum sempervivoides*, which are resistant to adverse environmental conditions throughout the city, especially drought and low air temperature. Also, with the use of native species in plant design may break the monotony of the composition consists of species from the plant cultured with foreign species. By increasing in the visual landscape quality of the city, urban spaces can be used actively. The production of *Prometheum sempervivoides* species with high landscape value revealed in the study should be increased in throughout the country; by means of taking it into culture, researching the reproduction techniques and transferring them to applications or doing breeding studies.

Oudolf and Darke (2017) stated that all landscape design features of natural plants to be used in design should be known. The vegetation period is short due to the fact that the studied taxon is located in the continental climate zone. The flowering period of *Prometheum sempervivoides*, which has eye-catching red flowers, is 3 months, and the star-shaped flowers, which are formed by yellow-colored pollen powders on red during the flowering period, become even more remarkable. In addition, the color turning to burgundy in the period after flowering and the fact that the plant makes a strong impression in this color for a long time is one of the features that can be effective in designs for a long time.

Conclusion

As a result of the study, it has been revealed that the species can be used in landscape studies due to its flower color and star-shaped flowers, and its ability to be effective with its bright red leaves and ground cover properties. In addition, since they have adapted to different environments (stony rocky habitats, steep slopes, arid soil conditions), they can be recommended for use in rock gardens, succulent collections and so on. Also, for future studies, it may be suggested to intensify the research on cultivation and urban adaptation of this species.

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REVIEW ARTICLE

Application of HACCP in Aquaculture Processing

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ABSTRACT

Haccp, food safety is one of the most important and critical points for human health. It can be defined as reducing the risks in biological, chemical, physical production systems that may arise in food safety and systematic preventive actions. HACCP can be shortly abbreviated as Critical Control Points Risk Analysis. Haccp is a series of food-specific standards. One of the most fundamental points in the Haccp standard is to make a hazard risk assessment and to determine critical control points and to take measures against these dangers. The Haccp standard is a set of conditions and systems for evaluating the risks that may occur during the transportation of food from the stage of production and during the use of foodstuffs. Processed seafood provides many advantages to consumers due to their long shelf life, following hygiene and sanitation rules at storage and usage conditions is crucial. Seafood processing industry is engaged in the efforts to increase the shelf life of the products, during the determination of critical control points (CCP), which is one of the important elements of the Haccp system. Correct determination of CCP will ensure that the high level quality of the product is kept for a long time. In this review, the importance and advantages of application of Haccp in aquaculture processing will be discussed.

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Introduction

Regarding food safety, every country has a regulation on microbiological criteria in its own food production codex. Every facility that produces food within the borders of the country must comply with these criteria. However, besides these criteria, other internationally accepted food safety analysis systems are also used. One of these systems is HACCP. HACCP can be shortly abbreviated as Critical Control Points Risk Analysis. Many manufacturing businesses in the food industry around the world also use this system (Anonymous, 2020a).

HACCP is an effective quality management system in which all the steps taken from the raw material of the food product until it reaches the consumer, the whole facility and personnel, all inputs and their producers are constantly monitored and controlled, and when properly operated, it is an effective quality management system that aims to prevent all possible dangers and protect consumers from health risks (Altun, 2011).

HACCP, which is a system for ensuring food safety, does not eliminate the risk of the control in the final product, instead, it mainly determines the pathogen contamination and possible risks of the contaminants in advance, determining critical control points at certain points and preventing these risks within acceptable limits. The purpose of HACCP is to ensure the production of reliable food and trust in the product and it is still used by many countries. HACCP has taken its place in food safety as an internationally accepted system. In this respect, it should not be forgotten that HACCP is not a sufficient system alone and must be implemented together with good hygiene practices (GHP) and good manufacturing practices (GMP) in order to comply with international regulations, gain consumer confidence and increase market share in domestic and foreign markets (Mert, 2012).

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The HACCP system is designed to be used in every stage of the food industry, from growing to harvest, from processing to production, distribution, consumption. It is the most effective approach adopted in ensuring food safety today (Balta, 2005; Anonymous, 2020b)

Seafood creates a suitable medium for microorganisms, due to the high proteins it carries, thus the risk of spoilage increases, and when consumed in this way, it causes various diseases and causes significant financial losses as well as death. In the face of this situation, aquaculture importer many countries have directly implemented systems related to quality control and food safety such as HACCP, which affect the fishery trade. The most prominent application of this is that the European Union (EU) held the producers responsible for the health of the products they produced with the 85/374 / EEC directive issued in 1985, in 1991, the 91/492 / EEC and 91/493 / EEC directives In 1993, the 93/43 / EEC directive required the implementation of the HACCP system in the EU food producing companies and the food products to be imported. The USA also implemented the HACCP system in 1992, followed by countries such as Australia, New Zealand, Brazil, Thailand and Morocco, harmonizing their fisheries control systems with the HACCP system (Mert, 2012).

History of HACCP System

The HACCP system was first developed in the 1960s by a group in the Pillsbury company, which produces healthy food for the 'Apollo' space program. In this space program, the food to be produced for astronauts was asked to be 100% safe by the American National Aeronautics and Space Administration (NASA) and the HACCP concept emerged for this purpose.

This system has been used in the official inspections made by the Food and Drug Administration (FDA) in the USA since the 1970s. It started to be settled in the aquaculture industry in the early 1970s. Towards the end of the 1980s, it was fully settled in the American National Food Preservation Conference by making it mandatory with the introduction of a new, revised fisheries law based on HACCP.

In December 1995, by the decision of the European Community, all fishery producers in the community were obliged to apply HACCP. In addition, with the directive of the EU, it has been decided to import fishery products only from the facilities in the countries implementing the HACCP program. In 1996, it was made a legal obligation that the entire food industry should implement, and countries such as the Netherlands, Denmark, New Zealand and Australia published their own standards.

In Turkey, November 16, 1997 Foodstuffs also not recorded officially as HACCP Regulation, identifying critical control points, monitoring and so on. It was stated that HACCP application would be sought. The HACCP requirements are clearly stated in the food hygiene section of the Decree Law No.560 on 'Production, Consumption and Inspection of Foods' published in the Official Gazette dated June 9, 1998 and numbered 233367, and 2 from 6 years (Alpay, 2002; Anonymous, 2020c).

Application and Planning of the HACCP System

As a reliable method to ensure food safety, HACCP provides many important advantages to the business, consumer and country. These advantages can be listed as follows (Özçiçek, 2002; Ertürk, 2003; Anonymous, 2004; Altun, 2011);

a) It enables safe food production,

b) Provides training of operating personnel on hygiene and HACCP,

c) It ensures that critical tests are carried out quickly and on site,

d) It ensures that records and documentation are kept in the enterprise,

e) It reduces costs,

f) Provides widespread exchange of information,

g) Increases the marketing power of the product,

h) It also reduces the economic losses of consumers and employers against diseases caused by food,

ı) Community health and protection of the environment are provided,

i) Cases resulting in death due to food poisoning are reduced,

j) It will provide an increase in exports and income by reducing barriers in international trade and helping businesses to compete more effectively in world markets, and thus, will also contribute,

k) Facilitates the understanding and compliance of the approach to the quality management system.

Basic Principles of HACCP System

To Make Hazard Analysis

The first principle in the application of the Haccp system is to make hazard analysis. In this principle, the dangers that may arise during the production or processing of food are determined. All hazards are analyzed and risk assessment of food is made.

Determination of Critical Control Points

At this point, the HACCP team determines the control points that may create potential danger.

Determining Critical Limits

The highest and lowest values of the chemical, biological or physical results to be obtained after the measurements to be made at the control points should be determined. Measurements must be made at each checkpoint. Therefore, it is important to determine the limits.

Monitoring Critical Control Points

In this step, questions such as how to take samples from control points, which methods will be analyzed, who will be responsible for taking samples from the HACCP team and how often to take samples should be formally defined.

Determination of Corrective Actions

The steps to be followed in case of encountering a problem that would prevent the health processing of food or the implementation of the HACCP system are determined.

Verification

Here, all actions taken in accordance with HACCP are checked. These actions include the settings of the machines used. Usually, the audit team set up in the HACCP team performs this step.

Recording

In this principle, all HACCP procedures, samples, results, control points and limits are recorded. In this way, both the sustainability of the food quality is ensured and new employees are guided (Anonymous 2020a, Anonymous 2020d).

Haccp Application in Aquaculture Processing

It is because the structure of seafood is very sensitive and microorganisms start to deteriorate quickly due to the high nutritional value of the product. Accordingly, Seafood processing industry is engaged in the efforts to increase the shelf life of the products, during the determination of critical control points (CCP), which is one of the important elements of the Haccp system. Correct determination of CCP will ensure that the high level quality of the product is kept for a long time (Alpay, 2002).

The aquaculture industry is an important position in the food industry, which is of great importance in terms of sea and inland water potential of our country. It is necessary to apply the Haccp principles, which have an important place worldwide, in order to progress successfully in the field of sea and inland waters (Balta, 2005).

The developing technology has brought new requirements and obligations to the agenda in the product, processing conditions and operating controls in the aquaculture industry. Today, within the concept of quality assurance and hygiene, this system has been accepted as a compulsory application rather than an optional one. This system is a Hazard Analysis System (Haccp) at Critical Control Points. The Haccp system is designed to identify and control dangers that may occur in a food processing. Haccp provides not only microbial quality, but also increase in sensory and nutritional quality, as well as quality assurance in service / service application (Balta, 2005).

The first step in creating the Haccp plan for the processing plant is the preparation of the team, the preparation of the raw material and the final product definition. The second step is the creation of control points by working on hazard analysis studies, flow charts and basic principles. The third and final step is to write down the record keeping system, standard health work procedure, return procedure and consumer complaints in terms of creating monitoring methods (Alpay, 2002).

The Haccp system has some objectives in aquaculture processing and recycling facilities. These (Balta, 2005);

- Continuously producing safe products,
- To ensure compliance with the legislation,
- Ensuring effective use of resources,
- Ensuring that food is processed reliably after reliable production,
- To direct the business in accordance with the quality control system,
- To ensure that a versatile discipline can be employed.

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