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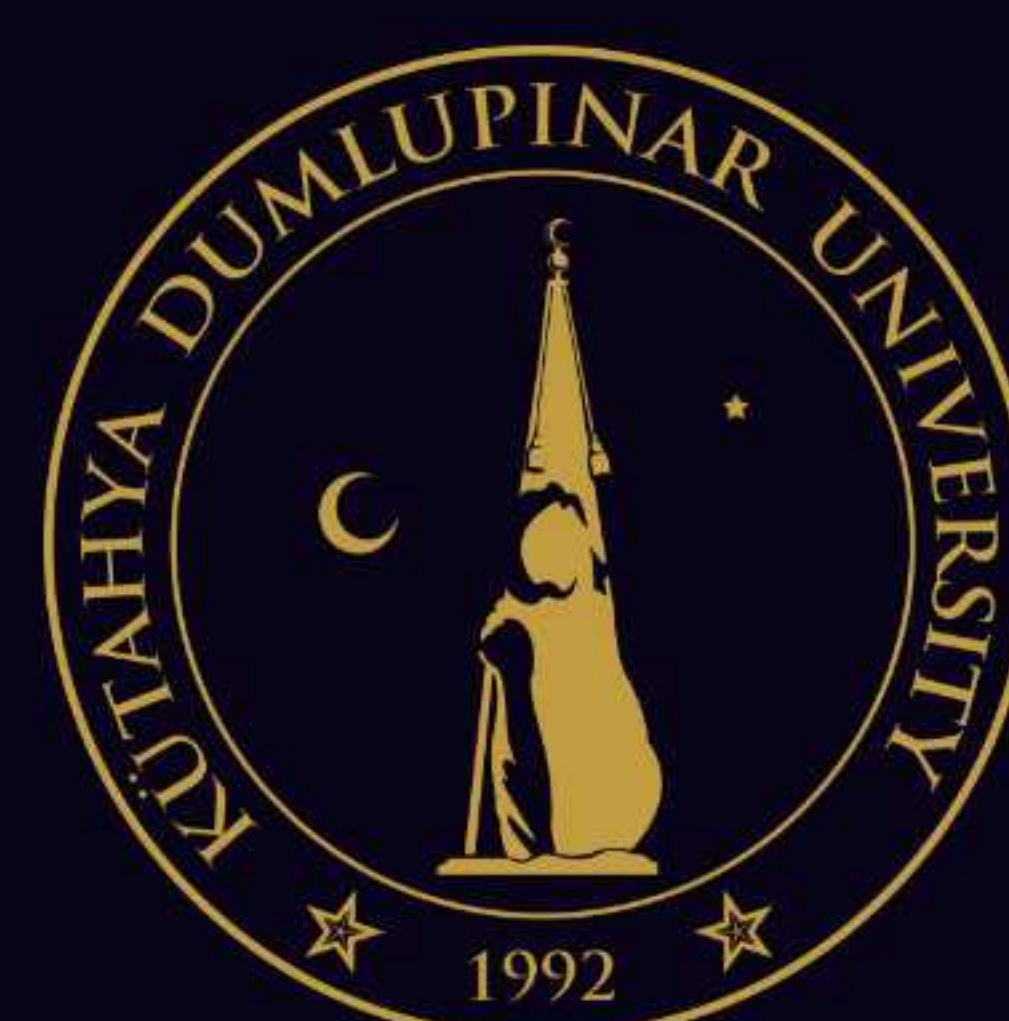
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Evliya Celebi Campus Tavsanlı Road 10 KM. 43270 Kutahya  
Phone : (0274) 443 19 29 - 30  
E-mail : [joursra@gmail.com](mailto:joursra@gmail.com)  
[www.gsjrsra.com](http://www.gsjrsra.com)

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**Correspondence Address:** Kütahya Dumlupınar Üniversitesi Evliya Çelebi Yerleşkesi Fen Bilimleri Enstitüsü  
43270 KÜTAHYA

**Phone:** 0 274 443 19 42

**E-mail:** [joursra@gmail.com](mailto:joursra@gmail.com)

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**Webpage:** [www.jsra.com](http://www.jsra.com)

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**RESEARCH ARTICLE**

**INVESTIGATION OF THE EFFECTION MECHANISM OF CINNAMIC ACID ON  
CONTRACTION AND RELAXATION OF SMOOTH MUSCLES OF ILEUM AND  
BLADDER OF RATS**

Fatih ALAN<sup>1</sup>, Hayri DAYIOĞLU<sup>2</sup>, Ayhan YILMAZ<sup>3</sup>

<sup>1</sup>General Directorate of Presidential Protection Services, Presidential Complex, Ankara, [fatihalan06@hotmail.com](mailto:fatihalan06@hotmail.com),  
ORCID: 0000-0002-0561-6192

<sup>2</sup>Kütahya Dumlupınar University, Faculty of Science and Letters, Division of Biology, Kütahya, [hayri.dayioglu@dpu.edu.tr](mailto:hayri.dayioglu@dpu.edu.tr),  
ORCID: 0000-0002-9270-8561

<sup>3</sup> Kütahya Dumlupınar University, Faculty of Science and Letters, Division of Biology, Kütahya, [ayhan.yilmaz@dpu.edu.tr](mailto:ayhan.yilmaz@dpu.edu.tr),  
ORCID: 0000-0003-0410-8687

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**ABSTRACT**

Cinnamic Acid is a phenolic acid derivative commonly found in *Cinnamomi cortex* (Cinnamon) plant, and it is named after cinnamon plant. It have Antimicrobial, Antitumoral, Anticancer and Antifungal properties and beyond that its vasodilator effect were also detected.

This study was performed separately for ileum and bladder in 7 different groups, namely atropine, phentolamine, propranolol, nifedipine, tetraethyl ammonium and atropine + phentolamine + propranolol applied groups. In the study, the contraction-relaxation responses to cinnamic acid of the relevant tissues were examined after application of different antagonists or channel blockers to KCl or carbachol pre-contracted ileum / bladder tissues.

Cinnamic acid caused constriction and relaxation of the ileum and bladder as dosage dependent manner. Atropine did not change the relaxation response while further contracting the ileum. It increased relaxation while inhibiting contraction responses in bladder. The contraction and relaxation responses of phentolamine on ileum and bladder was not changed. The propranolol inhibited the contraction responses of ileum, but did not change the relaxation responses. In bladder, the contraction responses increased, but relaxation responses did not change. Nifedipine did not alter the relaxation responses while inhibiting the response of contraction to cinnamic acid in ileum, and increasing relaxation responses without affecting the contraction responses in the bladder. TEA increased relaxation responses while it did not affect the contraction responses in bladder, but did not change contraction and relaxation responses in ileum. At the same time, the adrenergic and cholinergic receptor blockade performed at the same time altered the contraction and relaxation responses in the ileum and bladder, but did not significantly affect them.

In conclusion, it is thought that cinnamic acid applied on rat ileum and bladder smooth muscles, is also influenced by different receptors or pathways other than receptor antagonists and channel blockers preferred by us.

**Keywords:** Atropine, Bladder, Cinnamic Acid, Ileum, Nifedipine, Phentolamine, Propranolol, Tetraethyl Ammonium.

## 1. INTRODUCTION

Phenolic compounds in plant-based foods are related to human health with particularly epidemiological consequences that reduce the incidence of cancer. Many researchers' reports show that flavonoids are mutation and cancer-inhibiting. Hydroxycinnamic acids and hydroxybenzoic acids are bounded by OH and OCH<sub>3</sub> groups to form phenolic acids. The most important of these compounds are Hydroxycinnamic acids [2]. Hydroxycinnamic acids have different properties depending on the number and place of hydroxyl groups attached to the phenylpropane ring. Cinnamic Acid, Ferulic Acid, Caffeic Acid, O-kumaric Acid and P-Kumaric Acid are the known important acids. Hydroxycinnamic acids are free in trace amounts and usually found in the form of acid derivatives. Esters of hydroxycinnamic acids are commonly used in foods. Hydroxycinnamic acid glycosides and amides also occur in many plants. In cinnamic acid structures, there are 3 different free radical bonds. They are mostly compounds that come into play on the C<sub>6</sub>-C skeleton [2]. Cinnamic Acid (CA) is a phenolic acid derivative commonly found in *Cinnamomi cortex* (Cinnamon) plant. Antimicrobial, Antitumoral, Anticarcinogenic and Antifungal properties and vasodilator effect were determined [38], [63], [48], [25] and [41].

Former studies found that CA have anticancerogen [38] antifungal [63] effects. In addition, it has also the antitumoral effect in human tumor cells [63]. The role of Ca in spontaneous, ACh-stimulated and KCl-induced contractions of rabbit small intestine longitudinal and circular smooth muscles was investigated and found that atropine decrease the amplitude and tonus of contraction in both types of muscles and also reduces the frequency of contraction in the circular muscles and nifedipine reduced ACh and KCl-induced contractions and the role of extracellular Ca in spontaneous contractions in rabbit ileus and extracellular and intracellular Ca involvement in ACh and KCl-induced contractions were concluded [20]. The vasodilator action and mechanism of CA in the rat thoracic aorta were investigated and consequently endothelium-dependent vasodilatation through the nitric oxide-cGMP-PKG mediated pathway in the rat thoracic aorta was observed [25]. The effects of *Achillea millefolium* extract on rat ileum contractions were investigated. Contracted ileum created by applying KCl and ACh showed the relaxant effects of *A. millefolium* extract. It is proved that propranolol, a  $\beta$ -adrenoceptor antagonist, does not inhibit the relaxant effect of *A. millefolium*. It may be due to the fact that the relaxant effect of extract blocks the voltage-gated Ca channels [46]. The effect and possible mechanisms of *Rosa damascena* hydroalcoholic extract on rat ileum smooth muscle contractions have been investigated. The cumulative administration of *R. damascena* extract reduced the KCl-induced ileal contractions by dose-dependent manner. Propranolol has been found to reduce the inhibitory effect of the extract.  $\beta$ -adrenoceptors may play a role in ileal movement reducing activity of extract [59]. A study of CA on vascular smooth muscle cells and platelet-derived growth factors resulted that CA suppresses early signal transduction and downregulates cell cycle-positive regulatory proteins [41]. The ex-vivo study investigated the interactions of cholinergic, serotonergic and adrenergic receptor systems with *Aegle marmelos* leaf extract in the ileum, stomach and trachea. Acetylcholine ( $10^{-9}$ - $10^{-4}$  M), atropine ( $10^{-7}$  M) and *Aegle marmelos* extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) were used in the ileum. 5-HT ( $10^{-9}$ - $10^{-5}$  M), ketanserin ( $10^{-6}$  M) and *Aegle marmelos* extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) were used in stomach. Isoprenaline ( $10^{-6}$  M), propranolol (1 ng/ml) were used in the trachea, and doses of *Aegle marmelos* extracts were selected by starting from a dose of 1  $\mu$ g/ml and titrating various concentrations by increasing 10 times in each step. As a result, *Aegle marmelos* aqueous extract has agonistic effects on cholinergic, serotonergic and adrenergic receptors in isolated rat ileum, stomach and tracheal tissues [32].

Some organs show a response to the electrical stimulation of autonomic nerves and are not affected by the pharmacological inhibition of these systems and the existence of the residual response puts forth the presence of adrenergic or noncholinergic nerve axons in the nerves [61] and [26]. This third system in the neurochemical classification of the sympathetic and/or parasympathetic nervous system is described as non-adrenergic non-cholinergic nervous system (NANK) [27]. The first finding of the NANK system is the pelvic nerve stimulation by atropine-resistant excitation in the mesentery [35]. In the next few decades; Mc Swiney and Robson (1929), Ambache (1951) and Paton and Vane (1963) stated that ganglion stimulating nicotine induces intestinal relaxation after inhibition of contraction by atropine [62]. The atropine and adrenergic neuron blocker bretylium formed large hyperpolarizations by stimulation of the intrinsic nerves of the intestine; however, these hyperpolarizations were inhibited by tetrodotoxin. It may be inhibitory junction potentials in response to stimulation of NANK nerves conducted on the cat, adrenergic nerve inhibiting agents were found to be ineffective in the presence of atropine in the relaxation created by vagal nerve stimulation [62]. The NANK system has been expressed in the urogenital, pulmonary and cardiovascular systems as well as the gastrointestinal system of all vertebrates. Nine different neuron types were identified morphologically in the enteric plexuses and ATP, VIP, tachykinins, GABA and nitric oxide may have possibility to be neurotransmitters of the non-adrenergic non-cholinergic system [62]. Nitric oxide inhibits the secretion of acetylcholine from intrinsic cholinergic nerve endings in the gastric fundus of rats, dogs and rabbits [37]. The NANK system regulates loosening in the guinea pig colon and the contraction of the guinea pig with adrenergic, cholinergic and NANK nerves were also produced. Electrical stimulations of NANK transmission may produce relaxation or contraction in longitudinal and circular smooth muscles in the ileum [62].

The transient receptor potential (TRP) channels [9] are divided into 7 subgroups: TRPC (classical or canonical, TRPC1-7), TRPM (melastatin, TRPM1-8), TRPV (vanilloid receptor, TRPV1-6), TRPA (ankyrin-rich protein, TRPA1), TRPP (polysistin), TRPML (mucolipin), TRPN (NOMPC, “no mechanoreceptor potential” C) [9], [43], [10], [12], [44] and [45]. Most of these channels conduct  $\text{Na}^+$  and  $\text{Ca}^{+2}$  as cotransport. The TRPA1 group of channels has been proven to be present in the ileum [23], [50] and [47]. TRPA1 agonists stimulate contractions in the guinea pig ileum and colon [50] and [47]. TRPA1 [4], TRPV1 [33],[40] group channels present in the bladder. The TRPA1 group [4] and the TRPV1 group channels [33] contract bladder. TRPV1 antagonists have reduced or prevented bladder contractions [4]. There is evidence that cinnamic acid and its derivatives use TRPA1 [4], [21] and TRPV1 group channels.

Hydroxycinnamic acids and hydroxybenzoic acids are linked by -OH and -OCH<sub>3</sub> groups to form important phenolic acid derivatives. The most important of these compounds are Hydroxycinnamic acids [2]. Cinnamic Acid is a phenolic acid derivative commonly found in Cinnamomi cortex (Cinnamon) plant, and its name is derived from cinnamon. Antimicrobial, Antitumoral, Anticarcinogenic and Antifungal properties and vasodilator effect were determined [38], [63], [48], [25] and [41].

Atropine is a mixture of D and L hyoscyamine in equal proportions. It is obtained synthetically as well as by extraction from Solanaceae. Atropine is a non-selective muscarinic receptor antagonist. The affinities of the muscarinic receptor antagonists to receptors can be overridden among different subtypes. This is due to the fact that antagonists can not selectively discriminate receptor subtypes [3]. The selectivity of these compounds results from the level of receptor expression in the relevant tissue or cell and from the affinity constant of the receptor antagonists. The affinity of atropine for binding to M<sub>1</sub> receptors was 9.0-9.7, while for M<sub>2</sub> receptor 8.7-9.3, for M<sub>3</sub> 8.9-9.2, for M<sub>4</sub> 8.9-9.1 and for M<sub>5</sub>



8.9-9.7 and they are found to be very high [11]. Atropine has the following effects [3]; 1) It has psychological excitation causing euphoric effect on some regions of the central nervous system with little doses. 2) It has direct parasympatholytic effect in the autonomic nervous system. This effect follows an indirect sympathomimetic effect. 3) Atropine reduces muscarinic effect of acetylcholine by paralyzed action on smooth muscle fibers. Atropine causes relaxation of the bladder, urine accumulation and urine drainage difficulty [18].

Fentolamine is an imidazoline derivative non-selective  $\alpha$ -adrenergic receptor antagonist. Fentolamine has equal binding sites on both the  $\alpha$ -1 and  $\alpha$ -2 receptors. The effects of phenolamine are nonselective [7]. Phentolamine, an alpha-adrenergic receptor antagonist, has direct smooth muscle relaxant, cholinomimetic, histaminic and sympathomimetic activity [24]. In addition to blocking the alpha-adrenergic receptor, it also inhibits the effects of 5-HT. The phenolamine may induce an agonistic effect on muscarinic receptor, histamine H1 and H2 receptors [53].

Beta-blockers belong to the class of antagonists that block the effect of the adrenergic neurotransmitter on beta adrenoceptors. The most important of these are timolol, pindol, metoprolol and propranolol [36]. Beta receptors are examined in two groups,  $\beta$ -1 and  $\beta$ -2. Propranolol is a nonselective  $\beta$ -adrenoceptor antagonist that inhibits the function of both  $\beta$ -1 receptors and  $\beta$ -2 receptors [15]. Propranolol,  $\beta$ -adrenergic receptor antagonist, increase inhibitory effects of hydroalcoholic extract of *Allium ampeloprasum* plant extract on potassium chloride caused contractions in ileum[58]. They concluded that the hydroalcoholic extract of *Allium ampeloprasum* leaf should affect beta-adrenergic receptors and voltage-dependent calcium channels in order to influence rat ileum motor activity [48]. There was a significant difference between the effects of incubation with the Ileum  $\beta$ -adrenergic receptor antagonist propranolol and *R. damascena* isolate on propranolol presence and absence of ileum contraction caused by KCl [59]. The active substances in the extract are likely to induce inherent inhibitory activity by affecting the  $\beta$ -adrenergic receptor which the presence and inhibitory effects are determined [6]. Here, contraction in the KCl-induced depolarized smooth muscle is due to the presence of calcium in the environment [65].

Nifedipine's effect is displayed by blocking voltage-dependent calcium channels on the cell membrane [30]. Dihydropyridine-derived calcium channel blockers show antihypertensive effects in two pathways. One of these pathways is the direct relaxant effect due to the inhibition of calcium entry from smooth muscle L-type calcium channels [14].

The flow of calcium ions into the muscle fibers through the slow calcium-sodium channels via the KCl effect allows the formation of contractions [52]. Calcium channels are the main channels that cause muscle contraction [31]. Increase of the how cytosolic concentration of  $\text{Ca}^{2+}$  ions has been questioned because of the how L-type  $\text{Ca}^{2+}$  channel blockers significantly reduces CCh-induced contractions [39], [66] and PLC inhibitors are not able to prevent CCh-induced contractions [57]. Experiments with verapamil, an L-type  $\text{Ca}^{2+}$  channel blocker, and confirmed that  $\text{Ca}^{2+}$  entry via L-type  $\text{Ca}^{2+}$  channels is an important contributor to CCh-induced contraction[28]. The formation of carbachol and inositol phosphates is insensitive to nifedipine concentrations and therefore nifedipine normally blocks intracellular  $\text{Ca}^{2+}$  uptake via voltage-gated channels [56]. Hodgkin and Huxley described voltage-dependent  $\text{K}^{+}$  channels in their work as delayed rectifiers. The channel activation is rather slow compared to the sodium channels, current in one direction is easier to pass than the flow in the other direction. There is no inactivation as long as there is a stimulation in these currents [19], [64], [13] and [49]. In addition to these channels,  $\text{K}^{+}$  currents activated with depolarization but rapid inactivation were also observed. These are referred to as  $\text{K}^{+}$  currents or A currents that are rapidly



inactivated. The operation of delayed rectifier type potassium channels is inhibited by tetraethylammonium (TEA). Vasoactive agents such as ACh, bradykinin, ATP, adenosine and histamine lead to extracellular flow through hyperpolarization in endothelial cells. The  $K^+$  channels activated by  $Ca^{+2}$  are of two types: potassium channels with high and small conductance. The activation of these channels depends on intracellular ion concentration [17]. Activation of these potassium channels in endothelial cells changes the cell membrane potential through synthesis and secretion of endothelium-derived factors by intracellular  $Ca^{+2}$  ion concentration. Hyperpolarization and relapse associated with activation of  $K^+$  channels are lost in the presence of TEA, which is a high  $K^+$  or non-selective  $K^+$  channel blocker [7].

In this study, the CA contraction and relaxation responses on the rat's bladder and ileum were investigated, and the aim was to find out the mechanisms it reacted.

## 2. METHODS AND TOOLS

### 2.1. Animals Used in Experiments

Male Sprague-Dawley rats weighing 200-300 g 8 weeks old were obtained from Dumlupınar University-Animal Application and Research Center and used in experiments. Prior to the commencement of the study, the approval is received from Dumlupınar University Medical School-Local Ethics Committee of Animal Experiments (DPU HADYEK dated 26.10.2016 and decision no. 2016.10.03). Experimental animals were housed in Dumlupınar University-Experimental Animal Application and Research Laboratory with a 12 hour dark, 12 hour light cycle, 40-60% humidity and a constant room temperature of  $22 \pm 1$  °C.

### 2.2. Methods

#### 2.2.1. Isolated organ bath experiments

Male Sprague-Dawley rats were subjected to cervical dislocation followed by longitudinal abdominal and thoracic incision to open the abdomen and chest cavity [33]. Preparations were taken from the ileum and bladder organs and placed in a petri dish containing cold Krebs-Henseleit physiological solution. The composition of the Krebs-Henseleit physiological solution was prepared to be 119 mM NaCl, 4.7 mM KCl, 2.5 mM  $CaCl_2$ , 1 mM  $MgCl_2$ , 25 mM  $NaHCO_3$ , 1.2 mM  $KH_2PO_4$  and 11.1 mM Glucose. The organs taken from the experimental animals were ripped off from the surrounding fat and other tissues and then transferred to an isolated organ chamber aerated with 95%  $O_2$  and 5%  $CO_2$  at 37 °C. The one tip of the tissue sample is then ligated to isolated organ and the other tip to transducer. During the experiment, 1 gr tension was applied to ileum and bladder. Ileum and bladder responses were detected with an isotonic transducer and recorded via a recorder.

#### 2.2.2. Protocol studies

A total of 56 Sprague-Dawley male rats were used. Two different protocols were applied in each arranged group study as shown in Table 1. Animals were divided into 7 equal groups as shown in Table 2.



**Table 1.** Protocols. In the protocol 1, antagonist or channel blocker substance administration after each wash was performed and the 20 min incubation time was applied as shown. Unlike the groups, two protocols were applied in the control group study, but no any receptor antagonist administration was performed.

| Protocol Number | Protocol Procedure                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1               | <p>The organs (viable with KCI solution) washed and rested for 20 min</p> <p>↓</p> <p>the receptor antagonist administered</p> <p>↓</p> <p>Resting 45 min</p> <p>↓</p> <p><math>10^{-6}</math> M CA was applied and washed</p> <p>↓</p> <p>waiting 20 min</p> <p>↓</p> <p><math>10^{-5}</math> M CA applied and washed</p> <p>↓</p> <p>waiting 20 min</p> <p>↓</p> <p><math>10^{-4}</math> M CA was applied and washed</p> <p>↓</p> <p>waiting 20 min</p> <p>↓</p> <p><math>10^{-3}</math> M CA applied</p> <p>↓</p> <p>protocol 1 termination</p> |
| 2               | <p>The organs (KCI determined vitality) were left to rest for 20 min</p> <p>↓</p> <p>The receptor antagonist was applied</p> <p>↓</p> <p>Rested for 60 min</p> <p>↓</p> <p>Carbachol-contracted organs were cumulatively administered in order of <math>10^{-6}</math> M, <math>10^{-5}</math> M, <math>10^{-4}</math> M and <math>10^{-3}</math> M with CA</p> <p>↓</p> <p>Protocol 2 termination</p>                                                                                                                                             |



**Table 2.** Protocols. In the protocol 1, antagonist or channel blocker substance administration after each wash was performed and the 20 min incubation time was applied as shown. Unlike the groups, two protocols were applied in the control group study, but no any receptor antagonist administration was performed.

| Groups | Group Procedures                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1      | <p><b>In the Group 1 study, which was accepted as the control group, merely CA's effects on the organs (contraction-relaxation responses) were observed.</b></p> <p>↓</p> <p><b>Two different protocols were applied in the control groups.</b></p> <p>↓</p> <p><b>Two different pieces were taken surgically from all applied organs.</b></p> <p>↓</p> <p><b>Each piece of organ was applied separately in both protocols.</b></p> <p>↓</p> <p><b>A total of 8 animals were used in the group 1 study.</b></p>     |
| 2      | <p><b><math>\alpha</math>-adrenoceptors were blocked with a non-selective <math>\alpha</math>-adrenoceptor antagonist, fentolamine (<math>10^{-5}</math> M), and the response of organs was determined by administering each organ to CA.</b></p> <p>↓</p> <p><b>Two different pieces were taken from all applied organs.</b></p> <p>↓</p> <p><b>Each piece of organ was applied separately in two protocols.</b></p> <p>↓</p> <p><b>Two different protocols were applied in the group 2 study. (8 animals)</b></p> |
| 3      | <p><b>The <math>\beta</math>-adrenoceptors were blocked by propranolol (<math>10^{-6}</math> M), a non-selective <math>\beta</math>-adrenoceptor antagonist, and the response of organs was determined by administering CA to each organ.</b></p> <p>↓</p> <p><b>Two different pieces were taken from all applied organs.</b></p> <p>↓</p> <p><b>Each piece of organ was applied separately in two protocols.</b></p> <p>↓</p> <p><b>Two different protocols were applied in the group 3 study. (8 animals)</b></p> |
| 4      | <p><b>Cholinergic receptors were blocked by a non-selective cholinergic receptor antagonist atropine (<math>10^{-6}</math> M), and the response of organs was determined by applying each organ with CA.</b></p> <p>↓</p> <p><b>Two different pieces were taken from all applied organs.</b></p> <p>↓</p> <p><b>Each piece of organ was applied separately in two protocols.</b></p> <p>↓</p> <p><b>Two different protocols were applied in the group 4 study. (8 animals)</b></p>                                  |
| 5      | <p><b>The L-type Ca channel blocker, tetraethylammonium (<math>10^{-3}</math> M), blocked the L-type Ca channels and the response of the organs was determined by applying CA to each organ.</b></p> <p>↓</p> <p><b>Two different pieces were taken from all applied organs.</b></p>                                                                                                                                                                                                                                |



|   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|   | <p>↓</p> <p><b>Each piece of organ was applied separately in two protocols.</b></p> <p>↓</p> <p><b>Two different protocols were applied in the group 5 study. (8 animals)</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 6 | <p><b>The K-channel blocker, nifedipine (<math>10^{-6}</math> M), blocked the K channel and the response of the organs was determined by applying CA to each organ.</b></p> <p>↓</p> <p><b>Two different pieces were taken from all applied organs.</b></p> <p>↓</p> <p><b>Each piece of organ was applied separately in two protocols.</b></p> <p>↓</p> <p><b>Two different protocols were applied in the group 6 study. (8 animals)</b></p>                                                                                                                                                                                                                                                                                |
| 7 | <p><b>In this group, non-selective <math>\alpha</math>-adrenoceptor antagonist fentolamine (<math>10^{-5}</math> M), <math>\beta</math>-adrenoceptor antagonist propranolol (<math>10^{-6}</math> M) and cholinergic receptor antagonist atropine (<math>10^{-6}</math> M) were administered together to block both the adrenergic receptor and cholinergic receptor and the responses of the organs were determined by applying CA to each organ.</b></p> <p>↓</p> <p><b>Two different pieces were taken from all applied organs.</b></p> <p>↓</p> <p><b>Each piece of organ was applied separately in two protocols.</b></p> <p>↓</p> <p><b>Two different protocols were applied in the group 7 study. (8 animals)</b></p> |

### 2.3. Statistical Analysis

The contraction responses to CA in the presence or absence of trachea and mesenteric antagonists and blockers in the experimental groups were calculated as a percentage of 80 mM KCl-induced contraction responses. Relaxation responses to CA were expressed as a percentage of carbachol contractions. One-way ANOVA and Dunnett's post hoc test were used for statistical analysis. All evaluations were performed in computer environment with GraphPad Prism program. The data were expressed as mean $\pm$ standard error and  $p < 0.05$  was considered meaningfully significant.

### 3. FINDINGS

Experiments were performed separately for ileum and bladder in 7 different groups applied as control, atropine, phentolamine, propranolol, nifedipine, tetraethyl ammonium and atropine+phentolamine+propranolol. Two different protocols were applied in this study. The contraction-relaxation responses of related tissues to cinnamic acid after administration of different



antagonists or channel blockers to KCl or carbachol pre-contracted ileum/bladder tissues were examined. We preferred the logarithmic data conversion process for its normalizing effect as it was shown in all figures.

### 3.1. The Effects of Cinnamic Acid on Ileum

Cinnamic acid produced carboxylazole-induced relaxation response in dose dependent manner (Fig. 2), while it produced rat ileum smooth muscle contractions in dose dependent manner (Fig. 1).

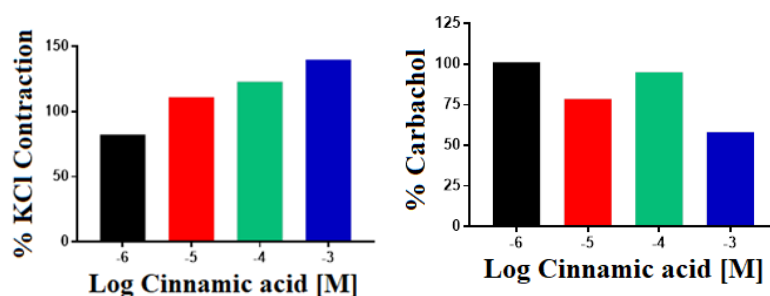


Figure 1(left). The effects of cinnamic acid on ileum

Figure 2(right). The effects of cinnamic acid on ileum pre-contracted by carbachol.

#### 3.1.1. The effects of non-selective muscarinic receptor antagonist atropine on cinnamic acid contraction-relaxation responses

The non-selective muscarinic receptor antagonist atropine-induced contraction responses to cinnamic acid were increased in dose dependent manner (Figure 3). There was no statistically significant difference between the atropine applied group and the control group contraction responses ( $p > 0.05$ ). The non-selective muscarinic receptor antagonist atropine did not alter cinnamic acid responses of charbacol pre-contracted ileum smooth muscle ( $p > 0.05$ ) (Fig. 4).

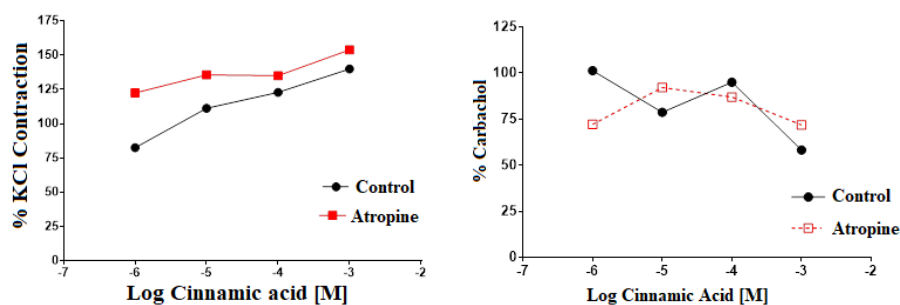


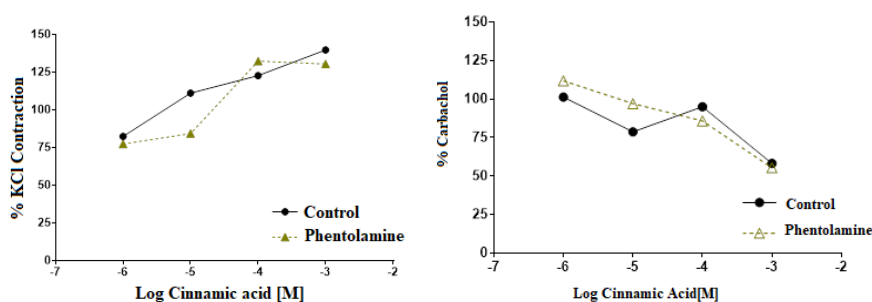
Figure 3(left). The effects of cinnamic acid on ileum with or without atropine

Figure 4(right). The effects of cinnamic acid on ileum pre-contracted by carbachol with or without atropine.



### 3.1.2. The effects of non-selective $\alpha$ -adrenergic receptor antagonist phentolamine on cinnamic acid contraction-relaxation responses

Contraction responses of cinnamic acid in a dosage dependent manner were not changed by the non-selective  $\alpha$ -adrenergic receptor antagonist phentolamine ( $p>0.05$ ) (Figure 5). The non-selective  $\alpha$ -adrenergic receptor antagonist phentolamine did not change cinnamic acid responses on carbachol-precontracted ileum smooth muscle relaxation ( $p>0.05$ ) (Fig. 6).

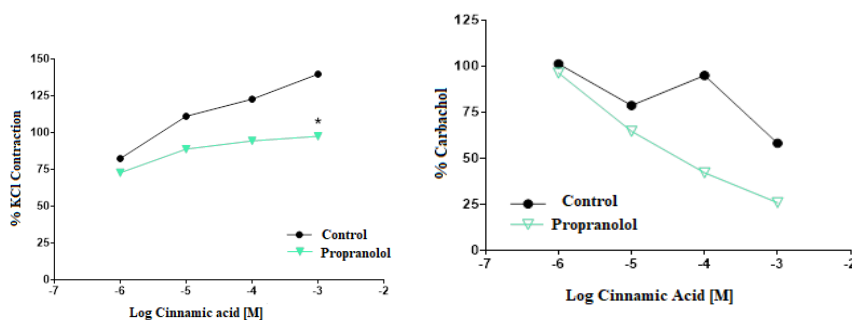


**Figure 5(left).** The effects of cinnamic acid on ileum with and without phentolamine.

**Figure 6(right).** The effects of cinnamic acid on ileum pre-contracted by carbachol with or without phentolamine.

### 3.1.3. The effects of non-selective $\beta$ -adrenergic receptor antagonist propranolol on cinnamic acid contraction-relaxation responses

The non-selective  $\beta$ -adrenergic receptor antagonist propranolol significantly inhibited the contraction responses of cinnamic acid in a dosage dependent manner ( $p<0.05$ ) (Figure 7). The non-selective  $\beta$ -adrenergic receptor antagonist propranolol did not alter carbachol pre-contracted ileum smooth muscle cinnamic acid responses ( $p>0.05$ ) (Fig. 8).



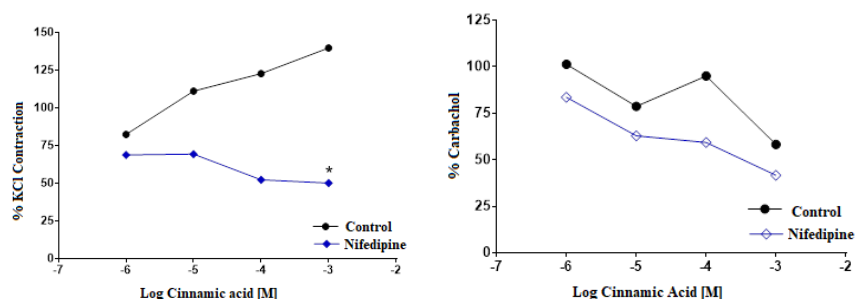
**Figure 7(left).** The effects of cinnamic acid on ileum with or without propranolol,

**Figure 8(right).** The effects of cinnamic acid on carbachol pre-contracted ileum with or without propranolol.

### 3.1.4. The effects of L-type $\text{Ca}^{2+}$ channel blocker nifedipine on cinnamic acid contraction-relaxation responses

The L-type  $\text{Ca}^{2+}$  channel blocker nifedipine significantly inhibited the contraction responses of cinnamic acid in a dosage dependent manner ( $p<0.05$ ) (Figure 9). The L-type  $\text{Ca}^{2+}$  channel blocker nifedipine did not change the carbachol pre-contracted ileum smooth muscle relaxation responses with cinnamic acid ( $p>0.05$ ) (Figure 10).

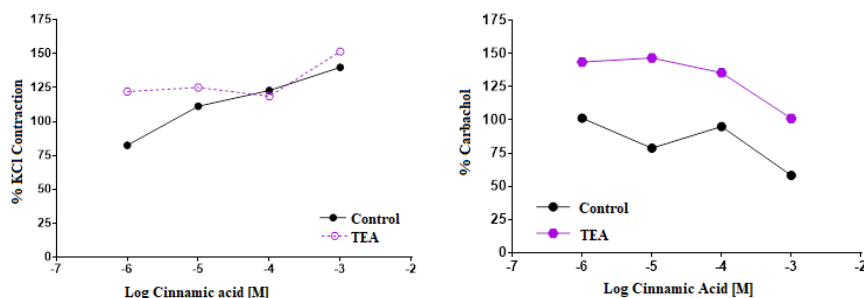




**Figure 9(left).** The effects of cinnamic acid on ileum with or without nifedipine, **Figure 10(right).** The effects of cinnamic acid on carbachol pre-contracted ileum with or without nifedipine.

### 3.1.5. The effects of $K^+$ channel blocker tetraethylammonium on cinnamic acid contraction relaxation responses

Contraction responses of the cinnamic acid in dosage dependent manner were not changed by the  $K^+$  channel blocker tetraethyl ammonium ( $p > 0.05$ ) (Figure 11). In the smooth muscle relaxation responses to cinnamic acid in carbachol-pre-contracted ileum, the  $K^+$  channel blocker tetraethyl ammonium provided contraction but no significant difference was observed between the groups ( $p > 0.05$ ) (Figure 12).

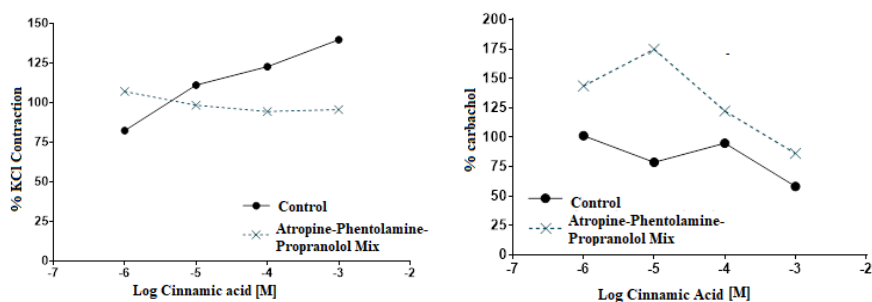


**Figure 11(left).** The effects of cinnamic acid on ileum with and without TEA, **Figure 12(right).** The effects of cinnamic acid on carbachol pre-contracted ileum with and without TEA.

### 3.1.6. The effects of atropine, phentolamine and propranolol mix on cinnamic acid contraction-relaxation responses

Contraction responses of cinnamic acid in a dosage dependent manner decreased by the non-selective muscarinic receptor antagonist atropine, non-selective  $\alpha$ -adrenergic receptor antagonist phentolamine and non-selective  $\beta$ -adrenergic receptor antagonist propranolol, but no statistically significant difference was found between the groups ( $p > 0.05$ ) (Figure 13). A mixture of non-selective muscarinic receptor antagonist atropine, non-selective  $\alpha$ -adrenergic receptor antagonist phentolamine, and non-selective  $\beta$ -adrenergic receptor antagonist propranolol resulted in a contraction of pre-contracted smooth muscle of ileum relaxation response to carbachol with cinnamic acid, but no statistically significant difference was found between the groups ( $p > 0.05$ ) (Figure 14).



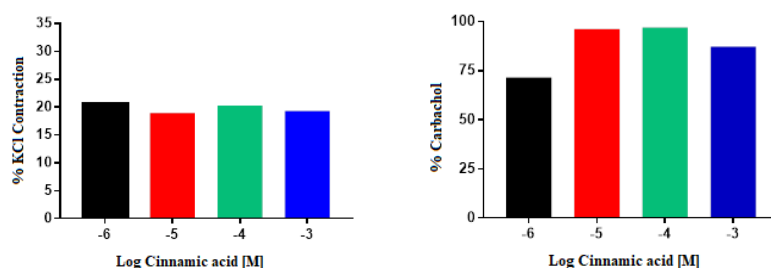


**Figure 13(left).** The effects of cinnamic acid on ileum with or without atropine- phentolamine-propranolol mix.

**Figure 14(right).** The effects of cinnamic acid on carbachol pre-contracted ileum with or without atropine-phentolamine-propranolol mix.

### 3.2. The Effects of Cinnamic Acid on Bladder

Sinnacic acid responded as contractions in rat bladder smooth muscles in a dose dependent maner (Fig. 15) but formed relaxation responses against carbachol in a dose dependent manner (Fig. 16).



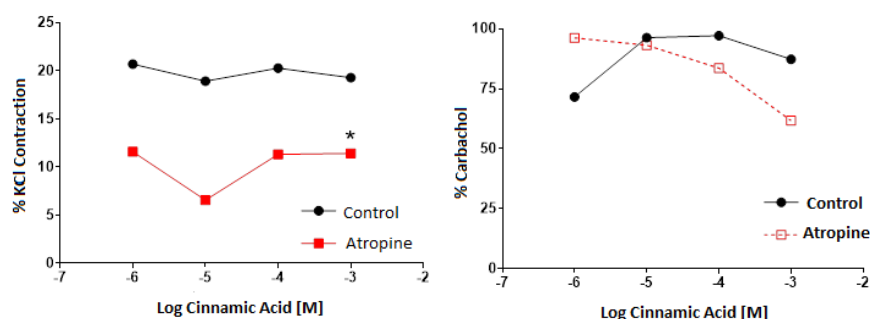
**Figure 15(left).** The effects of cinnamic acid on bladder,

**Figure 16(right).** The effects of cinnamic acid on carbachol pre-contracted bladder.

#### 3.2.1. The effects of non-selective muscarinic receptor antagonist atropine on cinnamic acid contraction-relaxation responses

The non-selective muscarinic receptor antagonist atropine significantly inhibited the contraction responses of cinnamic acid in a dosage dependent manner ( $p < 0.05$ ) (Figure 17). Carbachol-induced pre-contracted bladder smooth muscle relaxation responses by cinnamic acid were increased by non-selective muscarinic receptor antagonist atropine, but no significant difference between the groups was not observed ( $p > 0.05$ ) (Figure 18).



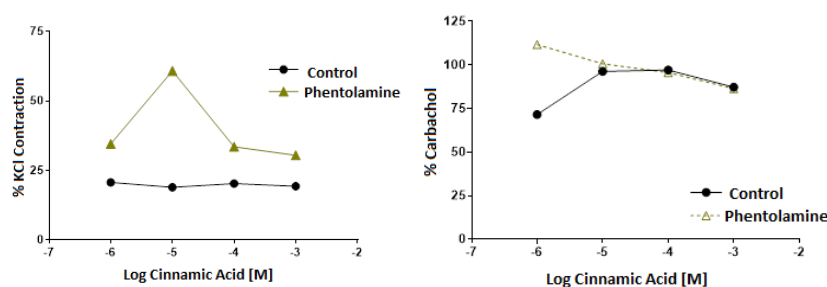


**Figure 17(left).** The effects of cinnamic acid on bladder with and without atropine.

**Figure 18(right).** The effects of cinnamic acid on pre-contracted bladder with or without atropine.

### 3.2.2. The effects of non-selective $\alpha$ -adrenergic receptor antagonist phentolamine on cinnamic acid contraction-relaxation responses

Contraction responses of cinnamic acid in a dosage dependent manner were increased by non-selective  $\alpha$ -adrenergic receptor antagonist phentolamine, but no significant difference was observed between the groups ( $p > 0.05$ ) (Figure 19). Non-selective  $\alpha$ -adrenergic receptor antagonist phenolamine did not change carbachol-preadministered with cinnamic acid pre-contracted bladder smooth muscle relaxation responses ( $p > 0.05$ ) (Figure 20).



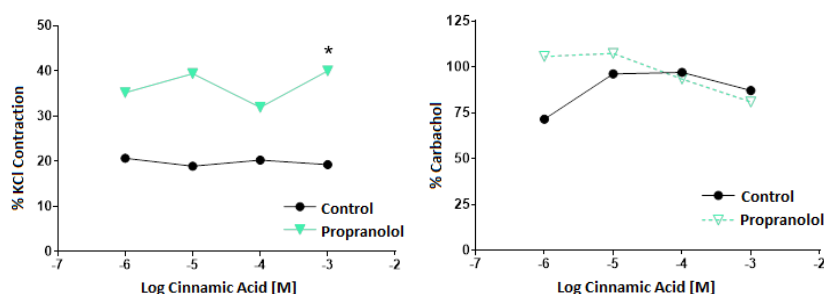
**Figure 19(left).** The effects of cinnamic acid on bladder with and without phentolamine.

**Figure 20(right).** The effects of cinnamic acid on carbachol pre-contracted bladder with and without phentolamine.

### 3.2.3. The effects of non-selective $\beta$ -adrenergic receptor antagonist propranolol on cinnamic acid contraction-relaxation responses

The non-selective  $\beta$ -adrenergic receptor antagonist propranolol significantly increased the contraction responses of cinnamic acid in a dosage dependent manner ( $p < 0.05$ ) (Figure 21). The non-selective  $\beta$ -adrenergic receptor antagonist propranolol did not change carbachol-preadministered with cinnamic acid bladder relaxation responses ( $p > 0.05$ ) (Fig. 22).

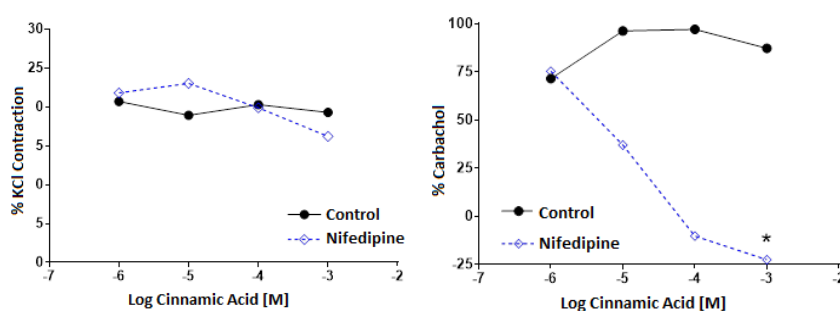




**Figure 21(left).** The effects of cinnamic acid on bladder with and without propranolol.  
**Figure 22(right).** The effects of cinnamic acid on carbachol pre-contracted bladder with and without propranolol.

### 3.2.4. The effects of L-type $\text{Ca}^{2+}$ channel blocker nifedipine on cinnamic acid contraction-relaxation responses

The L-type  $\text{Ca}^{2+}$  channel blocker nifedipine did not change the contraction responses of the cinnamic acid in a dosage dependent manner ( $p > 0.05$ ) (Figure 23). The L-type  $\text{Ca}^{2+}$  channel blocker nifedipine significantly increased the carbachol-preadministered pre-contracted with cinnamic acid bladder smooth muscle relaxation responses ( $p < 0.05$ ) (Fig. 24).

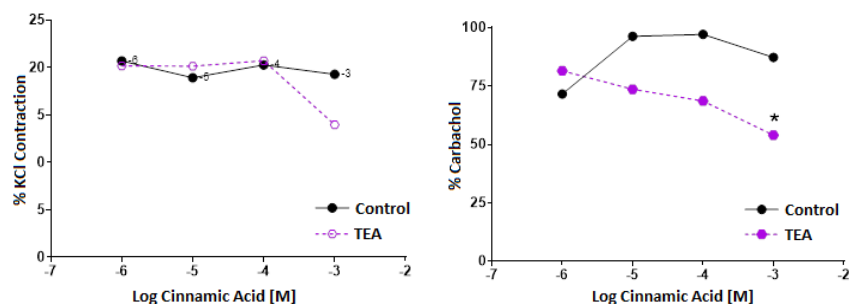


**Figure 23(left).** The effects of cinnamic acid on the bladder with and without nifedipine.  
**Figure 24(right).** The effects of cinnamic acid on carbachol pre-contracted bladder with and without nifedipine.

### 3.2.5. The effects of $\text{K}^{+}$ channel blocker tetraethylammonium on cinnamic acid contraction-relaxation responses

Contraction responses of the cinnamic acid in a dosage dependent manner were decreased by the  $\text{K}^{+}$  channel blocker tetraethylammonium but no significant difference was observed between the groups ( $p > 0.05$ ) (Figure 25). Carbachol-precontracted bladder smooth muscle with cinnamic acid relaxation responses were significantly increased by  $\text{K}^{+}$  channel blocker tetraethyl ammonium ( $p < 0.05$ ) (Figure 26).



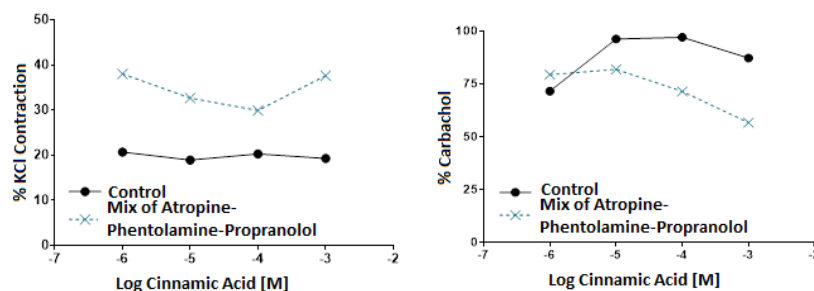


**Figure 25(left).** The effects of cinnamic acid on the bladder with and without TEA.

**Figure 26(right).** The effects of cinnamic acid on carbachol pre-contracted bladder with and without TEA.

### 3.2.6. The effects of atropine, phentolamine and propranolol mix on cinnamic acid contraction-relaxation responses

Contraction responses of cinnamic acid in a dosage dependent manner was increased by the mix of non-selective muscarinic receptor antagonist atropine, non-selective  $\alpha$ -adrenergic receptor antagonist fentolamine and non-selective  $\beta$ -adrenergic receptor antagonist propranolol, but no statistically significant difference was observed between the groups ( $p > 0.05$ ) (Figure 27). Cinnamic acid enhanced carbachol pre-contracted bladder smooth muscle relaxation responses were increased by a mixture of non-selective muscarinic receptor antagonist atropine, non-selective  $\alpha$ -adrenergic receptor antagonist fentolamine and non-selective  $\beta$ -adrenergic receptor antagonist propranolol, but no statistically significant difference was found between the groups ( $p > 0.05$ ) (Figure 28).



**Figure 27(left).** The effects of cinnamic acid on bladder with and without the mix of atropine, phentolamine and propranolol.

**Figure 28(right).** The effects of cinnamic acid on carbachol pre-contracted bladder with and without the mix of atropine, phentolamine and propranolol.

## 4. DISCUSSIONS

Cinnamic Acid's effect on aortic smooth muscles and vasodilator effect on rat aortic rings was determined [25]. However its vasodilator effect has not been investigated on the ileum and bladder smooth muscles and this fact has attracted our attention and led us to work on this topic. Thus, the mechanisms of action of CA on the ileum and bladder smooth muscles have been investigated at *in*

*vitro* conditions. In the study, pharmacological agents such as phentolamine, propranolol, atropine, nifedipine, tetraethylammonium (TEA) were used and the responses given to these agents were observed. The study was planned on the implementation of two different protocols. In both protocols, receptor antagonists were administered to the organs left to rest for 20 minutes and CA administration was performed at different doses. Next to that, in the second protocol, CA was applied to carbachol-contracted organs. Moreover, a total of 56 Sprague-Dawley rats were used in the control group. As a result of the study, the effects of CA on the bladder and tracheal organs and the mechanism or mechanisms by which this effect was carried out are intended to contribute to the production of this substance and other substances of similar activity.

In our study, 7 different groups applied as control, atropine, phentolamine, propranolol, nifedipine, tetraethyl ammonium and atropine+phentolamine+propranolol was performed separately for ileum and bladder. Two different protocols were applied in this study. The contraction-relaxation responses of related tissues to cinnamic acid after application of different antagonists or channel blockers to KCl or carbachol pre-contracted ileum/bladder tissues were examined. Cinnamic acid caused contractions and relaxations in ileum and bladder in a dose dependent manner.

Atropine did not change the relaxation response of the group more contracted by the treatment of cinnamic acid. It increases relaxation while inhibiting contraction responses in bladder. Cholinergic receptors have created a mechanism that causes contraction of bladder [1], [42]. The inhibition of contraction by the result of atropine administration is expected. Many researchers have mentioned atropine-resistant excitation in smooth muscles [55], [34]. We consider that the reason for more contractions of ileum by cinnamic acid is caused by the noncholinergic system even though atropine is applied, and we think that muscarinic receptors are not used for the cinnamic acid caused ileum contraction responses.

It is reported that TRPA1 channels increase contraction in the ileum [50], [47] and cinnamic acid and its derivatives use these channels [54]. We also think that the cause of contractions in the ileum may be the channels with transient receptor potentials. Atropine causes relaxation of bladder to occur [18]. It is observed that the bladder responses were normal responses to atropine. The bladder response is consistent with the general information given in Goth's book.

Contraction and relaxation responses of ileum and bladder were not changed in the phentolamine-treated group. Phentolamine is a non-selective  $\alpha$ -adrenergic receptor antagonist [24]. The  $\alpha$ -adrenoceptors exist in the neck of the bladder and exist in trigone more than other parts of the bladder [1], [42].  $\alpha_1$ -adrenoceptors are thought to be effective not only on the contraction of the bladder but also on the secretion of neurotransmitters [42]. Findings makes a point that  $\alpha_2$ -adrenoceptor agonists reduce bladder capacity [22]. They pointed out that  $\alpha_1$ -adrenoceptor agonists increase the frequency of bladder contraction [16] and [29]. It has been guessed that  $\alpha_1$ -adrenoceptor agonists inhibit the contractile responses of ileum but the  $\alpha_2$ -adrenoceptor agonists have no effect on the mechanism of contractility of the ileum [59]. In our study, the remained unchanging of response of ileum and bladder to cinnamic acid in the groups treated with phentolamine suggesting that the mechanism of action of cinnamic acid is via nonadrenergic pathways.

In propranolol administration, the inhibited relaxation responses of the contraction responses of the ileum were not changed. In the bladder, relaxation responses were not changed while the contraction responses increased. Propranolol is a non-selective  $\beta$ -adrenergic receptor antagonist that blocks both  $\beta_1$  receptors and  $\beta_2$  receptors [36], [51].  $\beta$ -adrenoceptors regulate relaxation in the bladder smooth



muscles [1], [42]. In our study, an increase in cinnamic acid responses in the presence of propranolol in the bladder is an expected normality. It has been shown that  $\beta_1$ -adrenoceptor agonists have no effect on the mechanism of contraction of the ileum but  $\beta_2$ -adrenoceptor agonists inhibit contraction responses [60]. The results of our studies of this group are incompatible with previous studies. Increased contraction responses are expected due to the antagonist usage. The inhibition of responses suggests that cinnamic acid is effective through nonadrenergic pathways.

Nifedipine did not alter relaxation responses while inhibiting contraction responses of cinnamic acid in ileum, and increased relaxation responses without affecting contraction responses in the bladder. Nifedipine is a calcium antagonist that blocks voltage-dependent L-type calcium channels in smooth muscle cell membranes [30]. The activation of the L-type Ca channels allows the smooth muscle to contract. Their inactivation inhibits contraction. In our study, the inhibition of cinnamic acid responses in ileum in the presence of nifedipine suggests that cinnamic acid uses this pathway. The fact that cinnamic acid relaxation responses of bladder to carbachol increased in the presence of nifedipine suggests that cinnamic acid uses this pathway. However, the relaxation responses to carbachol in the ileum and the unchanged contraction responses in the bladder suggests that cinnamic acid in the presence of nifedipine may have used transient receptor potential channels (TRPs) permeable to different cations beside the L-type Ca channels.

TEA administration increased relaxation responses without affecting contraction responses in the absence of changes in contraction and relaxation responses of the ileum. TEA is a non-selective potassium ion channel blocker. Hyperpolarization and relaxation associated with activation of  $K^+$  channels are lost in the presence of TEA, which is a high  $K^+$  or non-selective  $K^+$  channel blocker [7]. In our study, the unchanged contraction responses of cinnamic acid in the ileum and the bladder in the presence of TEA and the increase in relaxation responses of the bladder suggests that cinnamic acid did not use this pathway. We think that in the presence of TEA, cinnamic acid may have used transient receptor potential channels permeable to different cations beside  $K^+$  channels. Adrenergic and cholinergic receptor blockade made at the same time altered the contraction and relaxation responses in the ileum and bladder, but it did not affect significantly. The fact of the response of this group being not different from the response of the control group suggests that the mechanism of action of cinnamic acid in both organs is through non-adrenergic-non cholinergic pathways. In the study made in the rat thoracic aorta suggests that cinnamic acid acts through the NO-cGMP-protein kinase G pathway [25]. We support the idea that cinnamic acid uses the same pathway in ileum and bladder. In addition, TRP channels have been shown to increase contraction activity in the ileum and bladder [50], [47], [4], [33] and usage of these channels by cinnamic acid and its derivatives [4], [21] and [54]. We also assume that the reason for unchanged cinnamic acid responses to contraction-relaxation during cholinergic and adrenergic blockade compared to the control group's response experimented at the same time may be the transient receptor potential channels.

Based on our results, we can say that cinnamic acid also uses cholinergic, adrenergic, non-adrenergic non-cholinergic and TRP channels in the ileum and bladder smooth muscles. We believe that the future works to be performed using NANK inhibitors and TRP antagonists will explain these mechanisms much better.

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## ATTACHMENTS

### APPENDIX 1. Experimental Animals Local Ethics Committee Approval Certificate

| T.C.<br>DUMLUPINAR ÜNİVERSİTESİ<br>HAYVAN DENEYLERİ YEREL ETİK KURULU<br>ARAŞTIRMA BAŞVURUSU ONAYI                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                          |                                                                     |      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|------|
| <b>BAŞVURU BİLGİLERİ</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | ARAŞTIRMANIN ADI                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Sıçan İleumu, Mesanesi ve Trakeası Düz Kası Kasılma ve Gevşeme Cevapları Üzerine Sınnamik Asit'in Etki Mekanizmasının Araştırılması      |                                                                     |      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | ARAŞTIRMA YÜRÜTÜCÜSÜ KURUMU                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Doç.Dr.M.Kasım ÇAYCI<br>Dumlupınar Üniversitesi Fen Edebiyat Fakültesi Genel Biyoloji ABD                                                |                                                                     |      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | PROJE YÜRÜTÜCÜSÜ KURUMU                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Doç.Dr.M.Kasım ÇAYCI<br>Dumlupınar Üniversitesi Fen Edebiyat Fakültesi Genel Biyoloji ABD                                                |                                                                     |      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | YARDIMCI ARAŞTIRICILAR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Doç.Dr.M.Kasım ÇAYCI Yrd.Doç.Dr.Yasemin TEKŞEN<br>YL.Öğr. Emre KUNDAKÇI Öğr.Gör.Sinan DARCAN<br>Dr.Öğr.Fatih ALAN Prof.Dr.Hayri DAYIOĞLU |                                                                     |      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | ARAŞTIRMANIN TAHMİNİ SÜRESİ                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 12 Ay                                                                                                                                    |                                                                     |      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | KULLANILACAK HAYVAN TÜRÜ VE SAYISI                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Sprague Dawley (E) – 56 Adet                                                                                                             |                                                                     |      |
| DEĞERLENDİRİLEN İLGİLİ BELGELER                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Belge Adı                                                                                                                                | Tarihi                                                              |      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | DİLEKÇE                                                                                                                                  | 17.10.2016                                                          |      |
| <b>KARAR BİLGİLERİ</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Karar No : 2016.10.03 Tarih : 26.10.2016                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                          |                                                                     |      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Yukarıda başvuru bilgileri verilen dilekçe gerekçe, amaç ve yöntemler dikkate alınarak görüldü ve ilgili belgeler incelendi. 2016.03.06 karar nolu çalışmada hayvan soyunun değişmesi, gruplardaki hayvan sayısının azaltılması, destekleyici kuruluşlardan DPUBAP'ın çıkarılması, çalışmanın diploma derecesine yönelik kısımla Doktora derecesi eklenmesi, çalışmaya Dr.Öğr.Fatih ALAN ve Prof.Dr.Hayri DAYIOĞLU'nun eklenmesi ile ilgili dilekçenin etik açıdan uygun olduğuna,çalışmanın aşağıdaki hususlar dikkate alınarak yürütülmesine ve sorumlu araştırmacıya iletilmesine <b>ÖY BİRLİĞİ</b> ile karar verildi. |                                                                                                                                          |                                                                     |      |
| <ol style="list-style-type: none"> <li>1) Projede herhangi bir değişiklik gerektiğinde kurulumuzdan onay alınması,</li> <li>2) Projede çalışacağı bildirilen araştırmacılarda değişiklik olduğunda kurulumuzdan onay alınması,</li> <li>3) Deney hayvanları üzerinde yapılacak girişimin başlangıç ve bitiş tarihinin bildirilmesi,</li> <li>4) Çalışma süresinde tamamlanamaz ise ek süre talebinde bulunulması,</li> <li>5) Çalışma tamamlandığında sonuç raporunun gönderilmesi.</li> </ol> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                          |                                                                     |      |
| <b>ETİK KURUL BİLGİLERİ</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                          |                                                                     |      |
| <b>ÜYELER</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                          |                                                                     |      |
| Unvanı / Adı / Soyadı<br>EK Üyeligi                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Uzmanlık Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Kurumu                                                                                                                                   | İlişki (*)                                                          | İmza |
| Doç.Dr. Aynur GÜLCAN Başkan                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Tıp Fakültesi                                                                                                                            | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| Doç.Dr.M.Kasım ÇAYCI Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Genel Biyoloji Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Fen-Edebiyat Fakültesi                                                                                                                   | <input checked="" type="checkbox"/> E<br><input type="checkbox"/> H |      |
| Doç.Dr.Sermet İNAL Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Ortopedi ve Travmatoloji Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Tıp Fakültesi                                                                                                                            | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| Yrd.Doç.Dr.Yasemin TEKŞEN Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Farmakoloji Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Tıp Fakültesi                                                                                                                            | <input checked="" type="checkbox"/> E<br><input type="checkbox"/> H |      |
| Yrd.Doç.Dr.Sezer AKÇER Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Anatomi Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Tıp Fakültesi                                                                                                                            | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| Yrd.Doç.Dr.Hasan METİNİREN Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Ortopedi ve Travmatoloji Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Tıp Fakültesi                                                                                                                            | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| Yrd.Doç.Dr.Ahmet Haris Selçuk ÖZEN Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Zooloji Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Tıp Fakültesi                                                                                                                            | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| Yrd. Doç. Dr. Hasan Emre AYDIN Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Beyin ve Sinir Cerrahisi Anabilim Dalı                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Tıp Fakültesi                                                                                                                            | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| Vet. HEKİM Aydın AKCILAR Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Veteriner HEKİM                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Tıp Fakültesi DEHYUAM                                                                                                                    | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| Biyolog Mehmet ÖNCEBE Üye                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                          | <input type="checkbox"/> E<br><input checked="" type="checkbox"/> H |      |
| * Araştırma ile İlişkisi                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                          |                                                                     |      |



RESEARCH ARTICLE

FINITE ELEMENT METHOD BASED STRUCTURAL ANALYSIS OF QUADCOPTER UAV  
CHASSIS PRODUCED WITH 3D PRINTER

Serkan ÇAŞKA<sup>1</sup>, Kadir GÖK<sup>2</sup>, Mustafa AYDIN<sup>3\*</sup>, İkbâl ÖZDEMİR<sup>4</sup>

<sup>1</sup>Celal Bayar University, Hasan Ferdi Turgutlu Faculty of Technology, Devision of Mechanical Engineering, Manisa,  
[serkan.caska@cbu.edu.tr](mailto:serkan.caska@cbu.edu.tr), ORCID: 0000-0002-2157-8931

<sup>2</sup>Bakırçay University, Faculty of Engineering and Architecture, Division of Biomedical Engineering, İzmir,  
[kadir.gok@bakircay.edu.tr](mailto:kadir.gok@bakircay.edu.tr), ORCID: 0000-0001-5736-1884

<sup>3</sup>Kütahya Dumlupınar University, Faculty of Engineering, Division of Mechanical Engineering, Kütahya,  
[mustafa.aydin@dpu.edu.tr](mailto:mustafa.aydin@dpu.edu.tr), ORCID: 0000-0002-9150-4081

<sup>4</sup>Celal Bayar University, Hasan Ferdi Turgutlu Faculty of Technology, Division of Mechanical Engineering, Manisa,  
[181200001@ogr.cbu.edu.tr](mailto:181200001@ogr.cbu.edu.tr), ORCID: 0000-0002-6062-9973

Geliş Tarihi:11.06.2020

Kabul Tarihi:22.06.2020

ABSTRACT

In this study, the static analysis and manufacturing of a quadcopter type small unmanned aerial vehicle (UAV) design compatible with landing platforms was performed by using the finite element method. In static analysis, the weight of the quadcopter body is considered as the load and the base is fixed. Each part of the quadcopter used in the Finite Element Analysis (FEA) is manufactured with Acrylonitrile Butadiene Styrene (ABS) and Polyactic Acid (PLA) materials with a thickness of 0.05 mm with i3 prusa printer. As a result of the structural analysis, the stress produced in the quadcopter chassis produced from ABS material was 0,053 MPa and the stress on the quadcopter frame produced from PLA material was calculated as 0,065 MPa. In addition, the deformation in the quadcopter frame produced from ABS material was 0,014 mm, while the quadcopter frame produced from PLA material was 0,010 mm.

**Keywords:** 3D manufacturing, FDM, Quadcopter UAV, Finite element method

1. INTRODUCTION

Manufacturing methods are developing rapidly with technology. Traditional manufacturing methods are generally based on the principle of material removal from the workpiece [1,2]. Unlike traditional manufacturing methods, in additive manufacturing technologies, which have become more common in recent years, the basic principle is to add layers to the material, not to reduce parts from the material [1-5]. In contrast to casting and injection molding method, there is no initial investment and maintenance cost in additive manufacturing technology [6,7].

In the last 10 years, the application area of 3D manufacturing have increased in the production of final products or visual prototypes in many different fields from dental implant production to the construction of engine parts. In recent years, the use of 3D printers has increased considerably due to the reduced costs and widespread use. The use of these devices is not limited to industrial facilities



and research institutes. The use of 3D printers has reached the individual user. Desktop 3D printers are widely used in homes or hobby workshops [7,8]. In recent researches realized in 2018, it was stated that ten different methods have been developed for 3D manufacturing technologies. These methods are Fused Deposition modeling (FDM), stereolithography (SLA), Digital Light Processing (DLP), Selective Laser Sintering (SLS), Direct Metal Laser Sintering (DMLS), Selective Laser Melting (SLM), Electron Beam Melting (EBM), Material Jetting (MJ), Drop on Demand (DOD) and Binder Jetting (BJ) [9]. In addition FDM method has a feasibility of desktop usage in recent years.

In the FDM method, thermoplastic or thermoplastic derivative materials are used as building materials. In this method, ABS, PLA and rarely composite based filaments are used [5-7]. ABS (Acrylonitrile Butadiene Styrene) is an oil-based polymer. It is an inexpensive material for low cost prototyping. However, it spreads toxic gases after burning with high temperature. Therefore, it should not be in contact with food [8]. PLA (Polylactic Acid) material is obtained from renewable resources. PLA material has a medium cost price and is an excellent 3D printer material [8]. PLA material is made from organic materials like renewable, corn starch or sugar cane. PLA material is often used to make food packaging, medical devices and implants [10]. FEA which is significant method used in structural analysis of engineering designs [12]. In recent years, FEA was used to analyze the design of quadcopter chassis with PLA and ABS materials.

Today, there is a significant increase in applications related to the aerial surveillance system [13]. These systems are developed by companies that working with unmanned vehicles [14]. This study focused on structural analysis of a quadcopter chassis that is main simple component of aerial surveillance [15].

## 2. EXPERIMENTAL STUDIES

### 2.1. 3D Modeling and Analysis

In this study, the 3D model of a quadcopter unmanned aerial vehicle that is shown in Figure 1a. was created using the SolidWorks-2018. Computer-assisted analyzes were performed using AnsysWorkbench software. 3D CAD models were transferred into AnsysWorkbench software to prepare the finite element model. Load conditions, boundary conditions and material models are defined in AnsysWorkbench. In Figure 1b, the quadcopter chassis produced with PLA material by using FDM method is shown.



**Figure 1.** a) 3D model of quadcopter chassis b) 3D printed quadcopter chassis produced from PLA.

Quadcopter has 6 degree of freedom and its dynamic model has 6 equations of movement. Dynamic model of the quadcopter includes virtual inputs composed by angular velocities of rotors. The angular velocities ( $\omega_i$ ) of the rotors are the real input variables of the quadcopter UAV. However, at the mathematical model is used virtual inputs that consisting of angular velocities. Virtual inputs are given in equations 1-4.

$$u_1 = (\omega_1^2 + \omega_2^2 + \omega_3^2 + \omega_4^2) \quad (1)$$

$$u_2 = (\omega_1^2 - \omega_3^2) \quad (2)$$

$$u_3 = (\omega_2^2 - \omega_4^2) \quad (3)$$

$$u_4 = (\omega_1^2 - \omega_2^2 + \omega_3^2 - \omega_4^2) \quad (4)$$

In equations 1-4,  $u_1$  is the thrust force acting on the body of the quadcopter,  $u_2$  is the force that generates the rolling torque,  $u_3$  is the force that generates pitching torque,  $u_4$  is the force that generates the turning torque. Dynamic model quadcopter was shown in equations 5-10. In this equations,  $d$  (drag) is impact factor.  $L$  is distance from the propellers to the center of the quadcopter,  $m$  is total mass of quadcopter.  $I_x$   $I_y$   $I_z$  are moment of inertia on basic axes.

$$\ddot{x} = (\cos\psi\sin\theta\cos\varphi + \sin\psi\sin\varphi) \frac{u_1}{m} \quad (5)$$

$$\ddot{y} = (\cos\psi\sin\theta\sin\varphi - \sin\psi\cos\varphi) \frac{u_1}{m} \quad (6)$$

$$\ddot{z} = -g + \cos\theta\cos\varphi \frac{u_1}{m} \quad (7)$$

$$\ddot{\phi} = \dot{\theta}\dot{\psi} \left( \frac{I_y - I_z}{I_x} \right) + \frac{L}{I_x} u_2 \quad (8)$$

$$\ddot{\theta} = \dot{\phi}\dot{\psi} \left( \frac{I_z - I_x}{I_y} \right) + \frac{L}{I_y} u_3 \quad (9)$$

$$\ddot{\psi} = \dot{\theta}\dot{\phi} \left( \frac{I_x - I_y}{I_z} \right) + \frac{d}{I_z} u_4 \quad (10)$$

In Table 1, was given parameters and values of parameters that used in mathematical model design of quadcopters.

**Table 1.** Value of parameters used in the mathematical model of quadcopter.

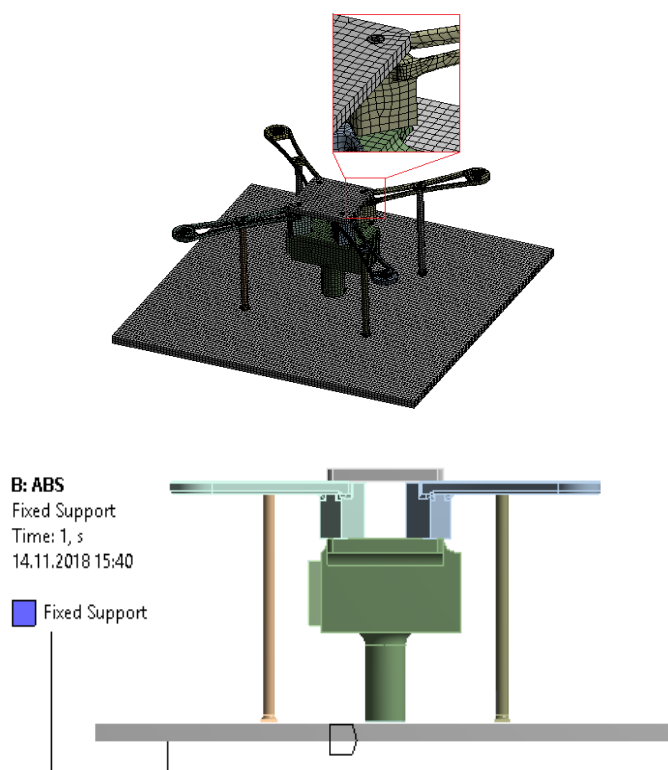
| Parameter | Value(PLA)            | Value(ABS)            |
|-----------|-----------------------|-----------------------|
| g         | 9,81 m/s <sup>2</sup> | 9,81 m/s <sup>2</sup> |
| m         | 0.81 kg               | 0.65 kg               |
| L         | 0.25 m                | 0.25 m                |



|       |                          |                          |
|-------|--------------------------|--------------------------|
| $I_x$ | 0.0132 kg m <sup>2</sup> | 0.0104 kg m <sup>2</sup> |
| $I_y$ | 0.0132 kg m <sup>2</sup> | 0.0104 kg m <sup>2</sup> |
| $I_z$ | 0.0257 kg m <sup>2</sup> | 0.0205 kg m <sup>2</sup> |

## 2.2. Loading and Boundary Conditions

Meshing was performed by using a hex-dominant finite element type for FEA as shown in Figure 2. The FEA model has 323719 nodes and 82601 elements. Element size was chosen to 5 mm. Total weight of the quadcopter have considered as load. Quadcopter was fixed on the base surface as shown in Figure 3.

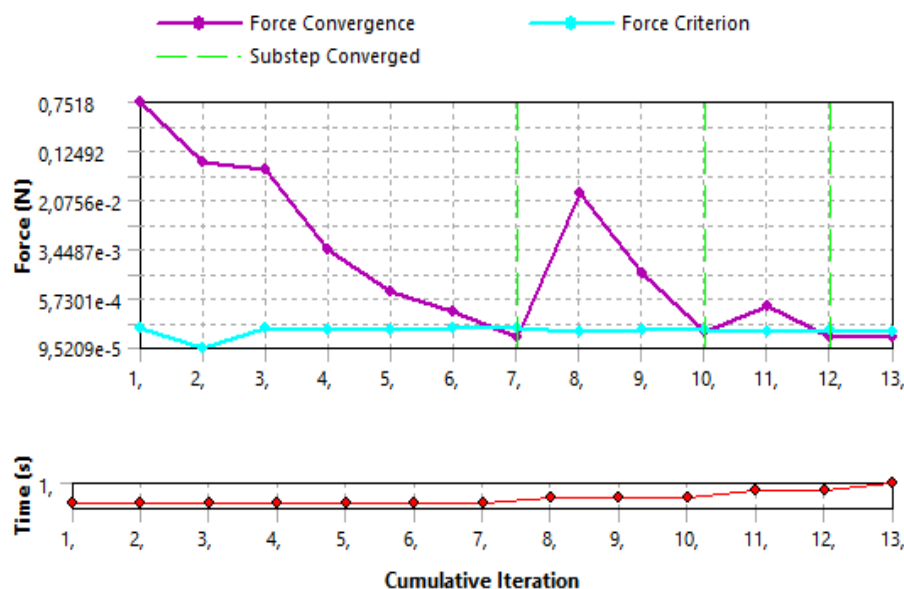


**Figure 3.** Loading and boundary conditions.

Mechanical properties of ABS and PLA plastic materials are given in Table 2. These values were taken from the library of Ansys Workbench. Soil was selected from Ansys Workbench material library for floor [8]. Convergence analysis is shown in Figure 4 [9].

**Table 2.** Mechanical properties of materials used in FEA.

| Parameters                   | ABS [10] | PLA [11] |
|------------------------------|----------|----------|
| Density (kg/m <sup>3</sup> ) | 1020     | 1252     |
| Elastic Moduls (MPa)         | 2230     | 3500     |
| Yield Strength (MPa)         | 33       | 70       |
| Poisson Ratio                | 0,34     | 0,36     |

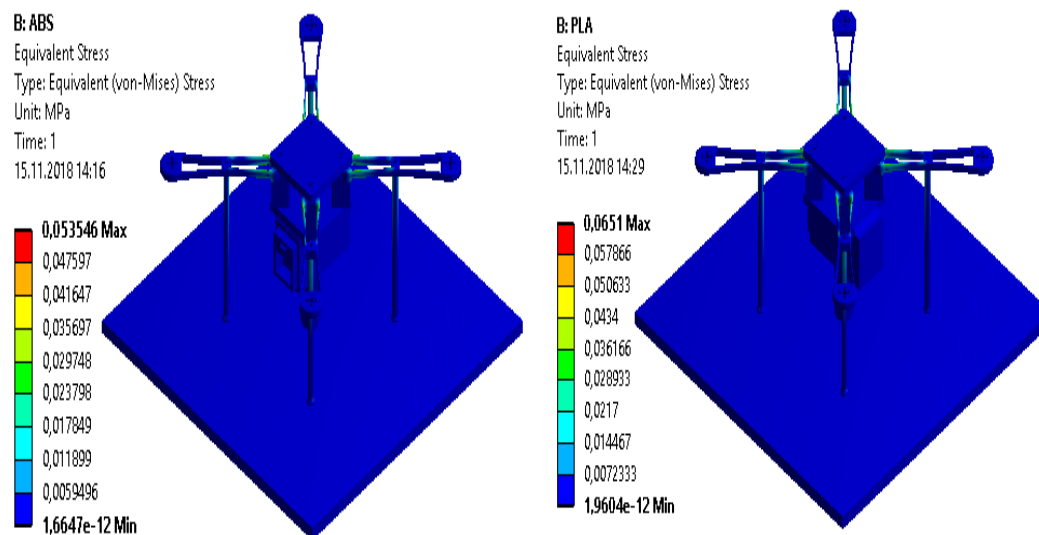


**Figure 4.** Convergence analysis.

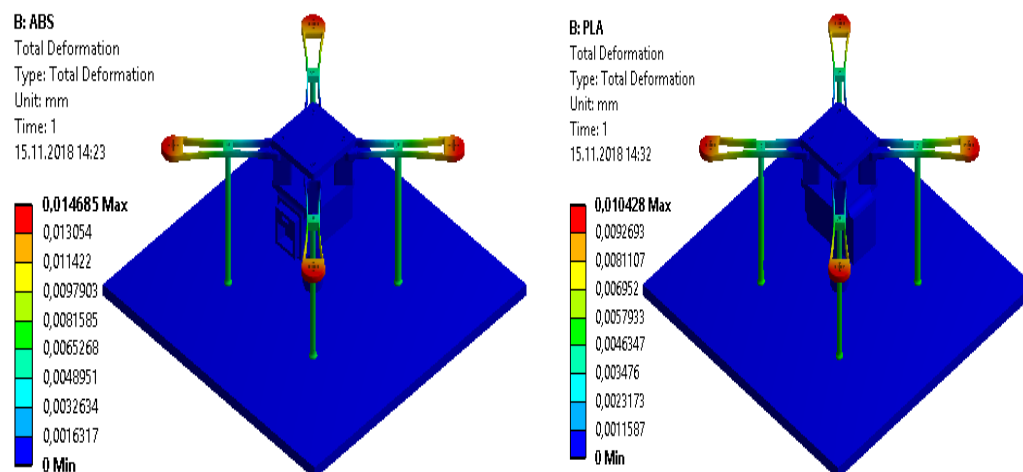
### 3. EXPERIMENTAL RESULTS

Finite element analyzes were performed after the loading and boundary conditions were defined. When quadcopters land on to soil ground, stress that was shown in Figure 5 was consisted with impact of its weight. Deformation that consisted with impact of its weight was shown in Figure 6. In Figure 5 and Figure 6. As a result of the structural analysis, the stress occurred in the quadcopter chassis designed with ABS material was 0,053 MPa, whereas design with PLA material was calculated as 0,065 MPa. In addition, the deformation in the quadcopter frame designed with ABS material was 0,014 mm, while the quadcopter frame designed with PLA material was 0,010 mm. In Table 3, stress and deformation values are given.





**Figure 5.** Tensile values, a) produce of ABS material, b) produce of PLA material.



**Figure 6.** Deformations values a) ABS and b) PLA.

**Table 3.** Deformation and stress values for quadcopter chasis.

| Material | Stress (MPa) | Deformation (mm) |
|----------|--------------|------------------|
| ABS      | 0,053        | 0,014            |
| PLA      | 0,065        | 0,010            |

#### 4. CONCLUSION

PLA material has a high modulus of elasticity and a high yield strength. Therefore, when contacting on the ground with a load of quadcopters own weight, strains occur at the contact points of the arms. These stresses are higher stresses according to ABS material. The modulus of elasticity is inversely proportional to elastic deformation as is seen in equation 11 and equation 12. The modulus of elasticity is the resistance of the material to elastic deformation. Therefore, PLA that have high modulus of elasticity is expected to constitute lower deformation.

$$E = \frac{\sigma}{\varepsilon} \quad (11)$$

$$\sigma = E \cdot \varepsilon \quad (12)$$

The stresses (0,053 MPa ve 0,065 MPa) formed in the quadcopters designed with materials ABS and PLA are very low ((ABS,  $\sigma_{ak} = 33 \text{ MPa}$ ), (PLA,  $\sigma_{ak} = 70 \text{ MPa}$ )). Since the stresses that occur in analysis did not exceed yield strength of material, any damage wasn't comprise in both designs. However, PLA material is economical, it does not create toxic materials during manufacturing, it prints without using table heating during printing and creates lower deformation during printing. Therefore, it has been determined that it is more appropriate to use filament obtained from PLA material.

#### ACKNOWLEDGEMENT

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& Computing And 2013 IEEE 10th International Conference On Autonomic & Trusted Computing, pp. 216-223, Vietri sul Mare.

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RESEARCH ARTICLE

FUTURE PROJECTION OF OLIVE PRODUCTION IN ÇANAKKALE

Mustafa ÖĞÜTCÜ<sup>1,\*</sup>, Akın KIRAC<sup>2</sup>

<sup>1</sup>\*Çanakkale Onsekiz Mart University, Faculty of Engineering, Department of Food Engineering, Çanakkale, [mogutcu@comu.edu.tr](mailto:mogutcu@comu.edu.tr), ORCID: 0000-0001-8686-2768

<sup>2</sup>Çanakkale Onsekiz Mart University, Çanakkale Vocational School of Technical Sciences, Çanakkale, [akinkirac84@gmail.com](mailto:akinkirac84@gmail.com), ORCID:0000-0001-5596-2256

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ABSTRACT

Global warming is one of the most important problems in the world due to its effects not only on human life but also on agricultural products and food safety, sustainability, and water resources. The present study aims to investigate the influence of climatic changes on olive cultivation in North-West Turkey for the next 50 years. In this context, the data were collected from 182 coordinates in olive cultivation areas in Çanakkale which is situated at the intersection of the Euro-Asian region. The data were analysed using MaxEnt software to determine the projection of olive cultivation for the next 50 years. The results show that the optimistic scenario is (representative concentration pathways) RCP 2.6 (2070) while the pessimistic scenario is RCP 8.5 (2070) for Çanakkale olive cultivation. When the results were compared with the current conditions of Çanakkale, the RCP 2.6 scenario indicated that potential olive cultivation areas would mostly be protected. On the other hand, according to the worst scenario, these areas would decrease in size. All of the scenarios, however, show that olive cultivation areas will spread towards the mountainous areas of Çanakkale. Additionally, total olive cultivation areas in Çanakkale will increase depending on climatic changes in 2070. In conclusion, even if climatic changes may lead to an increase olive production yield, their effects on olive and olive oil quality are unknown.

**Keywords:** *Climate change, Scenario analysis, Risk, Adaptation, Yield, Olive cultivation*

1. INTRODUCTION

Global climate change is one of the most important problematic issues of the 21st century. The effects of global climate change increase due to the increasing world population, technological advances, and changes in industrial conditions and human-based conditions [1,2]. According to the fifth report of the Intergovernmental Panel on Climate Change (IPCC), land and ocean temperature data show an increase of 0.9 °C between 1901 and 2012. Atmospheric concentrations of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O gases have increased more than ever in the last 800,000 years [3]. CO<sub>2</sub> concentration increased by 40% compared to the pre-industrial period. The main reason for this situation is the use of fossil fuels and the second reason is the change in land use. According to this report, 4 Representative Concentration Pathways (RCP) have been identified for climate change scenarios. It has been predicted that CO<sub>2</sub> concentration by 2100 would be 1370 ppm for RCP 8.5, 850 ppm for RCP 6.0, 650 ppm for RCP 4.5 and 490 ppm for RCP 2.6. By these scenarios, temperatures (1.5-5.8°C) increase and precipitation is

expected to decrease. Among these scenarios, RCP 2.6 is the scenario where the effects of climate change will be least seen [4]. The IPCC, currently in its 6th phase, published its 1.5 °C Report on Global Warming in October 2018 and emphasized that 1.5 °C warming would be relatively safe compared to 2 °C in terms of potential climatic effects. If the temperature rise cannot be limited to 1.5 °C, in other words, if CO<sub>2</sub> concentration (490 ppm) is exceeded in the RCP 2.6 scenario, the effects of climate change may have more devastating consequences [5]. As a result of this, forests and flora, drinkable water sources, and agriculture are directly or indirectly affected along with sea level, energy sources, human health and biodiversity [6]. Particularly, many researches have indicated that agriculture, livestock, fisheries and food will be most affected by global climatic changes [7]. Besides, agricultural production leads to climatic changes in itself due to greenhouse gases such as CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O. Climatic changes result in a potential change in rainfall, which reduces irrigation water and efficient agricultural areas and leads to desertification or relocation. Therefore, food safety is negatively affected as a consequence of the increasing climatic stress on agricultural production. This situation makes it harder to reach drinkable water, safer food, and healthier nutrition as well as leading to social and economic problems [8,9,10].

Moreover, local foods and patented foods or products will be negatively affected or will perish [11-19]. According to one theory, *Olea chrysophylla* and *Oleaster Olea sylvestris*, which are the ancestors of the olive tree (*Olea europea*), were cultivated in the big area of the Sahra before the Pleistocene epoch. The first cultivation of the olive tree was made thousands of years ago in Egypt; however, it perished because of an unknown reason in 2000 B.C. [20,21]. The olive tree is one of the most ancient cultivars in the world. Olive and olive oil, which are the products of the olive tree, are found in legends, ancient civilizations inscriptions and holy books [20,21]. In the literature, data indicated that olive had been cultivated even 6000 years ago [20]. It is generally agreed that olive originates in South East Anatolia, Mesopotamia and East Asia [20]. The olive tree cultivation is done between 30-45° latitude in the world where winters are soft and rainy, summers are dry, and springs are partly cold and rainy [21]. In literature, were reported that the climatic conditions of the olive tree were 16-21 °C of annual temperature, 500-1200 mm amount of total rain, 5000 h insolation time and enough chilling time [21]. According to the 2018 data, the amount of the world production of olive and olive oil was 21 million tons and 3.1 million tons in 10.5 million ha areas, respectively [22]. According to the 2019 data, there were 182 million trees in Turkey, while the amount of olive and olive oil production was 415,000 tons (table olives), 1.1 million tons (for oil) and 263,000 tons in 864,428 ha areas, respectively [23]. Moreover, there were 5.3 million trees in Çanakkale, the amount of olive and olive oil production in Çanakkale was 11,000 tons (table olives), 85,000 tons (for oil) and 16,995 tons olive oil in 325,731 da areas, respectively [23].

In order to predict how species would be distributed in climate change, it is necessary to determine the climatic conditions of those areas in which species are currently distributed. Afterwards, it is necessary to estimate in which areas the determined climatic conditions will continue or will not be continue. To make these estimations, we entered the analysis process through Maximum Entropy (MaxEnt) method. MaxEnt can predict the climatically suitable habitats of species in the present and future by means of bioclimatic data (bio1 - bio19) and the data of the target species [24-26].

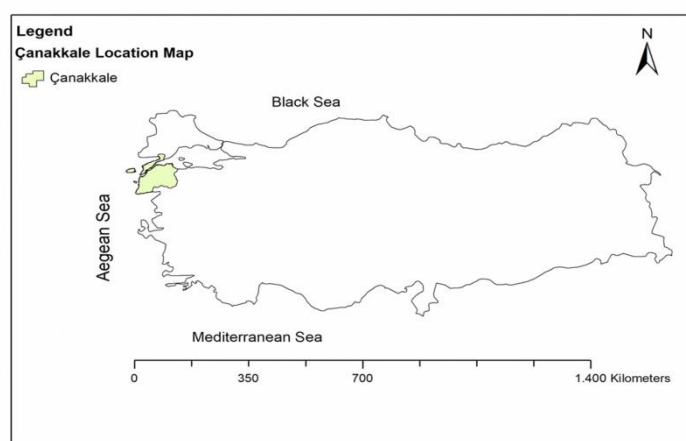
As mentioned above, olive and olive oil are one of the most important products of the human diet not only in Çanakkale province and Turkey but also in the world. This study aimed to investigate the potential effects of global climate change on olive and olive oil production in Çanakkale province.



## 2. EXPERIMENTAL DESIGN AND EVALUATION

### 2.1. Study Area and Species Data

Çanakkale province is located between longitudes 25° 35' and 27° 45' E and between latitudes 39° 30' and 40° 45' N (Fig.1). The average temperature of Çanakkale between 1970 and 2011 was 15 °C and had an increasing trend. The maximum daily temperature measured from 1970 to 2011 was 39 °C (23.07.2007), and the minimum daily temperature was -11.8 °C (14.02.2004). July is the hottest month with an average temperature of 24.6 °C, while January is the coldest month with an average temperature of 6.2 °C. The average rainfall and the difference in precipitation between the driest month and the wettest month were 637 and 104 mm, respectively [27]. One hundred and eighty-two coordinate data were collected from olive groves in Çanakkale where at least 40 trees were found. The data were obtained from the areas where both natural and planted olive trees were located. These olive trees are mostly belong to the domestic Ayvalık cultivar in Turkey Aegean seaside according to Öğütçü et al. (2008).



**Figure 1.** Location map of Çanakkale province, Turkey.

### 2.2. Bioclimatic Data and Habitat Suitability Model

Nineteen bioclimatic data were downloaded from <http://www.worldclim.org> [28]. In Version 1.4, HadGEM2-ES-based 19 bioclimatic data (Table 1) were downloaded on a global scale, with current (1950-2000) and future (2070) climate projections (RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5 scenario). Then, these data were processed at Çanakkale scale by using ArcGIS 10.2 software and made ready for analysis. At the end of all these processes, current and future bioclimatic data with a cell size of 874 x 874 m<sup>2</sup> (30 arc seconds) and a latitude/longitude coordinate WGS84 were obtained in "ascii" format.

MaxEnt software is an ecological niche modelling algorithm for estimating the probability of distributions based on the principle of maximum entropy [29]. The analysis was conducted using MaxEnt software v.3.4.1. MaxEnt use only presence data of species to determine environmental factors that make up the habitat of a species and to estimate its potential distribution. MaxEnt makes

the best estimation and the best results with the least data and the smallest areas compared to other modelling methods [24].

The Olive presence data (csv.), the bioclimatic data (ascii) and the future climate projection data (ascii) were entered under “samples,” “Environmental Layers” and “Projection layers directory/file”, respectively. In the settings section, the training data were set to be 90%, the test data were 10%, and the number of iterations was 10, and the analysis started [29,30]. The MaxEnt model results were evaluated in two ways. The first way was the receiver operating characteristic (ROC) curve and area under the curve (AUC). The other way was the Jackknife test which showed the contribution of each independent variable used in the model [31,32]. If the climatic variable were not contributing to the model, this variable was removed in the Jackknife test result. This analysis procedure would help determine the current climatic conditions which were essential for the survival of olives and allow us to predict where these conditions would end and continue in the future.

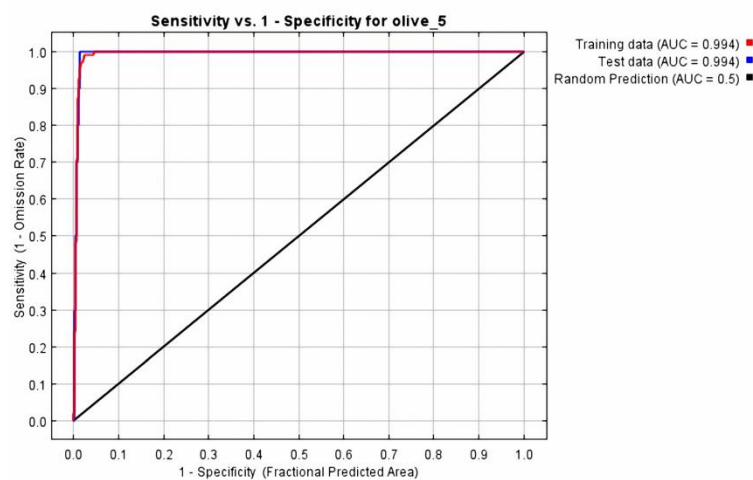
**Table 1.** Bioclimatic variables used for future projection of olive cultivation.

| Code         | Bioclimatic Variables                                      | Unit   |
|--------------|------------------------------------------------------------|--------|
| <b>Bio1*</b> | Annual Mean Temperature                                    | °C     |
| Bio2         | Mean Diurnal Range (Mean of monthly (max temp - min temp)) | °C     |
| Bio3         | Isothermality (Bio2/Bio7)* 100)                            | -      |
| Bio4         | Temperature Seasonality (standard deviation *100)          | C of V |
| Bio5         | Max Temperature of Warmest Month                           | °C     |
| Bio6         | Min Temperature of Coldest Month                           | °C     |
| Bio7         | Temperature Annual Range (Bio5-Bio6)                       | °C     |
| Bio8         | Mean Temperature of Wettest Quarter                        | °C     |
| Bio9         | Mean Temperature of Driest Quarter                         | °C     |
| Bio10        | Mean Temperature of Warmest Quarter                        | °C     |
| <b>Bio11</b> | Mean Temperature of Coldest Quarter                        | °C     |
| <b>Bio12</b> | Annual Precipitation                                       | mm     |
| Bio13        | Precipitation of Wettest Month                             | mm     |
| Bio14        | Precipitation of Driest Month                              | mm     |
| Bio15        | Precipitation Seasonality (Coefficient of Variation)       | C of V |
| Bio16        | Precipitation of Wettest Quarter                           | mm     |
| <b>Bio17</b> | Precipitation of Driest Quarter                            | mm     |
| Bio18        | Precipitation of Warmest Quarter                           | mm     |
| Bio19        | Precipitation of Coldest Quarter                           | mm     |

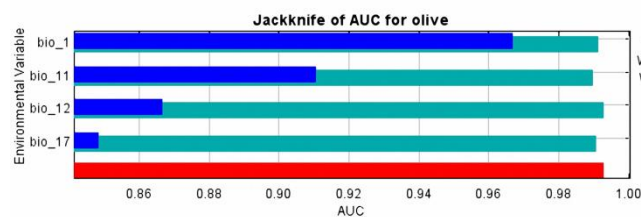
\*The highlighted variables were contributed in modeling.

### 3. RESULT AND DISCUSSION

Figure 2 shows the area under the ROC curve, or simply the AUC values of the training and test data. According to Fig. 2, AUC values of the training data and test data were 0.994 and 0.994, respectively. These results demonstrated that bioclimatic factors were affecting the distribution of olives (*Oleo europea L.*), especially Bio 1 (mean value of temperature per year). The other effective factors on the olive cultivation were Bio 11 (mean temperature of the coldest quarter), Bio 12 (Annual precipitation) and Bio 17 (average three arid months of precipitation) (Fig. 3).



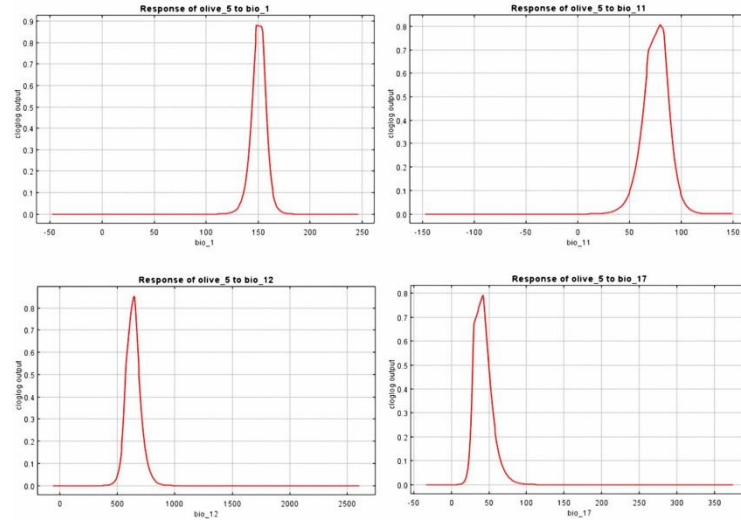
**Figure 2.** Receiver operating characteristics curve or simply area under the curve values of the training and test data.



**Figure 3.** Bio-climatic factors affecting the distribution of olive cultivation.

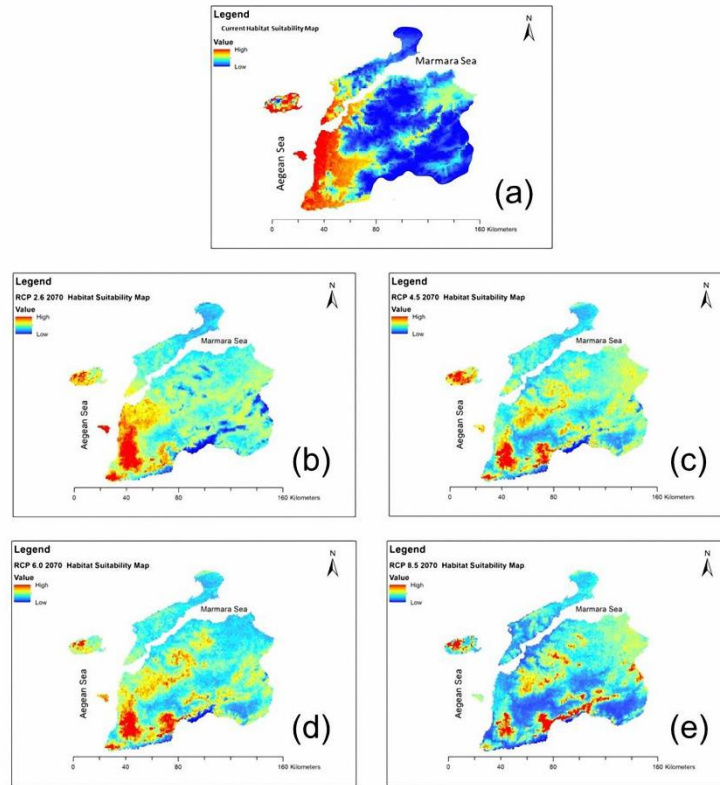
In the present day, olive cultivation demands habitats that have 15 °C as a mean value of temperature per year, 7 °C as an average temperature of the coldest three months, 600-700 mm for annual precipitation and 40 mm for average three arid months of precipitation (Fig. 4). Similar to our findings, Crisci et al. [33] reported that climatic changes were commonly dependent on an increase in minimum temperatures rather than on changes in maximum temperatures.





**Figure 4.** Factors affecting olive cultivation habitats in the present day.

According to bioclimatic changes, the olive cultivation suitable map of Çanakkale are given in Figure 5. Çanakkale is among the important olive producer provinces of Turkey in terms of olive production potential. Considering the conditions mentioned above, olive cultivation were mainly observed in the Aegean Sea side of Çanakkale as well as Bozcaada and Gökçeada islands (Fig. 5a). In the 2011 data reported, the olive production values of Çanakkale were 128.896.200 TL. Olive was substantially cultivated in the districts of Ezine, and Ayvacık in Çanakkale [34]. These results proved that olive cultivation was important in terms of agricultural economic input in Çanakkale. Orlandi et al. [35] and Galán et al. [36] demonstrated that there was a close relationship between climate and reproductive phenology in olive. In the same study, researchers indicated that olive flowering could be considered a good indicator of climate change [35,36].



**Figure 5.** The suitable olive cultivation map of Çanakkale, (a) current distributions, (b) RCP 2.6 (2070) scenario, (c) RCP 4.5 (2070) scenario (d) RCP 6.0 (2070) scenario (e) RCP 8.5 (2070) scenario.

The RCP 2.6 2070 scenario is shown in Figure 5b. According to this scenario, olive cultivation will mainly protect its cultivation areas, alongside it will be spread on the inland of Çanakkale which areas are currently mountainy. In this aspect, the scenario RCP 2.6 2070 is the better scenario among the other climatic changes scenarios for olive cultivation of Çanakkale. When today's conditions and RCP 2.6 results compared, it is seen that olive cultivation will reduce in Gökçeada and especially Eceabat districts. Indeed, the RCP 2.6 scenario is closely similar to the ICCP 1.5 °C report. RCP 4.5 2070 scenario shows that olive cultivation will not be suitable for the coastal areas near the seaside of Çanakkale (Fig. 5c). Additionally, olive cultivation will decrease in Bozcaada district, while it will be distributed to north-east Çanakkale. Similar results were observed RCP 6.0 2070 scenario (Fig 5d). The worst scenario is RCP 8.5 2070 in terms of Çanakkale olive cultivation. In this scenario, it is expected that the olive production will decrease due to the estimated reduction in the areas that are currently suitable for olive cultivation (Fig. 5e). According to these results, if especially the RCP 8.5 scenario happens, the olive farmers and olive oil producers of Çanakkale will be economically be damaged. It has been observed that new potential areas have emerged despite the reduction of existing olive growing areas for all scenarios. On the other hand, the most convenient olive cultivation areas compared to current conditions will decrease according to the 2070 RCP scenarios. Even if these results may lead to an increase in olive production yield, the effects on olive and olive oil costs, yield

and quality are unknown. Tanasijevic et al. [37] reported that olive cultivation areas extending to the North and to higher altitudes would increase by 25% in 50 years. Gutierrez et al. [38] reported that olive cultivation would spread to unfavourable cold areas in higher elevations in the Apennine mountains in central Italy and in the Po Valley in the north. Moreover, climate change is expected to contract the range of olive cultivation in southern desert areas and to spread it northward and alongside coastal areas in CA-AZ [38]. The fact that climate warming would affect olive yield and result in economic winners and losers at the local and regional scales in the Mediterranean were reported by Ponti et al. [39]. In the same study, researchers predicted that profitability of small olive farms in many marginal areas of Europe would decrease at the local scale [39]. Literature findings are close similar to our results.

#### 4. CONCLUSION

Even the pessimistic scenarios estimated that the olive cultivation of Çanakkale would continue and that it may not, however, be economically sustainable due to its spreading over rugged and high areas. Supply and demand balance of olive and olive oil cultivation will change in the future and olive and olive oil prices will, therefore, be higher than those of the current economic status. Indeed, olive and olive oil will be the most precious food products in the future scenarios. It is demonstrated that climatic changes affect agricultural products that are the raw material of food products. It is well known that the world population increases while the food and water resources of the world decrease day by day. In this aspect, the protection of agricultural products and food safety will be very important in the future.

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**RESEARCH ARTICLE**

**IMPROVEMENT AN COST ANALYSIS OF BLASTING OPERATIONS AT WESTERN LIGNITE OPEN CAST MINE**

Şahin YUVKA<sup>1</sup>, Emre DURAN<sup>2\*</sup>, Önder UYSAL<sup>3</sup>

<sup>1</sup>Kütahya Dumlupınar University, Department of Mining Engineering, Kutahya, [sahin.yuvka@dpu.edu.tr](mailto:sahin.yuvka@dpu.edu.tr),  
ORCID: 0000-0002-3219-2321

<sup>2</sup>Turkish Coal Enterprises, Garp Lignite Corporation, Kutahya, [durane@gli.gov.tr](mailto:durane@gli.gov.tr),  
ORCID: 0000-0001-7567-2790

<sup>3</sup>Kütahya Dumlupınar University, Department of Mining Engineering, Kutahya, [onder.uyisal@dpu.edu.tr](mailto:onder.uyisal@dpu.edu.tr),  
ORCID: 0000-0002-3640-3341

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**ABSTRACT**

Drilling and blasting operations have a great importance in mining operations. By means of drilling and blasting operations, ease of excavability is provided and production can be made in desired particle sizes especially for ore. In this study, state-operated blasting operations in Western Lignite Corporation mining sites and problems encountered in these blasting operations are investigated. It was aimed to solve the problem of encountering blocks after blasting by using nitroglycerine based cast boosters instead of emulsion based cast boosters. The blasting shots and excavation loading works in the blasting area were recorded. The muckpile images obtained after blasting operations were examined with WipFrag software and particle size distributions were checked. According to the data obtained, cost analyzes related to blasting and transportation were performed. It was found that after the change, the problems decreased and the company economically gained 25% annually in terms of blasting costs.

**Keywords:** *Blasting Costs, Particle Size Analysis, Problems With Blasting*

**1. INTRODUCTION**

Minerals and elements extracted from different depths of the earth's crust are called as ore. Mining works involve the exploration, project design, operation of the ore deposits and enrichment of the mined ore. [1]

Mining activities have a large share in the country's economies. Mining activities are basically carried out by two methods. These methods are Open Pit Mining and Underground Mining. Both production methods include development, drilling and blasting, excavation, loading and haulage. Depending on the properties of the mine and the method of production, the equipment to be used and the way it works varies.

Considering the expenditures made during mining activities, drilling and blasting costs are of great importance as they will affect consecutive operations such as loading, transportation and breaking. [2] In order to reduce these costs, the most suitable drilling and blasting design is required.

Drilling and blasting design varies according to the geological structure, the properties of the mine, the characteristics of the equipment used, the desired particle size and environmental effects. In addition to all these factors, the hole parameters (such as hole diameter and angle, zone thickness, hole length, hole pattern, toe share and stemming length, distance between holes, shape of bench face, dispersion and quantity of the explosive in the hole, cast booster, detonation type and order, delay type and duration) are also of great importance during the blasting design. Therefore, drilling and blasting processes should be evaluated as a whole. [3]

Many problems can be encountered during drilling and blasting operations. Mistakes made during drilling, such as the hole length not the same, hole diameters are not equal and angle of holes are wrong, may affect the yield to be obtained from the blasting process. [4] Problems such as obtaining blocks larger than the desired particle size, need for secondary blasting, toe formation at the slope bottom and working face that will negatively affect the excavation performance of machinery, rock bursts during blasting, occurrence of vibration and noise during blasting are problems that may be encountered in blasting operations. Regarding the solution of these problems, many factors such as blasthole patterns, technical properties of explosives, charging of explosives in the blasthole should be reviewed.

The most suitable blasting conditions are usually determined by comparing the different shot results with each other. The main indicators of a technically and economically successful shot are as follows; the muckpile obtained should be homogeneous and close to the desired particle size, there should be no flyrock, no toe formation at the bottom and no gases formed as a result of incomplete combustion, should not require a secondary shot. [5] In the blastings made at the mine before 2017, a number of problems have arisen that cause low productivity and create additional costs. The most important ones among these problems are the toe formation at the faces and bench floors, and the less frequently oversize boulder formation after blasting.

Secondary blasting or ripping is needed when the toe formation is encountered. The excavator stops working because there are no suitable conditions for excavation until ripping or secondary blasting is done. Accordingly, excavator operating efficiency decreases. In order to eliminate the toe, ripping is performed on the ground primarily with a dozer. If the toe problem still continues after the ripping operation, secondary blasting process takes place. However, secondary blasting creates an extra cost.

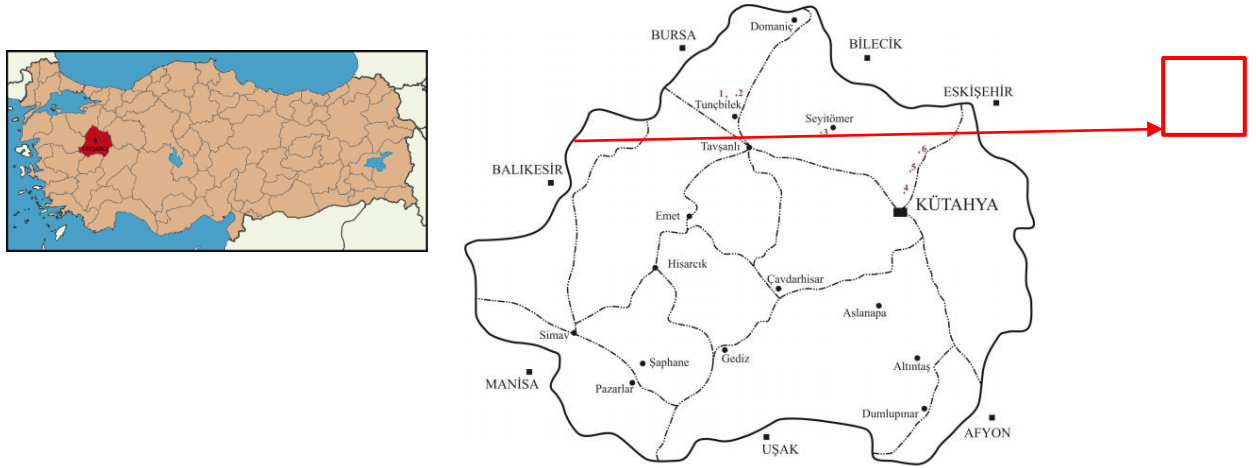
Another problem, oversize boulder formation, is a much less common problem in the mine. The oversize boulders, which are larger than the bucket volume of the excavators, are reduced by a pop-shooting process and divided into smaller particles of suitable size.

In this study, the particle-size distributions were checked by analyzing the muckpile images, which were obtained after state-operated blasting operations of Western Lignite Facility Directorate, by using WipFrag software. By means of the WipFrag software, image processing was performed in computer environment and in the light of the data obtained, cost analysis related to blasting and transportation processes were made. [6]

## 2. FIELD WORKS

### 2.1. Introduction of the Site

This study was carried out in the concession area of the Western Lignite Facility Directorate, which is located on the Tavşanlı Domaniç highway. Site location map of the study area is given in Figure 1.



**Figure 1.** Site location map of the study area.

The study areas consist of marl formation. Clay, sandstone and serpentinite distributions can be seen in the marl formation. During the studies, marl was taken as the main rock.

### 2.2. Current Blasting Applications at the Worksite

In the blasting operations carried out within the scope of this study, pattern change experiments were made using 2 different types of cast boosters. In the blastings, booster sensitive ANFO, emulsion-type dynamite and nitroglycerin-based dynamite as cast booster and nonel delays for hole and surface were used.

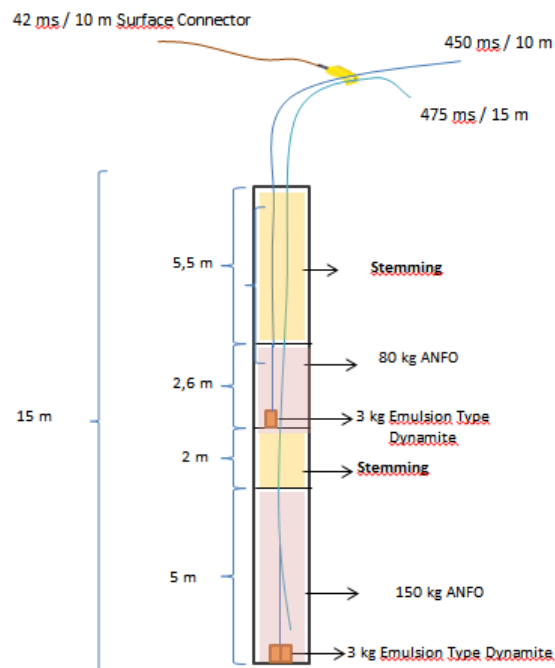
First of all, existing hole drilling system in the field and problems were identified. After determining these problems, studies have been done for the solution. Since it is not possible to change the formulation of cast booster, the booster sensitive explosive type was changed. In the existing 8m x 8m x 15m hole pattern, test shots were made by using emulsion type dynamite and nitroglycerin based dynamites. As a result of the trial blastings, it was determined that the problems disappeared. However, since the cost of nitroglycerin-based dynamite is higher than emulsion type dynamite, it has been observed that the amount of muckpile obtained per unit m<sup>3</sup> should be increased in order to reduce the costs. For this purpose, the current pattern was designed to be 9m x 9m x 15 m by changing the pattern. In this pattern, test shots were made using nitroglycerin based dynamite and emulsion type dynamite. After each shot, images were taken from the muckpile and grain size analysis was done with WipFrag software. [6] In the light of the data obtained as a result of analyzes, it was examined whether the muckpile had a suitable particle size for loading and transportation with excavators or created additional costs during transportation. As a result of all operations, total cost difference was determined by making a cost analysis.



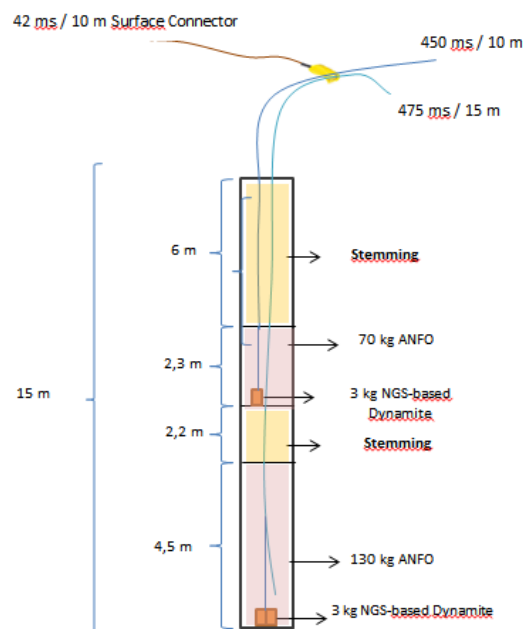
### 3. IMPROVEMENT WORK IN THE FIELD

Different problems were observed in the pattern used in the blastings operations before 2017 in the mine. Especially the toe formation problem at the bench floor increased the ripping times and the number of secondary shots. The increase of these shots also caused an increase in terms of costs. The machinery stops operating until the secondary shots are made, and indirectly the working efficiency decreases.

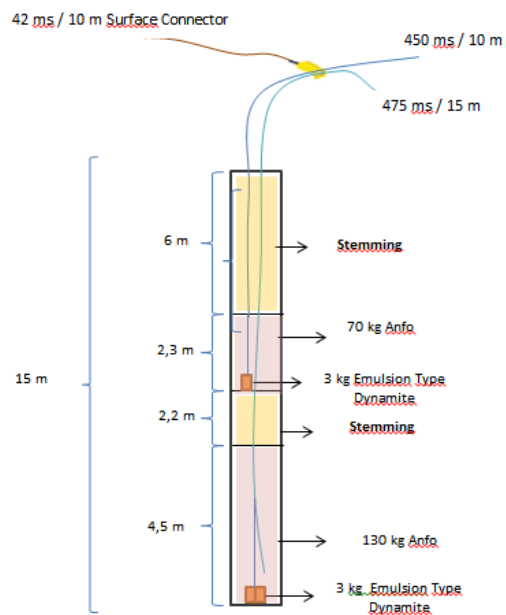
8m x 8m x 15m, 7m x 9m x 15m and 7m x 7m x 15m hole patterns have been tested in the facility before. Drilling and blasting costs have increased as the number of holes to be drilled increases as the distance between the holes and rows increases. To reduce explosive costs, the number of holes and the amount of per unit explosive used should be reduced. In order to reduce the amount of explosives used for the unit work, the distance between the holes and the rows must be increased. A number of cost analyzes were performed on the 9m x 9m x 15m, 9m x 8m x 15m and 10m x 9m x 15m, 10m x 10m x 15m hole patterns. As a result of trial shots, field observations and cost analysis, when the distance between the rows and the holes exceeds 9 meters, problems occur about particle size, the excavators have difficulties in loading and the need arise for pop-shooting process and therefore, it is thought that the most appropriate pattern will be 9m x 9m x 15 meters. Actually, this study has been commenced to determine the most suitable particle size-cost point, and within the scope of this study, holes have been drilled to be 9 meters between holes and 9 meters between rows. Emulsion type dynamite was first used in the drilled holes and another solution was found due to the problems about particle size and hard ridge protrudes at the floor. Emulsion type dynamite and NGS based explosive material were used in the drilled holes. In the trial shots, the specific charge for the 8m x 8m x 15m pattern was calculated as  $0.208 \text{ kg/m}^3$  and the specific drilling was  $0.0156 \text{ m/m}^3$ , the specific charge in the 9m x 9m x 15m hole pattern was  $0.165 \text{ kg/m}^3$  and the specific drilling was  $0.0123 \text{ m/m}^3$ . The data obtained were compared with the 8m x 8m hole pattern used previously and examined in terms of particle size and cost. Column charge designs of different patterns are given in Figure 2, Figure 3, Figure 4. and Figure 5.



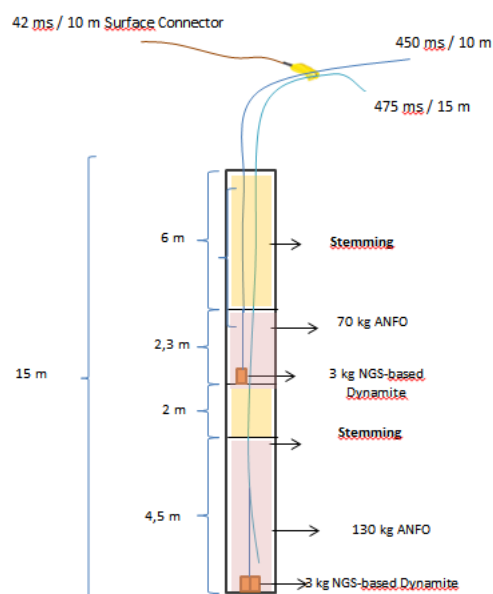
**Figure 2.** 8m x 8m x 15 m hole pattern, column charge for the use of emulsion dynamite.



**Figure 3.** 8m x 8m x 15 m hole pattern, column charge for the use of NGS-based dynamite.



**Figure 4.** 9m x 9m x 15 m hole pattern, column charge for the use of emulsion dynamite.



**Figure 5.** 9m x 9m x 15 m hole pattern, column charge for the use of NGS-based dynamite.



### 3.1. Particle Size Analysis

Determination of the particle size in the mining sector is generally used during the process of obtaining the final product. It is not possible to use sieve analysis methods for sizing during quarry production. Instead, image processing software is used.

WipFrag software was used in the image analysis made in this study, and there are many studies in the literature where particle size analyzes were performed with WipFrag software. A scaled object is placed in the muckpile where grain size analysis will be performed. Then the pictures of muckpile are taken and uploaded to the system. The system analyzes the image using different algorithms, and creates a net as an overlay on the original image, which encircles each grain. Depending on the quality of the picture, errors may occur in the process of identifying individual rock fragments. In this case, the user can check the boundaries of the pictures and reach the final result after making the necessary arrangements. [6,8]

One of the most important points to be checked in pattern changes is whether the particle size distribution resulting from blasting is suitable for the need. In terms of blasting economy, it is best to make blasting to obtain muckpile in the grain size ranges suitable for the need. In this study, test shots were made in different patterns and the images of the muckpile obtained were analyzed using WipFrag software. During the application, a ball with 225 mm diameter was used as the scale. Muckpile photos were taken after each shot and during machine operation and the size analyzes were performed with the program (Figure 7 and Figure 8). The data of the shot using NGS Type Dynamite and 8m x 8m x 15m pattern is not included in this article due to the high cost, and the data of the shot using the Emulsion Type Dynamite and 9m x 9m x 15m pattern is not included too due to continuation of problems in blasting operations.

As a result of the analysis, the data obtained from the shot using the 8m x 8m x 15m pattern and emulsion type dynamite is given in Table 1, and the data of the shot made by the 9 m x 9 m x 15 m pattern and NGS type dynamite is given in Table 2.

When the results are examined, it is seen that the grain size distribution between the 8m x 8m x 15 m pattern and the 9m x 9m x 15 m pattern shows some differences (Figure 6). However, these differences do not hinder the basic loading and carrying operations. The data obtained from the blastings is of great importance for 3 situations which are loading, transportation and filling of dumping areas. After the pattern change, if a muckpile is formed with oversized boulders and nonhomogenous grain size distribution, the excavators will have difficulty in loading this muckpile and the bucket will not be able to take material so that it is fully loaded. This will inevitably be resulted in the fact that the transport trucks will not homogenously loaded due to very large-sized materials and also increase the transportation costs. One of the basic needs of open pit mining is large dumping areas. Especially, in regions where the lands are valuable and feasible to production later on, the cost of creating new dumping sites for mining increases. Therefore, dumping sites should be created in the most efficient way. If the grain size distributions are not very homogeneous, the fillings to the dumping areas cannot be spread/piled up evenly. In other words, although the site is filled, gaps will remain between them. This will cause inefficient storage in dumping site over time.

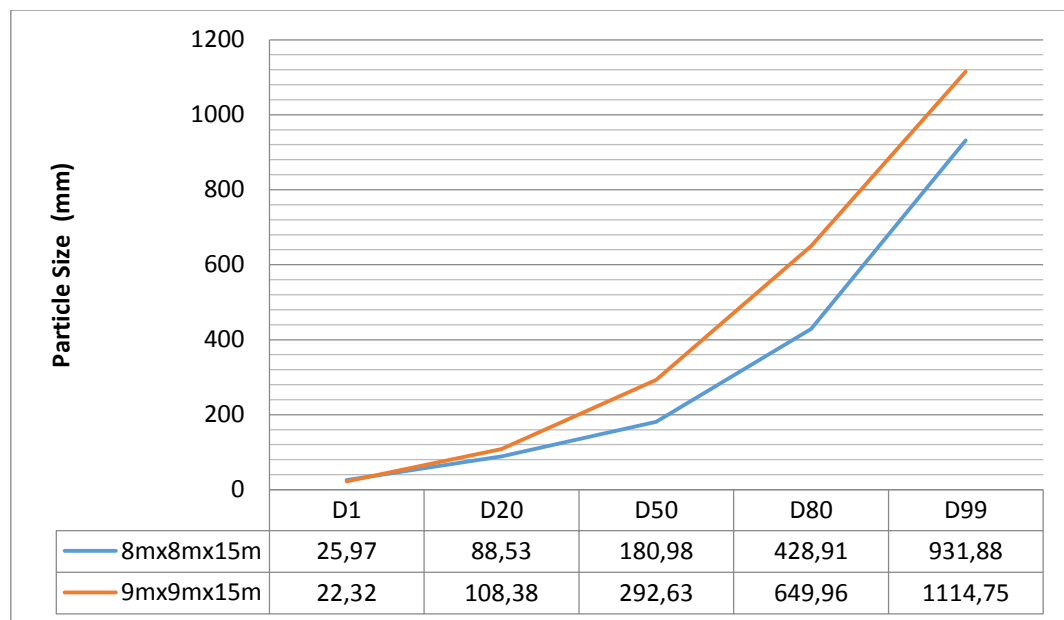
In addition, when the capacities and internal dimensions of the buckets of the PH 1900 (10 yd<sup>3</sup>) and PH 2300 (20 yd<sup>3</sup>) model excavators used for loading in the facility are examined, it can be seen that even the biggest piece (D99) obtained can be easily loaded.

**Table 1.** Particle size analysis results of 8m x 8m x15m emulsion type dynamite hole pattern.

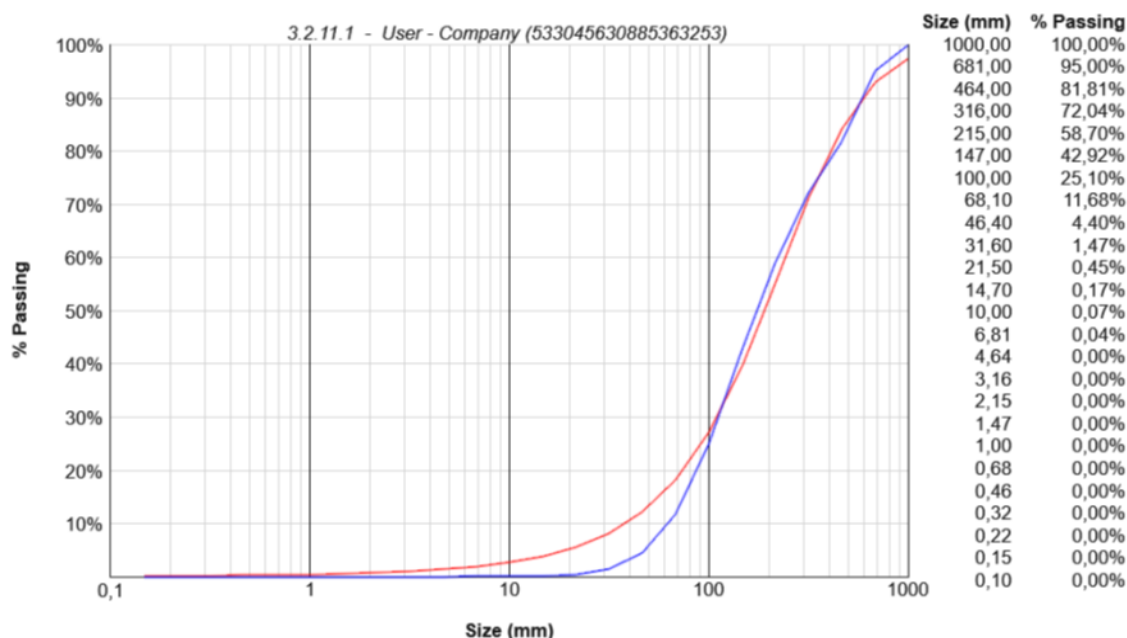
| SHOT NO        | D1 (mm)      | D20 (mm)     | D50 (mm)      | D80 (mm)      | D99 (mm)      |
|----------------|--------------|--------------|---------------|---------------|---------------|
| 1              | 27,65        | 101,84       | 237,93        | 566,41        | 973,27        |
| 2              | 26,62        | 91,09        | 179,45        | 394,99        | 967,3         |
| 3              | 24,27        | 86,01        | 170,98        | 398,49        | 904,54        |
| 4              | 26,97        | 87,89        | 177,49        | 436,60        | 936,26        |
| 5              | 24,34        | 75,86        | 139,07        | 348,08        | 878,05        |
| <b>Average</b> | <b>25,97</b> | <b>88,53</b> | <b>180,98</b> | <b>428,91</b> | <b>931,88</b> |

**Table 2.** Particle size analysis results of 9m x 9m x15m NGS type dynamite hole pattern.

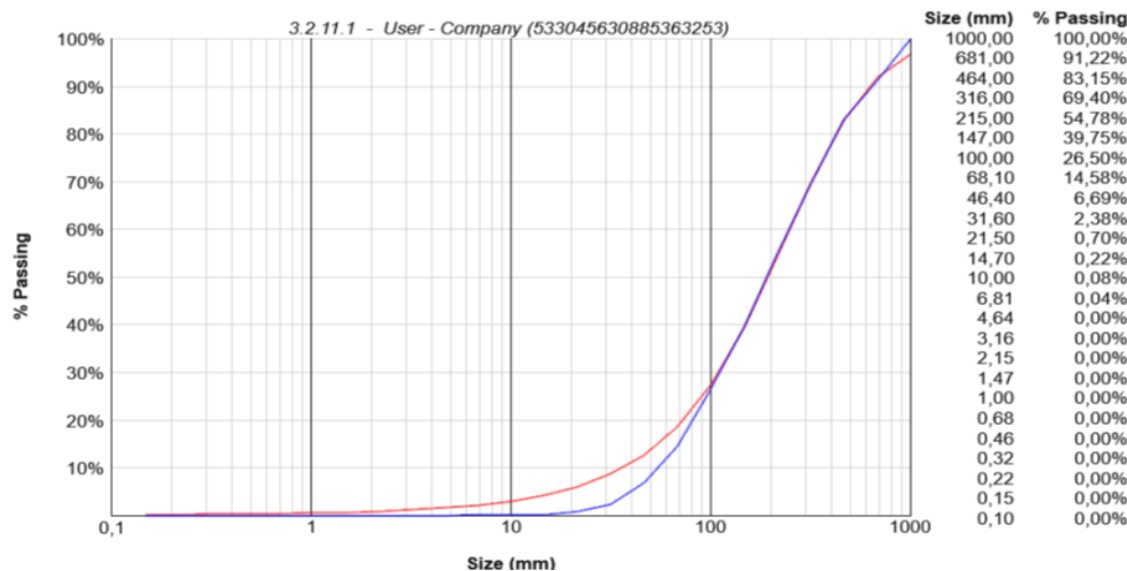
| SHOT NO        | D1 (mm)      | D20 (mm)      | D50 (mm)      | D80 (mm)      | D99 (mm)       |
|----------------|--------------|---------------|---------------|---------------|----------------|
| 1              | 19,07        | 83,33         | 197,88        | 425,71        | 970,35         |
| 2              | 23,31        | 82,6          | 193,38        | 430           | 963,65         |
| 3              | 22,83        | 102,79        | 291,34        | 754,75        | 1088,4         |
| 4              | 22,83        | 96,33         | 346,82        | 776,98        | 1353,74        |
| 5              | 23,58        | 176,89        | 432,39        | 862,35        | 1197,65        |
| <b>Average</b> | <b>22,32</b> | <b>108,38</b> | <b>292,63</b> | <b>649,96</b> | <b>1114,75</b> |



**Figure 6.** Comparison of two different parameters.



**Figure 7.** WipFrag analysis sample of 8m x 8m x 15m NGS type dynamite hole pattern.



**Figure 8.** WipFrag analysis sample of 9m x 9m x 15m NGS type dynamite hole pattern.

### 3.2. Dozer Ripping Costs

Dozers are one of the most important auxiliary equipments used in the facilities. Dozers are used for many different purposes. One of these is the ripping process of the toe formed at the bottom and the hard ridge protrudes as a result of the inefficient blasting. Although fuel consumption varies in different sizes and capacities, they generally have an important share in the fuel expenses of the facilities. [7,9] The fuel consumption of the dozers during ripping and other road construction processes etc. has also been measured and in the measurements made, it has been determined that a dozer consumes an average of 38.3 liters/hour in material displacement. Measurements were also made regarding fuel consumption during the ripping process of the same equipment (Table 3). According to the results, it was determined that the dozers consumed 51,7% more fuel during the ripping process compared to the material displacement works (Table 4).

In addition, the dozer provides the work of loosening with the ripper tooth attached to the rock. The ripper tooth is connected to the dozer with the help of a pin. These pins can break due to excessive strain during dozer operation. Unless it is noticed that the pin is broken, the ripper tooth mixes into the loosened soil and becomes difficult to find. If the situation is noticed and the tooth is found, it can then be replaced. Pin breakage and ripper tooth damage are the common problems. While making a cost accounting related to ripping operation, it constitutes an important cost item although they are generally ignored. With the use of NGS based dynamite, the toe problem at the bottom and the hard ridge protrude problem have also decreased. Accordingly, the ripping process has decreased compared to the areas where emulsion type dynamite is used.

**Table 3.** The amount of fuel consumed during ripping [1].

| Survey No                       | Working Hours (hour) | Fuel Consumed (lt) | Average Fuel Consumption (lt/hour) |
|---------------------------------|----------------------|--------------------|------------------------------------|
| 1                               | 5                    | 287                | 57,40                              |
| 2                               | 4,5                  | 249                | 55,33                              |
| 3                               | 3                    | 185                | 61,67                              |
| 4                               | 2,5                  | 146                | 58,40                              |
| 5                               | 3,5                  | 202                | 57,71                              |
| <b>Average Fuel Consumption</b> |                      |                    | <b>58,10</b>                       |

**Table 4.** Increase in fuel consumption during ripping [1].

| Survey No | Average Fuel Consumption During Ripping (lt/hour) | Average Fuel Consumption That should be Under Normal Working Conditions (lt/hour) | Increase in Fuel Consumption During Ripping (%) |
|-----------|---------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------|
| 1         | 57,40                                             | 38,3                                                                              | 49,87                                           |
| 2         | 55,33                                             | 38,3                                                                              | 44,46                                           |
| 3         | 61,67                                             | 38,3                                                                              | 61,02                                           |
| 4         | 58,40                                             | 38,3                                                                              | 52,48                                           |



|                                   |       |      |              |
|-----------------------------------|-------|------|--------------|
| 5                                 | 57,71 | 38,3 | 50,67        |
| <b>Amount of Average Increase</b> |       |      | <b>51,70</b> |

### 3.3. Repeating Shots in Areas Where Blasting Efficiency is not Achieved

Records of the repeating shots in the last 5 years are given in Table 5. The decrease in the number of repeating shots is clearly seen in the table.

**Table 5.** Repeating shot table [1].

| Years | Number of Shots | Number of Holes | Amount of Explosive Used (Kg) | Amount of Cast Booster Used (Kg) |
|-------|-----------------|-----------------|-------------------------------|----------------------------------|
| 2014  | 10              | 277             | 16100                         | 392                              |
| 2015  | 9               | 276             | 29900                         | 525                              |
| 2016  | 9               | 493             | 8050                          | 220                              |
| 2017  | 5               | 103             | 4950                          | 257                              |
| 2018  | 2               | 49              | 1490                          | 110                              |

### 3.4. Explosive Costs

200 kg of Anfo and 6 kg of cast booster were used per hole in the trial shots. In order to make an average of 10,000,000 m<sup>3</sup> of stripping annually; 10,416 holes should be drilled in the 8m x 8m x 15m hole pattern and 8230 holes should be drilled in the 9m x 9m x 15m hole pattern. Due to the decrease in the number of holes, 437,200 kg less Anfo is consumed in the 9m x 9m x 15m pattern. The costs incurred as a result of the trial shots are given in Table 6.

**Table 6.** Blasting unit cost table [1].

|                                                                 | 8m X 8m X 15m<br>Emulsion Type<br>Dynamite | 8m X 8m X 15m<br>NGS Type<br>Dynamite | 9m X 9m X 15m<br>Emulsion Type<br>Dynamite | 9m X 9m X 15m<br>NGS Type<br>Dynamite |
|-----------------------------------------------------------------|--------------------------------------------|---------------------------------------|--------------------------------------------|---------------------------------------|
| <b>Explosive Cost Per Unit m<sup>3</sup> (TL/m<sup>3</sup>)</b> | 0.61                                       | 0.57                                  | 0.42                                       | 0.45                                  |
| <b>Drilling Cost Per Unit m<sup>3</sup> (TL/m<sup>3</sup>)</b>  | 0.094751563                                | 0.094751563                           | 0.074865432                                | 0.074865432                           |
| <b>Labor Cost Per Unit m<sup>3</sup> (TL/m<sup>3</sup>)</b>     | 0.10216                                    | 0.10216                               | 0.08071                                    | 0.08071                               |
| <b>Unit Blasting Cost (TL/m<sup>3</sup>)</b>                    | 0.8044                                     | 0.7701                                | 0.5763                                     | 0.6085                                |

## 4. RESULTS

In this study, drilling and blasting operations in the areas concerned operated by the Western Lignite Facility Mine Directorate were examined. Within the scope of these investigations on drilling and blasting works, it was observed that the problem of toe formation was the leading problem in the facility. Firstly, the type of cast booster was changed. It was determined that the problem of toe

formation decreased after using the cast booster. Within the scope of cost study, changes were made in the hole pattern in order to reduce the costs. In this scope, the hole pattern, which is 8m x 8m, has been rearranged to be 9m x 9m. The muckpile images obtained after the trial shots were analyzed with the WipFrag software.

According to the results obtained;

- During the interviews with the operators and the examinations made in the working follow-up forms; it was observed that the ripping work with dozer was reduced in the blastings made by using 9m x 9m x 15m hole pattern and nitroglycerin based dynamite when compared to the blastings made by using 8m x 8m x 15 m hole pattern and emulsion type dynamite. In addition, there have been decreases in the number of repeating shots and pop-shootings. During ripping, a dozer consumed an average of 58,1/hour liters of fuel. This ~~data~~ means, on average, 51,7% more fuel consumption than that of the fuel consumed in normal operation. Considering the amount of stripping done and the hours spent for ripping, the reduction of ripping work provides a good profit to the business.
- When the costs of explosives are analyzed, a decrease of 0,196 tl / m<sup>3</sup> is observed in costs for per m<sup>3</sup>. Considering that the annual work is 10 million m<sup>3</sup>, a 25% financial gain is obtained [1].

The income/savings to be obtained from the blastings made for an average of 10 million m<sup>3</sup> of annual stripping is approximately 1,9 million TL. [1] Considering the additional costs due to the differences in grain size distribution, it has been determined that there is an increase in transportation costs of approximately 317686 TL. In addition, reductions in ripping operation and repeating shots caused a reduction in costs. According to these results, it was determined that the 9m x 9m x 15m hole pattern provided an important gain of approximately 1,5 million TL annually to the company. Therefore, It is recommended to continue working with this pattern in order to reduce operating costs.

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**RESEARCH ARTICLE**

**EVALUATION OF CAPPADOCIA PERLITES AS A BUILDING MATERIAL WITH  
NATURAL STONE RESIDUES**

Abdul Vahap KORKMAZ<sup>1,\*</sup>

<sup>1</sup> Afyon Kocatepe University, Dinar School of Applied Science, Afyonkarahisar, [avkorkmaz@aku.edu.tr](mailto:avkorkmaz@aku.edu.tr),  
ORCID:0000-0001-8691-1937

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**ABSTRACT**

The superior properties of perlite (thermal insulation, sound insulation, fire resistance, lightness) have led to the investigation of the possibilities of perlite usage in many areas. Although perlite has pozzolanic properties and does not have reserve problems in our country, it has not been a preferred material by cement manufacturers. The use of marble travertine residues in cement production is not very common in our country, although some cement manufacturers support it. In this study, the availability of cement samples produced by the use of natural perlite and marble travertine residues belonging to Nevşehir Acıgöl region in pozzolanic and doped Cements was investigated. In this study, the availability of cement samples produced by the use of natural perlite and marble travertine residues belonging to Nevşehir Acıgöl region in pozzolanic and doped Cements was investigated. Portland cement 5%, 10%, 15% and 20% marble travertine residues and perlite were replaced by pozzolanic and doped Cements and their properties were tested and their suitability investigated. In the experimental studies, 4 types of perlite-doped pozzolanic cement, 4 types of marble travertine-doped cement and composite Cements were produced. In the study, density, cement fineness, setting Times, water content, volume expansion, compressive strength tests were performed on the produced cements under laboratory conditions. The physical and chemical properties of the blended cements produced were compared with Portland cement. When the results of the experiment were examined, the increase in contribution rates decreased the pressure resistances of the concretes. The setting times of cement mortars increased and also contributed positively to the workability of concrete. There was no adverse effect on volume expansion. The pozzolanic and doped cements produced with perlite and marble travertine residues are within the standards according to TS en 197-1 and in cement production of both materials separately to 20 % and together up to 40% pozzolanic cement production up to 35% Portland Composite cement production can be used.

**Keywords:** *Marble and travertine residues, Perlite, Pozzolanic Cement, Physical Properties, Mechanical Properties*

**1. INTRODUCTION**

The United States is one of the world's largest producers and consumers of crude perlite and extended perlite, followed by China, Greece, Italy, the Philippines, Mexico and Turkey, respectively. Although 21% of the world's reserves are found in Turkey, 97% of perlite production is done by other countries.



Although most of the countries that have active role in the world cement sector have perlite reserves, there are very few studies on this subject [1]. Many of the academic studies have focused on the use and development of perlite as aggregates in the production of concrete rather than evaluating it as an additive material in the production of cement. It is known that pozzolanic materials are used as additive materials in cement production [2,3]. However, natural perlites also have pozzolanic properties and unfortunately the use of perlite in cement production is not very common. For this reason, researchers have great duties for the use of natural perlite in cement production [4].

Research shows that the use of mineral additives is widespread in cement production in order to reduce cement production costs. The use of Mineral additives has positive effects on concrete strength in parallel to the reduction of production costs and contributes to an environmental production by reducing CO<sub>2</sub> emissions [5,6]. Pozzolanic cements are of strategic importance in the concrete industry as they are cheaper than Portland cements and can be used for up to 50% of cement weight in the concrete mix. Moreover, the increasing workability of concrete, reducing the speed and amount of hydration temperature, increasing the final pressure resistance, providing affordability and finally reducing the alkali-aggregate reaction has greatly increased the preferability of pozzolanic cements in recent years [6]. In order to realize all these developments, pozzolanic materials such as volcanic ash, volcanic tuff, diatomite soil and baked clay, fly ash, granulated blast furnace slag, silica smoke, and rice husk ash are used. It is possible to achieve all positive developments shown and proven in both cement and concrete with natural perlite. The reason for this can be explained by the natural perlite has a pozzolanic property and sufficient reactive silica. In addition, fly ash, granulated blast furnace slag, silica fume and rice husk ash are artificial pozzolans while perlite has natural pozzolanic properties. Its features may vary depending on the region and the region it is located. The results from a natural pozzolan taken from a particular region are very difficult to predict. Therefore, it would be useful to determine the behaviour of natural pozzolans with limited variability and high reserve [7- 9].

International academic studies have focused on its use in concrete for natural perlite. Some of those; Erdoğan (2013), Early-age activation of cement pastes and mortars containing ground perlite as a pozzolan; Liu (2014), Thermal properties of lightweight dry-mix shotcrete containing expanded perlite aggregate; Yu (2003), Investigation on pozzolanic effect of perlite powder in concrete; Patchta ( 2019), Development and testing of grouts based on perlite by-products and lime are titled studies. Their common features are on improving concrete properties by using natural perlite or expanded perlite as the main product or by-product [9-11].

Erdem et al. [1] Use of perlite as a pozzolanic addition in producing blended cements, published a study called. The pozzolanic property of perlite, and grindability have investigated. For this purpose, natural perlites taken from two different regions of Turkey (Izmir and Erzincan) for the pozzolanic materials standards have been tested. Cement samples were produced with the addition of 20% or 30% perlite. Turkish perlites were found to have amorphous structure and conform to ASTM C 618. Therefore, it was concluded that perlite samples from Izmir and Erzincan region have sufficient pozzolanic properties for use in cement and concrete industry. The study concluded that grinding perlite was easier than Portland cement clinkers, and that less energy would be needed to produce blended cement by combining perlite and clinker. Cement standards allow the use of pozzolanic materials up to 55%. The study could be tried for higher contribution rates in this commitment. Moreover, due to its low cost, especially in Turkey, Cem IV 32,5/ R-B pozzolanic cements, which are cheap and can reach high strength in the long term, are preferred in many countries.

42% of the world's marble reserves are located in Turkey. Many academic studies have been carried out in recent years to evaluate the residues in marble quarries and processing plants. It is possible to use marble residues in almost all industrial areas. As with perlite, marble waste is predominantly considered as a concrete aggregate. However, the standards allow the use of limestone for some types of cement. Due to its similar chemical structure, marble and travertine wastes are the best competitors to limestone. The use of marble and travertine residues in cement production will help to protect the environment by reducing the use of limited natural raw material resources, preventing the destruction of nature and eliminating the problems associated with the deterioration of the natural balance that will occur as a result of accumulation of marble and travertine residues. In this way, it will be contributed to the green production approach, which has become a slogan recently [12-14].

For all these purposes, industrial raw materials from two different regions of Turkey (Cappadocia perlites; opium marble travertine mix residues) were tested in order to determine the suitability of their availability in cement production. Natural perlite and marble travertine mix residues were ground separately and together and cement samples were prepared. The performance of natural perlite as pozzolan in blended Cements has been investigated. Different amounts of perlite and marble travertine residual mixture (5%, 10%, 15% and 20% perlite by weight, marble travertine residual mixture, perlite + marble travertine residual mixture) cement mixtures were produced in different proportions. The following tests were conducted to determine the performance of the Cements: particle size distribution with grinding, normal consistency, robustness, setting time and compressive strength.

## **2. MATERIAL AND METHOD**

### **2.1 Materials Used in Experimental Studies**

Portland cement clinker, gypsum (Yıldızeli), marble travertine mix residues (Afyon), natural perlite (Nevşehir), standard sand and water were used in the preparation of cement mixtures. The sand used in the study is standard Rilem sand. The substance used was dilithium tetraborate (merck), lithium iodide (merck), ethylene glycol (merck), HCl (merck)  $\text{Ca}(\text{OH})_2$  and  $\text{CaCO}_3$ . Both the chemical test results and the mechanical strength prove that the material conforms to the limits specified in the relevant standard (TS EN 196-1:2009).

#### **2.1.1 Natural perlite**

The pozzolan tested was natural tuff taken from Cappadocia region that is generally used for brick production. These chemical analyses are observed by İstanbul University Central Laboratory. Before grinding, pozzolans are oven dried at 100°C. The chemical and physical analyzes of the natural perlite used in the experiments were carried out and their properties were determined. The chemical and physical analysis of the natural perlite sample is given in Table 1. According to ASTM C 311, to be called as pozzolan, a material should satisfy strength activity index parameters, so, the first test applied to material was strength activity. At the end of 7 and 28 days, the compressive strength of mortars those have 20% natural tuff in them, are tested and compared to the control cubes, without any pozzolan in it, and the results are given in Table 1. Its chemical composition is shown in Table 1. The chemical analysis result of the perlite sample is given in Table 4.

**Table 1.** Strength activity index of natural pozzolan.

| Blaine Fineness<br>(cm <sup>2</sup> /gr) | Flow<br>(%) | Strength Activity Index (%) |         |
|------------------------------------------|-------------|-----------------------------|---------|
|                                          |             | 7 days                      | 28 days |
| 4125                                     | 104         | 80                          | 81      |

According to ASTM, strength activity after 7 and 28 days, should be at least 75%. Also, according to Turkish and European Standards, the ratio is 70%. The pozzolan, as shown in Table 1. satisfied the requirements of both standards, and received the name of pozzolan after strength activity test. Also, it was observed that the ratio increased by the time passes.

#### 2.1.2 Portland cement clinker

The clinker is obtained from Çimsa Afyon Cement Factory. XRF; (Atomica technical panalytical axios model) method was used for chemical analysis and this clinker was used in experiments. The specific mass of clinker used was 3.17 g / cm<sup>3</sup>. The chemical analysis result of the clinker sample is given in Table 4.

#### 2.1.3 Gypsum

The gypsum was obtained from the Tokat Adoçim Cement factory and the chemical analysis was performed by XRF and the amount of crystal water was determined. The chemical analysis result of the gypsum sample is given in Table 4.

#### 2.1.4 Marble travertine mix residues

The marble travertine mix residues were obtained from the waste fields of Afyon natural stone factories. The marble travertine mix residues used in the experiments were blended in the laboratory and made composites. Chemical and physical analyzes of the prepared samples were tested and their properties were determined (Table 4).

#### 2.1.5. Cements and Codes

Using natural stone residues and perlite, 12 types of cement were produced. In order to define cement types, codes of each cement were determined. The codes of the cement types are shown in Table 2.

**Table 2.** The mixtures properties used in the tests and codes.

| Portland Cement                                | PC      |
|------------------------------------------------|---------|
| % 5 Natural Stone Residue                      | NSR-5   |
| % 10 Natural Stone Residue                     | NSR-10  |
| % 15 Natural Stone Residue                     | NSR-15  |
| % 20 Natural Stone Residue                     | NSR-20  |
| % 5 Natural Perlite                            | NP-5    |
| % 10 Natural Perlite                           | NP-10   |
| % 15 Natural Perlite                           | NP-15   |
| % 20 Natural Perlite                           | NP-20   |
| % 5 Natural Stone Residue, % 5 Natural Perlite | NSRNP-5 |

|                                                  |          |
|--------------------------------------------------|----------|
| % 10 Natural Stone Residue, % 10 Natural Perlite | NSRNP-10 |
| % 15 Natural Stone Residue, % 15 Natural Perlite | NSRNP-15 |
| % 20 Natural Stone Residue, % 20 Natural Perlite | NSRNP-20 |

## 2.2. Method

Experimental program of the study was divided into three main parts:

- i) Chemical analysis of the three types of main cements;  
Using XRF method, chemical ingredients of clinker, gypsum, and natural perlite were chemically analyzed.
- ii) Physical analysis of the three types of cements;  
Fineness (Both Blaine and 45 $\mu$  wet), specific gravity, particle size distribution, normal consistency, setting time, loss on ignition, heat of hydration, initial and final setting times
- iii) Mechanical tests of the three cements;  
2, 7, 28 days compressive strengths of the cement mortars (Table 3.).

**Table 3.** Tests performed on Cements.

| Test                         | Test Type          |
|------------------------------|--------------------|
| Chemical Composition         | X-Ray Fluorescence |
| Fineness by Blaine Apparatus | ASTM C204          |
| Fineness by 45 $\mu$ Sieve   | ASTM C430          |
| Normal Consistency           | ASTM C187          |
| Setting Time                 | ASTM C191          |
| Compressive Strength         | ASTM C109          |
| Strength Activity            | ASTM C311          |

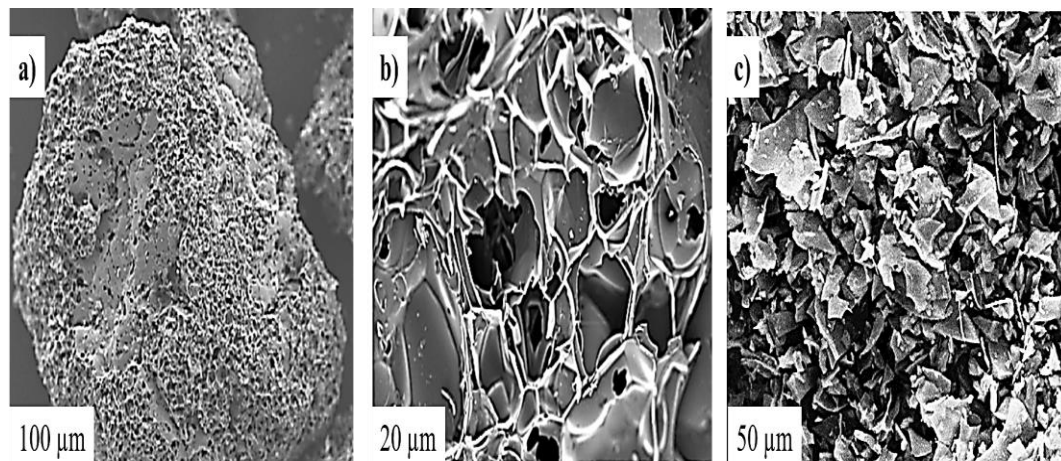
## 3. TEST RESULTS AND DISCUSSIONS

In this study, natural perlite and marble travertine mixture residues were blended separately and together and cements were produced using additives up to 40% in total and their physical and mechanical properties were investigated. The following results were obtained in the continuation of the studies for this article. The results were primarily compared with tables and graphs and the results were discussed.

### 3.1. Raw Material Mineralogical Analysis

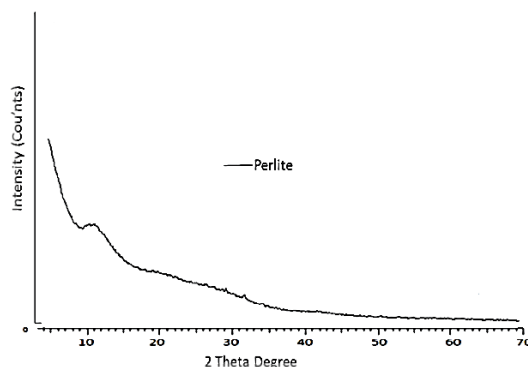
SEM micrograph of perlite and its magnified view (Fig. 1a, 1b and 1c) revealed the irregular morphology of perlite particles with broken or ragged edges. Similar pattern was observed in other reported micrographs of perlite.





**Figure 1.** Figure 1a. SEM image of the outer surface of raw perlite; Fig. 1b. SEM image of the inner structure of raw perlite [15]; Fig. 1c. SEM image of expanded perlite.

The low density of perlite raw material samples is due to the wide porosity between 20  $\mu\text{m}$  and 100  $\mu\text{m}$ , as can be seen in the SEM analysis images in Figure 1. Moreover, expanded perlite, which is used in many fields industrially, has a considerably high porosity. Crystal-like, high-porous and glassy structures are clearly visible in all three SEM images.



**Figure 2.** XRD profile of the natural perlite.

Silicate glass constitutes the main structure of the perlite sample. Perlite material can be characterized as rhyolite glass consisting of biotite, quartz and feldspar minerals. The most abundant element in its structure is Si (75%  $\text{SiO}_2$  by mass) followed by  $\text{Al}_2\text{O}_3$  and then alkaline metal oxides. More than 90% of its mineral composition is amorphous. It is seen in apatite, zircon, hornblende and ilmenite even though it is minor in its structure (Fig 2).

A large part (90-97%) of the volume of Perlite is glass and also contains crystallizing minerals such as feldspar and biotite. Some magnetite and apatite can be seen. The paste made of volcanic glass contains microlites and phenocrystals. Despite the glassy structure of perlite, it is thin from its onion peel-looking cracks somewhat dispersed zeolite crystallization, i.e. morderite formation, has been

identified. Perlite has an average refractive index value of 1,497. Perlite glass has an isotropic appearance when viewed in polarized light, but sometimes a light pair it may indicate fracture. X-ray analysis (XRD) showed that perlite contains up to 4% free silica [16].

**Table 4.** Chemical and physical analysis result of raw material samples.

| Component                      | Clinker | Gypsum | Perlite | Marble travertine residues |
|--------------------------------|---------|--------|---------|----------------------------|
|                                | %       | %      | %       | %                          |
| SiO <sub>2</sub>               | 20,83   | 0,7    | 70,96   | 10,91                      |
| Al <sub>2</sub> O <sub>3</sub> | 5,24    | 0,32   | 12,61   | 1,8                        |
| Fe <sub>2</sub> O <sub>3</sub> | 3,77    | 0,13   | 3,72    | 1,05                       |
| CaO                            | 66,83   | 33,71  | 1,8     | 47,31                      |
| MgO                            | 1,2     | 0,78   | 0,25    | 1,04                       |
| Na <sub>2</sub> O              | 0,39    | 0      | 1,22    | 0,18                       |
| K <sub>2</sub> O               | 0,84    | 0,03   | 3,77    | 6,2                        |
| SO <sub>3</sub>                | 0,61    | 41,58  | 0,25    | 0,6                        |
| LOI                            | 0,28    | 22,39  | 4,61    | 30,64                      |
| Total                          | 99,99   | 99,64  | 99,19   | 99,73                      |

### 3.2. Specific Gravity, Fineness, and Particle Size Distribution of Cements

It is known that the specific gravity of the pozzolanic cements with perlite added decreases the specific gravity of the cement as the amount of perlite increases. The biggest reason for this is that perlite has a very porous structure and thus its specific weight is low. It is stable as a result of previous studies that the density of the pozzolanic cements with perlite added produced will decrease, and the density will increase with the addition of limestone. Therefore, no density tests have been performed.

Fineness is an important criterion for increasing the reaction speed and capability of the cement. However, increasing the fineness of the cement is a costly task to increase the reaction rate, so it is always necessary to work with optimum fineness in order not to increase the production costs and energy consumption. The cement samples with additives prepared in line with the industrial experience obtained from previous years were grinded in a laboratory type ball mill for 45 minutes. Fineness and blaine tests were performed for each cement sample with additives. 45 $\mu$ , 90  $\mu$ m and 200  $\mu$ m fineness values were examined for each sample. grinding characteristics emerge better.

Travertine and marble residues are from the limestone and calcite family and they are very similar to each other mineralogically. They have revealed that harder, calcite and marble are hard to grind as a result of both hard grove and bond tests [17]. Perlite, on the other hand, resembles shaving due to its high silica content and glassy structure. Scientific studies conducted again revealed that the shade is easily grinded compared to clinker and limestone [18]. Therefore, it can be easily interpreted that perlite-added cements will reduce the grinding energy and decrease the production costs as the contribution rate increases. This study supports this.

Namely,

To compare the fineness of cement samples in the same time frame;  
cements produced with marble and travertine residues;  
NSR20>NSR15>NSR10>NSR5  
cements produced with natural perlite;  
NP20>NP15>NP10>NP5  
cements produced with marble and travertine residues and natural perlite mix  
NSRNP20>NSRNP15>NSRNP10>NSRNP5  
Final; NSR>NSRNP>NP screen sizes can be listed from high to low.

For the fineness values are also given in Table 5.

**Table 5.** Physical analysis result table of cement samples.

| Cement Type    | Fineness % |       |        | Blaine Fineness (cm <sup>2</sup> /gr) |
|----------------|------------|-------|--------|---------------------------------------|
|                | 45 µm      | 90 µm | 200 µm |                                       |
| NSR-5          | 9,2        | 0,8   | 0      | 3436                                  |
| NSR-10         | 11,9       | 0,9   | 0,1    | 3579                                  |
| NSR-15         | 13,4       | 1,2   | 0,2    | 3723                                  |
| NSR-20         | 14,1       | 1,6   | 0,2    | 3864                                  |
| NP-5           | 5,7        | 0,4   | 0      | 4174                                  |
| NP-10          | 6,0        | 0,4   | 0      | 4437                                  |
| NP-15          | 7,4        | 0,5   | 0,1    | 4383                                  |
| NP-20          | 8,8        | 0,7   | 0,1    | 4520                                  |
| NSR-5, NP-5    | 5,9        | 0,4   | 0      | 4061                                  |
| NSR-10, NP-10  | 6,8        | 0,6   | 0,1    | 4165                                  |
| NSR-15, NP-15  | 8,7        | 0,7   | 0,1    | 4388                                  |
| NSR-20, NP -20 | 9,8        | 0,8   | 0,2    | 4498                                  |

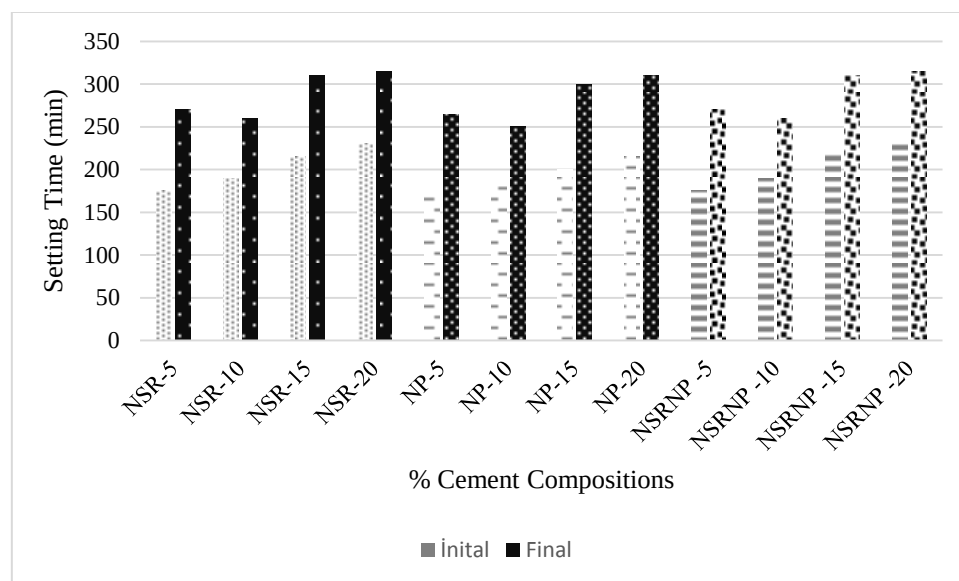
As can be followed from Table 5, while pozzolan added with increasing percentages by mass to the cements, the weight of the cements decreased. The aim in this study was increasing the surface area by the clinker decreases, so, the cements tested had 500 cm<sup>2</sup>/gr more surface area for every 25% increase in natural perlite mass.

### 3.4 Normal Consistency, Setting Time, Autoclave Expansion, and Heat of Hydration of Cement Pastes

Pozzolanic materials such as zeolite, pumice, trass used in place of cement increase the amount of binder paste since they have a smaller grain size. Because they are very fine grained, they prevent sweating by holding the water in fresh concrete. They also make a positive contribution to the workability of concrete. As they are very fine grained, they cause the gaps in the concrete to decrease and increase the water impermeability. It is preferred for pouring concrete in hot weather. Its

workability is better than Portland cement, it has long-lasting strength gains thanks to the pozzolanic materials it contains, and it has high durability. Pozzolanic materials reduce the need for water formed in concrete. The fine filling materials it contains tightens the micro pores in the concrete and increases its impermeability. It increases the quantity obtained thanks to its low density in the construction chemical production and contributes to the processability in ready-mixed concrete manufacturing. It provides long-term durability with its pozzolanic feature. It is possible to achieve all these properties because the natural perlite material also has pozzolanic properties.

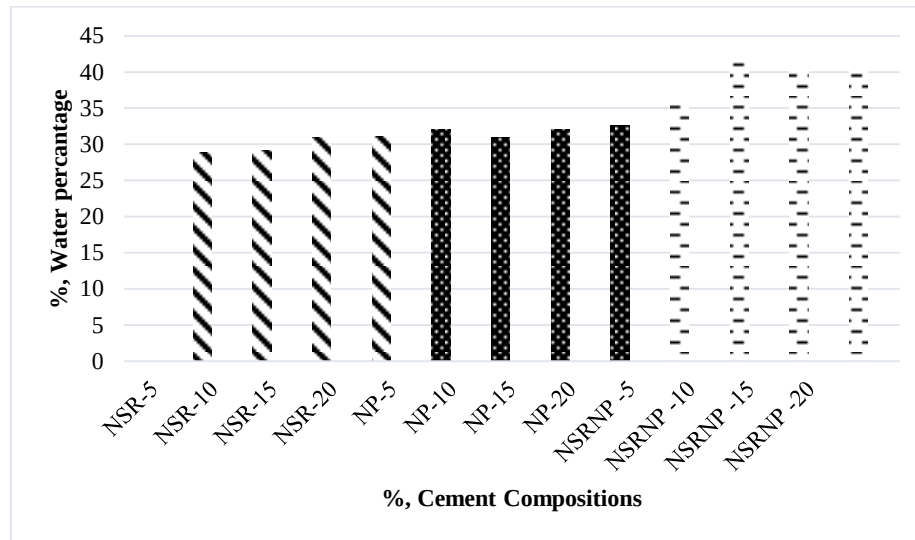
So far, scientific studies have shown that the water needs of pozzolanic cements increase with the increase in additive rates compared to Portland Cements, and the lengthening of the setting times. Erdem et al. [2] found that with the increase in the amount of contributions, the need for water and setting time increased. In addition, it has been proven that perlite species are not effective in improving cement properties, especially in setting times and water requirements. Moreover, in parallel with the values found in the study by Erdem et al. [1], it was observed that the plug-in times and water requirement increased with the increase in the contribution rate and thus had a positive effect on the workability.



**Figure 3.** Setting time of the cement samples.

According to TS-EN 196-3 in the standards for pozzolanic cements, the starting time of the setting time must be  $> 75$  minutes. Furthermore, according to TSE EN 196-3, the volume expansion amount of concrete must be  $< 10$  mm. Looking at Fig.3, it is seen that the cement samples obtained from the mixture of perlite, marble travertine residues and both materials (perlite and marble travertine residues) are below the standard values and meet the requirement. Especially, the setting times and workability of perlite-added cements are more prolonged compared to marble travertine-added cements and mixture cements setting time.



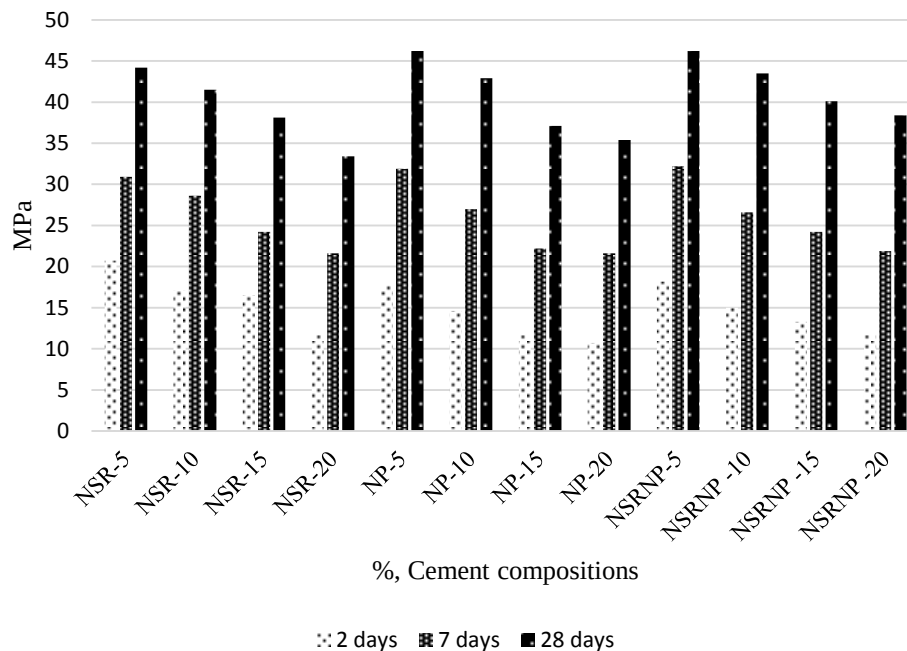


**Figure 4.** Water normal consistency of the cement samples.

Darweesh and Samhan [19] reported the longer initial and final outlet time of pastes by partially substituting the cement with perlite powder (PP) (63  $\mu$ m) at levels of 5%, 15% and 25% by weight. As the PP content increased, the setting time increased. Initial and final setting time increased by about 0.93% and 4.46%, including 15% and 25% PP, respectively. Virtue et al. [20, 21], Partly applied to 20% and 30%, EP (370 m by weight / kg) is reported in the long first and last setting of the paste by substituting cement with blaine surface area. With the increased EP content, the plug-in time was extended. Fig. 3-4. summarizes the effect of perlite on the workability and setting time of mortar and concrete.

### 3.5. Compressive Strength of Cement Mortars

According to TS EN 196-1, two-day compressive strength should be between > 20 MPa and 28-day compressive strength > 42.5 MPa and < 62.5 MPa in Portland cement, especially for Cem 1 42.5 R cement. Cement producers prefer Cem IV 32,5 R / B cement in order to reduce the cost and increase the durability in the long term by using more additives. According to TS EN 196-1, up to 55% pozzolanic additive material can be used to produce Cem IV 32,5 R-B pozzolanic cement. However, as the pozzolanic additive rate of the material used increases, Cem I 42,5 R decreases the strength compared to Portland cement. For this reason, according to TS EN 196-1, Cem IV 32,5 R-B pozzolanic cement has a two-day compressive strength > 10 MPa and a twenty-eight-day compressive strength value between > 32.5 MPa and < 52.5 MPa.



**Figure 5.** compressive strengths of the cement samples.

When the compressive strength values are examined in Fig. 5, it is observed that the perlite contribution rate increases and the pressure resistance decreases. Then the compressive strength values of the cement produced with mixture cement and then marble travertine residues are followed. When cement samples are compared with TSE EN 197-1 standard, it is seen from Fig. 5. that the strength requirements for Cements are met by all blended cements produced.

In the study conducted by Erdem et al. [1] it is observed that 20% perlite use produces higher strength than 30% perlite use when the strength of mortars containing Cements with the same fineness, same perlite and same grinding method is compared. The low strength of the Cements containing 30% perlite can be explained by the lower PC content (especially when pozzolanic reactions are not effective for early ages) and the more charged water cement ratio. The effect of finer particle size distribution has also been observed on the strengths of mortars containing different types of perlite. The strength values of the more finely ground and more homogeneous perlite-doped Cements were higher. However, due to the ongoing puzolanic reactions of perlite-doped cements, the differences will become smaller in later ages.

Topçu et al. [22] reported a decrease in compressive strength and bending strength of mortars including EP (size 0-2 mm). Lanzón and García-Ruiz [23] reported a reduction in the compressive strength and bending strength of mortars, including the entire composition EP (weight 0.08-1 mm). 28-day reduction in pressure strength, % 0.59, % 1.18, % 1.77, % 3.54 and including 7.08% EP respectively % 29.22, % 38.1, % 49.87, % 68.63 and is 80.97%. daily bending strength respectively % 32.51, % 45.95, % 53.15, % 71.83 and it was 85.5%. Fiat et al. [24] they reported that the inclusion of 5%, 7% and 8.5% EP (size 0-1 mm) in mortars led to a decrease in mechanical strength.

#### 4. CONCLUSIONS

Although there have been very few studies on the use of both natural perlite and expanded perlite in cement production in Turkey, it is known that perlite produced in Turkey has pozzolanic properties as a result of these studies. Turkish perlites generally have amorphous structure and have properties suitable for ASTM C 618. The Nevşehir-Acıgöl perlites used in this study also have amorphous structure and contain pozzolanic properties suitable for evaluation in cement production.

The use of perlite and marble travertine residues in cement production both separately and together increased the starting and finishing time of the cement outlet and the workability of the concrete. However, all these changes remained below the limit values according to TS EN 196-3 and met the requirements.

The strength values of the cement samples produced are low compared to Portland cement. Moreover, this is an expected result according to the literature. However, according to TS EN 197-1, cement samples meet the requirements of pozzolanic cement and composite cement concrete strength classes. That is to say;

| Portland calcareous cement |             | Pozzolanic cement |             | Composite cement |
|----------------------------|-------------|-------------------|-------------|------------------|
| Cem II/A-L                 | Cem II/A-LL | Cem IV/A          | Cem IV/B    | Cem V/A          |
| NSR-5                      | NSR-5       | NP-5              | NSR20, NP20 | NP-5             |
| NSR-10                     | NSR-10      | NP-10             |             | NP-10            |
| NSR-15                     | NSR-15      | NP-15             |             | NP-15            |
| NSR-20                     | NSR-20      | NP-20             |             | NP-20            |
|                            |             | NSR5, NP5         |             |                  |
|                            |             | NSR10, NP10       |             |                  |
|                            |             | NSR15, NP15       |             |                  |

As perlite is more easily milled than trass clinker and limestone, it reduces cement production costs and saves energy. It also provides thermal insulation thanks to its superior properties, it is resistant against fire, it can be used as a light building element due to its low density. Because of these reasons, more studies should be done about perlite.

The use of natural stone residues in addition to prolonging the life of Natural Resources and preventing the environmental pollution created by giant natural stone residues are ensured. In addition, since Portland cement will be substituted, cement production costs will be reduced again.

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