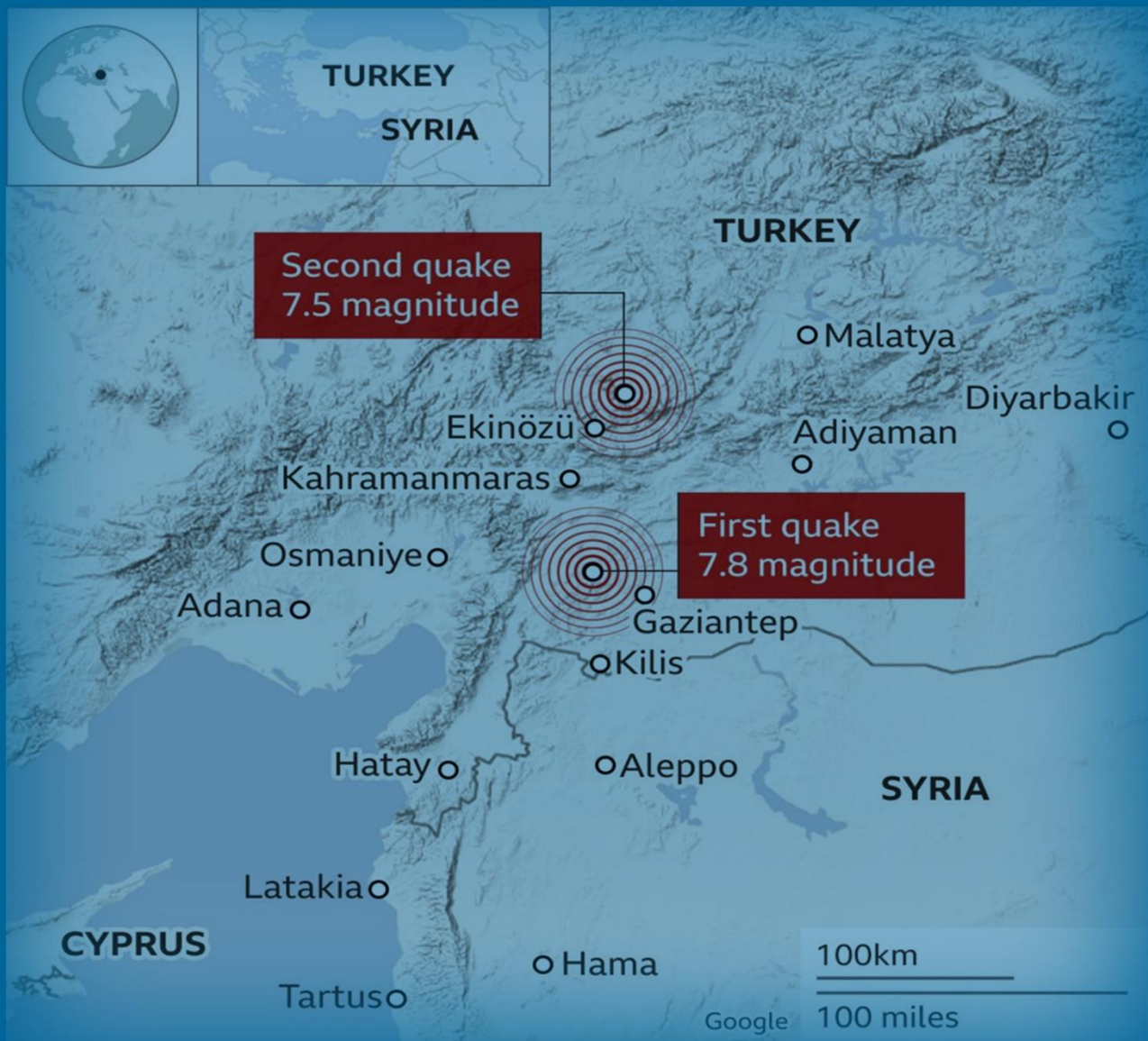


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Antifungal Activities of Essential Oil Obtained from *Mentha spicata* var. *Crispa* against Selected *Penicillium* Species

Hana Ďúranová^{1*}, Veronika Valková^{1,2}, Lucia Galovičová², Nenad L. Vukovic³,
Milena Vukic³, Miroslava Kačániová^{2,4}

Abstract: Attention of the scientific community has still focused on application of essential oils (EOs) as natural antifungal agents in the food industry to prolong the shelf-life of food products. In this regard, the current study was designed to evaluate chemical composition, antioxidant and both antifungal (*in vitro*, *in situ*) activities of spearmint (*Mentha spicata* var. *crispa*) essential oil (SEO) commercially obtained from Slovak company against selected *Penicillium* species. The EO was used in four concentrations (62.5, 125, 250, and 500 μ l/l) chosen, and gas chromatography–mass spectrometry, DPPH, agar disc diffusion and vapor phase methods were employed for such analyses. Results revealed that carvone (57.5%) and α -limonene (17.6%) were the principal constituents in the EO chemical composition. Although only a weak antioxidant activity (20.40 \pm 0.80% free radical-scavenging inhibition) was displayed by SEO, the highest EO concentration (500 μ l/l) was shown to be a moderate growth inhibitor of *P. expansum* (inhibition zone of 11.46 \pm 0.63 mm) and *P. crustosum* (inhibition zone of 12.93 \pm 0.46 mm). The growth of *P. citrinum* was only weakly inhibited by the SEO (\geq 250 μ l/l). Most importantly, the ability of the SEO to inhibit the mycelial growth of three *Penicillium* spp. tested was pronounced ($p < 0.05$) for all applied concentrations. Accordingly, the results from the current study complement our previous ones dealing with the possibility of utilizing diverse EOs commercially achieved from the same company in the food sector.

Keywords: Antifungal activity, DPPH assay, essential oil, *Mentha spicata*, vegetable, volatile substances

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1. INTRODUCTION

Due to microbial contamination of food products, the food sector is facing a great challenge to find a promising alternative to synthetic preservatives reducing food spoilage while being bio-incompatible, non-biodegradable, and environmentally unsustainable (Maurya et al., 2021). One of the major emerging technologies to extend the shelf-life

of foods seems to be the extraction of essential oils (EOs) from various plant organs and their application to food systems (Fernández-López and Viuda-Martos, 2018; Ribeiro-Santos et al., 2018; LaLonde et al., 2019; Rao et al., 2019; Santos et al., 2022). Indeed, as a valuable source of diverse biologically active compounds, EOs possess antioxidant and antimicrobial properties which participate in food shelf-life enhancement, thus making them an ideal

natural, eco-friendly, renewable, and cost-effective replacement of synthetic food additives (Fernández-López and Viuda-Martos, 2018; Basavegowda and Baek, 2021; Saeed et al., 2022).

Spearmint [*Mentha (M.) spicata*, equivalent to *M. viridis*] is a perennial herbaceous medicinal plant of the Lamiaceae family with a pungent smell, commercially cultivated in many regions of the world (Ounoki et al., 2021; El Menyiy et al., 2022). Leaves of the plant are traditionally used as tea (Dhifi et al., 2013) and an ingredient in a variety of mixed drinks including the mojito and the mint julep (Ounoki et al., 2021). Spearmint EO (SEO), belonging among the 10 most commercialized EOs (Delfine et al., 2022), is produced and stored in the glandular trichomes of the leaves. The EO is characterized by the high presence of monoterpenes (Chrysargyris et al., 2017), mainly of carvone as the major component responsible for its aroma (Dionísio et al., 2012). Hence, it is especially applied in the flavoring of chewing gums, toothpastes, and other oral products (Kokkini et al., 2003). There are many *in vitro* and *in vivo* experimental reports demonstrating *M. spicata* extracts and EOs as agents with remarkable antimicrobial, antiparasitic, antidiabetic, anti-inflammatory, and anticancer biological activities (El Menyiy et al., 2022). Additionally, SEO is reputed for its carminative, antispasmodic, and diuretic properties (Dhifi et al., 2013). Regarding its antimicrobial efficacy, common food-borne pathogenic bacteria, such as *Staphylococcus aureus*, *Bacillus (B.) subtilis*, *B. cereus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Shigella flexneri* (Ullah et al., 2012; Shahbazi, 2015; Horváth and Koščová, 2017) were shown to be sensitive to SEO actions. Also, antifungal activity of the EO is extensively tested (Şarer et al., 2011; Houicher et al., 2016; Bardaweel et al., 2018). However, it is well-known that chemical profile and relative proportions of organic compounds of any EO extracted from a selected single plant species depend on a plethora factors such as agricultural aspect (e.g., environment, climate, soil conditions, time of harvesting and postharvest handling prior to isolation) (Sankarikutty and Narayanan, 2003), extraction methods used (Berka-Zougali et al., 2012; Pintatum et al., 2020; Messaoudi et al., 2021), plant parts being used for extraction (Pintatum et al., 2020), and many others, which are principal causes of serious discrepancies identified among the studies concerning this field of research area. Taking into account this fact, the current study evaluated *in vitro* and *in situ* antifungal activities of the EO obtained from *M. spicata* var. *crispa* against selected *Penicillium* spp. inoculated on potato slices as a model of food substrate. In such a way, application of SEO in active food packaging to prolong shelf-life of foods can be considered. Furthermore, the report also adds another piece to our comprehensive view creation of various antifungal actions exerted by diverse commercial EOs achieved from the same company.

2. MATERIAL AND METHOD

2.1. Essential oil

Spearmint (*M. spicata* var. *crispa*) essential oil (SEO) was purchased from Hanus s.r.o Company (Nitra, Slovakia) to complement our previous studies (Galovičová et al., 2021a; Galovičová et al., 2021b; Kačániová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Galovičová et al., 2022; Kačániová et al., 2022a; Valková et al., 2022a; Valková et al., 2022b). The EO was prepared by steam distillation of flowering stems.

2.2. Fungal strains

Three *Penicillium (P.)* strains (*P. crustosum*, *P. citrinum*, and *P. expansum*) isolated from *Vitis vinifera* berries were employed to assess *in vitro* and *in situ* antifungal activities of the SEO. The strains were classified using a reference-based MALDI-TOF MS Biotyper followed by comparison with the taxonomic identification obtained by 16S rDNA sequences analysis.

2.3. Chemical characterization of SEO

Gas chromatography/mass spectrometry (GC/MS) analysis of the SEO was performed using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA) according to the methodology described by Valková et al. (2021a). The individual volatile constituents of the injected EO samples were identified based on their retention indices (Adams, 2007), and a comparison with reference spectra (Wiley and NIST databases). The retention indices were experimentally determined using the standard method which included retention times of n-alkanes (C6–C34), injected under the same chromatographic conditions. The percentages of the identified compounds (amounts higher than 0.1%) were derived from their GC peak areas.

2.4. Antioxidant activity of SEO

Antioxidant activity (AA) of the SEO was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as it was carried out in our previous experiments (Galovičová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Kačániová et al., 2021b; Kačániová et al., 2022b). The AA was expressed as the percentage of DPPH inhibition, and calculated according to the formula: $(A_0 - A_1)/A_0 \times 100$; where A_0 and A_1 were absorbances of the DPPH and the samples, respectively. The power of AA was assessed based on the following scheme: weak (0 – 29%) < medium-strong (30 – 59%) < strong (60% and more). Moreover, the value for total AA was expressed as Trolox equivalent antioxidant capacity (TEAC) according to the calibration curve as 1 µg of standard reference Trolox to 1 ml of the SEO sample. All analyses were performed in triplicate.

2.5. *In vitro* antifungal activity of SEO

In vitro antifungal activity of the SEO was performed using the agar disc diffusion method (Valková et al., 2021a; Valková et al., 2021b; Valková et al., 2022b). To prepare fungal media, the strains were inoculated in Sabouraud Dextrose Agar (SDA; Oxoid, Basingstoke, UK) and incubated for 5 days at 25 °C. Subsequently, small aliquots of the fungi were transferred to test tubes, each containing 3 ml of distilled water, and the inoculum concentration was standardized by comparison with the 0.5 McFarland scale (1.5×10^8 CFU/ml). For the analysis, an aliquot of 100 μ l of the culture media was firstly inoculated on the SDA, and the discs of filter paper (6 mm) impregnated with 10 μ l of the SEO sample (in four concentrations: 62.5, 125, 250, and 500 μ l/l; diluted in 0.1% dimethyl sulfoxide, DMSO) were applied on the SDA surfaces. Fungi were consequently incubated aerobically at 25 ± 1 °C for 5 days. After the incubation, diameters of the inhibition zones were measured in mm. The values for inhibitory activity were expressed in the following manner: weak antifungal activity (5 - 10 mm) < moderate antifungal activity (10 - 15 mm) < very strong antifungal activity (zone > 15 mm) (Valková et al., 2022a).

2.6. Moisture content and water activity of potato food model

Potato slices as a substrate for fungal growth were applied. The vegetable was purchased at the local market (Nitra, Slovakia). Its moisture content (MC) and water activity (aw) were measured using the Kern DBS 60-3 moisture analyzer (Kern and Sohn GmbH, Balingen, Germany) and the Lab Master aw Standard (Novasina AG; Lachen, Switzerland), respectively (Kačániová et al., 2020a; 2020b). All analyses were performed in triplicate.

2.7. *In situ* antifungal analysis

The vapor phase (contact) method was employed to assess *in situ* antifungal activity of the SEO (Valková et al., 2022a; 2022b). Firstly, sliced potato (5 mm) was placed on the bottom of Petri dishes, and the inoculum was applied by stabbing one time with an injection pin on the vegetable surface. Next, 10 μ l of the SEO in the same four concentrations was applied on the sterile filter paper disc (60 mm) which was consequently placed at the top of Petri dishes. The dishes were hermetically closed by parafilm and cultivated at 25 °C for 14 days. All analyses were performed in triplicate.

After the cultivation, the size of the fungal colonies with visible mycelial growth and visible sporulation (Kačániová et al., 2020a; 2020b) was evaluated using stereological methods. In this concept, the volume density of the colonies was firstly assessed using ImageJ software (National Institutes of Health, Bethesda, MD, USA), counting the points of the stereological grid hitting the colonies and those falling to the reference space (growth substrate used, potato). The antifungal activity of the SEO was expressed as the percentage of mycelial growth inhibition (MGI), which was calculated using the formula: $MGI = [(C - T)/C] \times 100$ (Sempere-Ferre et al., 2021), where C and T were

volume fractions of fungal colonies in the control (untreated) and treated samples, respectively.

2.8. Statistical analysis

Statistical analysis of obtained data was performed using Prism 8.0.1 (GraphPad Software, San Diego, California, USA). Significant differences between the analyzed groups of samples were assessed using one-way analysis of variance (ANOVA) followed by Tukey's test. A value for $p < 0.05$ denoted the level of statistical significance.

3. RESULTS AND DISCUSSION

Using GC/MS analysis, a total of 35 organic components were identified in our SEO, completely accounting for 99.7% of the EO chemical composition (Table 1). The dominant constituents were monoterpenes (98.5%), followed by sesquiterpenes (1%), and non-terpenic compounds (alcohols; 0.2%). Out of monoterpenes, oxygenated ones (77.9%), especially monoterpene ketones (63.8%) with carvone (57.5%) and monoterpene alcohols (8.1%) with dihydrocarveol (4.3%) have been detected in the highest amounts. Among monoterpene hydrocarbons (20.6%) as the second major class of compounds identified, α -limonene (17.6%) was the most prominent.

Table 1. Chemical composition of essential oil obtained from *Mentha spicata* var. *crispata*

No	RI ^a	Compound ^b	% ^c
1	938	α -pinene	0.6
2	977	sabinene	0.1
3	980	β -pinene	1.0
4	992	β -myrcene	0.4
5	993	3-octanol	0.2
6	1023	<i>p</i> -cimene	0.9
7	1028	α -limonene	17.6
8	1033	1,8-cineole	0.8
9	1060	γ -terpinene	tr
10	1068	cis-sabinene hydrate	tr
11	1148	isopulegol	tr
12	1151	menthone	1.1
13	1162	iso-menthone	0.4
14	1164	neo-menthol	0.3
15	1173	menthol	1.8
16	1178	4-terpinenol	tr
17	1192	cis-dihydrocarvone	4.1
18	1199	dihydrocarveol	4.3
19	1200	trans-dihydrocarvone	0.7
20	1217	trans-carveol	0.7
21	1229	cis-carveol	1.0
22	1241	carvone	57.5
26	1253	3-carvomenthenone	tr
27	1254	(<i>Z</i>)-anethole	tr
28	1297	menthyl acetate	0.9
29	1306	iso-menthyl acetate	0.5
30	1311	dihydrocarveol acetate	3.8
31	1379	α -copaene	tr
32	1385	β -bourbonene	0.2
33	1388	β -elemene	tr

34	1422	(E)-caryophyllene	0.6
35	1583	caryophyllene oxide	0.2
Total		99.7	

^a Values of retention indices on HP-5MS column; ^b Identified compounds; ^c tr - compounds identified in amounts less than 0.1 %

In general, biological activities of EOs being interested in the food and cosmetic industries, as well as in the field of human health are strongly dependent on their chemical composition (Dhifi et al., 2016). Diverse hydrocarbon and oxygenated monoterpenes compounds have been intensively studied in terms of their antimicrobial and antifungal activities against various foodborne pathogens (Badawy et al., 2019). Similarly to our results, carvone (65.33%), limonene (18.19%), and dihydrocarvone (2.97%) as the major compounds of *M. spicata* EO were reported by Liu et al. (2012). Additionally, the carvone and limonene being the predominant components of SEO have also been identified in the research by Bardaweel et al. (2018) and Snoussi et al. (2015). However, while first authors determined values for carvone (49.5%) and limonene (16.1%) to be close to our ones, the latter authors showed that both compounds participate in different proportions (40.8 ± 1.23% of carvone, 20.8 ± 1.12% of limonene) in the EO chemical profile in comparison with our study. Carvone (56.6%; 41.1%; 78.76%; 62.9%; 62–65%; 41.1%) and limonene (27.3%; 20.1%; 11.50%; 8.5%; 11–13%; 14.1%) as the major constituents of *M. spicata* EO have also been detected by other researchers (Aggarwal et al., 2002; Martins et al., 2012; Shahbazi, 2015; Ounoki et al., 2021; Piras et al., 2021; Giménez-Santamarina et al., 2022), respectively. Generally, the concentrations of monoterpenes (such as carvone) being extracted from the same species is strongly influenced by the plant parts and the method itself selected for such procedure (Bouyahya et al., 2021). Also, already above-mentioned (in the part “Introduction”) other factors must be kept in mind, all of them contributing to different findings identified between studies employed for data comparison as it was shown in our report.

Data from DPPH free radical-scavenging activity analysis has revealed a weak (20.40 ± 0.80% free radical-scavenging inhibition) AA of our SEO with a value of 107.66 ± 3.0 µg TEAC.ml⁻¹. This assay is commonly used for measurement of AA of diverse EOs (Bag and Chattopadhyay, 2015; Inaam et al., 2015; Anggraeni et al., 2018; Olmedo et al., 2018; Chambre et al., 2020; Galovičová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Kačániová et al., 2022a). According to many authors (Amiri, 2012; Bag and Chattopadhyay, 2015), the DPPH has been largely used as an easy, quick, reliable, and reproducible assay for screening *in vitro* antioxidant activity of EOs or plant extracts. Using the method, we have found that the SEO was able to scavenge the radical; however, only to a lesser extent indicating its weak AA. The same finding was also displayed by EO from *M. spicata* growing in Portugal (Martins et al., 2012) and Poland (Grzeszczuk and Jadczyk, 2009). By contrast, moderate inhibition of DPPH radicals (54.68%) of *M. spicata* EO from Oman have been observed by Alsaraf et al. (2021). Furthermore, Ahmad et al. (2012) have detected even higher values for AA (61-71%) of *M. spicata*

methanolic extract. As compared to our previous study, the EO from *M. spicata* exhibited lower AA than that from *M. piperita* (36.85 ± 0.49%) recognizing menthol, menthone, and menthyl acetate to be the major constituents (Valková et al., 2021a). The same fact was also reported by other researchers (Dorman et al., 2003; Nikavar et al., 2008).

In spite of only a weak AA displayed by our SEO, an *in vitro* antifungal activity of the EO against the growth of *Penicillium* spp. selected (Table 2) was observed. In effect, the disc diffusion method revealed a moderate inhibitory efficiency of the highest SEO concentration (500 µl/l) against the growth of *P. expansum* and *P. crustosum*, with inhibition zones of 11.46 ± 0.63 mm and 12.93 ± 0.46 mm, respectively. Against *P. citrinum* mycelial growth, the highest concentration of the EO induced only a weak inhibitory action; the same impact was also noticed for 250 µl/l of the EO against all three fungal species.

Table 2. *In vitro* antifungal activity of spearmint essential oil expressed as the diameter of the inhibition zone (in mm)

Fungi	SEO (µl/l)			
	62.5	125	250	500
<i>P. expansum</i>	1.73 ± 0.55 ^a	4.55 ± 1.03 ^b	9.05 ± 0.83 ^c	11.46 ± 0.63 ^d
<i>P. crustosum</i>	2.76 ± 0.57 ^a	5.14 ± 0.74 ^b	8.36 ± 1.09 ^c	12.93 ± 0.46 ^d
<i>P. citrinum</i>	2.88 ± 0.36 ^a	4.86 ± 0.77 ^b	6.56 ± 0.34 ^c	9.47 ± 0.59 ^d

Note: Mean ± standard deviation. SEO: spearmint essential oil. Values in the same line with different superscripts are significantly different (*p* < 0.05).

In addition, all concentrations of the SEO were able to considerably (*p* < 0.05) inhibit the mycelial growth of *P. expansum*, *P. crustosum*, and *P. citrinum* inoculated on potato slices as a food model substrate. The data from *in situ* analysis is summarized in Table 3. Moreover, there was a more pronounced inhibitory action of higher concentrations of SEO (≥ 125 µl/l; ≥ 250 µl/l) on the growth of *P. expansum* and *P. citrinum*, respectively, as compared to the lower concentrations. On the other hand, *P. crustosum* was equally sensitive to all the EO concentrations.

Table 3. *In situ* antifungal activity of spearmint essential oil expressed as mycelial growth inhibition

Fungi	MGI (%)			
	SEO (µl/l)			
	62.5	125	250	500
<i>P. expansum</i>	55.07 ± 5.15 ^a	91.35 ± 9.39 ^b	100.00 ± 0.00 ^b	91.67 ± 9.24 ^b
<i>P. crustosum</i>	100.00 ± 0.00 ^a	93.30 ± 10.17 ^a	97.03 ± 5.84 ^a	95.12 ± 5.99 ^a
<i>P. citrinum</i>	78.43 ± 3.69 ^a	92.80 ± 12.92 ^{ab}	95.96 ± 7.58 ^b	96.74 ± 9.15 ^b

Note: Mean ± standard deviation. MGI: mycelial growth inhibition; SEO: spearmint essential oil. Values in the same line with different superscripts are significantly different (*p* < 0.05).

The characterization of our EO from *M. spicata* from both *in vitro* and *in situ* antifungal activities has shown that the SEO is an effective inhibitor of the *Penicillium* spp. growth. Similarly to our study, low to moderate antimicrobial activity of SEO against pathogenic microorganisms including gram positive, gram negative bacteria, and fungi was also reported by Bardaweel et al. (2018). Spearmint EO has also been found to be active against *P. citrinum* (Liu et al., 2012), *Candida albicans*, *Aspergillus niger*, *Fusarium oxysporum* (Martins et al., 2012), and *Vibrio* spp. strains (Snoussi et al., 2015). Aggarwal et al. (2002) have tested the effect of SEO, as well as their main isolated components, carvone and limonene, on a wide spectrum of human pathogenic fungi and bacteria revealing their high *in vitro* bioactivity. The use of carvone as an antifungal agent against various fungal strains is suggested by many reports (Morcia et al., 2012; Boni et al., 2016; Hassan et al., 2017; Moro et al., 2017). From this aspect it can be assumed that the low to moderate antifungal activity of our SEO demonstrated by the disc diffusion method can be attributed mainly to the carvone abundance in its chemical composition.

Food spoilage is a very common phenomena in which genus *Penicillium* plays an important concern because of its ubiquity and mycotoxin production (Pitt, 2014). In the current study, potato slices as a food model substrate for *Penicillium* spp. growth were employed. Moisture content and aw of the potato substrate were estimated to be $75.18 \pm 1.23\%$ and 0.969 ± 0.002 , respectively; the data of these physical parameters demonstrates its suitability for microbial (fungal) spoilage. Indeed, food substrates with values for MC and aw being around 80% and higher than 0.60, respectively, are well suited for the *in situ* antifungal activity analysis of diverse EOs (Valková et al., 2022b). Moreover, our data of both physical parameters have been found to be in accordance with the study of the mentioned authors (Valková et al., 2022b). A strong antifungal potential of our SEO against the mycelial growth of all three *Penicillium* species could be explained by the presence of oxygenated monoterpenes, such as carvone that has emerged as a promising antifungal compound (Bouyahya et al., 2021) due to its disruptive impact on the cell membrane and fungal mitochondria (Zhang et al., 2022). Also, limonene (another major component of SEO) is able to inhibit the growth of *C. albicans* by generation of oxidative stress in the cell envelope and induction of oxidative DNA damage, leading to cell-cycle modulation and apoptosis (Sales et al., 2022). Thus, the *in situ* antifungal activity of SEO demonstrated in our study can be associated mainly with the two major compounds and their mutual interactions. However, the biological activities of EOs as a multicomponent mixture cannot be easily ascribed to only one or two specific components but they are rather a result of additive, synergistic or antagonistic actions of different constituents present in their chemical profile (Bardaweel et al., 2018).

5. CONCLUSION

The results from the present study showed carvone (57.5%) and α -limonene (17.6%) to be the principal constituents in chemical composition of the SEO which displayed only a weak AA ($20.40 \pm 0.80\%$ free radical-scavenging inhibition). On the other hand, the highest concentration (500 μ l/l) of the EO exhibited a moderate inhibition efficacy against the growth of *P. expansum* (inhibition zone of 11.46 ± 0.63 mm) and *P. crustosum* (inhibition zone of 12.93 ± 0.46 mm), whilst the growth of *P. citrinum* was inhibited by the SEO (≥ 250 μ l/l) only in a weak manner. Essentially, the ability of the SEO (in all four concentrations used) to act as an inhibitor against the mycelial growth of three *Penicillium* spp. tested was evident ($p < 0.05$). In conclusion, the findings from all our analyses suggest the SEO to be a promising natural agent for extending the shelf life of vegetables (including potato) which can be a very helpful aspect for the food sector in terms of active food packaging.

Ethics Committee Approval

N/A

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Author Contributions

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Antifungal Efficacy of Mint Essential Oil Against *Penicillium* spp. Inoculated on Carrots

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Abstract: Current research aims to investigate the chemical composition, antioxidant, and *in vitro* and *in situ* antifungal activities of commercially available *Mentha arvensis* essential oil (mint, MEO). The identification of the volatile substances was done using Gas chromatography–Mass spectrometry (GC-MS) analysis. A total of 42 components representing 99.5% of the total oil were identified. The main compounds in the oil were menthol (37.3%), menthone (17.4%), neo-menthol (14.1%), and 1,8-cineole (4.9%). Antioxidant assays (1,1-diphenyl-2-picrylhydrazyl radical) demonstrate only weak activity for the MEO in values $195.00 \pm 5.30 \mu\text{g TEAC.mL}^{-1}$, with $22.8 \pm 1.2\%$ free radical-scavenging inhibition. Evaluation of *in vitro* and *in situ* antifungal activities of MEO (in four concentrations: 62.5 $\mu\text{L/L}$, 125 $\mu\text{L/L}$, 250 $\mu\text{L/L}$, and 500 $\mu\text{L/L}$) against three strains of *Penicillium* (*P.*) spp. fungi strains (*P. expansum*, *P. citrinum*, *P. crustosum*) were assessed by disc diffusion method and vapor contact method on the carrot as model food, respectively. The suitability of carrots as a substrate for analyzes was verified by determining moisture content (MC) and water activity (a_w), which showed values of $82.80 \pm 2.33\%$ and 0.959 ± 0.001 , respectively. MEO exhibited promising antifungal activity against analyzed strains of test fungi as a diameter of zones of inhibition (from 2.88 ± 0.55 to 12.33 ± 1.14 mm), as well as the effectiveness of this oil was detected on the carrot model (from -5.41 ± 7.35 to $100.00 \pm 0.00\%$). Moreover, it can be concluded that the growth inhibition of fungi strains significantly depends ($P < 0.05$) on the concentration of the MEO used in both procedures. Our results suggest that MEO, as a promising natural antifungal agent, can be applied in the innovative packaging of food products including carrots.

Keywords: *Mentha arvensis*, DPPH assay, volatile compounds, antifungal activity, model food.

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1. INTRODUCTION

Currently, the cultivation of herbs and aromatic plants to derive essential oils (EOs) is greatly increasing primarily due to the expanding demand generated by the food, pharmaceutical, and cosmetics industries (Lubbe and

Verpoorte, 2011). Among medicinal plants, the production of mint (*Mentha* species) is very agriculturally profitable because of a large number of practical applications (Souza et al., 2014).

Mentha (M; Lamiaceae) is a well-known genus including approximately 30 reported species that grow across the world in temperate areas (Trucker and Naczi, 2007). The most common species of *Mentha* include *M. aquatica*, *M. arvensis*, *M. citrata*, *M. longifolia*, *M. piperita*, *M. pulegium*, *M. rotundifolia*, and *M. spicata* (Anwar et al., 2019) which are widely used in savory dishes, food, beverages, and confectionary products (Tafrihi et al., 2021). These plants exhibit a great chemical diversity with respect to their EOs and important biological activities (Trucker and Naczi, 2007) which are used in management of plant pathogens and insect pests, in traditional medicine, as well as in culinary and cosmetics (Singh and Pandey, 2018). In general, EOs are a highly volatile lipophilic mixture produced in plant secondary metabolism responsible for plant protection and communication (Saeed et al., 2022). At the laboratory scale, steam distillation and hydrodistillation are the most commonly used methods for their extraction (Ribeiro-Santos et al., 2018).

Mentha arvensis, popularly known as corn mint, wild mint or field mint (Nazim et al., 2020), has unique importance among the mint family due to its high concentration of menthol (Thawkar, 2016) ranging up to 71.40% (Pandey et al., 2003). In effect, menthol has antiseptic, carminative, refrigerant, stimulative, and diuretic properties (Thawkar et al., 2016). Other primary compounds of *M. arvensis*, responsible for its typical aroma, are menthone and its isomers, menthyl esters, and piperitone (Trucker and Zarowin, 2006). Generally, essential oil obtained from *Mentha arvensis* (MEO) is yellow in color with a very strong and persistent odor of mint (Makkar et al., 2018). Its chemical profile is affected by many factors, such as environmental and cultivar conditions, soil nutrients, humidity, temperature, and biotic and abiotic stress (de Sousa Barros et al., 2015). Regarding its biological properties, MEO was found to be a potential candidate for antimicrobial (Bokhari et al., 2016; Bibi et al., 2021), antioxidant (Benabdallah et al., 2018), and fungicidal activities (Makkar et al., 2018).

In this report, the antifungal activity of MEO and its possible application as a bio-preserver of carrots were evaluated. For a detailed description of the EO, its chemical profile and antioxidant properties were also taken into consideration.

2. MATERIAL AND METHOD

2.1. Essential Oil

Mint EO (MEO; *Mentha arvensis*) was extracted by steam distillation of flowering stems. This EO was obtained by a commercial producer Hanus Ltd. (Nitra, Slovakia), and was preserved at 4 °C in the laboratory refrigerator until their next application.

2.2. Chemical Analysis

The chemical composition of the MEO was analyzed using gas chromatography with mass spectrometry (GC-MS), as it was described by Valková et al. (2022a). In brief, the analysis was carried out by Agilent Technology 6890N (Agilent Technologies, Santa Clara, CA, USA) coupled to

quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA). Separation of compounds was carried out using HP-5MS capillary column (30 m × 0.25 mm × 0.25 m). The temperature program was as follows: 60 °C to 150 °C (increasing rate 3 °C/min) and 150 °C to 280 °C (increasing rate 5 °C/min), using helium 5.0 as the carrier gas with a flow rate of 1 mL/min. Samples of essential oils were dissolved in pentane, and injection volume was 1 µL. The split/splitless injector temperature was set at 280 °C. The investigated samples were injected in the split mode with a split ratio at 40.8:1. Electron-impact mass spectrometric data (EI-MS; 70 eV) were acquired in scan mode over the m/z range 35–550. The mass spectrometry ion source temperature was 230 °C, while the temperature of MS quadrupole was set at 150 °C. Solvent delay time of 3 min. After the separation, the components were identified based on the comparison of their relative retention index and compared with the library mass spectral database (Wiley and NIST databases). The percentage composition of compounds (relative quantity; amounts higher than 0.1%) was measured based on the peak area. The retention indices were experimentally determined by injection of standard n-alkanes (C6–C34) under the same chromatographic conditions.

2.3. Determination of MEO Antioxidant Activity

To measure the antioxidant activity (AA) of MEO, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used, as previously described by Galovičová et al. (2021). The AA was expressed as the percentage of DPPH inhibition, which was calculated using the following equation: $(A_0 - A_1)/A_0 \times 100$; where A_0 was the absorbance of DPPH and A_1 was the absorbance of the sample. The power of AA was recognized as follows: weak (0–29%) < medium–strong (30–59%) < strong (60 and more %). Moreover, the value for total AA was expressed according to the calibration curve as 1 µg of the standard reference Trolox to 1 mL of the MEO sample (TEAC).

2.4. Evaluation of MEO Antifungal Potential

2.4.1. Fungal Strains and Culture Media

In the current study, three strains of genus *Penicillium* (*P. expansum*, *P. crustosum*, *P. citrinum*), isolated from berry samples of *Vitis vinifera* were employed. Consequently, the microscopic filamentous fungi were classified using a reference-based MALDI-TOF MS Biotyper, and validated by comparison with the taxonomic identification using 16S ribosomal RNA (16S rRNA) gene sequences analysis.

2.4.2. In Vitro Antifungal Activity of MEO

Evaluation of the *in vitro* antifungal activity of the EOs was performed using the agar disc diffusion method, according to Valková et al. (2022a). For this purpose, an aliquot of 100 µL of culture media was inoculated on SDA. To prepare culture media, the strains were inoculated in Sabouraud Dextrose Agar (SDA; Oxoid, Basingstoke, UK) and incubated for 5 days at 25 °C. Subsequently, small aliquots of the fungi were transferred to test tubes, each containing 3 mL of distilled water. The inoculum concentration was

standardized by comparison with the 0.5 McFarland scale (1.5×10^8 CFU/mL). After that, the discs of filter paper (6 mm) were impregnated with 10 μ L of MEO sample (in four concentrations: 62.5, 125, 250, and 500 μ L/L), and applied on the SDA surfaces. Fungi were incubated aerobically at 25 ± 1 °C for 5 days. After the incubation, diameters of the inhibition zones in mm were measured. The values for inhibitory activity increased in the following manner: weak antifungal activity (5 - 10 mm) < moderate antifungal activity (10 - 15 mm) < very strong antifungal activity (zone > 15 mm).

2.4.3. *In Situ* Antifungal Activity of MEO

All three fungal strains (*P. expansum*, *P. crustosum*, and *P. citrinum*) were used to evaluate the antifungal activity of the EOs *in situ*.

2.4.4. Food Model

Carrot was applied as substrates for the growth of the fungi. This vegetable was purchased at the local market (Nitra, Slovakia).

2.4.5. Moisture Content and Water Activity of Food Model

To predict the suitability of substrates for fungal growth, moisture content (MC) and water activity (a_w) were determined, as reported by Valková et al. (2022a).

2.4.6. Vapor Contact Method

The experiment itself was performed as reported by Valková et al. (2022a). Firstly, sliced carrot (5 mm) was placed on the bottom of Petri dishes (PDs), and the inoculum was applied by stabbing one time with an injection pin on the vegetable surface. Further, 10 μ L of the MEO (in the same four concentrations) was applied on the sterile filter paper disc (60 mm), then, it was placed at the top of PD. Subsequently, PDs were hermetically closed using parafilm and cultivated at 25 °C for 14 days.

2.4.7. Determination of Fungal Growth Inhibition

In situ fungal growth was determined using stereological methods. In this concept, the volume density (V_v) of visible fungal colonies was firstly established using ImageJ software counting the points of the stereological grid hitting the colonies (P) and those (p) falling to the reference space (growth substrate used: bread, carrot, and potato). The volume density of strain colonies was consequently calculated as follows: V_v (%) = P/p. Finally, the antifungal potential of the EOs was expressed as the percentage of fungal growth inhibition (FGI) according to the formula $FGI = [(C - T)/C] \times 100$, where C and T is the growth of fungal strains (expressed as V_v) in the control and treatment group, respectively (Valková et al., 2022a).

2.5. Statistical Analysis

The data were submitted to one-way analysis of variance (ANOVA) and the means were compared by the Tukey test

at 5% of probability using statistical software Prism 8.0.1 (GraphPad Software, San Diego, CA, USA). All analyses were performed in triplicate.

3. RESULTS

3.1. Chemical Profile of MEO

GC-MS analysis revealed that a total of 42 substances, accounting for 99.5% of the whole constituents, were identified in the MEO chemical composition. The major compounds were shown to be menthol (37.3%), menthone (17.4%), neo-menthol (14.1%), and 1,8-cineole (4.9%), as presented in Table 1.

Table 1. Chemical composition of MEO

NO	RI ^a	Compound ^b	% ^c
1	926	α -thujene	0.1
2	938	α -pinene	2.3
3	948	camphene	0.3
4	977	sabinene	0.5
5	980	β -pinene	1.5
6	992	β -myrcene	0.4
7	993	3-octanol	0.6
8	1004	α -phellandrene	tr
9	1016	α -terpinene	0.1
10	1023	p-cymene	1.0
11	1028	α -limonene	3.6
12	1033	1,8-cineole	4.9
13	1047	(<i>E</i>)- β -ocimene	tr
14	1060	γ -terpinene	0.5
15	1088	α -terpinolene	0.4
16	1148	isopulegol	1.7
17	1151	menthone	17.4
18	1160	pinocarvone	1.2
19	1162	iso-menthone	1.0
20	1164	neo-menthol	14.1
21	1170	borneol	0.2
22	1173	menthol	37.3
23	1189	α -terpineol	0.6
24	1217	trans-carveol	0.1
25	1229	cis-carveol	tr
26	1239	pulegone	0.9
27	1241	carvone	0.2
28	1253	3-carvomethenone	1.0
29	1254	(<i>Z</i>)-anethole	0.4
30	1276	<i>p</i> -pent-1-en-7-al	5.2
31	1289	(<i>2E</i>)-hexenyl valerate	0.3
32	1297	menthyl acetate	tr
33	1298	<i>p</i> -menth-1-en-9-ol	tr
34	1378	α -ylangene	tr
35	1379	α -copaene	tr
36	1385	β -bourbonene	0.2
37	1388	β -elemene	tr
38	1422	(<i>E</i>)-caryophyllene	0.8
39	1443	aromadendrene	tr
40	1483	germacrene D	0.2

Table 1. Chemical composition of MEO (continue)

41	1525	δ-cadinene	0.4
42	1583	caryophyllene oxide	0.1
Total			99.5

Note: ^a Values of retention indices on HP-5MS column; ^b Identified compounds; ^c Percentage of identified compounds; tr - compounds identified in amounts less than 0.1%

3.2. Antioxidant Activity of MEO

It was found that values for AA of the MEO were $195.00 \pm 5.30 \mu\text{g TEAC.mL}^{-1}$, with $22.8 \pm 1.2\%$ free radical-scavenging inhibition linked to a weak AA.

3.3. In vitro Antifungal Activity of MEO

Results from the antifungal effects of MEO against *P. expansum*, *P. crustosum* and *P. citrinum* are shown in Table 2. It can be concluded that the growth inhibition of fungi strains significantly depends ($P < 0.05$) on the concentration of the MEO used. Concretely, moderate antifungal activities (12.13 ± 0.48 mm, 11.56 ± 0.86 mm, and 12.33 ± 1.14 mm) were observed at the highest concentration (500 $\mu\text{L/L}$) of MEO against the growth of *P. expansum*, *P. crustosum* and *P. citrinum*, respectively. On the other hand, 125 $\mu\text{L/L}$ and 250 $\mu\text{L/L}$ concentrations of MEO showed weak antifungal effects against all evaluated strains; whereas the lowest concentration of MEO resulted in only a very low inhibitory efficiency.

Table 2. In vitro antifungal activity of MEO in analyzed concentrations (inhibition zones in mm)

Fungi	MEO ($\mu\text{L/L}$)			
	62.5	125	250	500
<i>P. expansum</i>	2.88 ± 0.55^a	5.92 ± 0.39^b	8.13 ± 1.25^c	12.13 ± 0.48^d
<i>P. crustosum</i>	4.36 ± 0.78^a	6.23 ± 0.44^b	7.89 ± 0.96^c	11.56 ± 0.86^d
<i>P. citrinum</i>	3.71 ± 0.61^a	5.84 ± 1.06^b	8.56 ± 1.01^c	12.33 ± 1.14^d

Note: Mean \pm standard deviation. MEO - Mint essential oil. Values in the same line with different small letters are significantly different ($P < 0.05$).

3.4. Moisture content and water activity of carrot

The results from the moisture content (MC) and water activity (a_w) measurements showed that the parameters of carrot in our study had values of $82.80 \pm 2.33\%$ and 0.959 ± 0.001 , respectively.

3.5. In situ antifungal activity of MEO

The antifungal effectiveness of MEO on the growth of the *Penicillium* spp. inoculated on carrots are demonstrated in Table 3. From the findings it is clearly evident that with an increasing concentration, the MEO exhibited an enhancing

antifungal effects against all analyzed strains, with the strongest one in the highest concentrations (500 $\mu\text{L/L}$).

Table 3. In situ antifungal activity of MEO on carrot.

Fungi	MGI (%)			
	MEO ($\mu\text{L/L}$)			
	62.5	125	250	500
<i>P. expansum</i>	28.57 ± 4.31^a	88.89 ± 5.12^b	89.09 ± 6.13^b	98.08 ± 4.78^c
<i>P. crustosum</i>	-5.41 ± 7.35^a	3.68 ± 2.93^a	72.04 ± 6.71^b	98.43 ± 4.88^c
<i>P. citrinum</i>	-23.33 ± 6.09^a	71.81 ± 8.12^b	96.92 ± 5.13^c	100.00 ± 0.00^c

Note: Mean \pm standard deviation. MEO - Mint essential oil. Values in the same line with different small letters are significantly different ($P < 0.05$). The negative values indicate a profungal activity against *Penicillium* strains.

4. DISCUSSION AND CONCLUSIONS

Generally, the biological potencies of plant EOs were attributable to their chemical composition and especially to their major substances (Kasrati et al., 2015). Therefore, the detection of individual volatile components, which we implemented in our study, is an important tool for knowing the effect of EOs. In line with our findings Pandey et al. (2008), Chagas et al. (2020), and Mahn and Tuyet (2020) detected the major substance in EO obtained from *M. arvensis* menthol (71.4%, 86.1%, 66.04%, respectively). However, in our analyzed MEO was presented in lower concentration (37.3%). A similar lower concentration of this substance (21.35%) was also confirmed by Khan et al. 2019. The authors also find high content of menthone (29.42%) in its conception which also creates a high concentration of our oil sample (17.4%). We assume that differences in the percentage of the chemical components in MEO between mentioned studies may be related to varying cultivars of mint or different growing stages of plants (Verma et al., 2010).

DPPH assay is a widely employed procedure to estimate the free radical scavenging ability of materials due to its simplicity and rapidity (Gudimella et al., 2021). This method is based on the reduction of the commercially available radical (DPPH) and shows a color change from deep purple to pale yellow upon reaction (Higgins et al., 2021). Due to its properties, this technique is often used to analyze the antioxidant characteristics of EOs (Valková et al., 2022a,b,c). In our study, we found that despite the diverse chemical profile, MEO showed weak values for AA ($195.00 \pm 5.30 \mu\text{g TEAC.mL}^{-1}$, with $22.8 \pm 1.2\%$). Accordingly, in previous research works, we also noted weak antioxidant activity in green mandarin EO (Valková et al., 2021a), rosalina EO, fir EO and niaouli EO (Valková et al., 2022b). We propose that the weak AA of our MEO may be related to the high concentration of monoterpene and sesquiterpene hydrocarbons in its conception, which has low solubility in the assay medium and also does not have the ability to donate hydrogen atoms (Mata et al., 2007). These properties can be an essential limitation for the determination of the DPPH radical scavenging activity of some types of samples, including various EOs (Viuda-Martos et al., 2010).

Therefore, the choice of methodology largely affects the antioxidant activity of the samples. In the future, we plan to carry out the determination of antioxidant activity using several methods, including the FOMO and the ABTS methods.

Essential oils from *Mentha* spp. were screened for their antifungal activities (Saba and Anwar, 2018). Confirming our findings, Hussain et al. (2010) also detected the antifungal effects of *M. arvensis* EO against seven fungi strains including *Aspergillus* (*A.*) *flavus*, *Alternaria* (*A.*) *solani*, *Fusarium* (*F.*) *solani*, *Rhizopus* (*R.*) *solani*, *A. alternata*, *A. niger* and *Rhizopus* spp. Their results from the disc diffusion method indicated that MEO showed maximum antifungal activity with large inhibition zones varied from 16 to 30 mm against fungi strains. The antifungal efficacy of MEO may be related to its high menthol content, which we also detected in our study (37.3%). It is known that menthol exhibits antifungal effects against various fungi strains including *Candida* (*C.*) *albicans* (Piran et al., 2017), *Aspergillus* (*A.*) *niger*, *A. fumigatus*, *A. flavus*, *A. ochraceus*, *A. alternata*, *Botrytis* (*B.*) *cirenea*, *Cladosporium* spp., *P. citrinum*, *P. chrysogenum*, *F. oxysporum* and *Rhizopus oryzae* (Abbaszadeh et al., 2014). Although the exact mechanism of menthol action is not fully understood, its antifungal effect can be resulted from a perturbation of the lipid fraction of fungi plasma membrane, resulting in alterations of membrane permeability and in leakage of intracellular materials (Trombetta et al., 2005). Moreover, Samber et al. (2015) demonstrated the efficacy of menthol due to its integration with PM-H⁺ ATPase enzyme which possibility an electrochemical proton gradient across the cell membrane necessary for nutrient uptake. However, we assume that individual compounds of MEO and their interaction are crucial for their final inhibitory effects on mycelial growth. Therefore, the antifungal efficacy of our EO may be related to other volatile components present in its conception, as well.

MC and a_w largely influence the ability of microorganisms to grow on food products (Qiu et al., 2019). The presence of water in the form of MC is the major factor aiding the growth and activities of the microorganisms because it increases their metabolic activities. Without water or in the presence of a limited volume of water, agricultural products will become inhospitable to the microorganism and inhibit their growth (Rajeev et al., 2012). In this context, many fresh foods including vegetables are perishable due to their high MC (> 40%; Akdogan, 1999; Dagnas et al., 2017). Further a_w is defined as the ratio of the vapor pressure of water over a substrate compared to that over pure water at the same temperature and pressure (Cazier and Gekas, 2001). Concerning a substantial impact on the growth of microorganisms it was found out that a_w above 0.7 supporting the microbial spoilage (Syamaladevi et al., 2016). In line with our findings, similar values for MC ($82.80 \pm 2.33\%$) and a_w (0.959 ± 0.001) of carrot we detected in our last research ($86.83 \pm 0.42\%$; 0.945 ± 0.002 ; Valková et al., 2022c). Our results indicate the suitability of carrot as food model for *in situ* antifungal analysis of the MEO investigated.

Some studies have reported that vapor generated by EOs has a greater antifungal effect compared with EOs in liquid form applied by direct contact (Tullio et al. 2007; Fisher and Phillips 2008). Moreover, the vapor phase allows free attachment of EOs to microorganisms, unlike lipophilic molecules in the liquid phase associated to form micelles which restrain the attachment of EOs to microorganisms (Boukhatem et al., 2014). In this way, the vapor phase of EOs has a specific impact on fungi due to their superficial growth reflecting more susceptibility to EO volatile compounds (Edris and Farrag 2003). Furthermore, the composition of the food system impacts the antifungal effectiveness of EOs, and this activity is typically decreased in *in situ* conditions compared to *in vitro* ones. However, the low-fat content of vegetables can participate in the successful antifungal effects also on the food model (Burt, 2004). Therefore, in the current study, this effect has been investigated for carrots as food substrates. In accordance with our results, in our previous study we noted the antifungal effect of MEO obtained from *M. piperita* against the same fungi strains (*P. expansum*, *P. citrinum*, and *P. crustosum*) inoculated on bread (Valková et al., 2021b). In both cases, the effect of essential oils on food models was dependent on their concentration, with the highest efficiency recorded at the highest concentrations (500 $\mu\text{L/L}$), with the exception of *P. citrinum* inoculated on bread (Valková et al., 2021b), when the highest efficiency was recorded at concentrations 125 $\mu\text{L/L}$. Moreover, our findings are in accordance with our previous studies, in which the antifungal efficacies of various types of EOs, such as mandarin EO (Valková et al., 2021a), coriander EO (Kačaniová et al., 2020), fir EO, rosalina EO, and niaouli EO (Valková et al., 2022c), against the same fungi species analysed were confirmed.

From the results of all our analyses, it can be concluded that MEO may be a promising agent with potential use for extending the shelf-life of vegetables including carrots on the commercial scale of the food industry.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: V.V., H.Đ., M.K.; Investigation: V.V., H.Đ., L.G., N.L.V., M.V., M.K.; Material and Methodology: V.V., H.Đ., L.G., N.L.V., M.V., M.K.; Supervision: M.K.; Visualization: V.V., H.Đ.; Writing-Original Draft: V.V., H.Đ.; Writing-review & Editing: V.V., H.Đ., M.K.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Challenges in Lecturing Sport and Exercise Technology using Online Platforms during the COVID-19 Pandemic

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Abstract: This article details the development of transitioning of in-person diploma lecturing to online lecturing through the course of the COVID-19 pandemic. Due to the practical components of the course, specific challenges emerged involving course design and practical learning. This article considers the problem-solving process and strategies for remotely teaching, discussing and experiencing the global exercise trend. Specifically, accessibility, adjustments and attitudes as important pedag.

Keywords: COVID-19, Sport and Exercise Technology, accessibility, adjustments, attitudes.

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1. INTRODUCTION

One of the challenges faced in the pedagogy of higher education is our tendency to cling to habits, rituals, and routines. Lecturers tend to attribute students' lack of success to a deficit in their ability to cope with various delivery modes. The core objective of the Sport and Exercise Technology Diploma programme is to inspire conscious and physically active students, enabling them to think for themselves about different procedures and which procedure to use when working with clients. The overnight digitalisation of teaching and learning has had a major impact on access to education for students (Ivemark & Ambrose, 2021).

A once theoretical and practical diploma, where students had lectures face-to-face, followed by a visit to the laboratory, gymnasium or field for practical's, had overnight changed into a completely digitalised program, which meant no face-to-face lectures or practical's. With this sudden change in the mode of delivery, the staff lecturing the Sport and Exercise Technology Diploma programme only had a fortnight to figure out new methods to be implemented as the 'new normal', for the diploma had a major practical component which still needed to be attended to. The global Coronavirus pandemic also known as COVID-19 pandemic forced staff to look for other methods beyond the normal face-to-face method that has been implemented thus far. Both lecturers and students were required to migrate to a pedagogy

dialogically platform with specific times, spaces, and technologies available.

Stabilisation of the academic programme in the wake of COVID-19 was prioritised, to be able to continuously deliver the curriculum. Furthermore, an online technological solution was implemented by the University to assist students and lecturers to continue with the curriculum. While implementing the 'new normal', students' readiness to cope with online lecturing became the focus of course designers and managers. However, a major problem faced by students in South Africa is poverty and digital dis-connectivity, which is yet to be addressed in a highly disjointed social system (Maringe, 2017). The most important questions posed regarding the students changing to the 'new normal' were, how would the students embrace the shift to alternative pedagogical modes of delivery? what forms of adjustments were needed? and are there orientation sessions required? Furthermore, how would this new pedagogical mode influence our own attitude towards our new roles and responsibilities as higher education curriculum specialists and designers of our own pedagogy for future practice?

This study reflects on the adaptabilities of the lecturers, to optimally lecture and teach the Sport and Exercise Technology Diploma programme in the middle of the COVID-19 pandemic, and how the online mode of delivery was embraced through the use of Moodle, the official University of Zululand's online teaching and learning platform. Furthermore, this article centres three main

concerns, the conceptualisation of accessibility; emphasising adjustments towards the ‘new normal’; and concluding with a view on shifts in attitude to continue with quality higher education teaching.

2. MATERIAL AND METHOD

2.1 Search strategy

To ensure that a thorough review of literature was performed with regards to the challenges in lecturing a variety of programs using online platforms, an electronic search was conducted using the following databases: CISTI Source (2018–June 2022), International E-Catalogues, Current Contents, Science Direct, Cochrane Database of Systematic Reviews, EBM Reviews, PubMed (2018–June 2022), and Google Scholar. A keyword search yielded MeSH headings: “teaching”, “learning”, “teaching using online platforms”, “learning using online platforms”, “teaching and learning”, “teaching and learning during the online pandemic”, “accessibility to online platforms”, “adjustments to teaching and learning to online platforms”, “attitudes of using online platforms for teaching and learning”, which were fused and exploded. The searches were limited to peer-reviewed articles written in English. For the literature review and discussion, original articles were identified and grouped.

2.2 Data extraction

All the data that was relevant to the study was collected and compiled by the first author, which included the challenges in lecturing a variety of programs using online platforms. Additionally, research that clearly did not meet the inclusion criteria was excluded, and whenever there was an abstract from which a firm decision could not be made, the research was provisionally included for full-text analysis. Furthermore, the first author was also responsible for checking the eligibility of research papers for inclusion based on an analysis of full-text papers After which final validation was performed by at least one of the co-authors to make a final choice on inclusion. Any issues that were raised with regards to the inclusion of a paper was debated until a and agreement was reached.

Each study included was classified as being relevant to the challenges in lecturing a variety of programs using online platforms and was based on the journal or conference they were published in, as well as the associated keywords. The extraction of the data collection information was then performed. Window selection was used in order to effectively extract the data, after which analysis techniques and spatial aggregation features were used to review the contributions of all studies to the components of feature construction and modelling & analysis. Additionally, the problem definition or goal of the study was always kept in mind when extracting information to review the interpretability of all included studies. Lastly, a single framework was used to categorize all of the findings, which in turn will give context for our literature review and discussion.

3. RESULTS

This study used 9 full-text English-language papers from 105 citations found through electronic searches.

Table 1. Challenges in Lecturing Sport and Exercise Technology using Online Platforms

Challenges:	Description:	References:
Accessibility	Even though there was a wide spread of internet connectivity and devices for teaching and learning, it’s still found to be lacking in many households, especially in low-income households.	Bower & Chambers, 2017); Hubackova & Semradova, 2016); (Jowsey et al., 2020) (Woo et al., 2008); (Anderson & Kumar, 2019); (Asch, 2020); (Bali & Liu, 2018); (Ashour, et.al. 2021);
Adjustments	Adjustments had to be made by both lecturer and student once the pandemic started to impact the academic year in 2020. The government enforced lockdown regulations, which included student attendance, traveling limitations and no mass gatherings, which made face-to-face contact impossible.	(Godber & Atkins, 2021); (Murgatrod, 2020) (Rodríguez-Triana et al. 2020); (Nordmann et al. 2020)
Attitudes	Lecturers treats the new form of pedagogy as temporary, as they believe it will only last for as long as Covid-19 is still around. Even though the learning approached goals were experienced as a positive challenge for lecturers, and useful for competence development, there is still a sense of avoidance of goals in situations that were seen as threatening.	(Chandwani et al, 2021); (Lee et al, 2015); (Emmanuel, 2020); (Akcil & Bastas, 2021); (Algahtani, 2011); (Edwards & McKinnell, 2007)

4. DISCUSSION AND CONCLUSIONS

4.1. Accessibility

In 2019, the world was hit by the COVID-19 pandemic. In March 2020, South Africa started to experience the effect of the pandemic, which was a major turning point for a lot of universities. Students and lecturers that has never used online teaching, suddenly overnight had to become a custom with the ‘new normal’, as face-to-face teaching and learning was not possible due to most countries going into a lockdown period (Ashour, et al., 2021). Suddenly, the students that were used to teaching and learning taking place in person was forced to change into a distance learner. Even though the use of online teaching for higher education is becoming more popular in Universities (Woo et al., 2008), there were

immediate red flags, which raised concerns. According to Anderson & Kumar (2019), even though there was a wide spread of internet connectivity and devices for teaching and learning, it's still found to be lacking in some majority households, especially in low-income households. We also experienced this with our Sport and Exercise Technology students, as some of our students were living in un-urban areas and connectivity was a major concern. Connectivity will be available one minute and not available the next. Some students also mentioned that they don't have laptops or tablets to access the nodes, which made real time or synchronous online teaching very difficult in the beginning of the pandemic. According to Asch (2020), lecturers started questioning if it's fair to hold students that is only familiar with face-to-face teaching accountable for deadlines as some students were unable to access essential course work and materials. Additionally, the pedagogical changes embrace the need for students to take ownership for their own learning needs to assert agency over the curriculum (Bowyer & Chambers, 2017; Hubackova & Semradove, 2016; Jowsey et al., 2020). Furthermore, Bali & Liu (2018), also found that online courses offer less opportunities for interaction with other students and with the lecturer. With this said, we discussed the best way to assist the students taking in account connectivity and device problems. Some students did have connectivity and devices to access course material and synchronous online teaching, which enabled us to have live classes for the students in their normal timetable slots (timetable according to face-to-face classes). For the students that had interrupted connectivity, device problems or both, we developed voice embedded PowerPoints and voice embedded video PowerPoints. Other challenges followed, including that that some students also struggled to have accessed to Moodle. Fortunately, in these cases we developed WhatsApp groups for each module, where we posted all course work and PowerPoints, to give accessibility to all the students. As it was a requirement off the University to use the Moodle platform for tests, assignments and examinations, the students needed to gain access to Moodle. As we still had to consider that some students might have interrupted connectivity, we made all our assessments over two days and two attempts in case they lose connectivity while attempting the assessment. To stop students from assisting one-another, we added criteria to the assessments where there will be a time limit once they have logged in and all questions were randomly selected and shuffled by the system for each student.

4.2. Adjustments

Adjustments had to be made by both lecturer and student once the pandemic started to impact the academic year in 2020. The government enforced lockdown regulations, which included student attendance, traveling limitations and no mass gatherings, which made face-to-face contact impossible. This section will discuss the range of adjustments that the lecturers had to make, to make teaching and learning possible. This includes the pedagogical activities designed in preparation for the course, prior to and during the modules, and additionally placing attention on negotiating with students with regards to their own personal and public technological literacy spaces (Rodríguez-Triana et al. 2020; Nordmann et al. 2020). According to Godber &

Atkins (2021), the emergency shift to a remote teaching environment was complicated, as the response to a crisis's satiation has little resemblance on developing your own online teaching and learning platform. Furthermore, Murgatrot (2020), found that there were some challenges that came with the sudden, unplanned e-learning responses to a global pandemic. The following changes has been highlighted by research, flexibility, life-long learning, affordability, educational policy, accessibility, and the learning pedagogy. To make it slightly easier on our staff and to enable them to optimally prepare for their modules, the University started to offer a series of *staff-capacity development workshops* which were offered to assist lecturers to embrace online, blended learning approaches, and to be digitally flexible. Although there was the option of attending these developing programmes, a collective decision was made to attend as many courses as possible to assist ourselves, one another and to be able to assist the students to the best of our capabilities. Some courses we attended utilized the Zoom program to get their information across. The use of Moodle was also encouraged as a more interactive pedagogy, to redesign alternative assessment strategies online. Attending these online courses expanded our confidence to use these alternative models of delivery effectively. Due to us being placed in the role of the student, it allowed us to gain first-hand experience as to the vulnerabilities they had to address, as we had to start embracing the new learning and teaching modalities ourselves. We used our own pedagogical and curriculum design experiences to harness the technological potential of these new digital means. We collaborated with one another discussing what we have learned from these courses and what the best step forward will be to improve the online teaching methods. A realisation occurred during which we found that we also had to incorporate this kind of adjustments and connecting of our students' potential. Overall, we found that the pedagogical space became progressively more relaxed due to the focus being shifted from the technology towards foregrounding interest in learning deeply from the interaction of the learning/teaching moment. With this said, we found that the students were more present if we had online classes, and not merely to mark the attendance register as some did with face-to-face classes. Overtime, the students started adjusting themselves to see this space not just as a performance space where they were assessed, but also their own "technological space". Due to us being the facilitators of the teaching project, our personal experience became essential in noting that all learners move in and out of participation (due to different reasons). Being the hosts of the platform allowed us to "haul them back into the classroom interactivity learning mode". This caused students to become more alert, since they were unlikely to predict when they would be chosen to participate in a certain section of the content. We did this, as we found that some students in the early online classes will log on into a class with their cameras off and disappear. This highlighted the fact that the most important activity that ensures quality learning is not based on the technology alone, but the lecturer is essential in mediating that technology for his or her learners.

Although the global pandemic did force countries to adjust online teaching and learning at a rapid pace, some positives

did develop from this. We as lecturer's and our students were forced to learn a 'new skill', using new digital platforms and systems like Google Classroom, Microsoft Teams, Zoom, Moodle and social media (WhatsApp groups) in a short period of time, which most likely would still have been pushed to the side if not for the pandemic. Furthermore, the pandemic forced us to think out the box and create initiatives to overcome some of the limitations of virtual teaching.

4.3. Attitude

Due to the COVID-19 pandemic, institutions all over the globe were forced to ensure that learning does not get altered, which endorsed online teaching. Online teaching has been considered as the best substitution for lecturing learners across different places (Chandwani et al, 2021). Although, online teaching has been found to be the best alternative to face-to-face teaching, the 'new normal' cannot replace face-to-face teaching, as some modules will always have a practical component and in case of a professional course (Chandwani et al, 2021). Many lecturers seem to approach the shift towards the new form of pedagogy as a temporary measure to deal with the COVID-19 (Akcil & Bastas, 2021). The learning approach was found to be directly linked to the positive experience of online teaching and learning, while competence development as well as performance avoidance goals were seen as threatening to these learning situations. The lecturers who suffered burnout experiences, and provided learning opportunities of less quality (as assessed by student), were the ones whom felt threatened by online teaching during the Covid-19 pandemic. Training for online teaching became a priority for the University to assist the staff, as some staff felt that the online teaching made them negative and that they don't know what the next step will be (Algahtani, 2011; Edwards and McKinnell, 2007). According to Lee et al. (2015), a negative attitude towards e-learning had a 180-degree shift once staff started doing training and started developing and understanding the different digital platforms and systems like Google Classroom, Microsoft Teams, Zoom, Moodle and social media (WhatsApp groups). Furthermore, communication on new developments and new online courses from the faculty also played a major role to decrease stress and anxiety, which developed a positive attitude amongst the lectures that was still in two minds about online teaching.

As with South Africa, other countries experienced that a large part of the population does not have access to smart devices. Learners overnight suddenly needed smart devices to meet their academic needs, which made this a major problem for parents and guardians with low-income jobs. Learners experienced the online teaching similar to the lecturers lecturing them, as some found it easy and others a nightmare and wished the days past to be back at the University. This is seen by the unfortunate report of a girl, in Malappuram district of Kerala which committed suicide due to her inability to attend online classes (Emmanuel, 2020). Furthermore, Chandwani et al, (2021) reported of a 15-year-old student in Western Assam's Chirang district, which allegedly committed suicide, after he failed to attend online classes and examinations in the absence of a smart phone. With these tragic incidences, we can see to what extent the

smart divide has become, for uniform delivery of e-learning amongst learners around the world.

5. CONCLUSION

This article aimed to give major insight to the variety of methods that was used during the COVID 19 pandemic by a group of lecturers from the Sport and Exercise Technology diploma program. The COVID 19 pandemic has indeed been a revolutionary destabilising of the routines of our everyday world of university education. It has assisted lecturers and students in reassessing the values we hold dear and allowed us to re-question the foundational principles which underpin our practices. It has allowed us to test a variety of teaching methods that was not being used during normal face-to-face teaching. On a mundane level, it has required that university lecturers find a balance between the technology, their pedagogy and the content knowledges of our present times. But on a more profound level, this operational world will soon become unravelled by the dawn of a future era of multiple uncertainties and pluralities of technological revolutions. This will force both lecturers and students to come of age and use the variety of online tools at their disposal. We are rebuilding our beliefs, rethinking the knowledge we have gained over the years, as well as acknowledging our levels of under-preparedness to tackle the new world of a technological revolution. We have to re-educate ourselves to be willing to adapt when we do not know what to do or what the future will hold. Our success will not lie in whether we graduate hordes of existing graduates, or how we prop up our nationalistic or disciplinary pride or geographic xenophobic or culturally bounded prejudices, but by making sure that quality education is provided to the graduates that need to transition successfully into the work force.

5.1. Epilogue

The COVID 19 pandemic has forced lecturers and students to move into the future of teaching and learning at a very rapid rate. We acknowledge that not all parties involved were ready for the changes, however adjustments need to be made, as this will be how teaching and learning will be conducted in years to come. Whether conditions will ever return to normal remains to be seen, however by ensuring that the foundation is laid in terms of accessibility to online tools, as well as have the willingness to make the necessary adjustments, while doing so with the correct attitude, both the lecturer and the student will be ready for anything that happens in the future.

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Characterization Of Soil For Road Shoulders Mixed With Reclaimed Asphalt Pavement Waste

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Abstract: The use of recovered asphalt pavement can reduce the amount of new bitumen and aggregates used in pavement construction and rehabilitation (RAP). RAP is a waste product that results from the removal of an old or damaged pavement surface. Although it has been used since the 1970s, and numerous recommendations for using RAP in the new mixture have been made, there are only a few research available. Because the materials used are recycled, it is also cost-effective and environmentally friendly. The purpose of this study is to characterize soil that has been blended with reclaimed asphalt pavement (RAP) for use on the local road shoulder. RAP is one of the rehabilitation procedures used to repair a deteriorated surface by removing the upper pavement and replacing it with new pavement. According to earlier study, mixing dirt with different materials improves the finding. The goals of this study are to establish the material qualities of soil and RAP, determine the optimum moisture content of material and degree of compaction for road shoulders, and determine the appropriate mix proportion of soil and RAP for road shoulders using the California Bearing Ratio (CBR) test. The Atterberg limit, liquid limit, and plastic limit for soil were evaluated on a sample of soil and RAP. For soil and RAP, a sieve analysis was performed. Compaction tests for soil and RAP were carried out with a mixture of 10S, 2S8RAP, 4S6RAP, 6S4RAP, 8S2RAP, and 10RAP, as well as CBR tests for soil and RAP. . According to the results of the laboratory test, 20% of RAP (8S2RAP) had a better mixed proportion for the road shoulder. As a result, repurposed materials like as RAP can be used as a road shoulder material.

Keywords: Reclaimed, Asphalt, Soil, Road.

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1. INTRODUCTION

Since the events of September 11, 2001, there has been a street framework in Afghanistan connecting Kabul with the province's essentially alternate urban regions. The efforts to improve the street system have been done appropriately and through the quick improvement arrangement, particularly the provinces Plan at regular intervals which were promoted by the Afghan government. After the US force entered Afghanistan, the world paid attention to Afghanistan reconstruction.

The US and NATO governments spend a lot of money building streets in Afghanistan in accordance with requirements, advance planning, and street solidness requirements that depend on a variety of factors. The flexible asphalt is typically recommended for use for ten to fifteen years in asphalt plans. In order to ensure quality and improve

the street's usability, the recovery and support activities are absolutely essential.

Nowadays, the development sector offers maintainability a more significant attention. The methodologies used in development, the planning processes, the technology, and the materials used in development are only a few of the many factors that contribute to economic progress. As technology and the use of conventional resources grow, so do interest in bitumen and the overall cost of material development.

Asphalt pavement reuse has become a common activity in the transportation industry. The environmental, financial, and societal benefits are frequently cited as motivations for reuse. By wisely using common resources, the use of Reclaimed Asphalt Pavement (RAP) in the construction of new roads aligns with the broader goal of sensible transformation (Hoppe et al., 2015).

Bituminous pavement recycling is not a novel concept, but it is one of the options that supports sustainability while also lowering construction and material costs. The 1970s saw advances in recycling machinery and technology. It lowered the amount of waste that was routinely dumped into landfills, maintained the environment, reduced building costs, and improved the efficacy of the pavement overall (Asphalt Recycling and Reclaiming Association, 2001).

Using salvaged asphalt pavement is one of the common ways to recycle bituminous materials (RAP). RAP, or reclaimed asphalt pavement, was taken out of service via milling or full-depth removal. In view of the rising expense of asphalt and the necessity to protect the environment, using RAP has been preferred over using virgin materials. The use of recycled asphalt also lessens trash generation and aids in the solution of the issue of how to dispose of materials used in highway building.

1.1 Background of study

In order to meet the needs of road users, there is a significant need for new roads to be built as well as upgrades to existing ones. This expansion in road building and rehabilitation improves the workability of roads while accelerating socioeconomic development. Road construction has significantly aided in economic expansion and the eradication of poverty (Fan and Chan-Kang, 2005).

But one issue that frequently arises during road building and needs to be changed or repaired is pavement deterioration. As a result, road surface development, upkeep, and restoration are issues that are becoming more and more crucial both in Slovenia and around the world. In addition to the volume of traffic, additional elements that affect the longevity of roads and their durability include local weather conditions, road design, and the quality and performance of the materials used in their construction (Ossa et al., 2016).

Due to this circumstance, the road repair and reconstruction business has supported the disposal of road trash, consumed vast amounts of raw materials, and produced enormous volumes of waste while building and demolishing roads, walkways, and bridges. The main components of this waste are bitumen grinding, aggregate, and mortar.

1.2 Problem statement

Our natural resources have been plundered in recent years by the construction industry, which inevitably led to environmental damage. If there is constant demand for construction materials, which causes the depletion of natural resources and an increase in waste materials, contractors who lack environmental awareness have irreparably harmed the environment by disposing of waste materials.

In order to withstand expected loads over the course of the road's design life, pavements are specifically constructed for them. Concrete pavements come in a variety of types, from flexible to semi-stiff to hard. The pavement will erode and sustain surface damage after a number of years of use. In order to get out of this condition, restoration and maintenance can halt but not stop pavement deterioration.

It has been determined that recycling bituminous pavement materials is the best way to reduce the use of natural resources and address the problems associated with waste disposal. RAP can now be added to the combination for the first time, according to numerous research. For instance, only a few locations in the US have effectively implemented RAP in the on-site pavement mixing (Federal Highway 4 Administration, 2016).

However, researches continue to come to differing conclusions about the performance of RAP, particularly with the proper dosage of RAP to be added to the combination. For instance, Yang and Lee (2016) recommended that RAP make up less than 25% of the whole combination, although other researchers asserted that with correct handling, larger RAP content might still perform as well as the conventional mixture (Poulikakos et al., 2014).

By adding or replacing components in the current pavement structure, rehabilitation is required to address the consequences of deterioration. Resurfacing is one of the pavement rehabilitation techniques that involves adding new materials to an existing surface in order to strengthen the surface's structural integrity and enhance the way it rides.

Milling waste is the byproduct of renewing pavement or wearing course. By carrying out this resurfacing, a new issue will develop since milling debris is seen as a waste product from work done to restore the existing pavement. Because milling waste is a hazardous waste and cannot be disposed of, it poses a problem for road building and rehabilitation. The additional issues with milling are piles of waste at specific construction sites and dumping by the side of the road. According to this study, recycling milling waste for use in road shoulders will lessen roadside dumping and the stockpile of milling waste.

1.3 Aim and objectives

The following objectives of this investigation were to determine the bituminous mixture:

- i. To determine the soil and RAP's material qualities.
- ii. To determine the ideal material moisture content and level of compaction for the road shoulder.
- iii. To determine the optimum mix proportion under California Bearing Ratio (CBR) test for road shoulder.

1.4 Scope of the study

This study's objectives were primarily to be accomplished through experimental work. The testing processes and methodologies were established in accordance with those advised by the British Standard Institution, along with some that had been suggested by earlier studies.

1.5 Significance of the study

The importance of this study lies in its emphasis on the use of recovered asphalt pavement (RAP) as a material for the road shoulder as well as on RAP's great capacity to improve

its qualities. In addition, this study offers trustworthy experimental data because there haven't been many studies on RAP for road shoulders. The information and data from this study can be used for subsequent research and may aid another researcher in defining the needs and specifications for the development of the road shoulder. Additionally, because RAP is used in road shoulders and can prevent milling waste from being dumped along the side of the road, the cost of road shoulder construction can be decreased. Additionally, employing RAP as road shoulder materials will lessen the need for using natural resources like aggregate and sand, which are extensively used in the building sector.

2. MATERIAL AND METHOD

As part of an integrated transportation system, roads will be developed as a safe and dependable mode of transportation that will benefit society, promote economic growth, and facilitate the free movement of people, products, and services, all of which will improve national competitiveness. Road transportation, more than any other method of transportation, has increased the mobility and accessibility of the vast majority of people around the world, according to Starkey (2002). The road's specific function is to link to economic hubs including production, distribution, and marketing centers (Febiansyah, 2012).

As the road shoulder is crucial to the development of the street transportation framework, the provincial road shoulder was selected as the review case and combined with the proper blended extent to serve the perfect path for overhauling the road shoulder. Without street bears, there will be certain problems for those who use the streets, especially when a vehicle needs to stop suddenly or when a car problem arises.

2.1. Road Shoulder

The purpose of the road shoulder is to provide a strip of land along the edge of the roadway for stopping cars. It also acts as an emergency lane for moving vehicles and offers lateral support for base and surface courses. Even in damp weather, it must be sturdy enough to support the weight of a transport that is completely loaded or empty. Its width must be sufficient to provide extra room around a stopped vehicle. In the case of highways and large roads, it may be wrapped, but it is often unsealed, shallower, and made of a lower-quality material than the neighboring traffic lane.

The purpose of the road shoulder is to protect the sealed pavement from excessive deterioration and to provide a safety measure for drivers who unintentionally enter or exit the area of sealed pavement (Road Shoulder, 2008). If vehicles may safely stop on the road shoulder and return to the traffic track after having accidentally driven against it, the risk of an impact is reduced. Stopping and also re-directing the vehicle onto the roadway will be simpler if the vehicle tires can grab the shoulder surface, especially when this happens quickly.

If the shoulder is sufficiently wide and the tires of the car can hold the shoulder's surface, the vehicle will be better prepared to execute both of these tasks (Shoulder

Improvements, 1995). Tires are better able to grip a fixed street surface. The ability of the car tires to grip the shoulder surface will make it easier to stop and also steer the vehicle back onto the street when a vehicle leaves the road, especially when this happens quickly.

It is easier for a driver to steer the car back onto the street at a shallower edge with enough road shoulder width, which lowers the likelihood that the driver may "overcorrect" and enter an oncoming activity. Road shoulders that are excessively wide become dangerous if they are used as an additional walkway (Zegeer et al., 1980).

There are a few advantages of a good plan of the street shoulder, for example:

- Reduce street head-on and running off after accidents.
- Wider road shoulder allows vehicles to leave the road in an emergency and gives them room from traffic (however crashes can happen when vehicles endeavor to re-join the movement).
- Wrapped road shoulders provide a safe area for cycling and can be designated as designated riding path ways.
- Road shoulders that are wrapped provide crucial support for the street asphalt. Fixing can reduce "edge drop" (a distinction stature between street surface and the street bear). The edge drop may make it more difficult for vehicles to re-enter the street after leaving it (Balgowan, 2014).

There are some execution issues of street shoulder, which are:

- i. Shoulder broadening and fixing can be finished in the meantime to cut down the expenses.
- ii. Edge covering can be enhanced at the season of redesigning the shoulder (particularly when fixing).
- iii. Road shoulders must not be too wide in light of the fact that drivers may use them as an extra street.

2.2. Pavement Recycling

According to a Federal Highway Administration report released in the United States of America in 1997, asphalt pavement is the most often reused component (as compared to others (in terms of tonnage), such as newsprint, glass bottles, aluminum, tins, and plastic bottles.

Recycled asphalt pavement is compared to other products in Figure 2.1 (Asphalt Pavement Alliance, a Coalition of Asphalt Institute, 2004). Because of the following benefits, degraded asphalt pavements are now recycled more frequently than they are overlaid with new asphaltic concrete material or completely rebuilt (depending on the nature and severity of the distress).

- A decrease in building costs.
- Aggregate, binders, and transportation fuel preservation (for new materials).
- Maintenance of the pavement's geometrical patterns.
- Environment protection.
- Lessening the need for landfills by recycling existing materials rather than throwing them away.

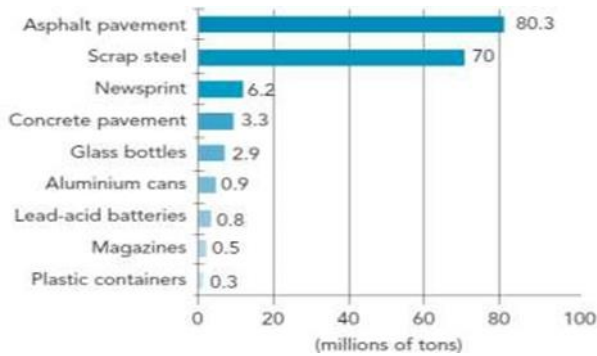


Figure 2.1: Comparison of Various Recycling Products

Poor ride quality, decreased vehicle traction due to reduced surface friction, and higher vehicle maintenance expenses are all effects of deteriorating pavement. Choosing the best recycling technique and, lastly, details on current recycling projects finished in Malaysia Recycling of old pavement can be used in conjunction with other preventive pavement management procedures to create a long-lasting, highly serviceable surface. The four basic types of recycling methods are defined by the Asphalt Recycling and Reclaiming Association (ARRA) (Kandhal et al., 1997). create a long-lasting, highly serviceable surface. The four basic types of recycling methods are defined by the Asphalt Recycling and Reclaiming Association (ARRA) (Kandhal et al., 1997).

- i. Hot in-place recycling.
- ii. Hot mix recycling.
- iii. Cold in-place recycling.
- iv. Complete depth reclamation.

One of the many solutions available for restoring pavements is recycling. The specific decision about the rehabilitation approach should be based on life cycle cost and designing considerations. The type, extent, and intensity of discomfort, an indicator of activity, and the structural state of the pavement should all be taken into consideration when designing. By determining the cost of the pavement using any of the several methods, such as present value or equal uniform yearly cost, economic considerations can be made.

The benefits of recycling pavement, different reusing strategies, and choosing a particular reusing strategy have all been looked at. The importance of pavement restoration (including the reuse technology) over new road building is becoming evidently more important as the country develops

and organizes its development strategies. Pavement recycling is expected to become increasingly significant in the coming years as resources become distinctly scarcer and environmental awareness becomes more pervasive. Table 2.1 lists the example of pavement recycling projects in Malaysia. (Malaysian Contractor Specializing in Pavement Recycling, 2004)

Table 2.1: Pavement recycling projects in Malaysia

Location	Types of recycling pavement method	KM
Jerangau - Jabor	Cold in-place recycling method mixed with cement	Federal Route 14 (km 99.5 - 105.0)
Bukit Sagu - Cerul, Kuantan	Cold in-place recycling using foamed bitumen	Route 1581 (km 21 - km 35)
Kapar - Sabak Bernam	Cold mix recycling (central plant)	Federal Route 5
Pulau Indah, Kelang	Hot in-place recycling	Route 181

Recycling techniques will likely replace the traditional overlay method or mill and pave methods for more road rehabilitation in Malaysia in the future. Pavement made on recycled asphalt (RAP). Reclaimed asphalt pavement (RAP) is commonly understood to be the fine bits of bitumen and inorganic material created by the mechanical grinding of asphaltic concrete components (often from dust to around 25 mm) (Balachanter, 2010).

RAP wastes often come from layers of the road's surface that are being removed to make room for resurfacing, according to Balachanter (2010). This material is removed from surfaces that are either too high to form an overlay, are deemed inappropriate, or cannot support the new surface layer. Bitumen typically ranges from 5% to 7% in RAP trash.

The precise composition and characteristics of the material, however, depend on a number of factors, including the age of the source asphalt mix, the type of mix, the bitumen and aggregate properties used in the mix, the design and operation of the RAP plant, and the clipping of underlying layers during the RAP process. Asphalt pavement recycling is a beneficial strategy for technical, economical, and environmental reasons (Kennedy et al., 1998).

Aging of the RAP binder is being significantly more viscous and having lower penetration values than the virgin bitumen.



Figure 2.2: Abandon Reclaimed Asphalt Pavement (RAP)

Resurfacing of flexible pavement's top layer is known as RAP. Binder and wearing courses make up the bottom and top layers of the surface layer, respectively. Using asphaltic concrete or hot mix asphalt (HMA) mixtures, this layer is cambered for drainage purposes. Aggregate, filler, and bitumen are the materials used. The majority of the mix, or 93%, is composed of aggregate.

Although reclaimed asphalt pavement materials, including mixes with moderate to high RAP proportions that are not inferior paving products, have been successfully designed and produced for many years, some people in the pavement community have a negative opinion about employing them. The performance is what counts: According to Long-Term Pavement Performance, a recent study evaluated the performance of recovered and virgin mixes (LTPP). According to the statistics, blends with at least 30% RAP perform equally well as virgin mixtures across all metrics (West, 2010).

Utilizing RAP would necessitate a connection to durability issues pertaining to the interactions between virgin and recycled materials. The degree of relationships between old and new asphalt binders is the key component that is still incoherent.

The layer that distributes course load to the road base and offers a level, even surface for the wearing of the course. The typical maximum aggregate size is 28 mm, and 3.5 to 5.5% of it is bitumen. The typical notation for this mixture is ACxx, where AC stands for asphaltic concrete and xx denotes the largest aggregate permitted in the mixture.

The pavement's top layer, which serves as a skid-resistant surface, protects the beneath layers and the drainage layers, offers smooth, safe riding, and supports traffic loading. The usual bitumen content ranges from 4.0 to 7.0%, with a maximum aggregate size of 14mm. This mixture is typically identified as ACxx, where xx represents the largest aggregate size in the mixture and AC stands for asphaltic concrete. The aggregate, bitumen, and mixture requirements must be met by all ingredients. The standard and construction layer thickness for the wearing course and binder course are shown in Table 2.2. (Hainin et al., 2016).

Table 2.2: Standard and construction layer thickness

Type of layer	Standard Thickness (cm)	One layer Lift (cm)
Wearing Course	4 - 5	4 - 5
Binder Course	5 - 10	5 - 10

The prior research on 100% RAP's stiffness and strength is shown in Table 2.3. However, collections of fine aggregate and asphalt mastic may tend to be brittle or flexible depending on the condition of the asphalt caused by aging, oxidation, and temperature exposure. Original coarse aggregate particles can be assumed to have good strength and be resistant to deform (Hoppe et al., 2015).

Table 2.3: Findings from the previous researcher for 100% RAP (strength and stiffness)

Researcher	Finding for 100% RAP
Bennert <i>et al.</i> , 2000	They found that 100% RAP samplings have a high stiffness and high resilient modulus amount, and lesser shear strengths than the dense-graded aggregate base course samplings. While RAP is stiffer than the thick evaluated total base course, 100% RAP material gathers the most noteworthy measure of stable strain. They also reported that the causing contrast in the middle of 100% RAP resilient modulus and its permanent deformation might be assigned to the advanced breakdown of asphalt binder under loading.
Dong and Huang, 2014	Showed that RAP materials tended to have a high resilient modulus and more permanent deformations when experimented as unbound aggregates. Triaxle creep experiment outcomes verified viscous properties and heat Dependency of unbound RAP base mixture.
Locander, 2009	Reported that the shear strength decreases as a number of RAP increases
Taha <i>et al.</i> , 1999	Indicated that the existence of RAP outcomes in the lower bearing capacity of the samplings matched to the virgin aggregates.

According to Ayan (2011), bitumen-coated aggregates slid over one another under load application, causing a drop in CBR values with increasing RAP concentration in unbound subbase. Ayan claimed that the performance with the 50/50 blend of RAP and recycled concrete aggregate was satisfactory (RCA). 15% RAP content was determined to satisfy the repeated load's triaxial criteria for application in the pavement in Australia by (Arulrajah et al., 2014) mixed RAP and RCA research. The best results were obtained between 59% and 78% of the ideal moisture level. The CBR readings for this blend, however, were just a little bit below the acceptable design value of 80.

Table 2.4 indicates the finding from previous research for RAP mixed with other materials and recommended a percentage of RAP mixed with other materials.

Table 2.4: Finding from the previous researcher for mixed RAP with other materials

Researcher	Finding for mixed RAP
(Bennert and Maher, 2005)	From this finding, it was concluded that the general trend of larger permanent deformations and lower CBR values as the RAP content increased in the granular mixture. The writers suggested that RAP is mixed with virgin aggregate to be limited to 50% by weight.
Bleakley and Cosentino, 2013	Granular RAP and lime rock mixtures without a stabilising agent can meet the strength and creep requirements for the base course if blended up to a maximum of 25% RAP and 75% lime rock. If the maximum of 50% of RAP is used but combined with a chemical stabilising agent, such as cement, the amount and type of the stabilising agent should be determined by a mix design method that results in a blend that meets the required performance specifications.

Table 2.5: Finding from the previous researcher for mixed RAP with other materials (Continue)

McGarrah, 2007	This author published her study on the properties of RAP blends and determined that 100% RAP does not construct a product of suitable base course quality and would not be allowable. This is because the more RAP content will decrease, the shear strength of the blend lower than the necessary level. She also suggested controlling the RAP quantity to 25% and mixing RAP with virgin aggregate at the mixing plant. Poor onsite mixing, resulting in base course separation.
Dong and Huang, 2014	The authors recommended that no 100% unbound RAP base be used under asphalt pavements.
Schaefer et al., 2008	The authors concluded that only 20% to 50% RAP contented is usually used in real construction.

Table 2.5: Finding from the previous researcher for mixed RAP with other materials (Continue)

Ooi, 2010	The author concluded that limiting RAP to 50% may be prudent as long as the material meets all other requirements in the specifications that a virgin aggregate would satisfy. In addition, he also recommended the minimum CBR values of 80 and 60 for the base and subbase aggregate blends, respectively. The intent was to provide performance specifications expressed in terms of CBR test results.
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2.2.1. Recycling technique.

Since 1915, technology has been developed to recycle old pavement material for road restoration and development projects. Recycling of pavements refers to the reusing of materials from existing pavements that have been treated to provide high-quality paving materials suitable for use in new pavement construction or pavement restoration. Recycling can aid in maximizing the use of energy and material resources while lowering the expense of maintaining streets, roads, and highways. The following categories are used by TRB (Transportation Research Board, 1980) to categorize road recycling techniques:

- I. Surface recycling involves employing a plane heater, heater- scraper, and hot or cold milling to recycle asphalt pavement with a thickness of less than 25 mm. This method works well to fix pavement problems such as raveling, rutting, flushing, and corrugation.
- II. In place surface and base recycling refers to the practice of implementing in-place recycling on pavement with a thickness of more than 25 mm, which is subsequently followed by compaction and reshaping. This method is employed to strengthen the pavement layers structurally. Lime, cement, asphalt, and other chemicals are used to stabilize the pavement that already exists. Cold-Mix Recycling Foam Bitumen (CMFRB) and Cement Treated Recycling Base were two technologies on this technology (CTRB).
- III. Central-plant recycling involves scraping the existing pavement and delivering it to the central plant as the first step in recycling. With or without the addition of fresh aggregate and modifier, the procedure is carried out in the central plant before being transported back to the site to be spread out, shaped, and compacted. CMFRB and hot mix recycling asphalt were two of the technologies used in this process (HMRA). A recycling technique called CTRB uses cement to stabilize the base layer's foundation, which consists primarily of aggregates. The stabilization of current asphalt pavement with foamed asphalt is known as CMFRB. RAP, foam bitumen, new aggregate (if necessary), and filler (cement or lime) are the components of CMFRB, which is spread out and compacted in cold weather. According to the results of the research center tests, (Saed, 2008) the following evaluation of

the suitability of RAP in unbound applications was made:

- IV. Strength test the findings demonstrate that RAP can be properly blended for application in high traffic areas in non-freezing temperatures or in low and medium traffic regions in freezing climates with low moisture surroundings.
- V. Test for Frost Susceptibility, the findings demonstrate that RAP can be correctly mixed for application in high-traffic environments.
- VI. According to the findings of the static triaxle test, RAP is suitable for use in locations with heavy traffic in non-freezing temperatures, medium traffic in freezing temperatures with little moisture present, and low traffic areas in freezing conditions.
- VII. Repeated triaxle loading test. Results indicate that RAP can be used in low traffic and medium traffic regions in non-freezing weather.
- VIII. Test for stiffness According to the results, RAP blends can be used in high traffic areas in non-freezing temperatures as well as in medium and low traffic areas in freezing temperatures.

2.2.1 Physical properties of reclaimed asphalt pavement (RAP)

RAP is the waste product that results from grinding the pavement surface. It could have both wearing and binder courses in addition to both. RAP may typically be re-used in roads as a base or surface material. The attributes and performance of the resulting recovered mixture, specifically the stiffness of its binder, have an impact on the physical characteristics of RAP. As a result of age, RAP binder coating is more viscous and has lower penetration values than virgin binders. The asphalt binder can be split into two categories: short-term and long-term, with chemical changes within the binder being the primary cause of aging (Sondag et al., 2002).

RAP has a distinct quality from virgin materials, however adding it to new combinations could often lower the cost of the materials (Asphalt Recycling and Reclaiming Association, 2001). Numerous variables, like the pavement's age, the amount of air voids, and the degree of moisture damage to the original asphalt pavement, all affect the varying quality.

RAP composition is typically described using applicable material standards. For instance, EN 13108-8: European Standard for Reclaimed Asphalt (European Committee for Standardization, 2005) permits the use of RAP in the manufacture of new HMA while incorporating the following requirements: no tar in the reclaimed asphalts; no foreign matter content; type of binder; binder properties; aggregate grading; and content of foreign matter.

2.2.2. Chemical properties

The fundamental knowledge that aids in a better understanding of controlling asphalt performance is how chemical parameters influence physical qualities. Bitumen is an organic mixture that is predominantly made up of highly

condensed polycyclic aromatic hydrocarbons. It is black, sticky, highly viscous, and completely soluble in carbon disulphide.

Asphalt cement typically consists of friable, dark brown particles. Determining the entire amount of asphalt cement depends on the kind of non-polar solvent used to precipitate it. As the component of asphalt cement that increases viscosity, asphaltenes have a significant impact (Roberts et al., 1996).

In other words, there is a connection between time and temperature impacts. The behavior at high temperatures over a brief period of time is equivalent to what occurs over a longer length of time and at the same temperature. This is sometimes referred to as the cement or asphalt transition time and temperature superposition notion.

The summarization of the prior research' recommendations for RAP is provided in Table 2.6. Studies on the application of RAP were heavily emphasized as a platform for this effort. Even though the use of RAP has been discussed and put into practice, numerous observations caused by the various materials, environments, and ambient temperatures have prompted further research into evaluating the use of RAP.

Table 2.6: Recommended usage of RAP

Researcher	Recommended RAP (%)
Poulikakos <i>et al.</i> , (2014)	40
Yang and Lee, (2016)	< 25
Valdés <i>et al.</i> , (2009)	60
Widyatmoko, (2008)	40

2.3. Soil

The soil is defined as an accumulation of broken rock put in the multi-phased interface section of the earth, created by weathering development, which occurs when rock buried in the earth layer is mechanically degenerated or biologically decomposed. The soil deposit is often divided into two basic categories: transported soil and residual soil. While transported soil is made up of material that has been transferred from another area by transporting agents like water, wind, glaciers, and others, residual soil is generated via the weathering of rock or a collection of organic material that is still present at its initial position.

Due to the nature of their pore fluids and fabric's mineralogy, several types of soil may yield diverse soil properties. The soil's weak circumstances made it easy for its stiffness to be impacted, which led to the soil's decline in strength. The most important aspect of any soil qualities is its strength. A big and high load could not be supported on soil with weak strength.

2.3.1. Classification of soil

Soil can typically be divided into two categories. The first type is referred to as coarse-grained dirt, and one of its distinguishing features may be seen clearly even without magnification. Sands and gravels can be found in these soils. Any soil component larger than gravel is referred to as cobbles or boulders. The element size itself is typically used to categorize coarse-grained soils. The second is fine-grained dirt, a component of which is very minute and some of which cannot be seen without the right tools. Silts and clays are present in these types of soils. These divisions are based on the soil's plasticity. Due to the variety in water content, clay soil is more malleable than silt soil, which has little to no plasticity. The general properties of soils are explained by the classification systems for soil, which are often based on soil parameters.

Depending on the void ratio achieved from the arrangement, David (2007) claims that stacking soil particle is similar to stacking marbles, either loosely or densely. According to David (2007), the organization of deposits of coarse-grained and silt soil is greatly influenced by the force of gravity because each particle's mass is significantly bigger than its surface area. On the other hand, due to their high surface to mass ratio, clay soil particles are more affected by the electrical forces operating on their surfaces.

The American Association of State Highway and Transportation Officials (AASHTO) system, founded in 1929 as the Public Road Administration classification system, has classified the area as stated in Table 2.7

Table 2.7: American Association of State Highway and Transportation Officials (AASHTO) classification

Criteria	Description
Grain Size	Gravel: Fraction passing the 75mm sieve and retained on the No.10 US sieve
	Sand: Fraction passing the No.10 US sieve and retained on the No.200 US sieve
	Clay and Silt: Fraction passing the No.200 US sieve

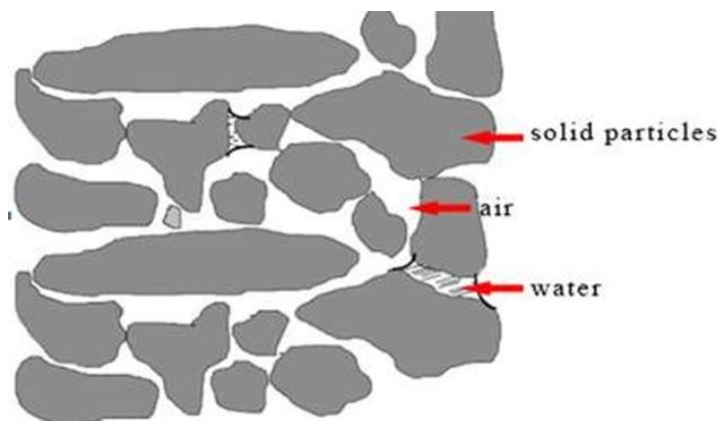


Figure 2.3: Component in soil (Hopmans and Rolston, 2000)

The Unified Soil Classification System's classification of soil is shown in Table 2.8. (USCS). According to the American Society for Testing and Materials (ASTM) D-2487 standard requirement, the geotechnical engineer uses this system. Whitlow (2001) asserts that soils are distinguished based on the letter of the group symbol.

Table 2.8: First and second letters of group symbols (Whitlow, 2001)

Soil Identification	First Letter of Group Symbol	Second Letter of Group Symbol
Coarse grained soil	G: gravel, S: sand	W: Well graded P: Poorly graded
Fine grained soil	M: silt, C: clay	L: Low plasticity (LL less than 50) H: High plasticity (LL more than 50)

Table 2.8: First and second letters of group symbols (Whitlow, 2001) (Continue)

Organic soil	O	L: Low plasticity (LL less than 50) H: High plasticity (LL more than 50)
Highly organic soils	Pt	No second letter

Figure 2.3 depicts the soils, which typically contain three elements: air, water, and solid particles. The interrelationship of the various elements is crucial for defining the state or physical characteristics of the soil.

Figure 2.3 depicts the soils, which typically contain three elements: air, water, and solid particles. The interrelationship of the various elements is crucial for defining the state or physical characteristics of the soil.

2.3.1. Laterite soil

Engineers frequently struggle with the limited bearing capacity of soft soils in geotechnical engineering design and are unable to meet the necessary specifications. Soil improvement is a crucial area of research in geotechnical engineering to address this issue. Therefore, site investigations should be done to determine the type of soil improvement on the site before any building begins. Soil stabilization or consolidation are two methods for improving the soil.

The laterite soil used in this study was collected from the region around Kuantan, Pahang. To achieve the best blended proportion, laterite soil was combined with RAP in this study. Typically, tropical and subtropical regions are home to lateritic soils. More than half of Peninsular Malaysia in Malaysia is covered by iron- and alumina-rich residual sedimentary rock soil, giving it a lateritic nature (Aun, 1982). Climate, parent rock, and the level of lateralization all have an impact on the geotechnical characteristics of these materials. Iron oxide and aluminum oxide cover and bind the clay particles during lateralization, changing the soil's microstructure. The geotechnical characteristics of lateritic soil are influenced by the mineralogy and microstructure (Mahalinga-Iyer and Willams, 1991).

Laterite is a substance that is highly worn and is rich in optional oxides of iron, aluminum, or both, according to Alexander and Cady (1962). It is virtually entirely devoid of bases and necessary silicates, yet it may be rich in quartz and kaolinite. When exposed to wetness and drying, it either solidifies or is hard. According to research by Nixon and Skipp (1957), Graft-Johnson et al. (1972), and Gidigas (1969), laterite soils may have molecule-measure qualities similar to those of any other type of soil. These qualities include the ability of the parent material's way of origin, degree of weathering, geologic origin, area in the topographic site, and depth in the profile. Bawa (1957) characterised all fine-grained laterite soils inside the accompanying fraction of size parts as appeared in Table 2.9.

Table 2.9. Ranges of size fraction for fine-grained laterite soil

Type	Grain size	Percentage
Sand	2.0 - 0.05 mm	50 ±

Table 2.9. Ranges of size fraction for fine-grained laterite soil (Continue)

Silt	0.05 - 0.002 mm	30 - 40
Clay	< 0.002 mm	20 - 30

Each type of soil behaves differently in terms of appropriate wetness and thickest layer. In this approach, each type of soil has unique requirements and controls for both fieldwork and testing.

When exposed to wetness and drying, it either solidifies or is hard. According to research by Nixon and Skipp (1957), Graft-Johnson et al. (1972), and Gidigas (1969), laterite soils may have molecule-measure qualities similar to those of any other type of soil. These qualities include the ability of the parent material's way of origin, degree of weathering, geologic origin, area in the topographic site, and depth in the profile. Bawa (1957) characterised all fine-grained laterite soils inside the accompanying fraction of size parts as appeared in Table 2.8.

2.3.1. Plasticity of laterite soil

Problem laterite soil often has very high natural moisture contents that are much beyond the plastic limit and frequently even beyond the liquid limit, but in their unaltered natural state, they resemble a solid mass rather than a plastic or liquid mass (Hirashima, 1948). Liquidity index should therefore be taken into account. Equation 2.1's derivation of the natural moisture content, plastic limit, and liquid limit yields the liquidity index:

$$L = (w - PL)/(L - +PL) \tag{2.1.}$$

When the natural moisture content of the sample is equal to the liquid limit, the liquidity index is equal to unity; and when the natural moisture content is equal to the plastic limit, the liquidity index is zero (Figure 2.5).

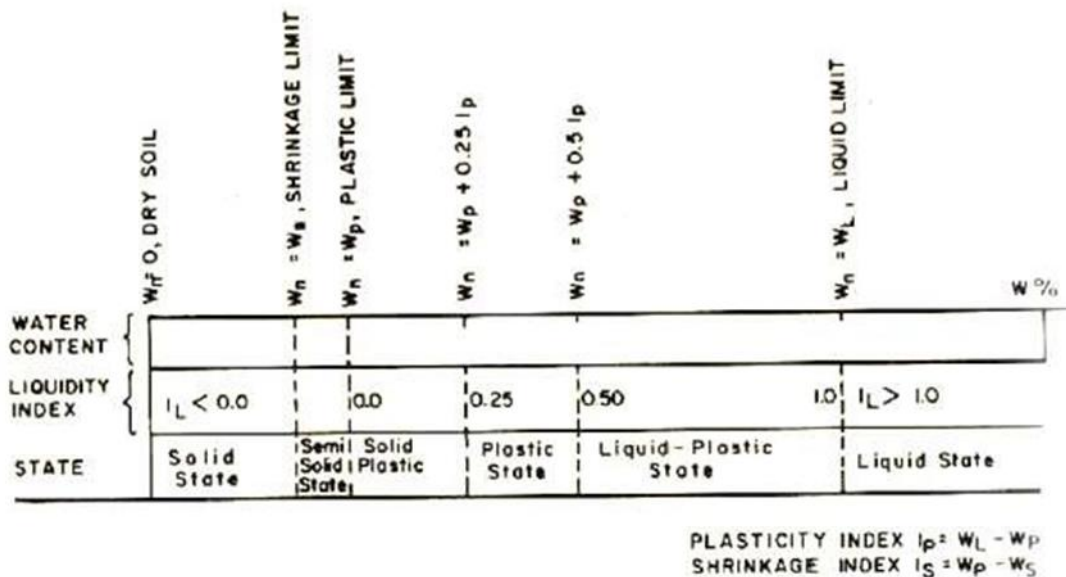


Figure 2.5: Plasticity characteristics and limits of soils (Gidigas, 196 distinctive grain sizes).

2.3.1. Compaction of laterite soil

By mechanically pressing the sturdy soil particles even more tightly together during the compaction process, the dry thickness of the soil is increased (Markwick, 1944). It is achieved by reducing the amount of air gaps in the soil, with almost no water content loss. The process of compaction works to reduce the soil's compressibility and porosity while increasing the soil's strength and bearing capacity.

For soil ranging in texture from red clays of volcanic origin (Hirashima, 1948; Newill, 1961; Tateishi, 1967) to gravels and gravelly soils of varied genetic origin (Brand and Hongsnoi, 1969; Gidigas, 1969; Gidigas and Teboa, 1972), the impact of the method of drying on the compaction characteristics of some laterite soil has been studied. It has been discovered that the initial moisture content of laterite soil has a significant impact on the shape of the moisture-density curve. It was discovered that oven drying, followed by air drying, generally tends to offer the highest maximum dry density and the lowest optimal moisture content. Additionally, the amount of swelling and the final moisture content of laterite soil are both influenced by the degree of drying. Therefore, a detailed research in the lab and on the ground is required to determine the importance of the influence of the pre-test preparations on the compaction properties.

2.3.2 Classes of Sub-Grade Strength based on CBR

The "Manual for the Structural Design of Flexible Pavement" (ATJ 5/85-Pindaan 2013), which is made available by Jabatan Kerja Raya Malaysia, serves as the basis for road pavement design and construction in Malaysia (JKR). This guide is intended to provide JKR and professionals working on asphalt construction projects in Malaysia with a uniform method of defining asphalts for all classes of movement. The California Bearing Ratio (CBR) has been widely used to classify subgrade support in asphalt planning.

The versatile solidness estimation of the subgrade is prescribed at whatever point plausible. The flexible firmness esteems utilized for the plan of the asphalt structures are introduced in Table 2.9.

Table 2.9: Classes of Sub-Grade Strength based on CBR (ATJ 5/85-Pindaan 2013)

Sub-Grade Category	CBR (%)	Elastic Modulus (MPa)	
		Range	Design Input Value
SG 1	5 to 12	50 to 120	60
SG 2	12.1 to 20	80 to 140	120
SG 3	20.1 to 30.0	100 to 160	140
SG 4	> 30.0	120 to 180	180

Pavements for Subgrade Category of SG 1 roads, which have very low traffic volume support, are advised to have a minimum CBR of 5%. The road will be supported by the high volume of traffic for subgrade category 4 (SG 4). With an elastic modulus between 120 MPa and 180 MPa, the CBR value must at least be 30%. The procedure and design of road construction can be more standard thanks to the manual provided by JKR.

3. RESULT AND CONCLUSION

The conclusions that can be made according to the result and analysis of this study are stated in the following section.

3.1. Material properties of soil and reclaimed asphalt pavement (RAP)

According to the results, the soil type was Brown Clayey Sand with a fineness modulus of 2.84 mm. The soil sample's plastic limit was in the 22–23 range and its liquid limit was in the 43–53 range, while the plastic index value was 21. While the sieve analysis from extraction was defined, the bitumen content from the extraction operations for RAP fulfilled the criterion from JKR/SPJ2008/S4.

3.2. Moisture content and degree of compaction

The OMC of soil from this investigation was 20% added to all mixed proportion.

3.3. Optimum mix proportion under california bearing ratio (CBR)

In this study, the 8S2RAP mixture provided the best mix proportion under CBR. As RAP increases, the MDD and OMC of this soil mixture will decrease. As the value content of RAP rises, the effectiveness of this combo will decrease.

In order to build a road shoulder, 20% RAP material is suggested. As more RAP is added, the road shoulder's strength and stability will deteriorate. Conclusion: RAP has the potential to be used as a replacement material to build the road shoulder with the right mix, which will substantially reduce RAP waste disposal and help to reduce environmental pollution.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conflict of Interest

The authors have no conflicts of interest to declare.

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Climate Change Will Cause a Pollination Crisis in the Mediterranean Basin

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Abstract: In the study, habitat suitability simulations of *Apis mellifera* species, which have great service in pollinating many natural and cultural plants, were made according to climate change scenarios that may occur in the Mediterranean Basin. The most striking result among the results obtained is that, for the pessimistic scenario, it shows that the Mediterranean Basin will face drought at the end of the century and *A.mellifera*, a pollinator insect species, will move away from the seacoast and seek suitable climatic conditions inland due to this drought. If this scenario is realized, it is predicted that coastal areas will be affected by the pollination crisis in Mediterranean countries.

Keywords: Pollination crisis, Climate change, Apis, Mediterranean Basin.

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1. INTRODUCTION

Today, more than 400,000 natural and agricultural plant species are known to be pollinated by pollinator insect species. Honey-bee colonies may include up to 80,000 individuals, making them one of the largest and most essential pollinator populations (Grünewald, 2010). It has been revealed as a result of experimental research that *Apis mellifera* is the most effective type of pollinator species (Abdulnabi, 2019). In addition, it has been determined that *Apis mellifera* plays an active role in the pollination of more than 300 plant species from 71 different families (Sıralı and Deveci, 2002).

It is possible that bee populations will decline as a result of global warming. Given the close evolutionary ties between insect-pollinated flowers and their pollinators, it stands to reason that a drop in local bee species could disrupt plant-pollinator networks, potentially setting off extinction cascades (Grünewald, 2010). For example, it is known that the *Paspalum* genus, which is the most important forage plant of the Mediterranean Basin, that is, the Mediterranean climate zone, is pollinated by *Apis mellifera*. The deterioration of this mutual relationship between the two due to climate change will cause a decrease in the grass plant, which is important for the pasture plant, or, in other words, a

decrease in the quality of forage pastures (Cho and Lee, 2015).

The aim of this study is to first determine the climatic conditions that *Apis mellifera* has adapted to from the recent past to the present in the Mediterranean Basin, and then to determine where the climatic conditions adapted in the climate change scenarios will survive on the map.

Finally, we will discuss how climate change can affect bee populations in the Mediterranean Basin and change the natural synchronization between pollinator and plant life cycles.

2. MATERIAL AND METHOD

2.1. Species Data

In order to obtain the species data of our target species, *A.mellifera*, the Wallace platform was opened by installing the relevant packages in Rstudio (Kass et al., 2018; 2022). On the Wallace platform, 581 records of *A.mellifera* species were obtained to cover the Mediterranean Basin.

2.2. Bioclimate Data

Historical climate data (1970–2100) and SSPs climate change scenarios data were downloaded from the www.worldclim.org data facility. The downloaded data is at 10 arc minutes of resolution. The 2081-2100 period of the SSPs 126, SSPs 245, SSPs 370, and SSPs 585 climate scenarios was downloaded from the CanESM5 climate model and made ready for analysis.

2.3. Pre-Statistics and Modeling

To get over the issue of multicollinearity amongst bioclimatic data, Principal Component Analysis has been used. Thus, 5 out of 19 bioclimatic data participated in the modeling phase (Table 1 and 2). These are Bio9 (Mean Temperature of Driest Quarter), Bio10 (Mean Temperature of Warmest Quarter), Bio11 (Mean Temperature of Coldest Quarter), Bio14 (Precipitation of Driest Month) and Bio19 (Precipitation of Coldest Quarter).

Table 1. Total Variance Explained

Component	Initial Eigenvalues			Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6,513	34,279	34,279	6,513	34,279	34,279
2	4,735	24,922	59,201	4,735	24,922	59,201
3	3,431	18,060	77,261	3,431	18,060	77,261
4	1,473	7,751	85,012	1,473	7,751	85,012
5	1,231	6,481	91,493	1,231	6,481	91,493
6	0,910	4,789	96,282			
7	0,304	1,599	97,881			
8	0,145	0,761	98,642			
9	0,098	0,518	99,160			
10	0,066	0,347	99,507			
11	0,048	0,254	99,761			
12	0,014	0,076	99,837			
13	0,014	0,073	99,909			
14	0,007	0,037	99,946			
15	0,005	0,028	99,974			
16	0,004	0,019	99,994			
17	0,001	0,006	99,999			
18	0,000	0,001	100,000			
19	2,529E-11	1,331E-10	100,000			

Extraction Method: Principal Component Analysis.

Table 2. Component Matrix

	Component				
	1	2	3	4	5
Bio1	0,756	0,543	0,288	0,171	-0,104
Bio10	0,380	0,583	0,680	0,192	0,016
Bio11	0,869	0,409	-0,124	0,159	-0,166
Bio12	0,744	-0,564	0,218	-0,087	0,210
Bio13	0,833	-0,219	0,070	-0,332	0,360
Bio14	0,208	-0,771	0,438	0,231	-0,144
Bio15	0,187	0,679	-0,426	-0,294	0,363
Bio16	0,827	-0,241	0,054	-0,329	0,366
Bio17	0,244	-0,770	0,441	0,237	-0,127
Bio18	0,716	-0,319	0,351	-0,417	-0,043
Bio19	0,123	-0,494	-0,131	0,485	0,613
Bio2	-0,314	0,632	0,125	-0,268	0,108
Bio3	0,466	0,198	-0,636	-0,107	-0,167
Bio4	-0,611	0,079	0,074	-0,004	0,200
Bio5	0,161	0,716	0,643	0,130	0,103
Bio6	0,885	0,238	-0,194	0,281	-0,145
Bio7	-0,626	0,331	0,640	-0,141	0,199
Bio8	0,642	0,218	0,505	-0,222	-0,346
Bio9	0,377	0,586	-0,145	0,550	0,263

Extraction Method: Principal Component Analysis.
a. 5 components extracted.

The climatic envelope model was developed using MaxEnt 3.4.1 (Phillips et al., 2017). MaxEnt software predicts, based on the presence data of organisms, which environmental conditions affect the distribution of organisms. Based on the model's AUC, it is rated as ">0.90: excellent, 0.90-0.80: good, 0.80-0.70: suitable, 0.70-0.60: poor, not informative." (Baldwin, 2009; Phillips vd., 2006). During the modeling process, 581 *A.mellifera* data were sliced as 75% training data and 25% test data. Each model was performed with 10 replications.

3. RESULTS

As a model output, receiver operating characteristic (ROC) curves showing the AUC value of the model, a Jackknife analysis graph showing the contribution of bioclimatic data to the model, and maps were obtained.

The training data AUC value of the obtained model is 0.938, and the test data AUC value is 0.937 (Figure 1).

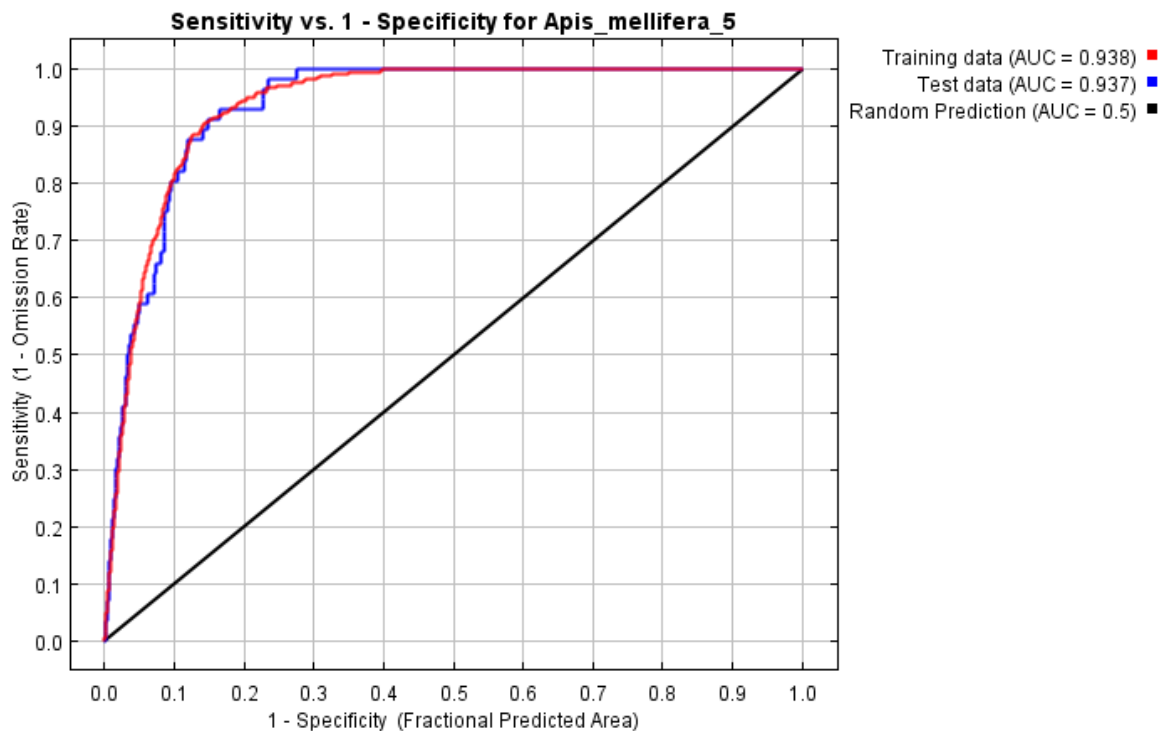


Figure 1. The receiver operating characteristic (ROC) curves for *Apis mellifera*

Inspecting the Jackknife analysis graph, we notice that Bio11 and Bio19 play a significant role in the dissemination of *A. mellifera* (Figure 2).

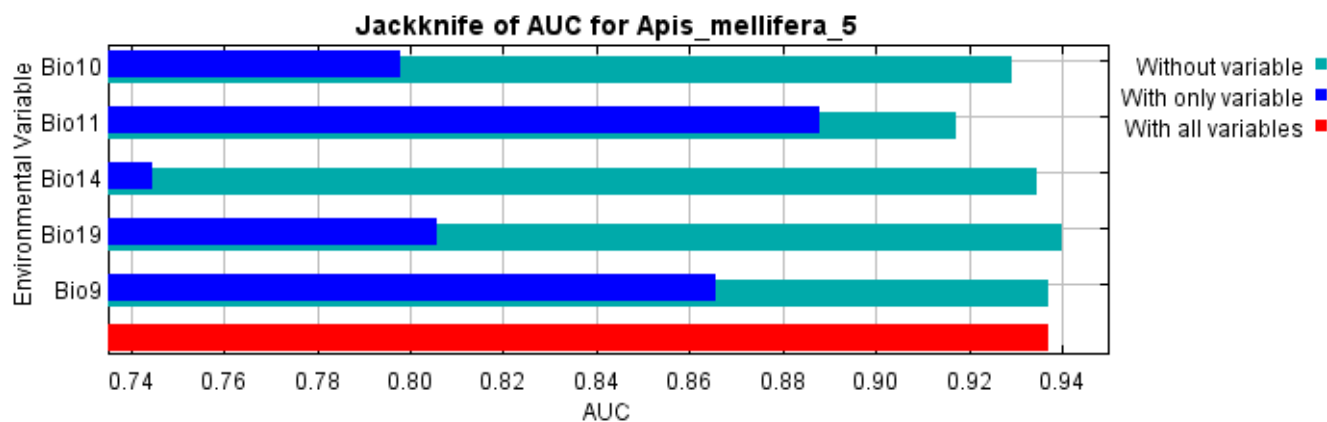


Figure 2. Results of the Jackknife test for evaluating the relative importance of environmental variables for *Apis mellifera*

When the maps from the model outputs are examined, the areas with suitable climatic conditions for *A. mellifera* in the coastal areas along the Mediterranean basin are shown in red

(Figure 3.A). At the end of the century, it is seen that suitable climatic conditions have shifted from the optimistic scenario to the pessimistic scenario towards the interior (Fig. 3. B-E).

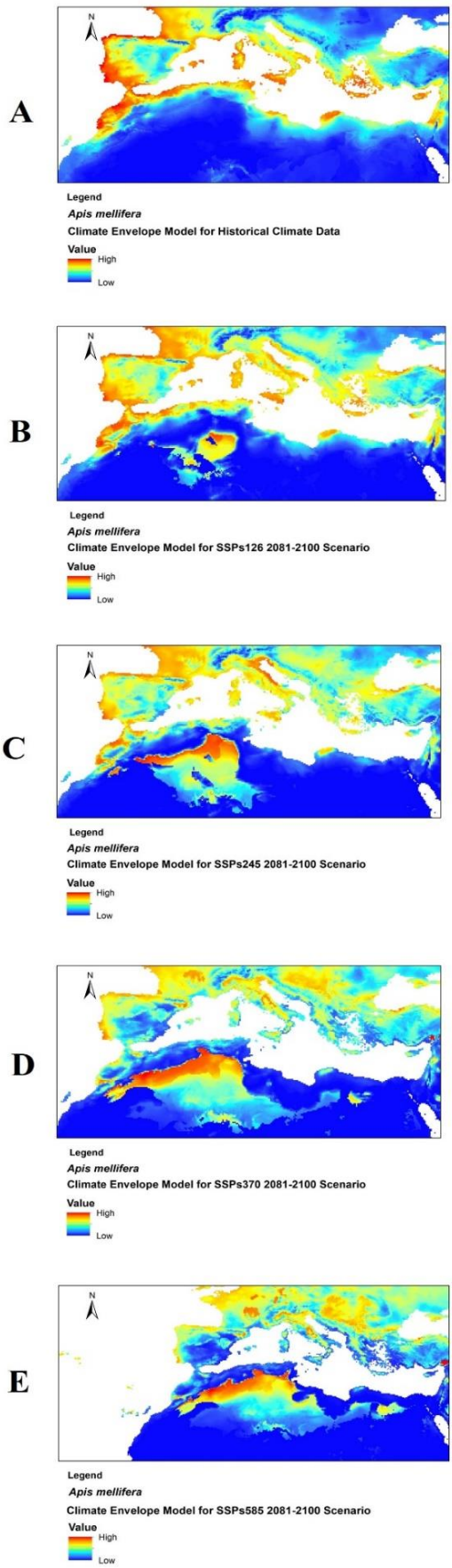


Figure 3. A: Climate envelope model for historical climate data. B: SSPs 126 2081-2100 scenario. C: SSPs 245 2081-2100 scenario. D: SSPs 370 2081-2100 scenario. E: SSPs 585 2081-2100 scenario

4. DISCUSSION AND CONCLUSIONS

A. mellifera is a species complex distributed all over the world. Most populations are adapted to many different habitats and climates. Therefore, climate change probably will not cause the extinction of honeybees. The life cycle of *A. mellifera* might be disrupted as a result of climate change. Because of the changing seasons, bee colonies in temperate areas of the world are unable to forage for food during extended periods of cold weather. In these places, the breeding season occurs only during the spring and summer months. If climate change causes winters to be warmer and springtime to arrive sooner, then there is a possibility that nutrition during the critical period of brood initiation will not be at its ideal level. However, honey bees, which are ectothermic creatures, may need special climatic conditions to keep their body temperatures within a certain range, and therefore changes such as extreme heat, drought, and decreased precipitation can be very harmful to *A. mellifera* species that experience habitat loss. Therefore, as a result of climate change, bees will most likely quit locations that have become too dry and will move towards regions that have a higher relative humidity. It is possible for them to colonize chilly locations that have seen warming and become more suitable for bees. There is a good chance that the distribution of different flower species will shift as a result of climate change (Grünewald, 2010; Kluser et al., 2010; Le Conte and Navajas, 2008). Migration towards suitable climatic conditions, displacing inland, seems to be the most logical option, as is the case highlighted in our results.

In a study conducted with *Paspalum* plants, known as watergrass, it was reported that these plants, which are distributed in the coastal areas of the Mediterranean Basin, will shift their distribution towards the interior in the future in the face of climate change (Cho and Lee, 2015). These results coincide with our study, considering the fact that *A. mellifera* plays a role in the pollination of *Paspalum* plants.

The literature and results show that, on the basis of species ecology, climate change seems to overcome the threat of extinction by changing species' distributions to more favorable conditions. However, drought along the Mediterranean coast, which we will call the old habitat before climate change, will bring a pollination crisis with it. The pollination crisis will lead to a decrease in rangelands, agriculture, and natural plant species, and thus a decrease in biodiversity. So, at least the steps that need to be taken to make the most optimistic climate change scenario happen will make sure that biological diversity will still be around at the end of the century.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: A.K., S.B.; Investigation: A.K., S.B.; Material and Methodology: A.K.; Supervision: A.K., S.B.; Visualization: A.K.; Writing-Original Draft: A.K., S.B.; Writing-review & Editing: A.K., S.B.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Evaluation of the Warehouse Location Alternatives for Possible Istanbul Earthquake

Asli Gul Yalcindag^{1*}, Mehtap Dursun¹, Nazli Goker¹

Abstract: The "Istanbul Earthquake" is expected to occur within the next ten years. Preparations have long been made for the Istanbul earthquake, which is expected to cause heavy loss of life and property. One of the important parts of earthquake preparedness is the disaster logistics warehouses, where emergency supplies and shelter equipment are stored to be dispatched to earthquake victims. The aim of this study is to determine the locations of the warehouses to deliver the aid materials to the points of need as soon as possible and to meet the needs in the event of a large earthquake in Istanbul. The model was set up in two steps to determine the number of warehouses to be opened with the set covering problem in the first step and to minimize the weighted distance with p-median in the second step. The established model was solved using The General Algebraic Modeling System (GAMS), and the optimum scenario was decided according to the results, and the scenarios were mapped.

Keywords: Disaster logistics warehouse, earthquake, location selection, p-median, set covering problem.

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1. INTRODUCTION

An earthquake is the event of sudden vibrations that occur due to fractures in the earth's crust, spreading in waves and shaking the environments they pass through (AFAD, 2019). An earthquake is a natural event that cannot be prevented. It is known that since the beginning of the world, millions of people have been dead and shelters have been destroyed by earthquakes. Turkey is located in the Alpine-Himalayan belt, which is one of the most critical earthquake zones in the world. In this context, it contains tectonic zones with high earthquake potential (Silahdar, 2022). Ms:8.0 1939 Erzincan, Ms:7.1 1957 Bolu, Mw:7.4 1999 Kocaeli and Mw:7.1 2011 Van earthquakes in the last century are the most important indicators of this activity (Tan et al. 2008; Utkucu et al. 2014).

One of the earthquakes expected to occur is the possible major earthquake in the Marmara Region that will also affect Istanbul. If we examine the major earthquakes that have occurred for the last one century along the North Anatolian fault line, starting with the Erzincan earthquake with a magnitude of 7.9 in 1939, the next significant earthquakes have always occurred further west on this line. This is because the released energy is transferred to the west when

an earthquake occurs on this fault line (Yamamoto et al. 2020). The 1999 Kocaeli earthquake, which was the last devastating earthquake on this fault zone, caused severe loss of life and property. Many studies by experts reveal that it is inevitable that a devastating earthquake will occur in Istanbul shortly (Yamamoto et al. 2020). Such an earthquake in Istanbul, Turkey's largest metropolis and economic center, will cause significant losses. This issue should be greatly important to reduce these losses and precautions should be taken to prepared for earthquakes.

According to the results obtained from the Istanbul Province Earthquake Loss Estimates Update Project analysis, a total of 120,115 low-rise residential buildings, 47,230 medium-rise residential buildings, and 1060 multi-story residential buildings will be moderately, heavily or very heavily damaged. Therefore, it is estimated that after the Mw:7.5 scenario earthquake, an emergency shelter need of about 640,000 households will occur. (İstanbul Büyükşehir Belediyesi, Kandilli Rasathanesi ve Deprem Araştırma Enstitüsü, 2020) Given the population of 3 people per household, approximately 2,000,000 people are expected to be in urgent need of shelter (İstanbul Büyükşehir Belediyesi, 2019). Various disaster logistics warehouse location selection studies have already been carried out. However, in

this study, current damage estimation data from Istanbul metropolitan municipality (I.M.M.) and Kandilli Observatory will be used, and the large Istanbul earthquake will be highlighted.

Various preparations are being made against the expected earthquake in Istanbul. One of them is disaster logistics warehouses. The centers where emergency aid materials are stored to be sent to the damaged areas in disasters and emergencies are defined as disaster logistics warehouses (AFAD, 2014). Disaster logistics warehouses contain materials such as medical supplies, tents, beds, blankets, heaters, and kitchen sets.

In this study, using mathematical programming methods, the focus will be on determining the optimum logistics warehouse locations to deliver the emergency aid materials to the points of need as soon as possible and to meet the requirements in case of a potential major Istanbul earthquake. Here, based on Aydın et al. (2017)'s research in the Maltepe district, the whole of Istanbul was emphasized instead of Maltepe. Facility location optimization model established by Boonmee et al. (2017) in their study was also employed while creating the model. According to the outputs obtained from this study, it can be decided where the warehouses should be established, the suitability of the existing warehouse and facility locations can be discussed, and arrangements can be made to ensure that the citizens suffer as minor damage as possible in a possible Istanbul earthquake.

2. MATERIAL AND METHOD

This study will establish a mathematical model to determine the optimum disaster logistics warehouse locations for a possible major Istanbul earthquake. The problem under consideration is modeled in two steps. In the first step, different scenarios with different coverage distances will be established, and the minimum number of warehouses designed with the set covering problem will be determined. In the second step, the results of the first step will be given as input to the p-median problem, and demand-weighted distance minimization and assignments will be made.

Indices

i: Index of the demand points (Districts)

j: Index of the facilities (Candidate logistics warehouses)

Parameters

n : Number of potential facilities

S : Coverage distance of the facilities planned to be opened (km)

a_{ij} : 1 if the distance between demand point *i* and facility *j* is less than or equal to *S*, 0 otherwise

w_i : Demand of the demand point *i*

d_{ij} : Minimum distance between demand point *i* and facility *j*

p : Number of facilities to serve

Decision variables

x_j : 1 if a facility is established at point *j*, 0 otherwise

y_j : 1 if a facility is opened at point *j*, 0 otherwise

g_{ij} : 1 if demand point *i* is assigned to facility *j*, 0 otherwise

Step 1:

Objective function

$$\min z = \sum_{j=1}^n x_j \tag{1}$$

Constraints

$$\sum_{j \in J} a_{ij} \cdot x_j \geq 1 \quad \forall i (i = 1, \dots, n) \tag{2}$$

$$x_j \in \{0,1\} \quad \forall j (j = 1, \dots, n) \tag{3}$$

Step 2:

Objective function

$$\min z = \sum_{i=1}^n \sum_{j=1}^n w_i \cdot d_{ij} \cdot g_{ij} \tag{4}$$

Constraints

$$\sum_{j=1}^n g_{ij} = 1 \quad \forall i (i = 1, \dots, n) \tag{5}$$

$$g_{ij} \leq y_j \quad \forall i, j \tag{6}$$

$$\sum_{j=1}^n y_j = p \tag{7}$$

$$g_{ij}, y_j \in \{0,1\} \tag{8}$$

In Step 1, Eq. 1 indicates that we want to minimize the number of facilities placed. Eq. 2 means that each demand point must be serviced by at least one facility. In this model, more than one facility can serve a demand point because not only one is assigned to a facility. Eq. 3 is the constraint of the decision variable being a 0-1 integer.

In Step 2, Eq. 4 minimizes the distance between demand points and candidate facilities. Eq. 5 ensures that each demand point receives service from only one facility. Eq. 6 provides that no demand point is assigned to the facility that is not open. Eq. 7 allows *p* units to be opened. Eq. 8 is the constraint for decision variables to be 0-1 integers.

3. CASE STUDY

For determining the locations of disaster logistics warehouses, the booklet of possible earthquake loss estimates in Istanbul by districts, prepared in 2020 by Istanbul Metropolitan Municipality and Kandilli Observatory, is used. In the booklet, the number of households in very heavy, heavy, and moderately damaged residential buildings is used to estimate the number of families that will need emergency shelter after a possible Istanbul earthquake. In accordance with this, number of households that will need temporary shelter were calculated for the Mw=7.5 earthquake scenario for each district. These values in the booklet are used as demands in the model. Districts are demand points. The district centers' coordinates were used to determine the demand points.

I.M.M. established a disaster response facility in Halkalı in 2006. Then, 40 potential locations were identified to set up additional facilities and warehouses. In this study, these candidate logistics warehouse locations compiled by Görmez (2008) were used (Figure 1).



Figure 1. Map of the candidate logistics warehouses (modified from Görmez, (2008))

In the study, the distances of each demand point (districts) to each facility (logistics warehouses) were calculated using the Euclidean distance formula (Eq. 9)

$$d(A, B) = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} \quad (9)$$

These data were entered while running our model. The first step considered seven scenarios with 40, 45, 50, 55, 60, 65, and 70 km coverage distances. Seven experiments were conducted by entering S as 40, 45, 50, 55, 60, 65, and 70. According to the test results, the number of warehouses to be opened for each scenario and which warehouses to be opened will be found. By using the results obtained at this step in the second step, it will be seen which warehouse will serve which districts and a decision will be made according to the results of the objective function that minimizes the total distance between the districts and the candidate warehouses. The GAMS program will be used to run the model.

4. RESULTS AND DISCUSSIONS

The problem is coded in GAMS, the results in Table 1 for Step 1 and Table 2 for Step 2 are obtained. The name of the districts are provided in Table 3.

Table 1. Results of Step 1

Scenario	Coverage distance S (km)	Number of storages to open	Storage no
Scenario 1	40	2	7, 21
Scenario 2	45	2	32, 36
Scenario 3	50	2	25, 35
Scenario 4	55	2	12, 25
Scenario 5	60	1	11
Scenario 6	65	1	10
Scenario 7	70	1	3

When we consider the objective functions, we see that the optimal scenario is Scenario 3, with a coverage distance of 50. According to this scenario, logistics warehouses should be established at location 25 in Kartaltepe, Küçükçekmece, and location 35 in Ümraniye, Fatih Sultan Mehmet. This scenario mapped in Figure 2.

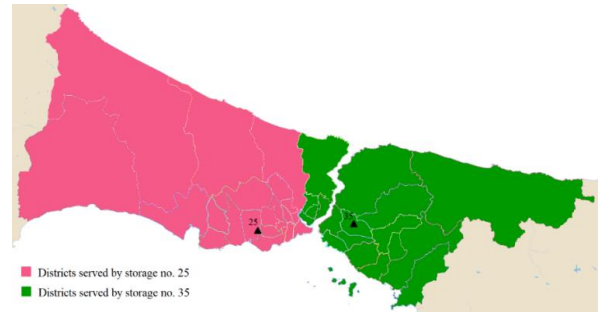


Figure 2. Map of Scenario 3

In real life, it can be decided that warehouses cannot be established in these locations for some reason. Therefore the areas of the other six scenarios can be evaluated. For example, since congestion and damage may occur on the Bosphorus bridges after the earthquake, it may be decided to serve the districts on the side where each warehouse is located, so that Scenario 2 can be implemented. The maps of the other six scenarios are in Figures 3-8.

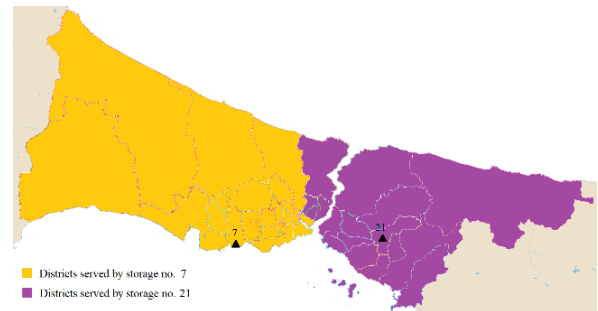


Figure 3. Map of Scenario 1

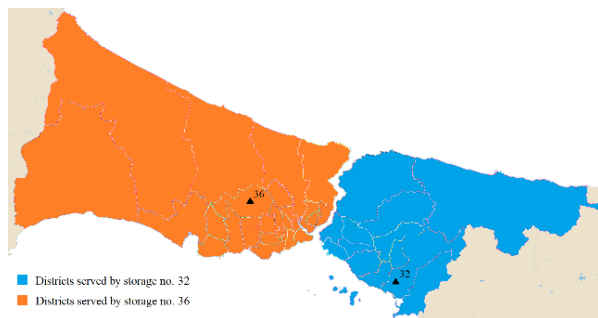


Figure 4. Map of Scenario 2

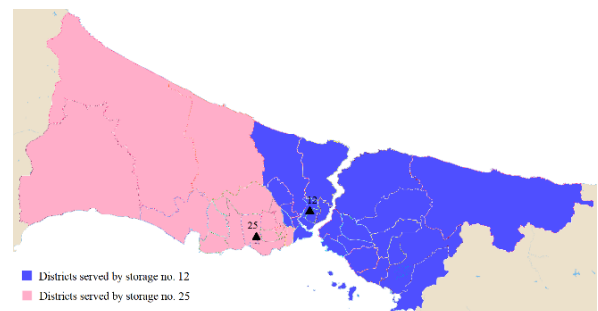


Figure 5. Map of Scenario 4

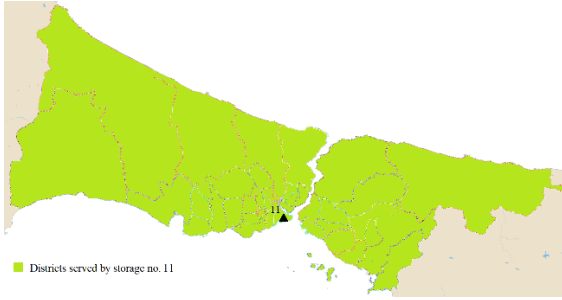


Figure 6. Map of Scenario 5

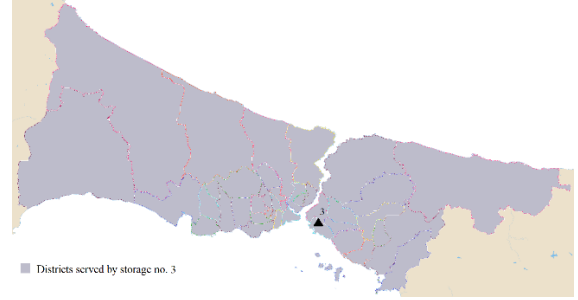


Figure 8. Map of Scenario 7

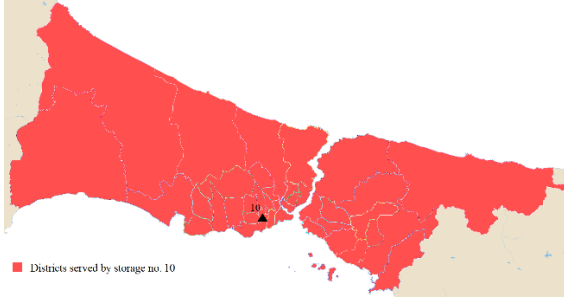


Figure 7. Map of Scenario 6

Table 2. Results of Step 2

Scenario	S (km)	Objective function	Storage No.	Districts to be served
Scenario 1	40	10.862.805,98	7	2:6, 9:13, 17, 25:30, 34:37
			21	1, 7, 8, 14:16, 18:24, 31:33, 38, 39
Scenario 2	45	10.513.175,91	32	1, 8, 14, 16, 18, 19, 22:24, 31:33, 38, 39
			36	2:7, 9:13, 15, 17, 20, 21, 25:30, 34:37
Scenario 3	50	8.468.443,53	25	2:6, 10:13, 17, 25:30, 34:37
			35	1, 7:9, 14:16, 18:24, 31:33, 38, 39
Scenario 4	55	9.615.695,88	12	1, 6:12, 14:16, 18:24, 30:33, 38, 39
			25	2:5, 13, 17, 25:29, 34:37
Scenario 5	60	13.290.759,13	11	All
Scenario 6	65	11.946.363,12	10	All
Scenario 7	70	16.690.656,53	3	All

Table 3. District no

1.	Adalar	11.	Fatih	21.	Şişli	31.	Sancaktepe
2.	Avcılar	12.	Gaziosmanpaşa	22.	Tuzla	32.	Ataşehir
3.	Bakırköy	13.	Güngören	23.	Ümraniye	33.	Çekmeköy
4.	Bağcılar	14.	Kadıköy	24.	Üsküdar	34.	Arnavutköy
5.	Bahçelievler	15.	Kağıthane	25.	Zeytinburnu	35.	Beylikdüzü
6.	Bayrampaşa	16.	Kartal	26.	Esenler	36.	Büyükçekmece
7.	Beşiktaş	17.	Küçükçekmece	27.	Silivri	37.	Esenyurt
8.	Beykoz	18.	Maltepe	28.	Çatalca	38.	Sultanbeyli
9.	Beyoğlu	19.	Pendik	29.	Başakşehir	39.	Şile
10.	Eyüp	20.	Sarıyer	30.	Sultangazi		

5. CONCLUSIONS

To prevent the chaos that may occur after the earthquake, it is vital to determine the locations of the warehouses where the aid materials will be stored with accurate analysis, to avoid that unnecessary traffic and lost time. Having access to help as soon as possible after the earthquake is important for the health of the citizens affected by the earthquake and their psychology. The sooner these earthquake victims get help, the sooner they can go on with their lives with less harm. The return of the earthquake victims to their daily lives also has a social significance. This is how the country's service and education systems can survive. We believe that the results of this study will contribute to these issues.

In this study, factors such as cost, highways, and traffic conditions in Istanbul were not considered. In addition, estimates of the number of households needing emergency shelter are considered deterministic, and it is assumed that there are no capacity limits. The study can be developed to be more realistic in the future, taking into account costs, highways, capacity, and traffic. Scenarios can be developed for the number of households that will need shelter, and different possibilities can be evaluated. Scenarios can be improved regarding the magnitude of the earthquake as well. The earthquake's location and the fault's rupture direction will also be considered.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: M.D.; Investigation: N.G.; Material and Methodology: A.G.Y., M.D.; Supervision: M.D.; Visualization: A.G.Y; Writing-Original Draft: A.G.Y, N.G.; All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Examination of Player Positions by Cluster Analysis

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Abstract: Today, the football industry stands out among the sports branches. Especially with the development of technology and its integration into football, different tactical understandings and formations emerge. With these developments, the current positions of the players and the other positions they are prone to play can be revealed as a result of the analysis. In this way, club management and technical team aim to establish the best team according to the current budget and tactical game understanding. Therefore, it is very important for the teams to play the players in the right position or to transfer the right player to the required position. In football competitions where 11 players are involved in the game, tactical changes can be made within the game according to the tactical arrangement and tactical understanding of the opposing team, and the player can be played in different positions. In this study, the player data of Turkey and the leagues of Germany, England, France, Spain, Italy, which are considered to be the five big leagues, for the years 2020-2021 were obtained from the website named “whoscored”. In the data set obtained, the players who stayed on the field for a minimum of 1500 minutes were taken as a basis and clustering analysis was performed with the data of 985 players. Players are clustered on four basic positions: goalkeeper, defender, midfielder and attacker. In the study, Expectation Maximization, one of the clustering analysis algorithms, was used and a success rate of 81 percent was achieved.

Keywords: Artificial Intelligence, Data Mining, Clustering.

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1. INTRODUCTION

Sports have an important place in the social life of today's societies. Sports activities, which develop day by day, have become a phenomenon that affects education, economy and social structure in the 21st century (Yetim, 2000).

One of the sports branches that increase the impact on human life day by day is football (Öntürk, et al., 2019). Football, which has increased its visibility with the development of technology, brings entertainment, sadness, joy, national and international achievements as well as management, technical team and licensed players (Talismiciler, 2008).

Football, which started with the first match played between Sheffield and London in 1966, managed to drag the masses after it and continued its development until today. According to official records, the first football match on Turkish soil was played in Thessaloniki in 1875 (İnan, 2007). In the

globalizing world, football has succeeded in creating its own economy by separating it from other sports branches with its fan base, advertising revenues, sales of team products, transfers and viewing (Aygün & Ulucenk, 2019). The main sources of income that make up this economy are; box office revenues, athlete transfers and rentals, membership revenues, donations and aids, broadcasting rights revenues, advertising revenues, sponsor revenues (İnan, 2007).

Behind the football competitions in which two teams of 11 people take place on the field, there are elements such as management, technical team and fans. The team structure planned with the right transfers is important for football teams who want to have a good season. The current management and technical team aims to establish the best team and achieve the best results, taking into account the current budget. The management and technical team, by determining the transfer policies in this direction, want to transfer the players in accordance with the game plan of the

team, to strengthen the team tactically and to bring the players to the team that will excite the fans. With the staff established at the end of the transfer period, the technical team aims to increase the quality of the team by creating a player group that can play in various tactical formations such as 4-4-2, 4-3-3, 3-5-2, and trainings such as attack, defense, technique and individual. It is very important for the teams to play the players in the right position within the squad planning or to transfer the right player to the needed position.

Football data is of great importance today. Many analyzes such as data mining are used to reveal meaningful information from these large data sets. With the developing technological innovations, it has become easier to store data and large-scale data has become available. The analysis of data with human skills has created an inverse proportion with the increasing amount of data, and the analysis of big data has become difficult. Computer technologies have been used to make the raw data information or meaningful as a result of the analysis, and data mining analysis methods have been developed. Data mining includes disciplines such as data visualization, artificial learning, and statistics. Data Mining models, which include predictive and descriptive models, are grouped into three main groups: Classification, Clustering and Association Rules, according to the functions they perform (Savaş, et al., 2012). Cluster analysis is a multivariate statistical analysis method that enables grouping individuals, units or objects according to their basic attributes. In this method, individuals, units or objects that are similar to each other are grouped in the same group according to the selected selection criteria. As a result of the analysis, while the data in the same cluster are similar, they are not similar to the data in the other cluster (Kangalli, 2014).

In their study in 2019, Narizuka and Yamazaki developed a clustering algorithm that enables to create a multi-team formation using the Delaunay method, which defines a team's in-game formation as the adjacent matrix of Delaunay triangulation (Narizuka & Yamazaki, 2019). Kawasaki et al., in their study in 2019, proposed a new method to create a passing net based on the measurement of passing passes in the game. In 2016 and 2017, 9 matches of Fagiano Okayama team in Japan Professional Football 2nd League were analyzed. The rust network was divided into clusters by the clustering method and the rust network between different clusters was formed. It has been abstractly demonstrated that the developed passing network can pass successfully (Kawasaki, et al., 2019). Iman Bahrevar and Sayed Mohammed Ravazi predicted the player transfer market value with 74% accuracy by using machine learning methods in their study in 2021 (Behrevar & Razavi, 2021). Johannes Stübinger and her friends conducted a study in 2019 on the prediction of match results using machine learning methods based on player characteristics. The study, which included the data set of Europe's five big leagues between 2006 and 2018, obtained statistically and economically significant results (Stübinger, et al., 2020).

In this study, the data of 985 players who played in the leagues of Turkey and Germany, England, France, Spain and Italy, which are considered to be the five big leagues, and took a minimum of 1500 minutes in the 2020-2021 season,

were taken from the website called "whoscored". In line with these data, it is aimed to cluster the football players considered according to their goalkeeper, defender, midfield and attacking positions by using the Expectation Maximization algorithm.

In the study, real data were used; The clustering of the football players according to four basic positions was carried out. Thanks to the results obtained as a result of the analysis, alternative positions where the players can be useful besides their original positions have been revealed.

2. MATERIAL AND METHOD

There are many different definitions for data mining. Data mining is the process of extracting valuable information from large-scale data. In this way, it is possible to reveal the confidential relationships between the data and to make forward-looking predictions when necessary. In other words, data mining can be defined as the process of obtaining meaningful information from large data sets.

The data mining process is similar to classical statistical applications. However, classical statistical techniques are mostly applied on regular and special data sets. In the data mining process, studies are carried out on very large data groups and features (Özkan, 2016).

Techniques used in the data mining process;

- Classification,
- Clustering,
- Association Rules

grouped under three main headings. Among these techniques, classification is among the predictive models, while clustering and association rules are among the descriptive models (Gemici, 2012).

In this study, cluster analysis, which is one of the descriptive models, and Expectation Maximization (EM), which is one of the cluster analysis algorithms, will be emphasized.

Cluster Analysis

Cluster analysis is a type of analysis that ensures that similar elements in the data set are included in the same clusters. In the cluster analysis, the elements in the same cluster are as homogeneous as possible; It is desired that the elements in different clusters be as heterogeneous as possible (Akpınar, 2014).

Looking at the literature, there are many different types of clustering techniques related to cluster analysis. It is possible to collect clustering techniques under five main headings. These; They are grid-based, hierarchical, density-based, probability-based, and partitional clustering methods (Choudhary, 2016).

In the study, many algorithms were tried in the clustering analysis made by using the football player data obtained from the whoscored website, and it was seen that the Expectation Maximization (EM) algorithm gave the most appropriate result. The EM algorithm used in the study is given in detail in the next step.

2.1.1. Expectation Maximization (EM) Algorithm

The EM algorithm is one of the clustering algorithms that aims to find the maximum likelihood solution for hidden variable models. The EM algorithm can be defined as an optimization algorithm based on iteration (Dempster, 1977).

The algorithm performs the basic steps iteratively until it reaches the best convergence in terms of working principle. These steps, which are applied iteratively, are two, namely the E-step and the M-step. The E-step is called the expectation stage, while the M-step is defined as the maximization step. In the e-step, first a random probability distribution function is created, then the logarithmic probability function is found by obtaining the logarithm of the current function. Upon obtaining the function, the EM algorithm estimates the parameters with the maximum likelihood of missing data. Then, using these estimations, the best probabilities for the missing data are estimated (Mahmoud & Xia, 2014). In the M-step, new estimates of the parameters are obtained by replacing the estimated missing data and calculating the maximum likelihood over all data (Bruzzone & Prieto, 2002). The EM algorithm iteratively continues the E-step and M-step until the convergence criterion is satisfied (Li, et al., 2019).

3. CLUSTER ANALYSIS OF FOOTBALL PLAYER POSITIONS

Today, the football industry stands out among the sports branches. Especially with the development of technology and its integration into football, different tactical understandings and formations emerge. With these

developments, the current positions of the players and the other positions they are prone to play can be revealed as a result of the analysis.

In the study, football players in Turkey (Turkish Super League) and Germany (Bundesliga), England (Premier League), France (Ligue 1), Spain (La Liga), Italy (Serie A) leagues, which are accepted as the five major leagues, according to four basic positions. clustering is intended. In this way, alternative positions where the football players can play alongside their original positions have been put forward based on the current statistical data. Thus, it is thought that it will provide convenience to the clubs and technical committees that want to strengthen their staff. In this direction, the football players discussed were analyzed by using the criteria based on real data on the website called "whoscored". During the analysis, the Weka 3.8 package program, a software developed by the University of Waikato in New Zealand, was used for machine learning.

The football players discussed in the study are divided into four basic positions: goalkeeper, defender, midfielder and attacker. Ancillary sites included in the main sites are given in Table 1. The criteria used during the analysis were determined as the most frequently mentioned attributes in the literature review. The abbreviations of these criteria are given in Table 2. In the literature review, whoscored, transfermarkt and goal websites stand out as providers of player statistics. The "whoscored" website was preferred as the provider that offers the most comprehensive information on player performances. The summary version of the data set used in the study is given in Table 3 and Table 4.

Table 1. Position Table

GOALKEAPER	DEFENSIVE	MIDFIELDER	FORWARD
GK	D	M	AMC
	DL	MC	AM
	DC	ML	AML
	DR	MR	AMR
	DMC		FW

GK: Goalkeeper

D: Defensive

DL: Defensive Left

DC: Defensive Center

DR: Defensive Right

DMC: Defensive Midfielder Center

M: Midfielder

ML: Midfielder Left

MC: Midfielder Center

MR: Midfielder Right

AM: Attacking Midfielder

AML: Attacking Midfielder Left

AMC: Attacking Midfielder Center

AMR: Attacking Midfielder Right

FW: Forward Wings

The football players considered during the analysis were clustered on the basis of 21 criteria in total. These criteria and their abbreviations, obtained from the Whoscored

website, are given in Table 2. In the study, the players who took 1500 minutes or more were determined as alternatives; Table 3 and Table 4 give an example of the data set of the study.

Table 2. Criteria and Abbreviations Used During Analysis

CRITERIA	ABBREVIATION	CRITERIA	ABBREVIATION
Goals Per Game	G.P.G	Clearance Per Game	C.P.G
Assists Per Game	A.P.G	Pass Drizzled Per Game	P.D.P.G
Total Number of Yellow Cards	T.N.Y.C	Outfielder Block Per Game	O.B.P.G
Total Number of Red Cards	T.N.R.C	Key Pass Per Game	K.P.P.G
Shooting Average	S.A	Drizzle Won Per Game	D.W.P.G
Pass Percentage	P.P	Dispossessed Per Game	D.P.G
Aerial Won Per Game	M.W.P.G	Total Pass Per Game	T.P.P.G
Tackle Per Game	T.P.G	Accurate Crosses Per Game	A.C.P.G
Interception Per Game	I.P.G	Accurate Long Pass Per Game	A.L.P.P.G
Fouls Per Game	F.P.G	Accurate Through Ball Per Game	A.T.B.P.G
Offside Per Game	O.P.G		

Table 3. Data Set Used in the Study

G.P.G	A.P.G	T.N.Y.C	T.N.R.C	S.A	P.P	M.W.P.G	T.P.G	I.P.G	F.P.G	O.P.G
4	8	4	0	0,9	92,6	0,9	0,9	0,9	0,9	0
0	5	2	1	0,3	78,4	0	0,9	0,5	0,8	0
4	2	3	1	1,4	80,3	0,9	0,2	0,1	0,7	0
0	1	4	2	0,4	81,6	1,6	0,5	1,3	0,6	0,3
2	6	6	1	0,7	89,2	0,2	0,8	0,1	1,2	0

Table 4. Data Set Used in the Study (Continued)

C.P.G	P.D.P.G	O.B.P.G	K.P.P.G	D.W.P.G	D.P.G	T.P.P.G	A.C.P.G	A.L.P.P.G	A.T.B.P.G
0,6	0,6	0,3	1	0,8	1,2	58,8	0	1,5	0
0,3	0,8	0,1	1,1	1	0,7	20,7	0,6	0,4	0
0,5	0,2	0	0,9	0,6	0,4	22,5	0,1	1,5	0,1
3,6	0,4	0,9	0	0	0	36,3	0	4,7	0
0	0,8	0,1	1,2	2,2	2,9	29,3	0,1	1,5	0,2

The data in Table 3 and Table 4 were adapted to the 'arff' format and loaded into the WEKA package program. Then those that fit the appropriate numerical data set are tried and then the most appropriate one is Expectation Maximization. Since there are four basic positions in football, namely goalkeeper, defense, midfielder and attack, four clusters were determined as the number of clusters in the study. When looking at Figure 1, the result interface that emerges as a result of the analysis using the EM algorithm is seen.

Looking at Figure 1, 91 (9%) of the football players clustered with the EM algorithm were assigned to Cluster 0, 234 (24%) to Cluster 1, 313 (32%) to Cluster 2 and 347 (35%) to Cluster. assigned to 3. In Table 5, the clusters obtained as a result of the analysis made with the EM algorithm and the

objects in the clusters are given. The results obtained as a result of the analysis made with the EM algorithm are given in Table 5. Looking at Table 5, it is seen that all of the 91 football players assigned to Cluster 0 belong to the goalkeeper position. Looking at Cluster 1, it was observed that 232 of the 234 football players assigned were players playing in the defensive position. Looking at Cluster 2, it is seen that 219 of the 357 football players assigned to this cluster consist of players playing in the midfield. Finally, when we look at Cluster 3, it is seen that 264 of the 313 appointed players took part in the attacking position. Looking at Table 6, 100% success was achieved in Cluster 1, while 95% success was achieved in Cluster 1, 63% in Cluster 2, and 84% in Cluster 3. In general, 806 of the 985 players were assigned correctly and a success rate of 81.82% was achieved.

Table 5. Result Table Obtained by EM Algorithm

Cluster	Position	Number Of Cluster Data	CLUSTERING RESULT				Percent
			Goalkeeper	Defensive	Midfielder	Attacking	
Cluster 0	Goalkeeper	91	91	0	0	0	100,00
Cluster 1	Defensive	234	0	232	2	0	99,15
Cluster 3	Midfielder	347	0	122	219	6	63,11
Cluster 2	Attacking	313	0	14	35	264	84,35

Table 6. Percentage of Success Obtained with the EM Algorithm

Cluster	Position	Number Of Cluster Data	Right	False	Percent
Cluster 0	Goalkeeper	91	91	0	100,00
Cluster 1	Defensive	234	232	2	99,15
Cluster 3	Midfielder	347	219	128	63,11
Cluster 2	Attacking	313	264	49	84,35
TOTAL		985	806	179	81,82

4. CONCLUSION AND EVALUATION

Football, which is one of the sports branches that attract the most spectators in the national and international arena, has increased its importance and popularity in recent years. Club managers make transfers to needed positions in addition to the existing staff in order to further increase this visibility. In this study, football players playing in Turkey and in the leagues of England, Italy, Spain, Germany and France, which are considered to be the five major leagues, in the 2020-2021 season are clustered according to four basic positions (goalkeeper, defender, midfielder and attacker). In the analyzes made using the Weka package program, algorithms such as Simple K-Means, Canopy, Expectation Maximization, which are clustering algorithms that can be applied to numerical data suitable for the use of quantitative data, have been tried; It has been seen that the Expectation Maximization EM algorithm gives the most appropriate result. In the evaluation made on the basis of the existing criteria, it was seen that all the players in Cluster 0 were goalkeepers. All 91 players in this cluster are goalkeepers. Looking at Cluster 1, 232 of the 234 players in this cluster are the players in the defensive position. The other two players in this cluster are players who play in the midfield position. According to the results obtained, 264 out of 313 players in Cluster 2 are players playing in the attacking position. While the other 35 players assigned to this cluster are players playing in the midfield, 14 of them are players playing in the defense position. When we look at Cluster 2, the fact that the players who play in the midfield are also included in this cluster is due to the fact that they reveal statistics close to the attacking football players in the data of the 2020-2021 season. Players in the midfield position have created richness for their teams in attack, especially in recent years, with their direct contributions to the score. This made the assignment to Cluster 2 tolerable. Looking at Cluster 3, it is seen that the players playing in the midfield position predominate in this cluster. 219 of the 347 players assigned to the cluster play in the midfield. 122 of the other football players who were appointed consist of players playing in the defense position and 6 of them playing in the attacking position. Considering the other clusters in the study, the highest deviation was experienced in this cluster. One of the reasons for this is the presence of defensive midfielders in football. Being close to each other statistically is one of the reasons why they are in the same cluster.

With the study, the current position of the players was determined and other positions that they can play in addition to their current position were determined. Thus, it will be possible to determine in which positions the current players or the players to be transferred can play based on the data of

the past years while making the roster planning of the teams. When there is a choice between more than one player in the transfer planning, the players will be analyzed and will be effective in the decision-making process of the player to be preferred to the desired position. In the training of young players and determining the position they will play, it will contribute to the determination of training programs suitable for the position that emerges with the analysis of the player.

In future studies, by increasing the number of leagues discussed, analysis can be made about the football players in these leagues and different results can be obtained. In addition, in future studies, different leagues can be included in the study, different results can be obtained based on different criteria and by trying different algorithms.

Ethics Committee Approval

N/A

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Author Contributions

All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Realization of a Bionic Hand Controlled via Bluetooth Communication Utilizing a Microcontroller

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Abstract: The ability of people without limbs to do their own work and their adaptation to life can be achieved with bionic hands. The aim of this study is to realize the design and application of bionic hand that can be controlled by mobile application for handicapped individuals. The aerobic designs necessary to solve this problem were made and a prototype was produced and controlled with Arduino Mega 2560 microcontroller. For the five fingers of the bionic hand designed with the work carried out, the independent movement of the five fingers from the fingertip to all the other joints has been successfully performed using five SG90 model mini servomotors. The movement of the servomotors is provided by the software developed with the MIT AI2 Companion program on the mobile phone. The software algorithm has been fulfilled separately for each finger and each joint. Microcontroller and mobile phone communication is done with HC-06 Bluetooth module. Servomotor fin connections are made with fishing line with the tip of each finger. The two joints of the thumb and three joints of the other four fingers are controlled by winding the fishing line, which is connected to the servomotor, according to the determined rotation angle. Thus, the control of the fingers on the hand was realized through the mobile application. Ultimately, the effective use of the bionic hand will have an impact on every individual's participation in society. Every individual who is brought into the society has the potential to be an individual who enters the business life at the same time. For this reason, this will allow for an increase in employment and working power. It is also more sustainable because it raises people's living standards.

Keywords: Bionic hand, SG90 mini servomotor, Microcontroller, Mobile application, HC-06 Bluetooth module.

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1. INTRODUCTION

Many of the disabled individuals living in our society cannot do many jobs due to their disabilities, and therefore their quality of life is seriously reduced. There are people in our lives who are born without a limb, who have lost their hands by accident or by amputation. They have difficulty in living their lives. In addition, they tend to keep themselves in the background psychologically in their work and social environments. Because this situation is not only organ loss for individuals, but also organ loss; means loss in function, body image, work and relationships (Özsoy & Okyayuz, 2016).

The ability of people without limbs to do their own work and to adapt to life can be solved with bionic hands. The bionic hand allows the individual to continue their daily lives like

other healthy individuals by completing the missing hand limb. In order to solve this problem, necessary aerobic designs must be made, formulas must be drawn up, and a prototype must be produced and controlled by controllers.

When a literature review is conducted, it is seen that various studies have been carried out on this subject from the past to the present. Karaçizmeli et al. (2014) carried out the control of the mechatronic-based robotic hand with the position information obtained from the human hand with the help of flexibility sensors mounted on the glove. They performed hand control with data from sensors connected to the glove in the other hand. However, they also stated that there is a disadvantage that hand control is not provided independently of the other hand. Şenli (2011) fulfilled a bioelectrical database of the activity of hand muscles and made interaction network and interface work between human and prosthetic

hand using this database. In addition, he subjected the recorded bioelectrical signals to a series of preprocessing, established the relationships between the electromyogram (EMG) signals and hand and finger movements, and provided the control of the bionic hand with a microcontroller. Aydın (2020) conducted high precision bio-electronic hand design work in his master's thesis. He filtered the EMG signals received from the forearm muscles and transmitted them to the microcontroller and provided the movements of the servomotors with this microcontroller. In another study, Paaßen et al. (2018) proposed an expectation maximization transfer learning that minimizes data classification that may occur in the bionic hand. With the algorithm they developed, data loss during transfer is minimized. Klug et al. (2005) designed a robot arm inspired by biological principles for industrial applications. They stated that one of the shortcomings in their studies was that they could not perform the sprains on their systems. Deng et al. (2016) carried out a study that takes into account the force applied while grasping soft and hard objects by the bionic hand. They improved the bionic hand grasp of objects with the polyvinylidene fluoride sensor they used. Lastly, Andrecioli and Engeberg (2013) developed an adaptive sliding mode prosthetic hand controller with a variable slope manifold. Considering all these studies, it can be predicted that bionic hand studies will continue without slowing down.

There are some considerations to consider in the design and realization of a bionic hand. One of them is to determine the hand limb design that is suitable for the individual. After that, the calculation of the power values of the motors suitable for this hand limb is made according to the designed limb. In addition, it is necessary for the preliminary design to determine the number of inputs and outputs in the control system to be used and to calculate the power switches for the system. Then, what needs to be done is to create the flow diagram of a hand limb and embed it in the microcontroller determined according to the system requirements. In the study carried out, a bionic hand study was designed and applied to facilitate people with no limbs. A microcontroller is included in the control of this bionic hand. The control signals of the microcontroller were sent from a mobile device using Bluetooth communication. System movements were realized by adjusting the angles of the servomotors. In the presentation of this study, after a short introduction given above, "Materials and Methods" are expressed in the second part. After showing the "Results and Discussion" obtained in the third part, "Conclusions" is given at the end of the paper.

2. MATERIAL AND METHOD

In this study, five fingers of the bionic hand were controlled by a mobile application. The movements of three joints in four fingers and two joints in the thumb from the fingertip to the root joints are controlled by signals sent from the mobile software. In this way, the movement and gripping feature of the desired finger(s) is realized.

Arduino MEGA 2560 microcontroller is used to control the whole system. It is also preferred for power sharing with Arduino UNO. Among the reasons why this microcontroller finds many applications today; programming with an integrated development environment (IDE) and coding with

commands similar to the C programming language. Arduino microcontrollers have at least one 5 V regulated integrated circuit (IC) and one 16 MHz crystal oscillator (Hidayanti et al., 2020). An external programmer is not needed for programming Arduino microcontrollers, because a bootloader program is already written to the microcontroller. Arduino Mega 2560 can be powered by an external power supply in the range of 6-20 V. However, when a supply under 7 V is made, the 5 V pin may output less than its value, and therefore the board may start to work unstable. When the board is supplied with a voltage greater than 12 V, the regulator may overheat. This may cause damage to the card. Considering all these, it is recommended to limit the supply voltage of the recommended microcontroller to the range of 7-12 V (Allahverdi et al., 2019).

HC-06 Bluetooth module is used in the communication of the microcontroller used with the mobile device. The Bluetooth module has been developed for short distance communications, and this communication protocol uses the 2.4-2.48 GHz industrial, scientific and medical (ISM) band. Communication can be provided up to 10 m, provided that there is no obstacle between devices. HC-06 Bluetooth module works at 2.4 GHz communication frequency. The sensitivity of the module is less than -80 dBm and the output power is less than 4 dBm. Asynchronous communication speed is 2.1 MBps/160 KBps, synchronous communication speed is 1 MBps/1 MBps. The operating voltage is in the range of 1.8-3.6 V. HC-06 Bluetooth module supports Bluetooth 2.0.

In the implemented system, RC (Radio Control) Tower Pro SG90 DC type mini servomotor, which provides position control at small powers, is used. Servomotors are closed-loop motors that can minimize position errors that may occur in the system, receive continuous feedback and are highly preferred in position control. They are divided into three according to their working angles; 120-140°, 0-180° and 0-360° servomotors. Servomotors with 0-180° and operating voltage of 5 V DC were preferred in the study. Their structures are small and their strain torques are high (Süzen et al., 2017). 20 ms period signals are sent for position control of servomotors. If there is a duty value of 2 ms within 20 ms, the position of the servomotor becomes 180°. If the duty value is 1 ms within 20 ms, the position of the servomotor becomes 0°. Angle values in other intervals are obtained by giving values between the duty value and 1-2 ms. The dimensions of these servomotors are 22.2 × 11.8 × 31 mm, their weight is 9 g, and their speed is 0.1/60° (VanHuy et al., 2017).

In the study, two methods were applied to obtain and compare the determined angle values of each finger and knuckles. The first one is obtained by making the relationship between the arc length and the angle with the method of finding the central angle in the circle. The second method is obtained by measuring the rotation time of the servomotor, that is, by the relationship established between the position of the servomotor and the pulse width.

The first method is related to the length of the servomotor fin and the length of the finger from the root to the knuckle. Therefore, the rotation angle of the servomotor blade was

calculated based on the formula for calculating the center angle in the circle. In Figure 1, the relation shape of servomotor blade rotation angle and finger joint length is given.

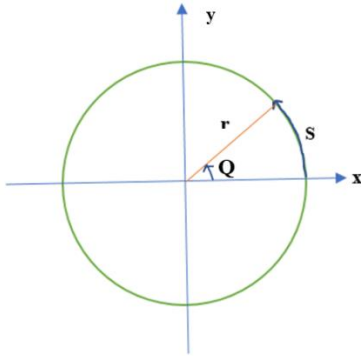


Figure 1. Relation of servomotor blade rotation angle and knuckle length

Here, Q represents the rotation angle of the servomotor vane, r the servomotor vane length, and S the length from finger root to knuckle. The ratio of arc length to radius gives the angle of rotation. The angle of rotation and the length of the arc of the circle are described as follows:

$$Q = S/r \quad (1)$$

$$|S| = (Q \times 2 \times \pi \times r)/360 \quad (2)$$

From here, the angle of rotation is again found in detail as follows:

$$Q = [(360/2) \times S]/(2 \times \pi \times r) \quad (3)$$

The fin length of the servomotors used in the realized bionic hand design is taken as $r = 1.5$ cm and the π value is taken as 3.14. Since the servomotor blade can provide a maximum of 180° rotation, the calculation was made over the semicircle and $360 / 2$ was written in the formula in (3). Finger knuckle lengths are shown in Figure 2.

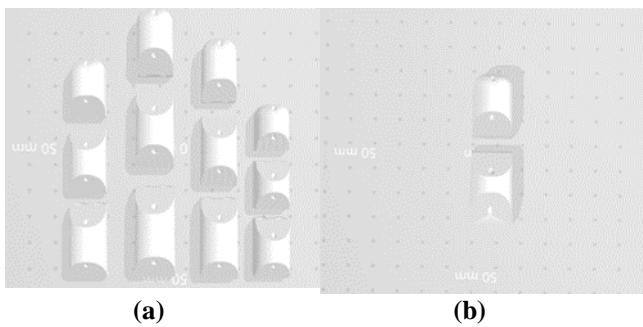


Figure 2. Finger knuckle lengths

The second method is the measurement of the servomotor rotation time, that is, the relationship between the servomotor position and the pulse width. After the electronic circuit connections were made with the bionic hand prototype, the code written in the Arduino IDE program was uploaded to the microcontroller board and Bluetooth connection was provided in the mobile application. Afterwards, 180° angle was entered for five fingers and the

rotation of the servomotor blade was ensured and the time was kept. As a result of this process, the time was stopped during the full closing movements of all nodes and noted. By using the relationship between servomotor position and pulse width, the duration obtained as a result of the experiment, the estimated realistic servomotor blade rotation angles were obtained by using the ratio/proportional method. The time taken for a rotation angle of 1° is $0.5/90 = 0.0056$ ms.

It was made in 3D printer for mechanical design and green polylactic acid (PLA) filament was used as material. The realized bionic hand design and electronic setup are presented in detail in Figure 3. The extra material in the servomotor bed was cleaned by melting it with solder. Support percentage rate must be entered to the printer when printing on the 3D printer. Normally, this rate can be between 10% and 15%. Since this rate is entered as 20% while printing, a filament layer has been created on the printer for extra support on the prototype. The extra support layer was removed with a side cutter and pliers. Thus, the unnecessary filament layer is eliminated. After cleaning the parts of the hand prototype, joint connections for each finger were made with fishing line and parachute rubber, and its connection to the hand body was ensured.

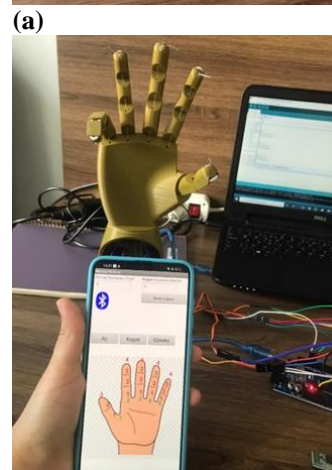
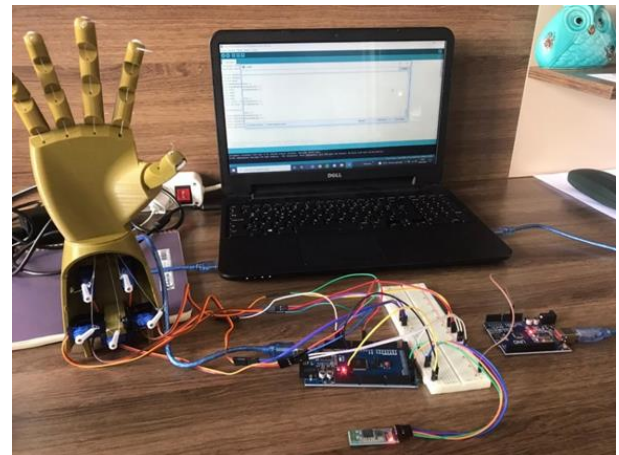


Figure 3. The performed bionic hand design and electronic setup

The free end of the fishing line is removed from the hand body to the wrist, which is the servomotor bearing. The rotations of the five servomotor blades are fixed to the

servomotor bed with the help of a silicone gun so that they do not interfere with each other. The fishing line, which is free on the wrist, is connected to the corresponding servomotor blades so that each of the five fingers is connected to a servomotor separately. Thus, when the servomotor blade rotates according to the adjusted angle value, the movement of the fingers will be realized depending on the rotation angle. Then, the finger joints, hand body and servomotor bearing connection of the hand prototype were made. Servomotors are fixed to the wrist and fingertip servomotor blade connection is made. The mechanical part has been completed and thus the stage of providing the electronic equipment has started.

In the electronic circuit design, each servomotor is connected to the PWM pins of the Arduino Mega 2560 microcontroller. The servomotor to which the thumb is connected is connected to the 13th pin, the servomotor to which the index finger is connected to the 12th pin, the servomotor to the 11th pin to which the middle finger is connected, the servomotor to the 2nd pin to which the ring finger is connected and the servomotor to the 3rd pin to which the pinky finger is connected. The 5 V terminals of the servomotors are connected to the 5 V pin of the microcontroller, and the ground terminals are connected to the GND pin of the microcontroller. However, after the Arduino Mega 2560 microcontroller was connected to the computer and the project was run. It was observed that only the Arduino Mega 2560 microcontroller was not enough to meet the power drawn by the servomotors, so a second microcontroller was added to the circuit to support and share power. By connecting the 5 V and ground ends of three servomotors to the power pins of the Arduino UNO microcontroller and two servomotors and the HC-06 Bluetooth module to the power pins of the Arduino Mega 2560 microcontroller, the desired operation of the servomotors has been achieved with two power connections. In addition, the RX end of the HC-06 Bluetooth module is connected to pin 50 of the microcontroller, the TX end to pin 51 of the microcontroller, the 5 V end to the 5 V pin of the microcontroller, and the GND end to the GND pin of the microcontroller, and the electronic circuit design has been completed.

The flow showing the operation of the system is given in Figure 4. Thanks to the codes uploaded to the microcontroller card of the bionic hand and the mobile application connected to the microcontroller card with the Bluetooth module, the desired finger and joint movements can be performed with the commands given. When the power connection is provided to the microcontroller card, the information entered through the buttons in the mobile application is transmitted to the microcontroller. Then, the servomotor movements were performed according to the situations given below.

First, the system was asked the question "Have the finger number and knuckle number been entered?" in the mobile application. If the answer is "Yes", the next step is taken, if the answer is "No", the initial state is returned. When the answer is "Yes", the next step, the finger information, is

read. Thumb, index finger, middle finger, ring finger and little finger were defined from 1 to 5, respectively, and it was determined which finger and which joint would move according to the finger and knuckle number read. Two conditions, namely the middle and root joints defined in the thumb, respectively, and three conditions were defined in the other fingers, namely the tip, middle and root joints. For example, if the entered finger number is 1 and the node number is 2, the loop containing the commands of the part up to the 2nd node of the 1st finger is executed. In the next step, according to the finger and knuckle number selected for each finger separately, the information about which joint will be moved starting from the end joint was sent, and thus all fingers were independently controlled.

The "Open" button to open all fingers and the "Close" button to close all fingers were added to the mobile application design. In addition, a number is defined for each finger that allows the fingers to be opened separately. The finger and knuckle number information entered in the mobile application is transmitted to the microcontroller and the rotation angles of the servomotors are determined according to the situations defined in the codes. The movement of the desired joints of the determined finger is provided by the rotations of the servomotor blades. The desired finger has the ability to open fully independently from other fingers, and it is also designed to have the ability to fully close and fully open all fingers at the same time.

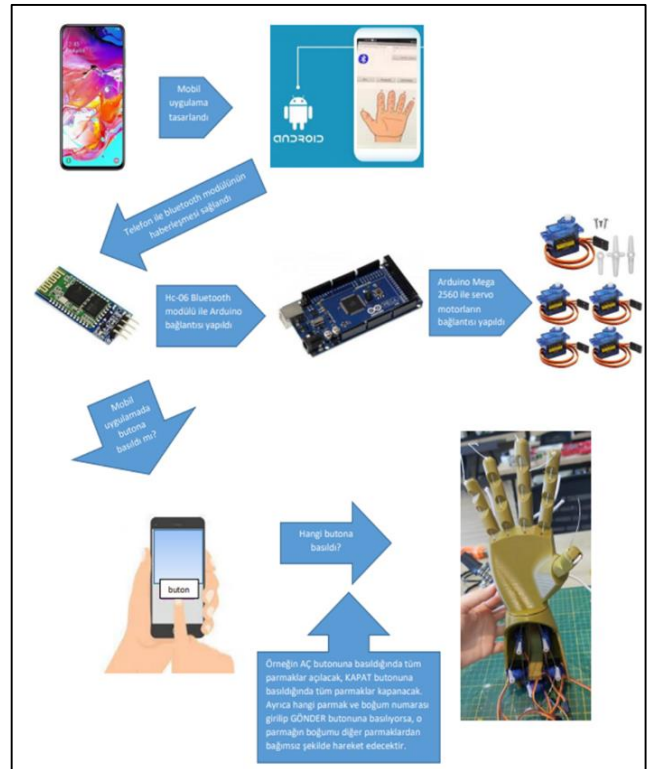


Figure 4. Flow showing the operation of the system

3. RESULTS AND DISCUSSION

(3) is used in the first method for the servomotor blade rotation calculation. In the second method, the PWM signal-

servomotor position relationship is considered. In the program calculated using these two methods, the servomotor blade rotation angles determined by the experimental practice-observation method are given in Table 1.

Table 1. Center angle calculation, rotation angle values found with PWM signal-servomotor position relationship and used in the program.

Finger name	Knuckle number	Length to finger knuckle, S (cm)	Servomotor blade length, r (cm)	Servomotor blade rotation angle found from equation (3) with center angle calculation, Q ($^{\circ}$)	Full closing time of finger knuckle (ms)	Rotation angle of servomotor blade found with PWM signal and servomotor position relationship, Q ($^{\circ}$)	Rotation angle values used in the program, Q ($^{\circ}$)
Thumb	First knuckle	2	1.5	38.216	0.594	106	112
	Second knuckle	3	1.5	95.541	0.924	165	170
Index Finger	First knuckle	2	1.5	38.216	0.235	42	50
	Second knuckle	2.4	1.5	84.076	0.532	95	100
	Third knuckle	3	1.5	141.401	0.918	164	170
Middle finger	First knuckle	2	1.5	38.216	0.202	36	40
	Second knuckle	2.5	1.5	71.656	0.375	67	80
	Third knuckle	3.5	1.5	152.866	0.941	168	179
Ring Finger	First knuckle	2	1.5	38.216	0.336	60	63
	Second knuckle	2	1.5	76.433	0.610	109	115
	Third knuckle	3	1.5	133.758	0.969	173	179
Little finger	First knuckle	1.5	1.5	28.663	0.459	82	91
	Second knuckle	1.75	1.5	62.102	0.717	128	135
	Third knuckle	2.5	1.5	109.873	0.958	171	179

In the second method, when the PWM signal and servomotor position relationship is calculated using the ratio proportional method, since the time taken for a 90° rotation angle of the servomotor blade is 0.5 ms, the time taken for a 1° rotation angle is 0, It is calculated that $5/90 = 0.0056$ ms. The exact closure times of the knuckles of each finger were found by timing with a stopwatch. Servomotor blade rotation angles were calculated for each finger and knuckle using the found times rotation angle/rotation time ratio.

For the rotation angles of the servomotor blade given above, when the angles resulting from the two methods were compared, the closing times of the knuckles for each finger were determined by the second method, the observation method, and it was observed that the corresponding servomotor rotation angle gave the most realistic and most reliable results. Because when the angle values obtained in the calculation of the center angle and rotation angle calculation, which is the first method, are entered into the system, it has been determined that the targeted healthy finger movements have not been achieved. The second method was compared with the first method and it was observed that the second method gave values close to the true angle values. In order to obtain exact values, the values in the second method were optimized by experimental observations. When the angle values calculated as a result of the first method are compared with the angle values selected in the application, it is seen that there are serious deviations. For this reason, angle values were selected based on the second method, which has the closest angle values to reality. Afterwards, since the servomotor rotation angles were aimed to be sensitive, the healthiest angle values were obtained by increasing-decreasing and observing the calculated angles. Thus, precisely precise turning angles were determined.

By applying the study, the joint movements of the five fingers of a targeted hand were provided by servomotors. The designed application is mobilized with the Bluetooth module.

4. CONCLUSIONS

In this study, a bionic hand and its control were successfully carried out with the mobile application, which is the target of the project. The project was designed and implemented as closely as possible to the normal human hand size, based on joint control of the fingers attached to a hand body prototype. In the applications made, it has been observed that the PWM method, which adjusts the angle of the servomotor in the control of the servomotor blade rotation angles, gives more successful results. Achieving precise grip angles of the fingers has not been fully realized due to tolerances in mechanical designs. In order to adjust this sensitivity, the angle values were improved with the experimental apply observation method.

In the following periods, it is possible to provide project development with research and development studies by making use of the development of technology. However, one of the next aims of this project is to develop the project not only with a hand prototype, but also to imitate all the structures and movements of the human hand. There is also the development of the bionic hand for different purposes. For example, in the next stages, it is aimed to detect the image of the object that the bionic hand has grasped with the image processing method and to determine which substance it consists of depending on the color-shape-tissue structure and to provide the gripping movement by applying the appropriate pressure to the object that the bionic hand has grasped. Providing the movements of the bionic hand with sound or brain signals can be counted among other work that

can be done. The demand for bionic hands is increasing both in the health sector and in companies that design medical products. With the advancement of technology, the project is open to improvements in line with demand. Ultimately, the project has sustainability and has the potential to be turned into a product.

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Author Contributions

Conceptualization: H.M., Investigation: C.D., M.A.Ü.; Material and Methodology: C.D., M.A.Ü.; Supervision: H.M., Visualization: C.D., M.A.Ü.; Writing-Original Draft: C.D., M.A.Ü.; Writing-review & Editing: H.M., C.D., M.A.Ü.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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The Use of an Artificial Neural Network for Predicting the Machining Characterizing of Wood Materials Densified by Compressing

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Abstract: In this study, an approach for artificial neural network (ANN) was presented to predict and control arithmetical mean surface roughness value (R_a), machining properties of wood materials densified by compressing in a computer numerical control (CNC) machine. Black poplar (*Populus nigra* L.) tree species were used as the experimental material. After specimens were densified by Thermo-Mechanical (TM) method at 0%, 20%, and 40% ratios, machining process of specimens were performed at 1000, 1500, and 2000 mm/min feed speeds and in 12000, 15000, 18000 rpm rotation speed on a CNC vertical wood machining center by using two different cutters. Data used for the training and testing of an ANN. Cutter type, compression ratio, feed rate, and spindle speed were selected as Four parameters. While hidden layer of the R_a model has ten neurons, one hidden layer was used, Compression ratio is the most significant parameter, followed by feed speed for R_a values. surface roughness increases with increased feed rate. R_a values in training, validation, and testing the data set for R_a were 0.97122, 0.8538, and 0.76685, respectively. The Mean Square Error (MSE) value was determined as 0.0019914 test of the network. The proposed ANN model came to agreement with the measured values in predicting surface roughness R_a values of MAPE. The MAPE value was calculated as 6.61, which can be considered a very good prediction (MAPE < 10 % = very good prediction). The study showed that obtained ANN prediction model is a practical and efficient tool to model the R_a of wood. For reducing energy, time and cost in the wood industry (densification and CNC wood machining), current research results can be implemented.

Keywords: Artificial neural networks, Thermo-Mechanics, densification, black poplar, machining, roughness

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1. INTRODUCTION

From the past to the present, development of different "Wood Modification Methods" have been performed because of all scientific studies carried out to rule out some of the unfavourableness of wood material. Wood modification applications were carried out to change or improve wood material properties (Şenol and Budakci, 2016; Senol, 2018).

The solid wood is mainly considered too soft or too weak for use in construction, which requires high strength, hardness, and durability. However, using wood material by increasing density can be an option in comparison to other materials (Blomberg and Persson, 2004; Pelit et al., 2014). The density of solid wood is mechanical (Rautkari, 2012) and machining (Lin et al., 2006; Malkocoglu and Ozdemir, 2006; Malkocoglu, 2007; Zhong et al., 2013; Pinkowski et al., 2018; Sofuoglu et al., 2022) significantly affect its properties. There is a relation between the surface quality of

machined solid wood and its density, denser wood presents the best quality of the machined surface (Lopes et al., 2014; Sofuoglu et al., 2022). When examined in general, hardness, mechanical and physical properties increase, surface roughness and wettability decrease, and occurrence of spring back as a negative situation may be seen, contingent on the increase in density in compressed densified wood species.

Before the compressed wood materials are converted into the final product, they must be machined with the machines used in the machining of classical solid wood, as well as with modern computer-aided machining centers. The results obtained in this study will determine the parameters to obtain the highest surface quality. Efficiency will increase, and the next step, such as sanding, will need to be omitted or minimally applied.

For this purpose, in this study, the poplar tree species that is frequently produced and used worldwide were machined by using Computer Numerical Control with today's technology, after intensification. Wood machining parameters with different values affecting the surface quality were used; determination of densification effect for the processing properties and determination of optimum parameters for obtaining the smoothest surface were aimed.

Neural networks are frequently applied in many industrial applications. They are suitable for modeling various manufacturing functions due to their ability to learn complex non-linear and multivariable relationships between process parameters (Karayel, 2009). Using artificial neural networks (ANNs) have been applied in wood and wood-based materials science

and the wood machining industry, such as in recognition of wood species (Esteban et al., 2009), the drying of solid wood (Wu and Avramidis, 2007) the mechanical properties (Fernández et al., 2012; Tiryaki and Aydin, 2014), machining parameters optimization (Sofuoglu, 2015; Gurgun et al., 2021), wood surface roughness (Ayanleye et al., 2021; Gurgun et al., 2021) the classification of wood and wood-based materials defects (Avramidis and Iliadis, 2005; Pan et al., 2021), the analysis of moisture (Zhang et al., 2016), noise emission in the machining of wood (Ozşahin and Singer, 2022) and fracture toughness of wood (Samarasinghe and Jamieson, 2007).

Investigation and evaluation of R_a CNC machining experiments for black poplar wood species were carried out in this study. Modeling the effects of some machining parameters on the R_a in CNC machining densified by compressing is the main objective of the present study.

2. MATERIAL AND METHOD

Sample preparation

Black poplar (*Populus nigra L.*) with low density and widely grown was selected for the experimental material in the study. Specimens were all randomly chosen from Afyonkarahisar, Turkey. Conditioning of samples were carried out at 20 ± 2 °C and 65 ± 5 °C, with relative humidity to moisture content (MC) of about 12%. The density of poplar solid wood material at 12% humidity was specified as 0.85 g / cm^3 (ISO 13061 2014; ISO 13061-2 2014). Figure 1 shows the experimental process of the study.

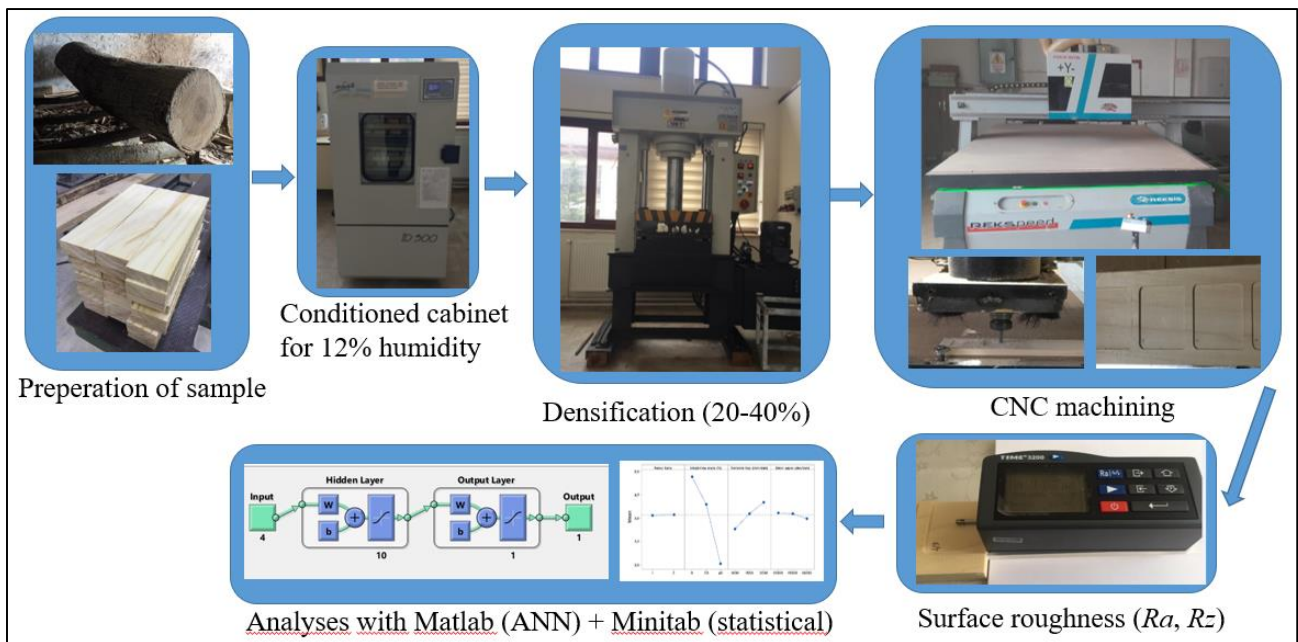


Figure 1. Schematic representation for experimental design

The densification process by compressing with the thermo-mechanical method (Total time = Heating time + 15 min, 0%, 20 %, and 40% ratios) for samples in the dimensions given in Table 1 were performed by a designed hydraulic press (Gazi University, Ankara / Turkey).

Table 1. Pre-compression dimensions of test samples (Tosun, 2021)

Compression ratio	Length (mm)	Width (mm)	Thickness (mm)
Control	430	85	20
20%	430	85	25
40%	430	85	33.3

After the densification process, a Reksis Rekspeed 2137 3-axis CNC milling machine (Çözüm Ahşap, Afyonkarahisar, Turkey) was used to carry out experiments. Experiments were carried out with different two router cutters (Figure 2). Using new cutters for each machining test was provided. Four machining parameters were used in the experiment (Table 2)

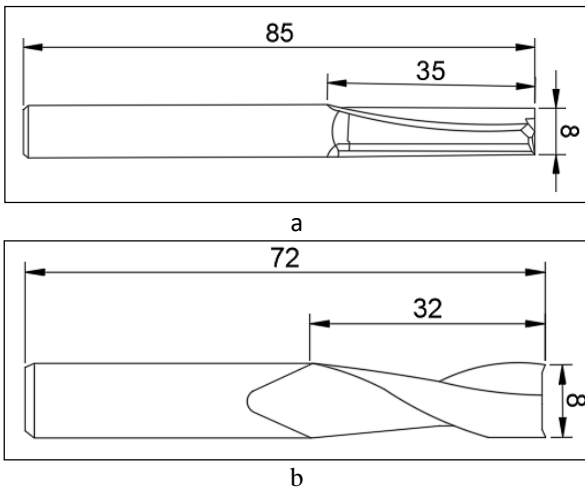


Figure 2. High-speed steel end mills (mm) a) Two-flutes straight end mill (Netmak), b) Two-flutes helisel end mill (Knob)

Table 2. Assignment of levels to factors (parameters used in the face milling of black poplar) (Tosun, 2021)

Machining parameter	Coded levels		
	Level 1	Level 2	Level 3
Cutter type	1	2	
Compression ratio (%)	0	20	40
Feed (mm/min)	1000	1500	2000
Spindle speed (rpm)	12000	15000	18000

Cutter 1: Two-flutes straight end mill, Cutter 2: Two-flutes helisel end mill

A total of 54 pieces with dimensions of 55x55 mm² were grooved on wood materials by a CNC router (Figure 3).

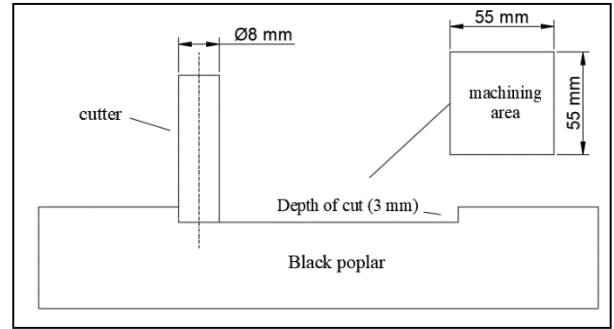


Figure 3. CNC process parameters

The optimal network structure for surface roughness CNC machining experiments is in Figure 4.

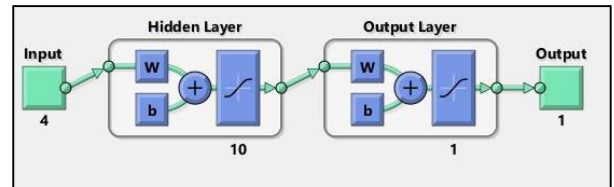


Figure 4. Optimal network structure for surface roughness CNC machining experiments.

Mean absolute percentage error (MAPE), root mean square error (RMSE), and correlation coefficient (R²) was used to measure performance of the network.

3. RESULTS AND DISCUSSION

Comparison of the measured values and predicted values by the neural network model of the R_a is presented in Figure 5. Measured and predicted values of surface roughness (R_a) and their errors are given in Table 3.

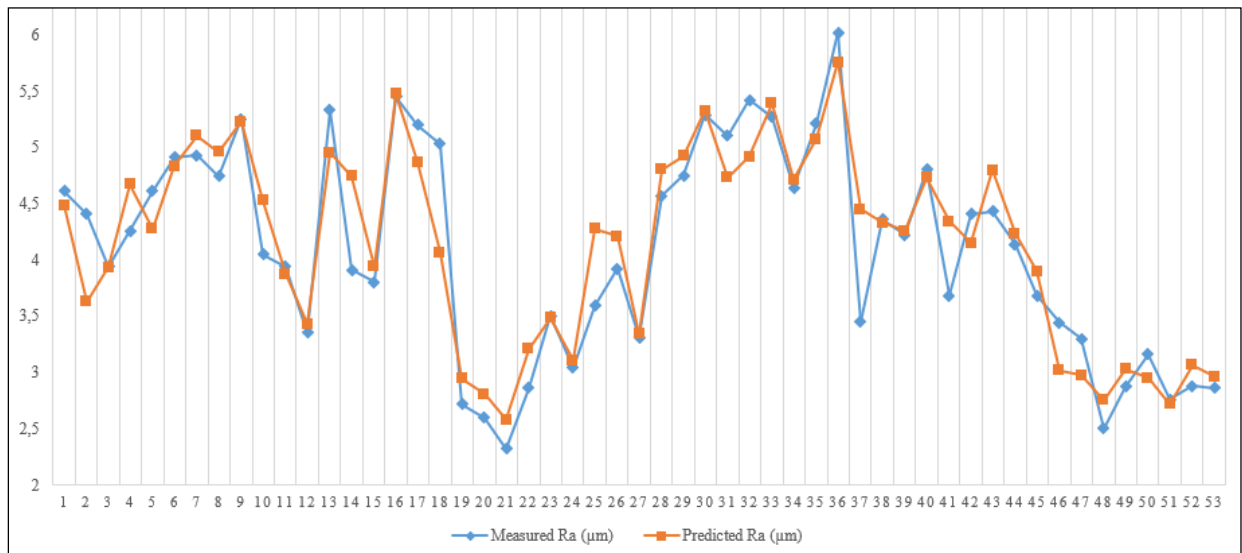


Figure 5. Comparison of measured and predicted results of R_a

Table 3. Measured and predicted values of R_a and their errors

Process No	Cutter type	Compression ratio (%)	Feed (mm/min)	Spindle speed (rpm)	Measured R_a (µm)	Predicted R_a (µm)	Error %
1	1	0	1000	12000	4.61	4.48	2,82
2	1	0	1000	15000	4.41	3.63	17,69
3	1	0	1000	18000	3.94	3.94	0,00
4	1	0	1500	12000	4.26	4.67	-9,62
5	1	0	1500	15000	4.61	4.27	7,38
6	1	0	1500	18000	4.91	4.83	1,63
7	1	0	2000	12000	4.93	5.10	-3,45
8	1	0	2000	15000	4.74	4.96	-4,64
9	1	0	2000	18000	5.25	5.23	0,38
10	1	20	1000	12000	4.05	4.53	-11,85
11	1	20	1000	15000	3.94	3.88	1,52
12	1	20	1000	18000	3.35	3.43	-2,39
13	1	20	1500	12000	5.33	4.95	7,13
14	1	20	1500	15000	3.91	4.74	-21,23
15	1	20	1500	18000	3.80	3.94	-3,68
16	1	20	2000	12000	5.45	5.47	-0,37
17	1	20	2000	15000	5.20	4.87	6,35
18	1	20	2000	18000	5.03	4.07	19,09
19	1	40	1000	12000	2.72	2.94	-8,09
20	1	40	1000	15000	2.60	2.81	-8,08
21	1	40	1000	18000	2.32	2.58	-11,21
22	1	40	1500	12000	2.86	3.21	-12,24
23	1	40	1500	15000	3.50	3.49	0,29
24	1	40	1500	18000	3.04	3.11	-2,30
25	1	40	2000	12000	3.59	4.27	-18,94
26	1	40	2000	15000	3.92	4.21	-7,40
27	1	40	2000	18000	3.31	3.34	-0,91
28	2	0	1000	12000	4.57	4.80	-5,03
29	2	0	1000	15000	4.75	4.92	-3,58
30	2	0	1000	18000	5.29	5.33	-0,76
31	2	0	1500	12000	5.10	4.73	7,25
32	2	0	1500	15000	5.42	4.92	9,23
33	2	0	1500	18000	5.27	5.39	-2,28
34	2	0	2000	12000	4.64	4.71	-1,51
35	2	0	2000	15000	5.21	5.07	2,69

36	2	0	2000	18000	6.02	5.76	4,32
37	2	20	1000	12000	3.45	4.45	-28,99
38	2	20	1000	15000	4.36	4.33	0,69
39	2	20	1000	18000	4.22	4.26	-0,95
40	2	20	1500	12000	4.80	4.73	1,46
41	2	20	1500	15000	3.68	4.34	-17,93
42	2	20	1500	18000	4.41	4.15	5,90
43	2	20	2000	12000	4.43	4.80	-8,35
44	2	20	2000	15000	4.14	4.23	-2,17
45	2	20	2000	18000	3.68	3.90	-5,98
46	2	40	1000	12000	3.44	3.02	12,21
47	2	40	1000	15000	3.30	2.97	10,00
48	2	40	1000	18000	2.50	2.75	-10,00
49	2	40	1500	12000	2.87	3.03	-5,57
50	2	40	1500	15000	3.16	2.95	6,65
51	2	40	1500	18000	2.76	2.71	1,81
52	2	40	2000	12000	2.88	3.06	-6,25
53	2	40	2000	15000	2.86	2.96	-3,50
54	2	40	2000	18000	2.78	2.75	1,08

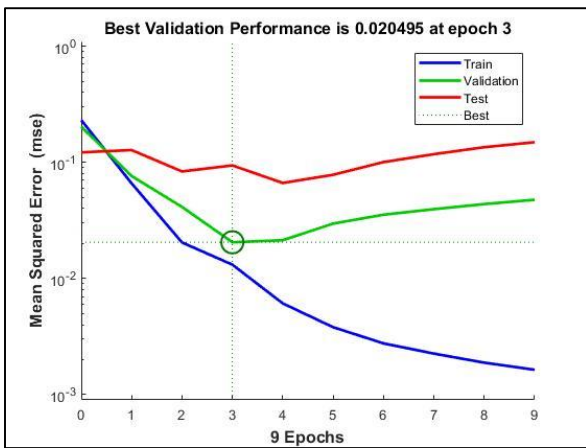


Figure 6. Performance of ANN model

In the literature, The MSE values were calculated as 1.05 and 3.70 surface roughness for solid wooden edge-glued panels (Sofuoglu, 2015) and the MAPE, RMSE, and R^2 values of the testing period of the ANN model were found as 8.556, 1.245, and 0.9814%, respectively (Ozsahin and Singer, 2021). In this study, The Mean Square Error value was determined as 0.0019914 test of

the network. MSE value is satisfactory for the accuracy of models. The performance of the ANN model for black poplar was shown in Figure 6.

One of the values to measure network ability to predict correctly is mean absolute percentage error (MAPE). MAPE values of artificial networks estimating the surface roughness of different materials under different machining conditions were reported as 3,866 for solid wood material (*Pinus sylvestris*) Gurgun et al. (2022) and 20,18 for massive wooden edge-glued panels (Sofuoglu, 2015). If the (MAPE) values are less than 10%, it is considered acceptable for a prediction with high accuracy (Nazerian et al., 2020). In this study, MAPE value was calculated as 6.61, which can be considered a good prediction. Figure 7 presents the relationship between the experimental results and the ANN-predicted results. The measured Ra values of the samples show similarity with the values predicted by the ANN model. While the R-value is high in training ($R=0.97122$), it is lower in validation ($R=0.8538$) and Test ($R=0.76685$). Wood material has a heterogeneous structure. The roughness data obtained from a heterogeneous structure may cause this.

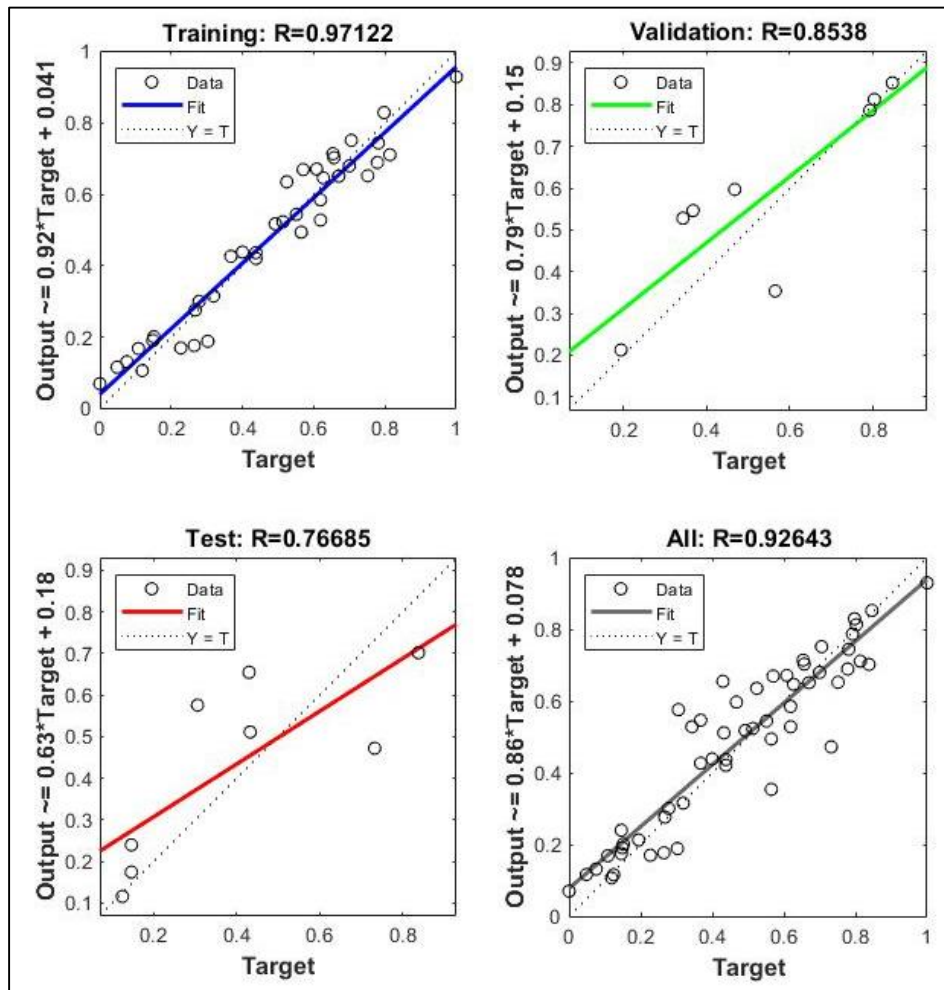


Figure 7. Relationship between experimental results and ANN-predicted results

4. CONCLUSIONS

In this study, the effects of cutter type, compression ratio, feed, and spindle speed on the R_a of wood were investigated and modeled using the ANN. The predicted R_a from the model is close to the values measured experimentally. The conclusions were summarized as follows:

1. Compression ratio is the most significant parameter, followed by feed speed for R_a values.
2. Feed rate is an important parameter, and surface roughness increases with increased feed rate.
3. The ANN modeling approach can be applied in predicting the R_a of wood samples under given conditions when the training of the model is properly completed.
4. R^2 values in training, validation, and testing the data set for R_a are 0.97122, 0.8538, and 0.76685, respectively.
5. The Mean Square Error (MSE) value was determined as 0.0019914 test of the network. MSE value is satisfactory for the accuracy of models.
6. The proposed ANN model came agreement with the measured values in predicting surface roughness R_a values of MAPE. The MAPE value was calculated as 6.61, which can be considered a very

good prediction (MAPE < 10% = very good prediction).

7. In further research, the ANN approach can be used to predict the surface roughness of different wooden materials.

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In the calculations in this study, the data in the master's thesis titled "Effect of thermo-mechanical densification on machining properties of massive wooden material" were used.

Ethics Committee Approval

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Author Contributions

M.T.: Construction of experiments, analysis of results. SDS: Experiment design, article writing and analysis of results. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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The Effects of the Traditional Data Augmentation Techniques on Long Bone Fracture Detection

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Abstract: Image collection and preparation phases are highly costly for machine learning algorithms. They require the majority of labeled data. Hence, the image pre-processing method, data augmentation, is commonly used. Since there are so many proposed methods for the augmentation task, this comparison study is presented to be a supporting guide for the researchers. In addition, the lack of studies with animal-based data sets makes this study more valuable. The study is investigated on a comprehensive medical image data set consists of X-ray images of many different dogs. The main goal is to determine the fracture of the long bones in dogs. Many traditional augmentation methods are employed on the data set including flipping, rotating, changing brightness and contrast of the images. Transfer learning is applied on both raw and augmented data sets as a feature extractor and Support Vector Machine (SVM) is utilized as a classifier. For the classification task, the experimental study shows that changing the contrast is the outstanding method for accuracy manner, while the rotation method has the best sensitivity value. The classification accuracy of the raw data, which was 0.817, improved to 0.845 with augmented data by changing the contrast values. The findings of the study also demonstrate that the transfer learning method is highly effective on the animal-based data set.

Keywords: biomedical image processing, bone fractures, convolutional neural networks, deep learning, data augmentation.

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1. INTRODUCTION

The recent development of deep learning studies has allowed the use of deep learning methods in many various fields. One of the most popular field is biomedical (Li, 2022). In the area of orthopedics, these deep architectures are utilized for assisting in fracture detection (Jangid et al., 2021), bone disease detection (Rajesh et al., 2022) and age assessment (Deshmukh et al., 2021).

Despite of all advances, deep architectures require majority of labeled data for learning phase. Accessing a comprehensive data set, especially in the field of medicine, is an important problem for deep learning analyzes. That problem leads us to one of the most preferred pre-processing methods, data augmentation.

Data augmentation is a transition from limited data to more data. On one hand, it helps to reduce overfitting problem, on

the other hand, it equalizes unbalanced data sets. In order to prevent overfitting problem, it is possible to modify the network structure. Batch normalization and drop-out can be given as examples of the modifications. Data augmentation techniques are different from them inasmuch as they are basically a pre-processing step (Shorten & Khoshgoftaar, 2019).

In the literature, data augmentation methods are frequently used in deep learning analyzes. In a work, authors explored and compared the data augmentation methods for image classification by applying simple techniques, such as cropping, rotating, and flipping the images. Also, they experimented Generative Adversarial Neural Networks (GANs), and a proposing method named neural augmentation. Their experiments show that the traditional augmentation methods are more effective than the others (Perez & Wang, 2017).

Shijie et al. used some data augmentation methods in their paper include: GAN/WGAN, flipping, cropping, shifting, PCA jittering, color jittering, noise, rotation, and some combinations. According to the results of the study, the four individual methods (cropping, flipping, WGAN, rotation) perform generally better than the others, and some appropriate combination methods are slightly more effective than the individuals (Shijie et al., 2017).

In the recent years, GANNs have become very popular for synthesizing images (Calimeri et al, 2017, Frid-Adar et al, 2018, Shin et al., 2018). GANNs are one of the machine learning method purposed by Ian Goodfellow (Goodfellow et al., 2014). GAN Network is formed by two neural networks competing with each other. The network has an ability to generate new data from given training set.

In another research paper, a variety of augmentation strategies, horizontal flips, random crops, and principal component analysis (PCA) are investigated. Their work shows that augmentation strategy greatly affects classification performance (Hussain et al., 2018).

The works related traditional methods can be exemplified further (Jia et al., 2017; Hernández-García & König, 2018; Sajjad et al., 2019; Shunjiro et al., 2020). Nevertheless, these methods can be highly impacted by data sets, and it is difficult to find studies in the literature using comprehensive data sets of animal-based X-Ray images.

In the literature there are few studies based on X-Ray images of pets. In a research, 143 X-ray images of dogs are used due to detect the tibia fractures. They used SSD MobileNet-v2 and obtained the F-score value as 0.68 (Baydan & Ünver, 2020). After that publication, the researchers applied different deep learning architectures for the same task by increasing the number of X-Ray images. They emphasized that the results of the study are promising for fracture detection of the tibia bone (Baydan et al., 2021).

Another paper based on animal medical image analysis is a lesion identification problem. Arsomngern et al. examined 2862 thoracic X-ray images obtained from both dogs and cats to classify lung lesion. They achieved 79.6% success with the CNN model they used (Arsomngern et al., 2019).

McEvoy et al used partial least square discriminant analysis and artificial neural networks as machine learning methods for the classification problem of canine pelvic radiographs. Their dataset consists of 256 images of dogs. Due to the results, their study can be useful in the veterinary field. (McEvoy et al., 2013).

The literature review showed that augmentation methods can be remarkably beneficial for the classification and detection tasks. In addition, it is noticed that related works are inadequate in the veterinary field. Therefore, this comparison study can be useful for the researchers.

In this study, a comprehensive data set created from dogs in Ankara Metropolitan Municipality Stray Animals Temporary Care Home is employed. For more detail about

the data set, readers can be referred in our previous studies (Ergün et al., 2021; Ergün & Güney, 2021). The aim is to investigate the effect of the traditional augmentation techniques on detecting long bone fractures of the dogs. Deep architectures are utilized for realization the task. Since the task needs a lot of labeled data, data augmentation techniques are investigated including flipping, rotating, changing brightness and contrast of the images. These methods are traditional data augmentation techniques and simple to apply. After performing the pre-processing step, deep neural models are carried out for the feature extraction, then SVM is used for the classification.

2. MATERIAL AND METHOD

2.1. Long Bones of Canine (Dog)

The canine's skeleton is made up from an average of 319 bones. These bones are divided into five types depending on their function: short, long, flat, irregular and sesamoid (The Skeleton, Ch. 4, 2016). In this work, the data set is created by X-Ray images of long bones. Long bones of dogs are also divided into six classes: femur, humerus, radius, ulna, tibia and fibula. The types of the long bones are given in Fig.1.

Although long bones of the dogs are investigating in six types, four classes are employed in this paper. According to the orthopedist veterinarian, any bone fracture occurred on radius or ulna bones, occur on both bones. Thus, radius-ulna is considered as a single bone class named 'Radius-Ulna'. Besides, the fibula bone fractures are not considered in the work. Because fibula bone is an accessory bone and carrying body weight is not a duty for it.

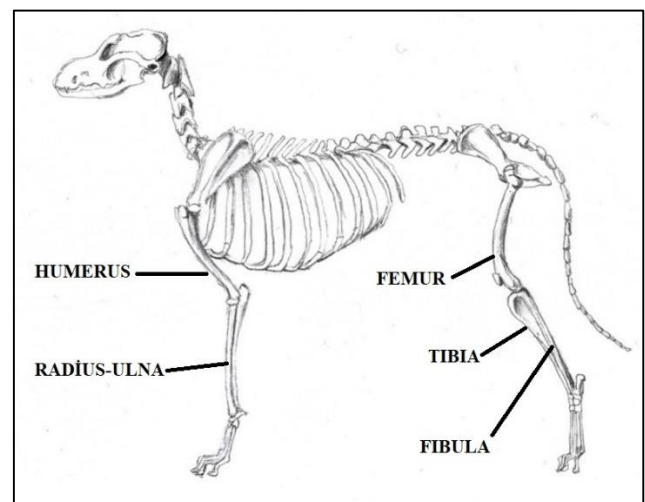


Figure 1. Labelled Long Bones of Canine (Lewis, 2019).

2.2. Data Set

The data set consists of 2028 X-Ray images of long bones of many different dogs taken from Ankara Metropolitan Municipality Stray Animals Temporary Nursing Home. 479 images of the data set were labeled as fractured, and the remaining 1549 images were labeled as no fracture. For better understanding of the data set, an example is given in Fig.2. Both images in the figure belong to radius-ulna.

2.3. Methods for Data Augmentation

Some of classical image data augmentation techniques can be divided into two categories. These are:

- Position augmentation: cropping, flipping, padding, rotation.
- Color augmentation: brightness, contrast, saturation, hue.

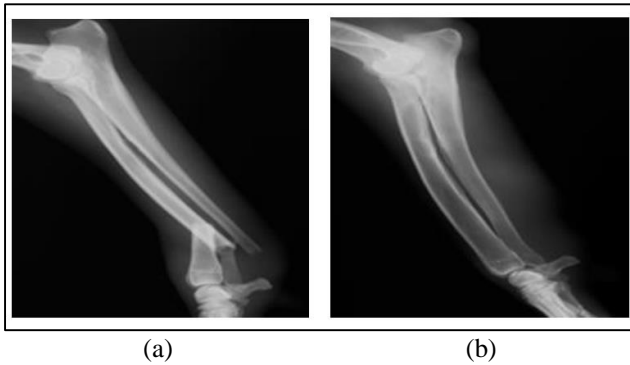


Figure 2. Sample images from the data set.
a) fractured, b) no fracture

Although, there are various methods for augmentation, four common data augmentation methods are investigated in this study because of the simplicity.

2.3.1. Flipping: Images can flip horizontally and vertically. In this study, three flipping options are applied: horizontally, vertically and both.

2.3.2. Rotation: It is rotating the images at specific angles. In this study, three different angles are applied: +15, -15 and -30 degrees. Positive angles rotate images to the left and vice versa.

2.3.3. Brightness: Another way for augmentation is changing the brightness of the image. The resultant images become lighter or darker depends on the application.

In a gray-level image matrix, pixel values can be between 0-255. Values closer to 0 mean darker, while values closer to 255 mean lighter.

Changing the brightness is done by increasing or decreasing the value of each pixel of the image. Therefore, adding a coefficient to each pixel value of the image increases the brightness of the image. Likewise, the process of subtracting the coefficient has a reducing effect on the brightness.

In this study, three brightness settings are applied:

- Adding 50 of each pixel value of the images.
- Adding 75 of each pixel value of the images.
- Subtracting 50 of each pixel value of the images.

2.3.4. Contrast: Changing the contrast of the image is another method for color augmentation. The resultant images become more or less distinguishable.

In gray-level input images, the desired contrast to match the values in the output image must be between (0-1). These

limits were processed in three different ways by trial and error method in this study:

- Contrast Setting 1: taking the contrast limits of the images as (0.1 - 0.9).
- Contrast Setting 2: taking the contrast limits of the images as (0.15 - 0.85).
- Contrast Setting 3: taking the contrast limits of the images as (0.2 - 0.8).

After the process, size of the data set increased from 2028 (479 broken, 1549 non-broken bones) to 8108 (1916 broken, 6192 non-broken bones) for each technique. After augmentation process, the outputs from all techniques are given in Fig.3- Fig.6.

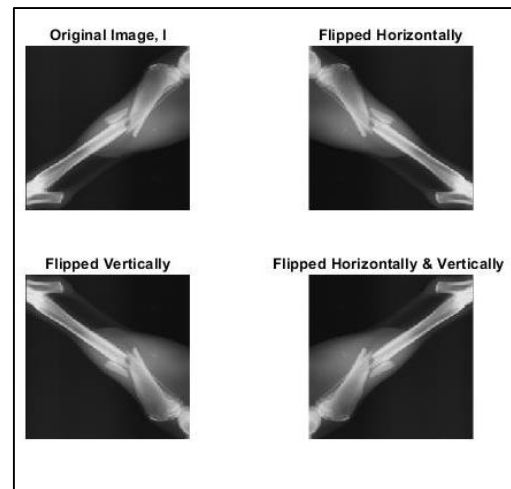


Figure 3. Output images after augmentation process for flipping.

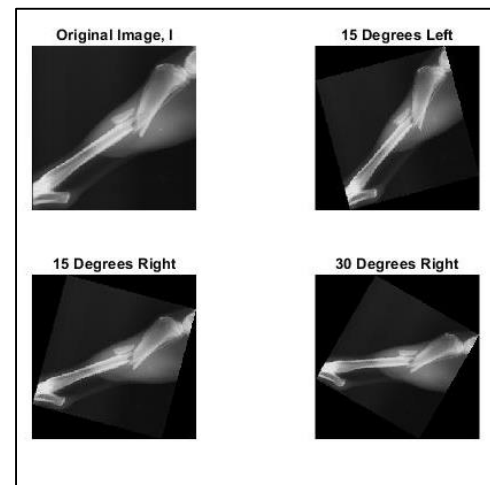


Figure 4. Output images after augmentation process for rotation.

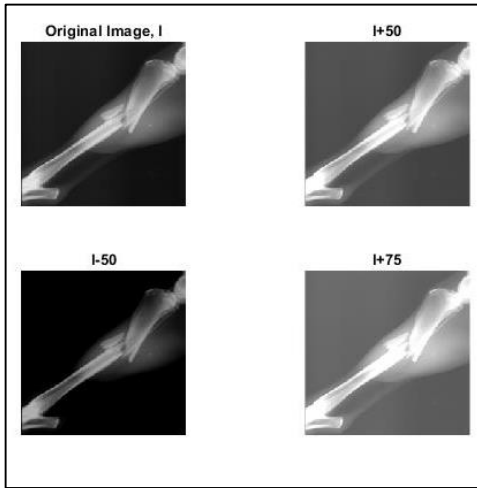


Figure 5. Output images after augmentation process for brightness.

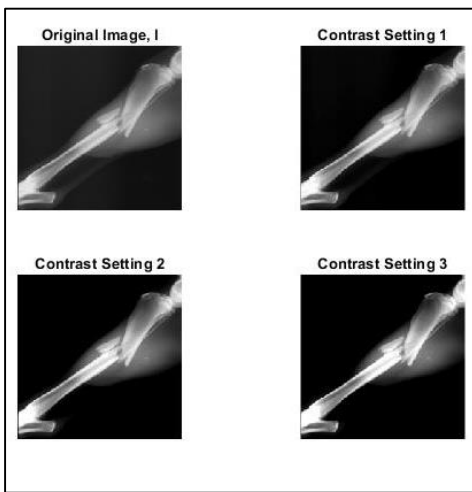


Figure 6. Output images after augmentation process for contrast.

In this study, because of the high performances on image processing problems, convolutional neural network(CNN) is used for both training and validation. (Guo et al., 2016). A convolutional neural network consists of 5 primary layers: An input layer, convolution layers, pooling layers, fully connected layers and an output layer (LeCun et al., 1989). The purpose of the convolution layer is to extract features from the input image by performing a dot product between images and filters. Pooling layer reduces dimension of image obtained from the previous layer. The matrix obtained by passing through all the determined layers is turned into a flat vector in the fully connected layer.

For the classification process, transfer learning is applied. One of the most popular deep architecture, Inception-v3 is employed for the feature extraction. Afterwards, Support Vector Machines (SVMs) is used for the classification task.

Inception-v3 is a deep convolutional neural network for assisting in detection and classification tasks. The architecture is the third edition of Google's Inception Convolutional Neural Network (Szegedy et al., 2015.). Inception-v3 has 316 deep layers with 350 connections. A faster training process is achieved by choosing number of

smaller convolutional filters. The network is presented in Fig.7.

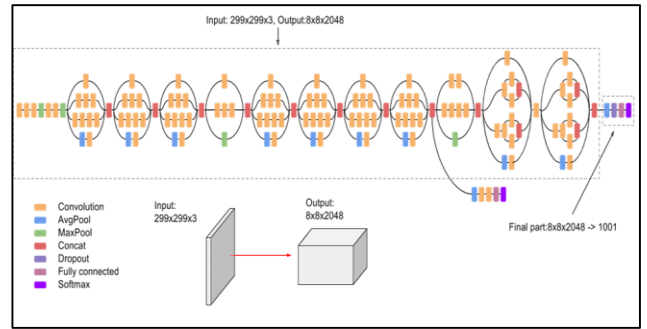


Figure 7. Inception-v3 Architecture (Szegedy et al., 2015).

In the study, the training and test sets are randomly selected 0.8 and 0.2 from the data set, respectively. Dimensions of each image in the data set are set to 200x200.

2.4. Performance Metrics

Many performance metrics are utilized due to measure the success of the models. In this study, accuracy and sensitivity are preferred. The accuracies of all models are calculated using (1).

$$Accuracy = \frac{TP+TN}{TP+TN+FN+FP} \quad (1)$$

where the true negative (TN) parameter shows the number of negative examples classified accurately. Similarly, true positive (TP) indicates the number of positive examples classified accurately. False positive (FP) means the number of actual negative examples classified as positive; false negative (FN) is the number of actual positive examples classified as negative.

Sensitivity parameter is calculated using (2). This metric is often used when false negatives are more attention than false positives.

$$Sensitivity = TP / (TP + FN) \quad (2)$$

3. RESULTS

The objective of the study is to classify the images as fractured or no fracture. In order to achieve this goal, Inception-v3 network and SVMs are utilized. While realizing the objective, some image augmentation methods are applied and compared their effect of the classification success. The results of the classification are given in Table 1 for each augmentation technique.

Despite the diversity of the augmentation techniques, in this study four of them are studied. It is because, these methods are easy to implant and they have very low cost.

From Table 1, it can be seen that the classification accuracy of the raw data is 0.8177. After the augmentation methods, the accuracy is increased except for the rotation process. The most effective method is changing the contrast of the images in data set, as it has 0.8450 classification accuracy. Although

the rotation process seems to decrease the classification success, it is observed that it increases the sensitivity.

In medical cases, false negatives are more substantial than false positives. Consequences can be more severe if actual positive cases are missed. Therefore, sensitivity comes to the front. Although the accuracy of rotation method is low, the sensitivity rates are more promising.

Table 1. Classification accuracy for raw and augmented data sets

Data Set	Accuracy	Sensitivity
Raw data	0.8177	0.8210
Augmented data with flipping	0.8425	0.8374
Augmented data with rotating	0.7700	0.9029
Augmented data with brightness	0.8225	0.8524
Augmented data with contrast	0.8450	0.8136

4. DISCUSSION AND CONCLUSIONS

After literature review, it is easily seen that the veterinary medicine problems are not adequately studied using deep neural networks. Although deep neural architectures need majority of data sets, it is hard to find comprehensive animal-based medical image data set. In order to make the deep models well trained and more robust, data augmentation is used.

Recently, data augmentation has been made using deep models. Using deep models can achieve great success, however they have complex structures as well as being highly dependent on hardware systems. For these reasons, traditional methods are applied in this study. For the future studies it would be intriguing to implement the modern approaches based on deep learning.

Despite recent advances in deep models, classical augmentation methods still remain popular. In a study which exemplifies this popularity, the authors aimed to classify different types of fractures in the proximal humerus bone of humans. 1891 plain shoulder radiographs with five labels were investigated in the study. The size of the training data set was increased by using traditional augmentation methods such as shifting, scaling and rotations (90°, 180°, 270°). After the pre-processing step, the authors used ResNet-152 network and obtained promising performance. It is claimed that using 90°, 180° and 270° rotations for augmentation process might lead to overfitting and they suggested changing the degree slightly. Furthermore, using JPEG images may affect the image quality because of the lossy compression process. For this reason, they also suggested to use lossless compression images, such as PNG and TIFF (Chung et al., 2018.).

In this study, using PNG images and rotating the images with small angles seems to be assisting to the study of Chung et. al. Nevertheless, it was observed that the rotation method reduced the classification accuracy. It appears that some

image information may have lost somehow during the rotation process.

One of the keys to the success of augmented data sets is the selection of the appropriate augmentation method according to the data set. Because, application of some augmentation techniques may add misleading information to the data. In the study, the authors emphasized that using shear, strain or spot noise augmentation can result in a misclassification situation. Thus, they prefer to perform mirroring, sharpness, brightness and contrast augmentation (Yahalomi et.al., 2019.). It can be thought that a similar situation may occur for the rotation method in this study.

What's more, the researchers of the regarding study aim to classify wrist fractures as broken or unbroken. Their data set was composed of 695 fractured and 694 unbroken labeled images. They augmented images by applying flipping, rotation, shifting, shearing and zooming. They used InceptionV3 as a transfer learning method and demonstrated that transfer learning from deep CNN pre-trained on non-medical images are effective just as this study. They computed offline data augmentation in their study, and the same augmented images were used to train deep model. The authors implied that it could be better to use different augmented images for each epoch (Kim and MacKinnon, 2018.). Hence, it may be considered to conduct a study for online data augmentation in future studies.

In conclusion, data augmentation is a very powerful technique for creating more comprehensive data sets. In the literature, numerous related works have been done, however all of these methods depend on the data sets and to the best of authors' knowledge, no similar research exists on the data set consisting canine's X-Ray images. For this reason, authors wanted to contribute to the literature with the original data set. The results of this study are promising for future works.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Simulation of Load Behavior Based on Perturb-Observation Method in Non-Isolated Boost Converter for Maximum Power Point Tracking of Thermoelectric Generators

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Abstract: The efficiency of thermoelectric generators (TEGs) is quite low. To operate the TEGs at the maximum power point (MPP), the internal resistance of the connected load and the TEG must be equal. This is not always possible. For this, converters containing the maximum power point tracking (MPPT) algorithm tracking MPP are placed between the TEG and the load. These converters cannot perform MPPT on every connected load value. The aim of this study is to investigate and highlight at which load values MPPT can be performed in non-isolated boost converters by using perturb & observation (P&O) method. For this purpose, a 50 W converter was designed with a 45.76 W TEG in MATLAB/Simulink environment. Load resistances have been increased starting from the minimum value up to 5.84 ohm being the internal resistance value of the TEG. For this case, the amount of error in MPPT was large up to the internal resistance value of the TEG. In other words, the P&O algorithm could not perform MPPT. When the load resistance value started from 5.84 ohms and increased to larger values, MPPT could be performed by means of the non-isolated boost converter with the P&O algorithm.

Keywords: Thermoelectric generator, MPPT, Boost converter, Load limit.

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1. INTRODUCTION

Thermoelectric generators (TEG) used to recover waste heat are semiconductor elements. Their output power raises in direct proportion to the temperature difference between their surfaces (Mamur et al., 2021). Since the efficiency of TEGs is low, it is important to generate maximum power for improving their working performance and ensure that they work close to full capacity (Mamur and Ahiska, 2015). To generate maximum power from the TEG, the overall internal resistance of the TEG setup and the external load resistance linked to the TEG must be equal (Bond et al., 2015). Ahmet et al. (2022) used a load resistor and a boost converter under different conditions to compare MPPT algorithms in photovoltaic (PV) systems. In the load resistance calculation and converter design, they did not specify the exact value of the resistance value in the PV system model. Dileep and Singh (2017) focused on the connection between converters and load resistors. They explained the relationship between the load resistors and the input impedance according to the converter type in their study. Attar et al. (2020) conducted a

study to find the optimum electrical load resistance in TEG systems without converters with MPPT. Khan et al. (2022) developed different algorithms for MPPT tracking by using PV and TEG systems together and used boost converter in their systems. Zafar et al. (2022) developed an algorithm using machine learning for MPPT in TEG system and used a boost converter here. They did not mention which loads can be connected to the boost converter and at which load values maximum power point (MPP) will follow. In another study, Benhadouga et al. (2022) performed MPPT with a boost converter using the sliding mode technique. They started their load from the lowest value and increased it up to about 50 Ω . They obtained the best MPP value around 5 Ω . Again, these researchers did not emphasize which loads are suitable for the boost converter.

Although many studies have been done using some converters, the relationship between selected load resistors, converter and input impedance has not been mentioned. Unlike other studies, in this study, a simulation of load behavior based on perturb-observation (P&O) method in

non-isolated boost converter for maximum power point tracking of thermoelectric generators has been carried out by means of a TEG setup in MATLAB/Simulink.

2. MATERIAL AND METHOD

2.1. Principle of Maximum Power Point

The circuit diagram of TEG and load connection is given in Figure 1. The load resistance in the circuit varies continuously. The load in the TEG system is connected to obtain power from the TEG. MPP is reached when the load resistance and TEG internal resistance are equal (Bhuiyan et al., 2022). This situation cannot always be achieved because the load is not always the same and the overall resistance of the TEG varies depending on the temperature difference (Mamur and Çoban, 2020a). But this equality is always desired.

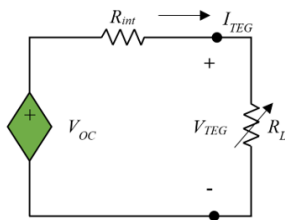


Figure 1. TEG load connection

The derivative of the power from TEG when MPP is zero:

$$\frac{dP}{dV_{TEG}} \Big|_{max} = \frac{V_{OC} - 2V_{TEG}}{R_{int}} = 0 \quad (1)$$

Where, V_{OC} is the open circuit voltage of TEG in (V), V_{TEG} is the voltage obtained from TEG in (V), and R_{int} represents the internal resistance of TEG in (Ω) (Montecucco et al., 2014). The value of $V_{TEG/MAX}$ in MPP is half of the value of V_{OC} and is given by the below equation (Armin Razmjoo et al., 2020).

$$V_{TEG/MAX} = \frac{V_{OC}}{2} \quad (2)$$

The maximum power from the TEG is explained by the following equation, depending on the open circuit voltage and internal resistance of the TEG:

$$P_{TEG_MAX} = \frac{V_{OC}^2}{4R_{int}} \quad (3)$$

Where, P_{TEG_MAX} is the maximum power of TEG in (W). TEG current and TEG voltage vary linearly depending on the load. MPP can also be extracted from the half of short-circuit current or open-circuit voltage of the TEG. Hence, the MPP voltage and MPP current are formulated by the given equation (Mamur and Üstüner, 2021):

$$V_{MPP} = V_{TEG/MAX} = \frac{V_{OC}}{2} \text{ or } I_{MPP} = I_{TEG/MAX} = \frac{I_{SC}}{2} \quad (4)$$

Where, V_{MPP} is TEG MPP voltage in (V), I_{MPP} is the MPP current of the TEG in (A), and I_{SC} is the short-circuit current of the TEG in (A). When the load is directly linked to the end

pins of the TEG, the efficiency of the TEG is further reduced if the internal resistance of the linked load and the TEG are not equal. This is called impedance imbalance. For minimizing the problem, DC-DC converters that carried out both maximum power point tracker (MPPT) and power arrangement are employed with TEGs. At that rate, the load of TEG becomes DC-DC converter (Bijukumar et al., 2019). Since the DC-DC converter linked between the TEG and the load is the load of the TEG, making the TEG overall internal resistance and the DC-DC converter resistance equal is carried out by the MPPT program. Thus, both the highest efficiency from TEG is obtained and voltage regulation is ensured (Tsai and Lin, 2010). Figure 2 illustrates the MPPT principle.

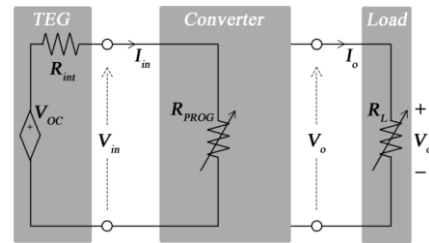


Figure 2. MPPT principle

When the converter is connected between TEG-load, now the load resistance of TEG is this DC-DC converter. This is expressed by R_{PROG} (Mamur et al., 2022).

2.2. Boost converter

A DC-DC converter is a circuit that converts DC voltage from one value to a higher value and operates with electronic switching. (Mamur and Çoban 2020b). As seen in Figure 3a, it consists of a coil, a switch, and a diode. Boost converters can be studied in 2 modes, Mode1 and Mode2, as seen in Figures 3b and 3c.

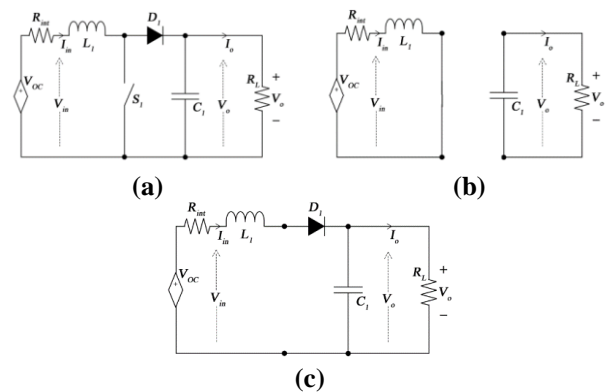


Figure 3. Boost converter (a) scheme, (b) Mode1, and (c) Mode2

In mode1, when the switch is on, the current comes from the source and flows through the coil and the switching element. In this way, energy is stored on the coil. In Mode2, when the switch is in off, the current circuit is completed via the diode and capacitor, not through the switching element. When the switch is conducting, the coil transfers the energy stored on the capacitor and the load via the diode. On the other hand, the capacitor charges when the switch is off, and discharges when the switch is on. When the switching process is

fulfilled very fast, the coil is not completely discharged during charging and discharging and is always loaded. In this way, the energy of the source and coil is loaded into the capacitor (Taghvaei et al., 2013). The load is fed from the capacitor when the switch is in the conduction position, and from the source and coil when it is cut. Thus, a low DC voltage is converted to a higher DC voltage.

The transformation equation of a typical boost converter is given below:

$$\frac{V_o}{V_{in}} = \frac{1}{1-D} \quad (5)$$

$$V_{in} = V_o \times (1 - D) \quad (6)$$

$$I_{in} = \frac{I_o}{1-D} \quad (7)$$

Where, V_o , V_{in} , D , I_{in} , and I_o are the output voltage in (V), the input voltage in (V), the duty cycle, the input current in (A) and the output current in (A) of the converter, respectively. The value of R_{PROG} in Figure 2 is the internal resistance of the inverter. Using the boost converter equations, the following result is obtained:

$$R_{PROG} = \frac{V_{in}}{I_{in}} = \frac{V_o \times (1-D)}{I_o / (1-D)} = \frac{V_o \times (1-D)^2}{I_o} = R_L \times (1 - D)^2 \quad (8)$$

Where, R_L is the load resistance in (Ω). (8) shows that in boost converters, the resistance of load can be increased up to an infinite resistance non depending on the TEG internal resistance.

While calculating the boost converter, the D value is given by the following equation:

$$D = 1 - \frac{V_{in(min)} \times \eta}{V_o} \quad (9)$$

Where, $V_{in(min)}$ is the minimum input voltage of the converter in (V), and η is the efficiency of the converter. The ripple current of the coil is shown in the following equation:

$$\Delta I_L = \frac{V_{in(min)} \times D}{f_s \times L} \quad (10)$$

Where, ΔI_L is the ripple current of the coil in (A), f_s is the switching frequency in (Hz), and L is the inductance of the coil in (H). Hence, the output current of the converter is given as below:

$$I_o = \frac{D \times (1-D) \times V_{in}}{2 \times L \times f_s} + (1 - D) \times I_{min} \quad (11)$$

The maximum allowable output current of the converter can be calculated by two different equations. These are given below:

$$I_{o(max)} = \left(I_{LIM(min)} - \frac{\Delta I_L}{2} \right) \times (1 - D) \quad (12)$$

$$I_{o(max)} = \sqrt{P_{TEG_MAX} / R_L} \quad (13)$$

Where, $I_{o(max)}$ and $I_{LIM(min)}$ are the maximum allowable output current in (A), minimum allowable switching current in (A) of the converter, respectively. On the other hand, the maximum allowable output voltage of the converter is given below:

$$V_{o(max)} = \sqrt{P_{TEG_MAX} \times R_L} \quad (14)$$

Where, $V_{o(max)}$ is the maximum output voltage value of the converter in (V). Along with these, the maximum switching current is given by the following equation:

$$I_{SW(max)} = \frac{\Delta I_L}{2} + \frac{I_{o(max)}}{1-D} \quad (15)$$

Where, $I_{o(max)}$, and $I_{LIM(min)}$ are the maximum allowable output current (A), minimum allowable switching current (A) of the converter, respectively. $I_{SW(max)}$ is the maximum switching current. The coil inductance equation of the converter is given below:

$$L = \frac{V_{in} \times (V_o - V_{in})}{\Delta I_L \times f_s \times V_o} \quad (16)$$

Since the coil value is not known in (10), the value of ΔI_L cannot be calculated. Therefore, the following equation is used:

$$\Delta I_L = (0.2 \sim 0.4) \times I_{o(max)} \times \frac{V_o}{V_{in}} \quad (17)$$

ΔI_L value fluctuates between 20% and 40% of the output current and is taken between these values. On the other hand, the required minimum capacitor value to be used in the boost converter is found with the following equation:

$$C_{out(min)} = \frac{I_{o(max)} \times (1-D)}{f_s \times \Delta V_o} \quad (18)$$

Where, $C_{out(min)}$ is the output capacitor (F). The output ripple voltage of the boost converter is given below:

$$\Delta V_o = ESR \times \frac{I_{o(max)}}{1-D} + \frac{\Delta I_L}{2} \quad (19)$$

Here, ΔV_o , and ESR are the output ripple voltage in (V) and the equivalent series resistance in (Ω) of the output capacitor used, respectively. The values calculated according to the equations used are shown in Table 1.

Table 1. The boost converter calculated values

Parameter	Value	Unit	Description
P_{TEG_MAX}	45.76	W	Power at MPP
P_{conv}	50	W	Power of converter
V_{in}	16.4	V	Input voltage
R_L	1-45	Ω	Load resistance
$I_{o(max)}$	$\sqrt{P_{TEG_MAX} / R_L}$	A	Max output current
$V_{o(max)}$	$\sqrt{P_{TEG_MAX} \times R_L}$	V	Max output voltage
ΔI_L	30% of I_o	A	Max ripple current
ΔV_o	5% of V_o	A	Max voltage ripple
η	0.8		Converter efficiency
f_s	20	kHz	Frequency
L_1	~62	μ H	Inductance
C_1	~2400	μ F	Capacitance

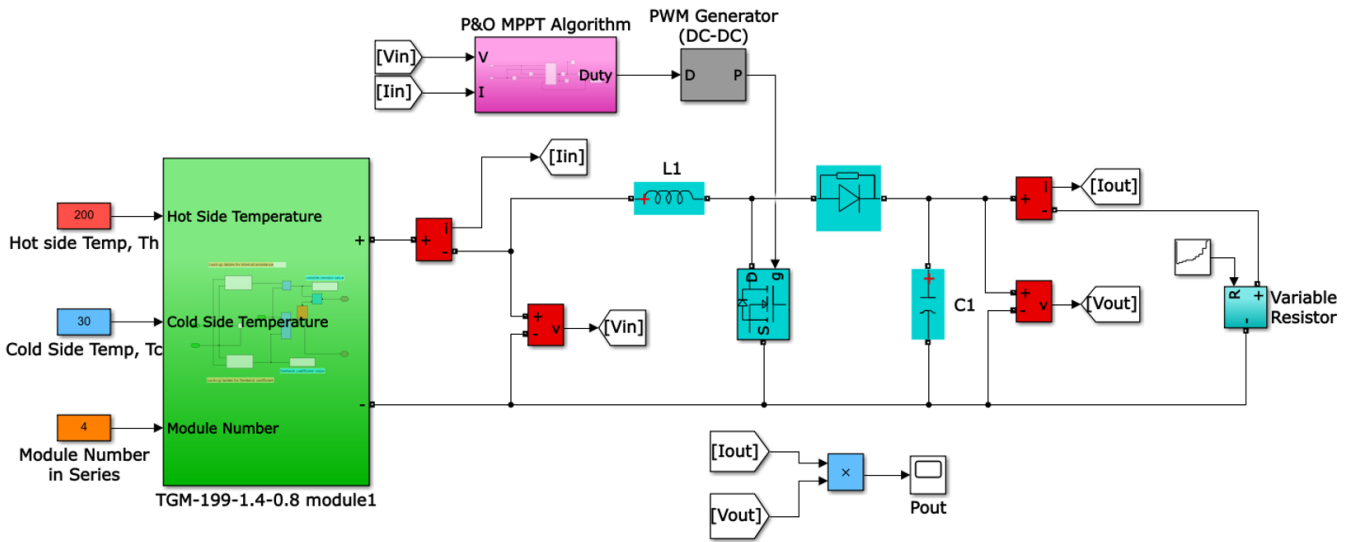


Figure 5. TEG system-converter and load connection

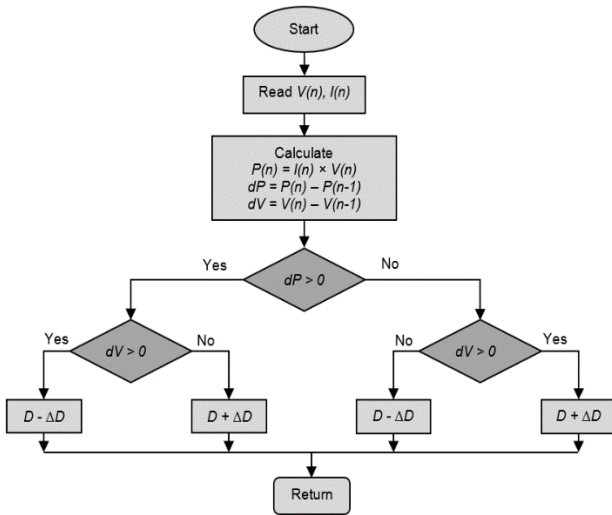


Figure 4. P&O MPPT algorithm

2.3. Perturb and observe algorithm

The most popular of MPPT methods is the P&O algorithm (Qasim et al., 2021), whose flow diagram is given in Figure 4. MPPT is required because when a power is generated from TEGs, the change in load and the change in temperature causes a difference in the generated MPP value. Power conditioning methods are used to capture MPP in TEGs. One of the power conditioning methods is impedance matching. The P&O MPPT algorithm fulfils the impedance matching. The algorithm adjusts the duty cycle of the switch in the converter operated in the setup. First, current and voltage values are obtained by means of current sensor and voltage sensor. Using the measured current and voltage value, the power, the changes of power, and of voltage are calculated. Then, the power change is questioned (Pilakkat et al., 2019). If the power change is positive, the voltage change is questioned. If this is also positive, the D value is lowered by ΔD . Here, if the voltage change is negative, the D value is raised by ΔD . It's back to the beginning. By making a new measurement, the power value is figured out, and new power

and voltage changes are found. According to these found values, if the power change is negative, the voltage change is again questioned. If the voltage change is positive, the D value is increased by ΔD . If the voltage change is negative, the D value is reduced by ΔD . The process is continued until the MPP value (Sarbu and Sebarchievici, 2018).

Although this P&O algorithm has a disadvantage MPP, oscillations occur around the MPP as the process is still running. Large ΔD ranges result in rapid MPP capture, resulting in ample oscillations in MPP (Al-Diab et al., 2010). On the other hand, small ΔD intervals result in slow MPP capture while reducing the magnitude of MPP oscillations. Another drawback is that when the temperature difference changes, the MPP will change and in this case a separate oscillation will occur to find the new MPP (Jouhara et al., 2018).

2.4. TEG setup

A TEG setup was designed in the MATLAB/Simulink software to carry out the study. In this TEG system, as many TEG modules as desired can be linked in series and parallel. The amount of power obtained varies according to the serial and parallel connection. A boost converter has been added to the TEG system, whose design calculations have been made. A gradually adjusted load is connected to the end pins of the boost converter. Figure 5 shows this TEG system-converter and load connection.

The internal resistance values of these TEG modules change depending on the temperature. Desired temperature ranges can be given with input values. The P&O MPPT method is operated to switch the boost converter. In the simulation study, the power obtained from the TEG to the converter and the power given by the converter to the load resistor were measured. The overall internal resistance of the TEG system is 5.84Ω . The load resistors linked the converter were gradually changed to 1, 3, 5.84, 7, 9, 11, 15, 20, 30 and 45Ω .

The hot surface temperature of the TEG setup was hold steady at $T_H = 200^\circ\text{C}$, and the cold surface temperature at $T_C = 30^\circ\text{C}$. Then, the variation of the output power obtained according to the changing loads was observed. The duty cycle D values produced by means of the P&O MPPT method depending on the variability of the load have been changed (Mamur et al, 2022).

3. RESULTS

In the simulation study performed in MATLAB/Simulink software, the load values of the TEG system were changed gradually. It would be better to think of these modified values as values above and below the overall internal resistance of the TEG system. In the study carried out, these values below 5.84Ω are these values below the overall internal resistance of the TEG system. These values 5.84Ω and above are these values above the overall internal resistance of the TEG system. In addition, analysis was made for the case of $R_{int} = R_L$, where it is equal. The MPP value of the modeled TEG system is 45.76 W .

The D value produced by the P&O MPPT algorithm has to be zero according to equation (8) to obtain the maximum load value at load resistance values below 5.84Ω . In simulation studies, the minimum D value was determined as 0.05 to prevent the converter from turning off or to keep it on continuously, as given in Table 2. In cases where the resistance of load is less than the overall internal resistance, the boost converter switch is expected to operate with a minimum value of D . When the value of the resistor of load is increased, it is hoped that the P&O MPPT algorithm will keep the $R_{int} = R_L$ state continuously by increasing the D value of the boost converter switch, so that the system will remain at the MPP value, since the overall internal resistance of the TEG setup is equal to the resistance of load.

The curves of change in current, voltage, and power in the TEG system under constant surface temperatures and variable loads of the TEG, performed in the MATLAB/Simulink simulation software, are illustrated in Figure 6. Depending on these load changes, the change in the D value of the boost converter switch generated by means of the P&O MPPT algorithm to catch the MPP value is given in Figure 7. In addition, the average D values produced according to the load changes are presented in Table 2. It would be appropriate to evaluate this Figure 6 and Figure 7 together.

There are two cases where the resistance of load is less than the overall internal resistance in the TEG setup. These are 1Ω and 3Ω values. The D value in these two cases was 0.05. For impedance matching, the system worked with a minimum D value. In these two cases, they are 19.88 W and 37.42 W , respectively, as given in Table 2.

In the third case, the $R_{int} = R_L$ condition is desirable. The P&O MPPT algorithm produced an average of 0.06 D , keeping the impedance matching state and reaching the MPP value with 43.4 W . When the resistance of load was changed to 7Ω , 9Ω , 11Ω and 15Ω , the P&O MPPT algorithm was able to perform impedance matching, producing D values of 0.097, 0.199, 0.27 and 0.348, respectively. The MPP values

obtained from the TEG system in these cases were 43.38 W , 42.76 W , 42.11 W and 40.93 W , respectively.

When the value of the load resistance in the stepped TEG system applied in MATLAB/Simulink environment is changed to 20Ω , 30Ω and 45Ω , the obtained power values are 39.55 W , 37.32 W and 34.53 W , respectively. D values produced depending on these load values are 0.414, 0.496 and 0.547, respectively.

4. DISCUSSION AND CONCLUSIONS

In this study, which was carried out in MATLAB/Simulink environment, a 50 W converter was designed for the TEG system with a maximum power of 45.76 W and a converter with P&O MPPT was used to prevent it from being affected by different loads. The boost converter was operated with the D values produced by the P&O MPPT algorithm according to the connected load values.

At the resistance values of load lower than the internal resistance of the TEG system, MPP cannot be followed due to the working principle of the boost converter. In the study, this situation is clearly seen in the first two resistance values of the MATLAB/Simulink simulation studies. In cases where the load resistance values connected with the overall internal resistance of the TEG system are equal and large, the boost converter working with the D values generated by means of the P&O MPPT algorithm provides the MPP value by matching the impedance. However, approaching the full MPP value is difficult due to the losses in the boost converter. However, at high load values, the power value that drops considerably without utilizing a boost converter embedded MPPT enables the follow-up of MPP when utilizing a boost converter embedded P&O MPPT algorithm. However, when high load values are reached, the output voltage of the boost converter increases with increasing D values and the MPPT error percentage increases. In R_L of 5.84Ω , D , P_{MPP} and error values are 0.06 and 43.4 W and 5.16, respectively.

As a result, in this study, a boost converter embedded P&O MPPT algorithm by a program software is linked between TEG and load to reach the power obtained from TEG to MPP value. The task of this boost converter is to ensure that the power obtained from the TEG is not affected by load changes. The load resistance value of the boost converter with P&O MPPT algorithm does not function from the minimum to the internal resistance value of the TEG. However, when the TEG reaches its overall internal resistance and rises above this value, the P&O MPPT algorithm starts to function. While the best MPP tracking is close to the internal resistance value, MPP tracking is performed at the load resistance values above it. However, as the load resistance increases, the MPPT error increases. As a result, MPP monitoring can be made between the TEG overall internal resistance and the infinite load resistance with the boost converter with P&O MPPT. The most efficient MPPT can be made up to about three times the internal resistance of the TEG.

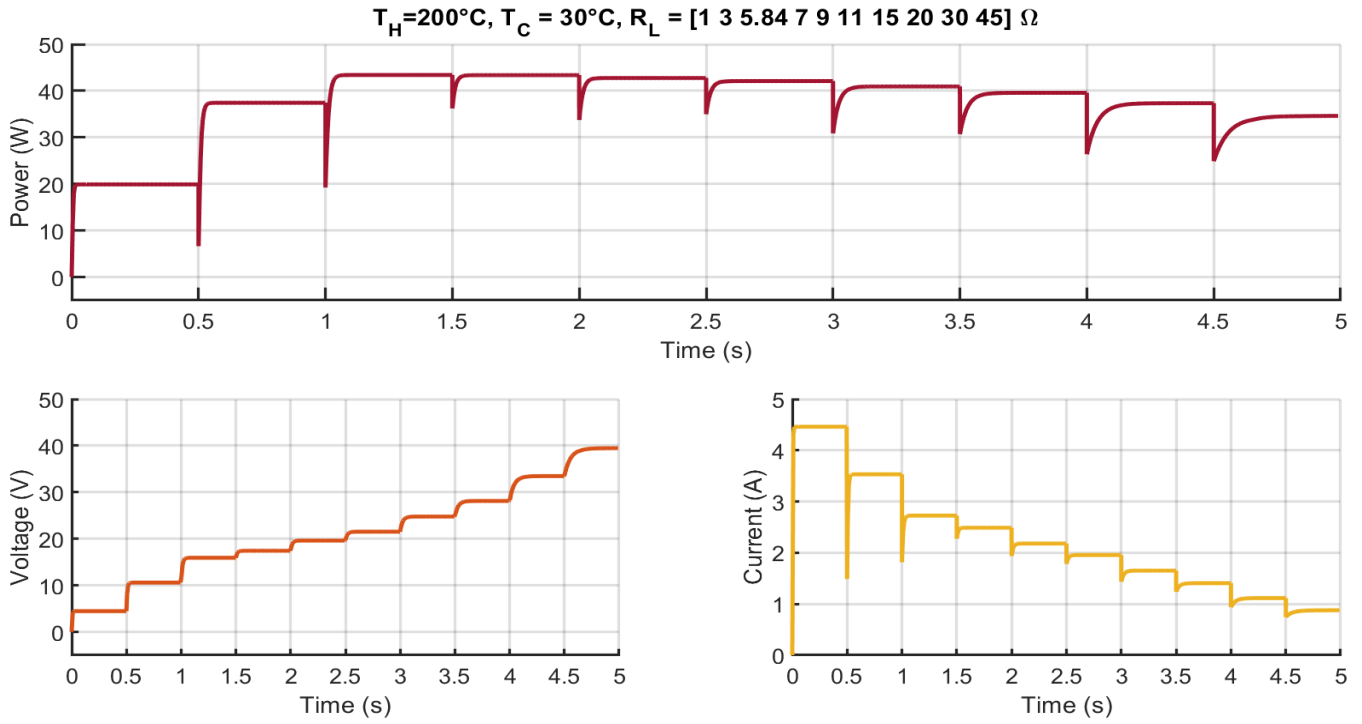


Figure 6. The curves in power, voltage and current of TEG system under variable loads

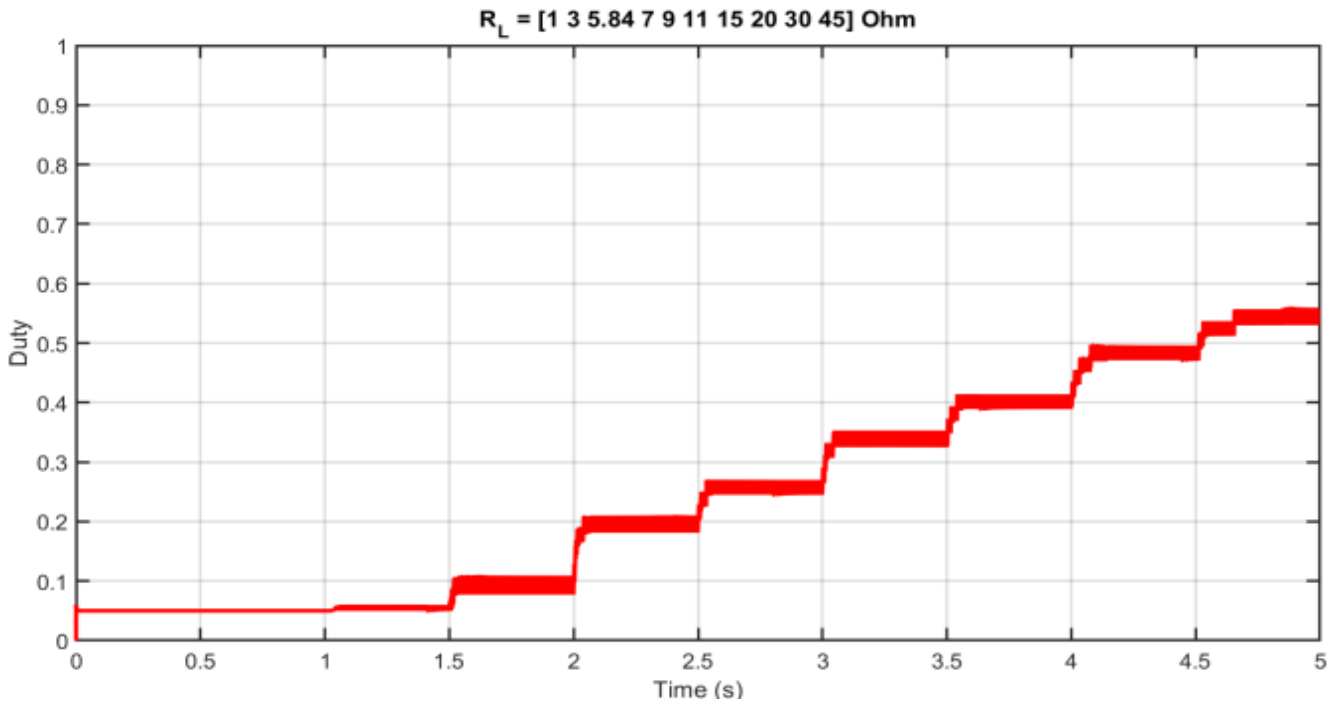


Figure 7. Duty cycle rate under variable loads

Table 2. Average D values produced according to load changes.

	R_L values (Ω)									
	1	3	5.84	7	9	11	15	20	30	45
D	0.05	0.05	0.06	0.097	0.199	0.27	0.348	0.414	0.496	0.547
P_{MPP} (W)	19.88	37.42	43.4	43.38	42.76	42.11	40.93	39.55	37.32	34.53
Error (%)	56.56	18.23	5.16	5.20	6.56	7.98	10.56	13.57	18.44	24.54

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Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: H.M., Investigation: Ç.A., M.A.Ü.; Material and Methodology: H.M., Ç.A., M.A.Ü.; Supervision: H.M., Visualization: Ç.A., M.A.Ü.; Writing-Original Draft: Ç.A., M.A.Ü.; Writing-review & Editing: H.M., Ç.A., M.A.Ü.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Post-Pandemic Spatial Decisions with University Students' Awareness of Covid-19 Measures in The Built Environment

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Abstract: Coronavirus, also called COVID-19 by the World Health Organization (WHO), was announced worldwide as a respiratory disease on 11 February 2020. In Türkiye, the first COVID-19 case was seen on 11 March. However, it is assumed that the world has not yet recovered from its effects and will not recover for many years. Pandemic coordination boards, science boards, and operation centers were established worldwide to prevent the pandemic's spread. These centers have published guidelines, posters, and brochures for public transportation places such as COVID-19 Risk Assessment, COVID-19 Guide, hospitals, workplaces, and public transportation to inform the communities. Measures to cover the whole society, especially in closed areas, have been implemented to a large extent. In this study, by examining the guide titled "Strategies from the WELL Building Standard to support the fight against COVID-19" published by the International WELL Building Institute (IWBI), thematically, the awareness by the students against COVID-19 was evaluated through the WELL criteria, and the need to be developed in the post-pandemic period. In this study, it is stated that the measures taken to reduce the impact of the pandemic on university students, the WELL criteria set is important for the built environment while conducting awareness research about the measures with the questionnaires made to the students, and also contributes to developing project ideas for designers, architects, and engineers against pandemics encountered worldwide. It is a preliminary study for spatial decisions.

Keywords: WELL certification, health, and well-being, COVID-19, spatial precautions.

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1. INTRODUCTION

Today, while the life quality of cities is rated, it consists of components for the quality of the physical, social, and economic environments. Physical environment quality is the presence of open and green areas, transportation network, transportation type, public transportation, infrastructure, and municipal services, communication, socio-cultural activities, protection of natural and historical values, planning of housing and living environment, housing type and quality, working environment (Kalaycı Önaç and Birişçi 2019). It can be defined by features such as reducing the environmental impact of areas and the presence of recreation areas (Emür and Onsekiz 2007). In this context, the limits of the conceptual framework are determined by today's living conditions and technology.

Today, this framework has surrounded almost every area of our lives with its different variations.

Coronavirus, also called COVID-19 by the World Health Organization (WHO), was announced as a respiratory disease worldwide on 11 February 2020, and the first Covid-19 case in Türkiye was seen on 11 March. Pandemic coordination boards, scientific boards, and operation centers have been established worldwide to prevent the pandemic's spread. These centers have published guides, posters, and brochures for many publishers. However, it is assumed that the world has not yet recovered from its effects and will not recover for many years, even though it has been almost two years since the onset of the pandemic. With the COVID-19 epidemic, negative effects have occurred on public health, the economy, and social and cultural activities. In addition, environmental effects such

as indoor air quality problems and school energy consumption have been observed due to the pandemic (Pekdogan, 2022). The epidemic severely affected public buildings, especially inadequate educational buildings.

With the continuation of online education, disruptions have also occurred due to the lack of technological infrastructure. Compared to face-to-face education, the efficiency obtained has created great debates in the education community and has been the research subject. (Şahin 2021) examined university students' views on online education during the Covid-19 Pandemic (Casacchia et al. 2021). 47% of the participants stated they had problems accessing the distance education system, and 38% did homework and stated that using projects and presentations is not beneficial. In addition, 30% of the participants declared that they had communication problems with the instructors throughout the process.

In Türkiye, a circular was published by the Ministry of Health on 3 March 2022, and a guide was prepared on the precautions to be taken by deciding to end the break in education. With the slowing effect of the COVID-19 pandemic and the spread of vaccination, the measures taken have ceased to be social and have turned into individual restrictions and measures. Along with these rules, the education, carried out online for almost two years, was decided to be fully face-to-face education again in the 2021-22 academic year.

Beginning the emergence of the COVID-19 pandemic, a new awareness has emerged about the precautions to be taken in the built environment to protect people from the virus. For example, buildings with good ventilation and air filtration are becoming more desirable than those without. That's why increasing awareness of the measures taken to protect against COVID-19 in the built environment is important. These measures include increased cleaning and sanitation, as well as social distancing. Following these simple guidelines can help keep ourselves and others safe as we return to our daily lives.

In this study, the conditions of learning and living spaces during the coronavirus pandemic and students' behavior in these spaces are examined within the scope of WELL. In this context, the findings regarding the conditions of learning and living spaces and students' behaviors are discussed by analyzing the questionnaires made by the students. The questionnaire based on the WELL criteria set is applied to 100 students, and further information is collected as additional criteria are created to prevent the COVID-19 pandemic in architectural spaces. Moreover, students were surveyed qualitatively to determine whether they had a general knowledge of the criteria influencing awareness of COVID-19. In this case, the study is based on analyzing physical documents and websites for all WELL Criteria.

IWBI guide (2020) in the WELL COVID-19 Standard section highlights that more work is needed to prevent infection and raise awareness by changing how people behave when using the space. Accordingly, this study aims to contribute to WELL criteria for architectural design,

which is accepted as a support material for adaptation to COVID-19 conditions. Also, IWBI is a member of the United Nations Global Compact, the world's largest corporate citizenship initiative, and works with companies to help them achieve the United Nations Sustainable Development Goals (SDGs) by implementing WELL (International WELL Building Institute, 2018).

University students' use of space and awareness of COVID-19, as well as the problems and solutions, are a suggestion against pandemics that may be encountered in the future. It will contribute to developing project ideas for future architects and raise awareness against pandemics such as COVID-19.

2. STRATEGIES FROM THE WELL-BUILDING STRATEGIES AGAINST COVID-19

The research was carried out using the qualitative content analysis method. The WELL guide evaluates spaces in 5 main categories: 1) Air and Ventilation, 2) Water, 3) Nutrition, 4) Light, and 5) Fitness. These recommendations are based on the criteria of air quality, water quality, food quality, light quality, vitality, comfort, and mental qualities defined in the WELL standard. Supporting design interventions and nutrition policies for all, WELL certification is a holistic rating system that places general public health and well-being at the center of decision-making, with a focus on nutrition and fitness as well as the construction and operation of spaces built to improve health (Mikic, 2019).

The guideline proposed by the IWBI aims to ensure that buildings and facilities are designed in a healthy way for their occupants, as well as to guide the healthy use of these buildings in pandemic situations (Labartino 2018). In line with this purpose, the following conditions were also noted in this study: increasing security awareness, providing appropriate ventilation, and operating hygiene and filtration systems effectively and efficiently. The headings used in the analyses are figured out in detail in Table 1.

3. MATERIALS AND METHODS

3.1. Data collection

This study investigates university students' awareness through the criteria prepared by the WELL building standard to reduce the effects of COVID-19 on both the university and the environment they live in. It researches the measures taken in the educational environments of the participants who are adapting or trying to adapt to the new normal in quarantine through WELL building standards. The measures included in the data collection tool were made under the headings determined by the certification system.

The data set was applied to 100 participants that are university students from different cities and departments selected. The sample group was generally selected from students of architecture and civil engineering, and in addition to having knowledge about the space and the built environment, the surveyed students consisted of second and

third-year students who studied online for about one and a half years at the university with the pandemic that started in 2020.

In this study, survey questions prepared based on the WELL Building Standard Strategies to support the fight against COVID-19 criteria set were asked to determine (1) the awareness levels of university students on the precautions taken for COVID-19 and (2) the spatial decisions that should be taken during the post-pandemic period. The survey questions were applied to 100 university students from different cities and departments in Türkiye to collect the research data. The data obtained from the questionnaires were applied to the study's sample group and were coded over the questionnaire results via SPSS statistical program. Finally, the correlations between the variables were examined.

3.2. Data analysis

The purpose of the data analysis in the study is to conduct awareness research on the measures taken and the measures put into practice to cope with the COVID-19 epidemic and reduce the effects on university students. As a result, all questionnaires were analyzed using descriptive statistics and Spearman correlation analysis for the correlation between criteria.

4. RESULTS

4.1. Descriptive statistics

Descriptive statistics for the characteristics are presented for all subgroups of the WELL Building Standard. Descriptive statistics were calculated for all variables (Table 1). The percentages were calculated for categorical data according to the survey. 100 completed questionnaires were processed to analyze the WELL Building Standard COVID-19 Criteria. The authors gave the codes to the criteria set in later data analyses.

Here, 100 students were asked whether they knew about these criteria and whether there was an example in their built environment about these criteria. Almost 80% of students know about A1, A2, and B1 criteria, and they know these criteria in the built environment during the pandemic. It has been seen in these analyses that they have 70% knowledge about C1, D1, E1, F3, F5, and G2 criteria. G2, H1, F6, and B2 are the criteria that students obtain the least information from the media, such as posters, advertisements, etc., in their built environment.

Table 1. WELL, Building Standard Strategies, COVID-19 Criteria Analysis

	Code	WELL, Building Standard Strategies, COVID-19 Criteria	Mark, if you know about the criteria %	Mark if there are examples in your built environment %	Percentage s %
A	A1	Handwashing	90	68	76
	A2	Cleaning Products & Protocol	82	65	79
B	B1	Reduce indoor air quality issues by providing adequate ventilation and filtration 1. Ventilation Effectiveness 2. Enhanced Ventilation 3. Operable Windows 4. Air Filtration 5. Microbe and Mold Control	74	46	62
	B2	Manage humidity and control sources of indoor moisture 1. Moisture Management 2. Humidity Control	51	17	33
C	C1	Test your water to ensure it's safe for use 1. Fundamental Water Quality 2. Water Contaminants 3. Enhanced Water Quality 4. Water Quality Consistency	66	30	45
D	D1	Prepare for emergencies 1. Emergency Preparedness 2. Family Support	67	19	28
	D2	Be informed by evidence. 1. Occupant Survey 2. Enhanced Occupant Survey	56	19	34
E	E1	1. Visual and Physical Ergonomics 2. Active Furnishings 3. Enhanced Ergonomics 4. Self-Monitoring	65	27	42
F	F1	Offer physical and mental health benefits 1. Health Services and Benefits 2. Mental Health Support	60	31	52
	F2	Cultivate a culture of health 1. Health Promotion	59	19	32
	F3	Mitigate the risks of smoking	70	36	51

		1. Smoke-free Environment 2. Tobacco Prevention and Cessation			
	F4	Stay nourished and hydrated. 1. Fruits and Vegetables 2. Drinking Water Promotion	77	34	44
	F5	Enable physical activity throughout the day 1. Physical Activity Opportunities 2. Physical Activity Spaces and Equipment 3. Physical Activity Promotion	72	37	51
	F6	Promote healthy sleep habits 1. Circadian Lighting Design 2. Sleep Support	53	16	30
G	G1	Maintain mental health 1. Mental Health Promotion 2. Mental Health Education 3. Stress Support 4. Restorative Opportunities 5. Restorative Programming	52	23	44
	G2	Provide access to nature and natural light 1. Access to Nature 2. Enhanced Access to Nature 3. Light Exposure and Education 4. Enhanced Daylight Access	65	17	26
H	H1	Support local communities and foster community connections 1. Civic Engagement 2. Community Access and Engagement	45	17	38
	H2	Provide access to localized food sources 1. Food Production 2. Local Food Environment	53	20	38

4.1. Spearman correlation statistics

Correlation analysis was performed to determine whether there is a relationship between the two data discussed above according to the descriptive analysis and, if so, the severity of this relationship. First, the normal distribution test of the variables was performed. Accordingly, the correlation to be applied was decided. Since the normality test is greater than 30, Kolmogorov-Smirnov was applied, and the data

distribution was not normal. In this case, where the data are not normally distributed, the Spearman Rank correlation coefficient is preferred.

Table 2 presents the results of the Spearman Correlation test applied to determine the relationship between WELL's Building Standard Strategies COVID-19 Criteria and its sub-dimensions. The relationship between the variables is weak if the correlation coefficient is between 0-0.29; medium if it is between 0.30-0.64; and strong if it is between 0.65-0.84 (Ural & Kiliç, 2005). Here, the correlation test prepared for two separate questions is presented together. The upper cross shows the analysis for the question "Mark if you know about criteria." In contrast, the lower cross shows the correlation within the question "Mark if there are examples in your built environment." All values processed to this figure are significant at the 0.01 level ($p=0.000<0.01$).

There is a strong, positively significant relationship between "Offer physical and mental health benefits" F1 and "Maintain mental health" G1 and between "Handwashing" A1 and "Cleaning Products & Protocol" A2. In addition, there is a strong, positive relationship between G1 and H1/H2. A weak and positively significant relationship exists between A1 and F3, F4, and F5. In general, the relationships are moderate, positively significant relationships.

Table 2. Correlation analysis for WELL Building Standards COVID-19 Criteria

	A1	A2	B1	B2	C1	D1	D2	E1	F1	F2	F3	F4	F5	F6	G1	G2	H1	H2
A1		0.68																
A2	0.31																	
B1																		
B2			0.48															
C1			0.34	0.35														
D1			0.31	0.39	0.45													
D2			0.45	0.51	0.34	0.52	0.4											
E1			0.45	0.39	0.47	0.27	0.56	0.39	0.48	0.41								
F1			0.44	0.41	0.44	0.44	0.27	0.55	0.46	0.29								
F2					0.37			0.39										
F3					0.31	0.29					0.32							
F4					0.35	0.41	0.28	0.29										
F5	0.29				0.48	0.58	0.28	0.46										
F6			0.28	0.32	0.42	0.47	0.51	0.43	0.59	0.3	0.35							
G1			0.5	0.37	0.31	0.45	0.39	0.43	0.41	0.47	0.51	0.43	0.59	0.3	0.35			
G2	0.29				0.39	0.34	0.34	0.43	0.37	0.31	0.29	0.35	0.41	0.28	0.29			
H1					0.44	0.41	0.44	0.43	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
H2					0.35	0.39	0.43	0.35	0.35	0.6					0.43	0.59	0.44	0.47
F1					0.53	0.42	0.43	0.35	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
F2					0.59	0.43	0.43	0.35	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
F3	0.27				0.29	0.43	0.43	0.35	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
F4	0.29	0.27			0.48	0.43	0.43	0.35	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
F5	0.29	0.27	0.27		0.48	0.43	0.43	0.35	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
F6			0.28	0.32	0.27	0.43	0.43	0.35	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
G1			0.5	0.37	0.31	0.45	0.39	0.43	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
G2	0.29				0.39	0.34	0.34	0.43	0.37	0.31	0.29	0.35	0.41	0.28	0.29			
H1					0.44	0.41	0.44	0.43	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
H2					0.51	0.52	0.43	0.35	0.44	0.41	0.44	0.41	0.47	0.51	0.43	0.59	0.37	0.49
F1					0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
F2					0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
F3					0.41	0.43	0.41	0.43	0.41	0.43	0.41	0.43	0.41	0.43	0.41	0.43	0.41	0.43
F4					0.45	0.31	0.39	0.39	0.45	0.31	0.39	0.39	0.45	0.31	0.39	0.39	0.45	0.31
F5					0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
F6					0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
G1					0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
G2					0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
H1					0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
H2					0.62	0.47	0.67	0.55	0.36	0.3	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31

5. DISCUSSION

In the COVID-19 period and in the post-pandemic period, many studies have been conducted on compliance with the rules, perception, awareness, and adherence to measures. Opinions about the COVID-19 preventive measures of university students were taken against the measures taken to improve public health discussed in this study, and the results are in line with the literature discussed below.

According to the study conducted at Purdue University in the USA, online survey results of university students' perception, awareness, and adherence to COVID-19 prevention measures were investigated, and in general, students accepted protective measures. However, the protocol violation effectiveness of those who tested positive for COVID-19 was higher. Women and graduate students were found to have a higher percentage of participation and compliance with the instructions (Akhter et al., 2022).

In a study about self-reported productivity before and after relocation into WELL-certified office buildings, a statistically significant improvement was observed in indoor air quality satisfaction, thermal comfort, and awareness of WELL certification, according to the study conducted on an office building upgraded with Well certification (Licina & Langer, 2021).

In the study conducted in Uganda, the effects of awareness of COVID-19, the adoption of prevention measures, and the COVID-19 quarantine on mental health and socio-economic disruptions were evaluated on the participants. According to this study, the participants reported that health problems increased due to the COVID-19 quarantine, balanced nutrition was difficult, disturbed sleep patterns, and socio-economic disruptions and sedentary life were caused. In addition, while 40% of the participants washed their hands more, 17.6% started to avoid entering public areas (Akhter et al., 2022).

According to the awareness research conducted on dentistry students, they pay more attention to precautions such as washing their hands frequently, maintaining a safe distance, and wearing masks (Akhter et al., 2022).

According to the study, which analyzed the demographics associated with awareness and compliance with Vermont in USA adult residents and identified characteristics associated with non-compliance, most respondents listed their primary motivation for following recommended public health actions as protecting their health, the health of their family and friends, and the health of other Vermonters. While Vermont residents demonstrated a high level of awareness of the COVID-19 guidelines and adherence to actions to limit transmission, the emergence of the COVID-19 Delta variant has shown that those who do not take the guidelines seriously or adhere to limiting actions are not in the minority (Vatovec & Hanley, 2022).

In addition to these studies, many studies show that COVID-19 measures cause some environmental problems. Considering these studies, it is seen that water use has

increased more. According to research on the increase in water use during and after COVID-19, water consumption in the DMA increased by more than 50% during the lockdown, with a corresponding increase in electricity bills (Almulhim & Aina, 2022). In the study of Abu-Bakar et al, water consumption increased by 46% in England during the lockdown. In another study, the increase in household water consumption was calculated to be between 15% and 20% (Abu-Bakar et al., 2021).

When we look at the studies mentioned above, similar trends are seen in university students in this study. It was understood that the samples received more information, especially about promoting clean contact and air quality, saw posters and brochures and were more informed about the COVID-19 pandemic. It is seen that students have information about "water quality," but only 45% get information about it via communication tools. When we look at the results of the "movement," the "stay at home" rule is more dominant, and only half of the students do sports during the pandemic period. Whereas supporting the immune system is of great importance for all diseases. In this period, 77 students declared that they paid attention to the issue of nutrition. 53 out of 100 students experienced sleep disorders. Moreover, according to the survey results, 52 students received psychological support throughout this period.

As a result of this analysis, it can be concluded that besides informing students to create awareness, spaces should be designed accordingly so that they can also experience precautions' benefits in their daily lives. For example, more health spaces may be designed to improve physical and mental health; more cleaning zones can be designed to achieve clean contact; the more mechanical system can be improved using technological improvements in the living spaces, especially in study areas. With these spatial and design precautions, students know and experience this knowledge in space.

6. CONCLUSION

Referred to the more rigorous period of lockdown in Turkiye as a security measure adopted to contain the COVID-19 pandemic, our study highlights the awareness by the university students against the Pandemic by WELL Building Standard COVID-19 Criteria. This certificate brings the health dimension to the green building concept much higher than the known certificate systems for the design and operation stages. It is a performance-based certification system that measures, certifies, and monitors the elements of the building and built environment that affects human health and is audited by independent organizations. This certification system is examined in 10 concepts focused on human health. Air, Water, Nourishment, Light, Movement, Thermal Comfort, Sound, Materials, Community and Mind. By transforming buildings into tools that help improve public health and allow users to protect themselves better indoors, this certification system can help reduce the risk of spreading the contagious disease. For this reason, the criteria determined by this certification system were asked of 100 students living in different cities in Turkiye. It was

analyzed by surveying whether there is information about the criterion and whether there are posters, information etc., in their built environment.

The results show that many respondents had high levels of awareness regarding COVID-19 measures in the built environment, such as social distancing and proper ventilation. Moreover, most participants reported they are more likely to follow safety protocols when they know it is an architectural decision designed to reduce pandemic spread. It is clear from the responses that post-pandemic spatial decisions will be greatly influenced by the level of knowledge and understanding university students have concerning architecture's role in keeping them safe during times of global health crisis.

Consequently, architects must not only continue to build spaces with improved hygiene and enhanced air quality but also employ creative and innovative solutions that foster increased awareness of safety measures among university students. These may include designing colorful instructional signage, using social media to highlight the connection between health protocols and architecture or even encouraging hands-on workshops for student groups to learn about air filtration systems and healthy building materials. With greater visibility surrounding safety precautions in the built environment, university administrators can empower students to make informed choices while navigating the built environment.

Finally, the WELL criteria set, accepted as a support material for adaptation to pandemic circumstances, using space and creating awareness of COVID-19, is an important proposal for the built environment against pandemics encountered worldwide. Users' behaviors and spatial requirements of built environments considering COVID-19 precautions will contribute to developing project ideas for designers, architects, and engineers by raising awareness against pandemics such as COVID-19.

Ethics Committee Approval

Ethics committee approval was received for this study from the Social and Human Scientific Research Ethics Committee ----- Science and Technology.

Peer-review

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Author Contributions

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Antibacterial Activity of The Vapor Phase of *Thymus mastichina* Essential Oil

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Abstract: Essential oils have a diverse spectrum of biological activities, they are also low-toxic, and easily degradable in the human body. These properties make them suitable candidates for the protection and shelf-life extension of agricultural products. The aim of our study was to evaluate the efficacy of the vapor phase of *Thymus mastichina* essential oil against microorganisms on model fruit and vegetable crops. We focused on comparing the efficacy of the essential oil in the vapor phase and contact application using the disk diffusion method against the tested microorganisms. Based on the methods disc diffusion method for contact application and antimicrobial activity of vapor phase on model crops for vapor phase we used, we concluded that *Thymus mastichina* essential has higher efficacy in a vapor application. For most of the tested microorganisms and on all the tested crops, the most significant inhibition was detected at the lowest tested concentration of 62.5 µL/L. Only moderate antimicrobial activity was detected in contact application and lower efficacy compared to antibiotics. These findings suggest that in the future *Thymus mastichina* essential oil could find application in crop storage to prevent crop deterioration due to microbial pathogens. Due to the need for low concentrations, it is assumed that the sensory properties of the crop for the consumer will not be affected. The replacement of synthetic fungicides and bactericides with natural alternatives could have a positive impact on the environment.

Keywords: essential oil, *Thymus mastichina*, vapor phase.

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1. INTRODUCTION

Thymus mastichina is also known as "Spanish Thyme" is native to the Iberian Peninsula. *Thymus mastichina* belongs to the Lamiaceae family and is a semi-woody shrub growing to a height of about 0.5 meters (Arantes et al. 2019). This plant is very resistant to frost, diseases, and pests. *Thymus mastichina* is mainly used for its antimicrobial, antioxidant, antirheumatic, and antitussive properties (Méndez-Tovar et al. 2015). For centuries, *Thymus mastichina* has been used as a spice to flavor food. From this mother plant, the essential oil of *Thymus mastichina* (TMEO) is extracted which is used in the pharmaceutical, food, cosmetic, and fragrance industries (Taghouti et al. 2019).

Essential oils (EOs) are volatile, aromatic compounds extracted from different parts of plants that possess diverse biological properties (Ishaq et al. 2017). EOs, due to their pronounced vapor-phase biological activities, low toxicity to humans, easy degradability, and antimicrobial effects, could find application in protecting and extending the shelf life of agricultural products during storage (Gutiérrez et al. 2009).

Vegetables and fruits are among the perishable products with a short shelf life after harvesting. These commodities are at risk of mechanical damage, variation in physical parameters, and contamination during handling by various microbial pathogens (Ding and Lee, 2019). Control of postharvest microbial pathogens is largely provided by synthetic fungicides and bactericides (Palou 2018). The use of these synthetic products can lead to the emergence of

resistance of pathogens to the products used as well as high residues on agricultural products posing a high risk to consumer health and the environment (Hosseini et al. 2020).

Our study aimed to evaluate the efficacy of the vapor phase of *Thymus mastichina* essential oil against microorganisms on fruit and vegetable crops model. To compare the efficacy of vapor phase essential oil with contact application against the microorganisms tested.

2. MATERIAL AND METHOD

2.1. Essential oil

Thymus mastichina essential oil was purchased from the Slovak company Hanus S.R.O. The essential oil was stored in the dark at 4 °C throughout the experiment. The composition of the essential oil were stated by the manufacturer is cineol 45-65 %, linalool 8-30 %, 4-terpineol, β -pinene, α -pinene, limonene, α -terpineol.

2.2. Tested microorganisms

The tested microorganisms were obtained from the Czech collection of microorganisms Brno. Gram-negative bacteria *Yersinia enterocolitica* CCM 7204 and *Haemophilus influenzae* CCM 4454 were used. Gram-positive bacteria were *Listeria monocytogenes* CCM 4699 and *Enterococcus faecalis* CCM 4224.. *Candida tropicalis* CCM 8264 was used as yeast. The biofilm-producing bacterium *Pseudomonas fluorescens* was obtained from a fish sample.

2.3. Antimicrobial activity of TMEO contact application

The antimicrobial activity of the contact application of TMEO was determined by the disk diffusion method. The bacterial inoculum was cultured for 24 hours in Tryptone soy agar (TSA, Oxoid, Basingstoke, UK) at 37 °C. The yeast inoculum was cultured for 24 h in Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK) at 25 °C. The inoculum was adjusted to an optical density of 0.5 McFarland's standard (1.5×10^8 CFU/mL). The disc diffusion method was analyzed on Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK). 100 μ L of modified inoculum were applied to Petri dishes (PD) containing MHA, to bacteria and yeast, respectively. Sterile paper discs (Oxoid, Basingstoke, UK) with a diameter of 6 mm were placed on the PDs. The discs were saturated with 10 μ L of TMEO. Samples were incubated for 24 h at 37 °C for bacteria and 25 °C for yeast. Two antibiotics (cefoxitin for gram-positive bacteria, gentamicin for gram-negative bacteria; Oxoid, Basingstoke, UK) and one antifungal (fluconazole; Oxoid, Basingstoke, UK) were used as positive controls for gram-negative, gram-positive bacteria and yeasts. An inhibition zone above 10 mm was determined as very strong antimicrobial activity, an inhibition zone of 5-10 mm was determined as moderate activity, and an inhibition zone below 5 mm was determined as weak activity. Antimicrobial activity was measured in triplicate (Kačániová et al. 2020).

2.4. Antimicrobial activity of vapor phase TMEO on model crops

The antimicrobial activity of vapor phase TMEO on model crops (apple, pear, carrot, white radish) was tested on gram-negative, gram-positive bacteria and yeast. SDA for yeast and MHA for bacteria was poured into 60 mm PD and capped. Sliced model crops (0.5 mm) were placed on agar. Using a microbial needle, three injections of inoculum were applied to the sliced model foods. TMEO was diluted in ethyl acetate to concentrations of 500, 250, 125, and 62.5 μ L/L. A sterile filter paper was placed in the lid onto which 100 μ L of the appropriate concentration was injected using a micropipette. The filter paper was dried for 1 min to evaporate the remaining ethyl acetate, then the plates were sealed and incubated at 37 °C for bacteria and 25 °C for yeast for 7 days.

Growth inhibition was assessed by stereological methods. Volume density (Vv) was estimated using ImageJ software. Colony (P) and substrate (p) stereological grid points were calculated. Growth density was calculated as % by the formula $Vv = P/p \times 100$. The antimicrobial activity of EO was expressed as growth inhibition $BGI = [(C - T)/C] \times 100$, where C was the growth density in the control group and T was the growth density in the treated group (Aman 2015; Talibi et al. 2012). Negative results represented growth stimulation.

2.5. Statistical data processing

One-way analysis of variance (ANOVA) followed by Tukey's test at $p < 0.05$ was used to statistically process the data using Prism 8.0.1 (GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1. Antimicrobial activity of TMEO contact application

Based on the disc diffusion method, we found that TMEO achieved moderate inhibitory activity on how many inhibition zones in the range of 5-10 mm were observed for all tested microorganisms (Table 1). The highest zone of inhibition was detected against the yeast *C. tropicalis* (8.66 mm) and the gram-negative bacteria *H. influenzae* (8.33 mm). Compared to the antibiotic control, the effect of TMEO was lower.

Table 1. Antimicrobial activity of TMEO contact application.

Microorganisms	Inhibition zones (mm)	ATB (mm)
<i>Yersinia enterocolitica</i>	5.66±0.58	29.66±0,58
<i>Haemophilus influenzae</i>	8.33±1.15	30.54±1,53
<i>Listeria monocytogenes</i>	6.33±1.66	30.67±1.15
<i>Enterococcus faecalis</i>	7.54±0.58	28.88±0.33
<i>Pseudomonas fluorescens</i> -biofilm	5.33±1.15	25.66±0.58
<i>Candida tropicalis</i>	8.66±0.58	31.66±1.15

The inhibition zone above 10 mm was determined as very strong antimicrobial activity, the inhibition zone 5-10 mm was determined as moderate activity, and the inhibition zone below 5 mm was determined as weak activity. Antimicrobial activity was measured in triplicate.

3.2. Antimicrobial activity of vapor phase TMEO on model crops

In the analysis of the antimicrobial activity of vapor phase TMEO on the model apple crop (Table 2.), we detected inhibition in all tested microorganisms and at all tested concentrations, except for *E. faecalis* with a concentration of 250 µL/L and for *P. fluorescens* biofilm with a concentration of 125 µL/L where we observed stimulation of growth. The most pronounced inhibition was observed at the lowest tested concentration, 62.5 µL/L, for all tested microorganisms.

Table 2. Antimicrobial activity of TMEO vapor application on model food apple.

Apple	BGI (%)			
	Con. (µL/L)			
Microorganisms	62.5	125	250	500
<i>Y. enterocolitica</i>	54.40±0.08 ^a	44.23±0.99 ^b	36.39±2.12 ^c	26.02±2.09 ^d
<i>H. influenzae</i>	35.25±1.07 ^a	22.65±0.89 ^b	15.83±0.83 ^c	12.68±0.67 ^d
<i>L. monocytogenes</i>	44.74±0.95 ^a	34.35±0.98 ^b	13.32±1.05 ^c	8.32±0.52 ^d
<i>E. faecalis</i>	34.71±0.95 ^a	6.73±1.20 ^b	-9.28±0.55 ^c	2.76±0.42 ^d
<i>C. tropicalis</i>	53.40±1.15 ^a	-24.46±2.06 ^b	24.68±0.42 ^c	8.74±0.57 ^d
<i>P. fluorescens-biofilm</i>	36.55±2.03 ^a	21.06±0.63 ^b	12.03±1.60 ^c	2.99±0.59 ^d

Mean ± standard deviation. Values followed by different superscripts within the same line are significantly different ($P < 0.05$). Con.—concentration. BGI – bacterial growth inhibition.

In the analysis of the antimicrobial activity of vapor phase TMEO on the model crop pear (Table 3.), we detected inhibition in all tested microorganisms and at all concentrations. The most pronounced inhibition was

observed at the lowest tested concentration, 62.5 µL/L, for all microorganisms.

Table 3. Antimicrobial activity of steam application of TMEO on model crop pear.

Pear	BGI (%)			
	Con. (µL/L)			
Microorganisms	62.5	125	250	500
<i>Y. enterocolitica</i>	36.63±2.04 ^a	33.72±5.34 ^a	12.36±0.84 ^b	6.84±0.95 ^c
<i>H. influenzae</i>	36.55±2.03 ^a	25.36±0.11 ^b	15.17±0.61 ^c	0.81±0.56 ^d
<i>L. monocytogenes</i>	44.09±2.28 ^a	23.85±2.03 ^b	12.56±0.48 ^c	5.37±0.44 ^d
<i>E. faecalis</i>	54.78±1.46 ^a	36.39±2.12 ^b	26.52±2.39 ^c	8.48±0.59 ^d
<i>C. tropicalis</i>	36.39±2.12 ^a	23.32±0.95 ^b	14.45±0.86 ^c	5.37±0.40 ^d
<i>P. fluorescens-biofilm</i>	64.89±1.00 ^a	36.46±2.72 ^b	17.43±0.06 ^c	6.98±0.97 ^d

Mean ± standard deviation. Values followed by different superscripts within the same line are significantly different ($P < 0.05$). Con.—concentration. BGI – bacterial growth inhibition.

In the analysis of the antimicrobial activity of vapor phase TMEO on a model carrot crop (Table 4.), we detected inhibition in all tested microorganisms and at all concentrations. The most pronounced inhibition

concentration for all microorganisms except *L. monocytogenes* was observed 62.5 µL/L, and for *L. monocytogenes* 125 µL/L

Table 4. Antimicrobial activity of steam application of TMEO on carrot model crop.

Carrot	BGI (%)			
	Con. ($\mu\text{L/L}$)			
Microorganisms	62.5	125	250	500
<i>Y. enterocolitica</i>	19.32 \pm 0.95 ^a	14.05 \pm 1.50 ^b	8.28 \pm 1.47 ^c	2.16 \pm 0.86 ^d
<i>H. influenzae</i>	56.18 \pm 1.17 ^a	43.66 \pm 1.02 ^b	27.47 \pm 2.13 ^c	8.27 \pm 0.52 ^d
<i>L. monocytogenes</i>	16.34 \pm 1.85 ^a	25.54 \pm 0.39 ^b	7.16 \pm 1.63 ^c	4.44 \pm 0.46 ^d
<i>E. faecalis</i>	44.21 \pm 0.48 ^a	28.17 \pm 5.90 ^b	13.24 \pm 0.55 ^c	3.43 \pm 0.83 ^d
<i>C. tropicalis</i>	56.05 \pm 0.95 ^a	33.72 \pm 1.06 ^b	12.47 \pm 0.45 ^c	4.96 \pm 0.34 ^d
<i>P. fluorescens-biofilm</i>	47.87 \pm 1.35 ^a	27.46 \pm 1.06 ^b	14.00 \pm 1.16 ^c	5.21 \pm 0.30 ^d

Mean \pm standard deviation. Values followed by different superscripts within the same line are significantly different ($P < 0.05$). Con.—concentration. BGI – bacterial growth inhibition.

In the analysis of the antimicrobial activity of vapor phase TMEO on a model crop of white radish (Table 5.), we detected inhibition in all tested microorganisms and at all tested concentrations. The most pronounced inhibition was observed for *Y. enterocolitica*, *E. faecalis* and *C. tropicalis* at the lowest concentration tested, 62.5 $\mu\text{L/L}$. Against other

microorganisms, efficacy was observed at higher concentrations for *H. influenzae* and *L. monocytogenes* at 250 $\mu\text{L/L}$ and biofilm-producing *P. fluorescens* at 125 $\mu\text{L/L}$.

Table 5. Antimicrobial activity of steam application of TMEO on a model crop of white radish.

White radish	BGI (%)			
	Con. ($\mu\text{L/L}$)			
Microorganisms	62.5	125	250	500
<i>Y. enterocolitica</i>	43.62 \pm 0.56 ^a	23.65 \pm 1.53 ^a	17.67 \pm 0.48 ^b	8.87 \pm 0.59 ^c
<i>H. influenzae</i>	15.68 \pm 0.57 ^a	5.20 \pm 3.68 ^b	35.30 \pm 1.06 ^c	35.21 \pm 0.76 ^c
<i>L. monocytogenes</i>	25.93 \pm 1.80 ^a	23.49 \pm 18.65 ^{ac}	87.67 \pm 1.48 ^b	14.26 \pm 0.57 ^c
<i>E. faecalis</i>	86.97 \pm 1.34 ^a	4.26 \pm 1.08 ^b	16.52 \pm 2.02 ^c	37.10 \pm 1.85 ^d
<i>C. tropicalis</i>	55.85 \pm 1.14 ^a	32.80 \pm 1.00 ^b	23.65 \pm 1.53 ^c	16.37 \pm 0.79 ^d
<i>P. fluorescens-biofilm</i>	35.28 \pm 0.95 ^a	75.47 \pm 1.33 ^b	24.45 \pm 2.41 ^c	8.43 \pm 0.71 ^d

Mean \pm standard deviation. Values followed by different superscripts within the same line are significantly different ($P < 0.05$). Con.—concentration. BGI – bacterial growth inhibition.

4. DISCUSSION AND CONCLUSIONS

Rodrigues et al. (2020) in their work found inhibition zones of TMEO against *L. monocytogenes* of 9.7-12.3 mm and for *P. fluorescens* of 9-10 mm, depending on the origin of the EO and the plant part used. The findings for *L. monocytogenes* are in agreement with our results for *P. fluorescens* we detected a lower zone of inhibition which could be because it is a biofilm-producing strain that is more resistant to inhibition. Faleiro et al. (2003) detected in their work the inhibitory activity of TMEO against *L. monocytogenes* with a zone of inhibition of 9.7 mm and also report that TMEO is most effective against the *Candida* genus with a zone of inhibition above 10 mm. These findings confirm our results. Ballester-Costa et al. (2013) in their work found a zone of inhibition against *P. fluorescens* of 9.0 mm. In our work, we detected a lower zone of inhibition which could be because it is a biofilm-producing strain which is more resistant to inhibition.

To our knowledge, TMEO has not yet been tested in the vapor phase on food models. In a previous study, we analyzed the vapor phase effect of *T. vulgaris* detecting a very good antifungal as well as antibacterial effect (Galovičová et al. 2021). Paris et al. (2020) in their work analyzed the antifungal and antimicrobial effect of EOs in washed and contact applications against fruit spoilage

pathogens and concluded that the vapor phase is more effective than the contact phase as EOs are rich in volatile compounds which confirms our findings. In contrast, Ács et al. (2018) in their study reported that their EOs were stronger inhibitors in liquid form, which is probably due to direct contact with the pathogen. This finding contradicts our results which may be due to the different chemical compositions especially the content of volatile compounds which are more effective in the vapor phase.

The results of our study show that TMEO has higher efficacy in vapor application where we detected the most significant inhibition at the lowest tested concentration of 62.5 $\mu\text{L/L}$ for most of the tested microorganisms and on all crops. In contact application, only moderate antimicrobial activity was detected, and lower efficacy compared to antibiotics. These findings suggest that in the future TMEO could find application in the storage of horticultural crops to prevent their deterioration due to microbial pathogens. In the future, it would be advisable to test even lower concentrations of TMEO as the trend of antimicrobial effect showed that in most cases the lowest concentration was the most effective. Due to the need to use very low concentrations, it is assumed that the sensory properties of the crop for the consumer will not be affected. The replacement of synthetic fungicides and bactericides with

natural alternatives could have a positive impact on the environment.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Characteristics of Some Warm Climate Grass Plants Commonly Used in Green Field Plant

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Abstract: The aim of this study is to investigate the characteristics of *Cynodon*, *Zoysia* and *Paspalum* genera and species, which are widely used in multi-purpose turf field facilities in temperate climate zones. All of these varieties are defined as warm climate grass. The genus *Cynodon* includes the most widely used species, especially with its resistance to high temperatures. Compared to other hot climate grass plants, *Zoysia* is successfully used in the formation of green areas with species that have very good shade tolerance and low temperature tolerance. *Paspalum* includes species that stand out with their resistance to salinity, drought and oppression, and it is successfully applied in erosion control as well as creating turf fields for different purposes.

Keywords: *Turf, Cynodon, Zoysia, Paspalum, characteristics*

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1. INTRODUCTION

The global water crisis and the increase in temperature have now become a common problem for all countries. For a livable world, all societies must act together and implement urgent environmental action plans. The World Economic Forum states that the water crisis is one of the three biggest global risks since 2012. The most significant impact of water scarcity has been on the agriculture sector, which accounts for 70% of global freshwater resources (Food and Agriculture Organization of the United Nations [FAO], 2012). Pollution of existing freshwater resources and irregularities in precipitation patterns strain the water resources needed for the growing world population, limiting the availability of irrigation water for the turf industry and increasing the need for drought-resistant varieties. This need has made the use of warm climate grass plants widespread in the greenfield facility, especially in the regions located in the temperate climate zone. The stress and ability of a plant to survive water deficiency is called drought resistance (Levitt, 1980). Grass plants contain 75-85% water by weight (Beard, 1966) and begin to wilt with a 10% reduction in water content (Beard, 1973).

In the Mediterranean coasts of Italy, France and Spain (Miele et al., 1995) and in the states of California, Arizona, Texas, which form the Mediterranean climate-like belt of the USA (Duncan and Carrow, 2007), successful and sustainable green areas are created by using grass species such as

Cynodon dactylon, *Zoysia japonica*, *Paspalum vaginatum*, *Paspalum notanum*, *Pennisetum clandestinum*, *Stenotaphrum secundatum*. The aforementioned grasses are preferred according to their usage areas (Martiniello and Andrea, 2006) and are used for grassing sports fields (football, tennis, baseball, golf, etc.). In this preference, the herbs in question, besides their superior resistance to heat and drought stress, compared to cool climate grass plants, the capability of performing two times more photosynthesis while using much less water under the same conditions, which plays a fundamental role. This is the basis of grass plant breeding studies that have been carried out for many years. (Hubbard, 1987; Gibeault et al., 1989; Goatley et al., 1994; Cockerham et al., 1998; Zhou and Abaraha, 2007; Geren et al., 2009). Today, species belonging to the *Cynodon*, *Zoysia* and *Paspalum* genera are successfully and widely used in lawn planting, especially in the hot climate zone. In this review, the main characteristics of the main grass species of the mentioned genera will be explained.

2. CYNODON

Bermuda grass (*Cynodon* (L.) Rich) has higher drought and temperature tolerance and fewer disease and pest problems than most grass species in tropical and warm climates. In addition to these features, it is a type of grass that is frequently used in sports fields, golf courses, parks, home gardens and other green area arrangements due to its very good resistance to pressing and chewing (Beard, 1973).

2.1. *Cynodon dactylon* (L.) Pers.

Bermuda grass creates a very dense, strong and dense layer of turf. Since the leaf blades are narrow in width, they form a thin, very thin or medium textured structure, the color varies from very light green to dark green, while the growth takes place in a completely horizontal form with stolons and rhizomes. The root system is fringed, dense and quite deep. While the production of all Bermuda types is carried out vegetatively with plucked or cut cuttings and rooted cuttings, only Bermuda grass can be produced with its seeds, which cover the area where it is applied in a short time with their rapid development (Emmons, 2000; Christians, 2004). Since Bermuda grass is adapted to temperate-rainy and temperate-semi-arid climates, it is extremely resistant to heat and drought but very sensitive to cold. For this reason, it enters the dormant period to protect itself from the winter cold and turns completely yellow. This yellowing and dormancy begin when the soil temperature drops below 15 °C and end when it rises above this temperature again in the spring, and plant growth resumes. The shade tolerance of the plant, which loves the light very much, is almost nonexistent (Avcioğlu, 2014). Bermuda grass, which can adapt to a wide variety of soil conditions, achieves its highest performance in fertile, relatively loose, well-drained soils; however, the coarse (sandy) soil structure gives negative results due to the lack of nutrients. The plant is also resistant to water accumulation, salinity and soil acidity conditions of pH: 5.5–7.5 (Uzun, 1999). Bermuda grass, which forms a very dense, uniform and high quality green cover under suitable conditions, is successfully used in parks, sports fields, athletic fields, home gardens, road slopes and golf areas due to its rapid regrowth feature in temperate climates such as the Mediterranean climate (Duble, 2013). This feature of it gives the most positive results in sports fields, even if the vegetation, consisting of partially dead stems and leaves, is damaged, sports can be done for a long time on the yellowed vegetation in winter. Because in the spring, Bermuda grass can cover the area again and quickly. In order to create a more balanced green cover on sports fields, Bermuda grass can also be grown mixed with cool-climate grass plants. Another technique used to give the sports fields of Bermuda grass, which turns yellow during the winter period, a green appearance in this period; top seeding and some cool climate grasses (perennial grasses), which are sprinkled on the yellowed vegetation at the end of summer. The area is covered with a green cover throughout the winter (Avcioğlu, 2014). Practices have shown that this type of grass requires moderate and, depending on the situation, intensive care. 1.25-2.50 cm cutting height is best because it is resistant to cutting from the bottom and good vegetation is provided with dense cutting. Some varieties of Bermuda grass can even withstand being mowed every day at a height of 0.5-0.65 cm (Emmons, 2000). Bilgili et al., 2017 say that mats and bald spots can be avoided by giving Bermuda grass, which grows well with water and fertilizer, an average of 4 kg/da of nitrogen each month and cutting it deep.

2.2. *Cynodon transvaalensis* Burt-Daw

The common name of Uganda grass, which has successful application areas in the Mediterranean climate zone, is African Bermuda grass (Taliaferro, 2003). Uganda grass,

which has a finer structure and slower growth than Bermuda grass, produces good results, particularly in hot climate sports fields (Cockerham, 2008).

3. ZOYSIA

Grown in temperate rainy and transitional climate zones, Zoysia produces a uniform, dense, short, high-quality green grass cover, while its growth rate is low. There are differences in leaf color and texture according to species. The leaves and stems are very rough and hard, making shaping difficult. Zoysia species spread horizontally with hard, strong stolons and rhizomes, this dense and strong vegetation prevents foreign plants from entering the area and is very durable (Ntoulas et al., 2013).

3.1. *Zoysia japonica* Steud

Z. japonica (japanese grass) is the most commonly used species in the genus Zoysia (Braun, 2011). Among the warm climate grass species, Japanese grass, which has the best tolerance to low temperatures in winter, retains its color in winter longer than other types. If the temperature drops below 10-15 °C, it enters dormancy and turns yellow, turning brownish white throughout the winter (Avcioğlu, 1997). Due to its good shade tolerance compared to other warm climate grass types, Japanese grass is also a suitable choice for use in turf areas with penumbra conditions that do not receive full sun (Wherley, 2011). The root system of Japanese grass is strong and fringed at medium depth. Because it produces a limited number of seeds, it is usually propagated by rooted and rootless cuttings as a spread planting method. Since the growth rate of the shoots is very slow, Japanese grass covers the plots very slowly, and the damage to the vegetation can be removed very slowly (Avcioğlu, 2014). The hard and strong stem and leaf structure greatly increases the resistance of the green areas formed by this plant to pressing and crushing, Japanese grass, which has a very good resistance to salinity, can adapt to different soil types, in heavy and fertile soils with a pH of 6-7 and high drainage, it gives the best results by applying 2.5-5 kg/da nitrogen fertilizer monthly and mowing at a height of 1.25-2.5 cm (Avcioğlu, 1997). With these features, Japanese grass is used in areas such as home gardens, playgrounds, parks, horse racing and athletic tracks, which are intensively used in the states of the USA with a temperate climate zone, Japan, Korea, Australia and the Mediterranean zone of our country (Boyd et al., 2003). It is resistant to diseases and pests compared to many green-field grasses. Sometimes helminthosporium, nematodes, gray worms, rust and leaf spots can cause significant damage. Japanese grass is also highly tolerant of herbicides such as Simazin and Atrazine. Many varieties and types of Japanese grass are available in commercial markets (Avcioğlu, 2014).

4. PASPALUM

Pistachio (*Paspalum* sp.) is found in natural vegetation with approximately 400 species in countries in the subtropical belt of North and South America, such as Brazil, Argentina, Cuba, Uruguay, Paraguay (Pessarakli ve Kopec, 2007).

4.1. *Paspalum vaginatum*

In recent years, it has been determined that *Paspalum vaginatum* of the *Paspalum* genus, which has been examined in all aspects with the intensive studies of breeders, is resistant to extreme salinity and shows superior grass performance, and as a result of intensive breeding studies, very effective varieties have been bred and presented to the consumer (Pessarakli ve Kopec, 2007). *Paspalum vaginatum* varieties can withstand water-saturated soil conditions under rain for 250–300 days, can stay under water for days, and can be irrigated with treated wastewater. When sea water is used in irrigation, it has a "herbicide" effect on *Paspalum vaginatum* plots. This grasshopper, which can form shoots as small as 2.5 cm, also has a highly competitive performance. It has higher shade and drought tolerance than other hot climate grasses. It can be cut up to 3-4 mm stubble height (Avcioğlu, 2014). *Paspalum vaginatum* has a variety of uses in the grass industry. Due to its leaf texture, quality and resistance to printing, it contains properties suitable for many different applications. Golf course applications, sports field applications, residential and landscape areas and erosion control can be counted among these. Under appropriate care conditions, salted pseudo-millet outperform other warm climate grasses in terms of resistance to multi-directional pressures (drought, salt, water ponding, low light intensity, extremely high and low pH).

4.2. *Paspalum notatum* Flugge

This perennial grass species, resistant to adverse conditions, adaptable to mild-rainy and temperate-arid climates, gives the best results in sandy soils in the coastal belt (Nelson et al., 1993). *Paspalum notatum*, which forms a dense vegetation with its horizontally growing thick rhizomes and relatively deep roots, can grow in almost any type of soil and is based on a dense form. This type of grass, which can be produced with seeds or rhizomes, is very successful only on road slopes, airports and areas where soil protection measures are required due to its coarse texture (Avcioğlu, 1997).

4.3. *Paspalum dilatatum* Poir.

It is an important pasture plant and fodder plant in the hot climates of the world and has the characteristics of being resistant to heat, drought and cold by adapting to temperate-rainy climates (White et al., 1975; Jones, 1985). *Paspalum dilatatum* is also called "water grass" because it loves water. It is produced by seeds and has proven to be effective in erosion control. Since *Paspalum dilatatum* is stronger than Bermuda grass in terms of resistance to adverse conditions, it provides successful lawn formation in mixed plantings (Avcioğlu, 2014).

4. DISCUSSION AND CONCLUSIONS

In this review, some important characteristics of the main plants preferred for turfgrass plantations in the temperate climate zone are discussed. Especially today, when the climatic conditions have changed significantly with a significant decrease in useful water resources, very high and low temperature values, and factors that complicate living

conditions such as salinity increasing, it is very important that grass areas, which are an indispensable element of residential areas, continue to exist. It can be said that *Cynodon*, *Zoysia* and *Paspalum* genera and species, which are highly resistant to adverse conditions for the sustainability of parks, gardens and sports fields, where people can spend a peaceful and happy time, will give successful results in the grass field facility.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Yazar rehberi

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Ana metin: Makale ana metni tek satır aralıklı olarak yazılmalı, çizelge ve şekillerle birlikte toplam 15 sayfayı geçmemelidir. Konu başlıkları 1., 1.1., 1.1.1., şeklinde numaralandırılmalıdır.

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