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**Research Article**

# Chemical composition and biological activity of *Salvia officinalis* essential oil

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The study was aimed at analyzing chemical composition, and biological and antibiofilm activity of *Salvia officinalis* L. essential oil (EO) with MALDI-TOF MS Biotyper. The main compounds of *S. officinalis* EO were a-thujone 24.6%, camphor 20.6%, 1,8-cineole 12.1%, and a-humulene 5.8%. Free radical scavenging activity was medium high. The highest antimicrobial activity was observed against *Bacillus subtilis*. Changes in the biofilm structure confirmed the inhibitory action of *S. officinalis* and the most pronounced effect was observed in *B. subtilis* biofilm. The highest inhibition *in situ* in antimicrobial activity was 78.45% at 125 $\mu.L^{-1}$ on apple for *B. subtilis*.

**Keywords:** sage essential oil, antimicrobial activity, antibiofilm profile, flavonoids, microorganisms

## 1 Introduction

*Salvia officinalis* L. (sage) has been used for medical purposes for several thousand years. The plant is used for many medical conditions (e.g. insomnia, measles, sea sickness, sexually transmitted diseases, worms) and it has a historical background dating to ancient Greek and Roman times. Sage was used for medicinal purposes against itching in Ebers Papyrus (1500 BC) period. It was used for mental clarity and to strengthen memory toward the end of 17$^{th}$ century (Altindal & Altindal, 2016). Sage is used due to its diuretic, anti-inflammatory, antimicrobial, antiseptic effects, as an expectorant, and for hyperhidrosis. Sage, which was used against plague in the past, is used for various clinical conditions today; thanks to its sedative, antimicrobial, antioxidant, antitumor, antihypertensive effects, for perspiration, coronary heart disease, chronic bronchitis, asthma, chronic renal failure, cirrhosis, dysmenorrhea, insomnia, infantile colic, and dyspepsia (Aríca et al., 2010). Sage has been the subject of various studies due to its phenolic substances. These studies reported that sage can be used as an antiperspirant, antifungal, antiseptic, antibiotic, astringent, antispasm, estrogenic, hypoglycemic, diuretic, carminative, and tonic agent (Cenic-Milosevic et al., 2013). Essential oil obtained from *S. officinalis* has medicinal effects against respiratory and digestive syndromes, heart and blood circulation, metabolic conditions, and endocrine diseases (Badiee et al., 2012; Oliveira et al., 2019). Moreover, sage leaves are used in medicine due to their antiseptic and anti-inflammatory effects (Bauner et al., 2012); in addition to this, sage has

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shown anticancer activity (Mohammad, 2011) and has inflammatory, estrogenic, and sedative effects. Sage can be helpful against muscle pains and chronic stress or mental tension. In addition, sage can be used as a mouthwash for sore throat, infected gingiva, mouth ulcers, and colds. Sage oil has aroma like camphor and thujone; therefore, it is used in the perfume industry. This plant has sedative effects on sweat glands, which decreases sweat secretion in the hand, foot, armpit, and the whole body (Mohammad, 2011). Sage treatment has shown promising and beneficial effects for slowing the progress of bone loss, thus indicating its usefulness as a potential therapeutic agent in humans (Abdallah et al., 2010). Moreover, it was reported that sage reduces the injury area and hastens the recovery period of the injury (e.g. skin breaks, muscle tears or bone fractures) (Anitha et al., 2013). Sage can be used in different forms such as tablets, as tea or droplets of essential oil. It can also be used for colouring carpets or rug yarn (Olmez & Kayabasi, 2002). The aim of this study was to investigate chemical composition, antimicrobial and antibiofilm activity of *Salvia officinalis* essential oil.

## 2 Material and methods

### 2.1 Essential oil

*Salvia officinalis* L. EO was purchased from Hanus, s.r.o. (Nitra, Slovakia), and previously prepared by steam distillation of dried flowering stalk. It was stored in the dark at 4 °C before the analyses.

### 2.2 Tested bacteria

The biofilm-forming bacteria *Bacillus subtilis* and *Stenotrophomonas maltophilia* were obtained from the dairy industry, identified with 16S rRNA sequencing and MALDI-TOF MS Biotyper. These bacterial species were tested for antimicrobial and antibiofilm activity.

### 2.3 Chemical characterization of the essential oil by gas chromatography/mass spectrometry (GC-MS) and gas chromatography (GC-FID)

GC-MS analyses of the selected essential oil sample was performed using Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to quadrupole mass spectrometer 5,975B (Agilent Technologies, Santa Clara, CA, USA). A HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used. The temperature program was: 60 °C to 150 °C (increasing rate 3 °C.min$^{-1}$) and 150 °C to 280 °C (increasing rate 5 °C.min$^{-1}$). The total run time was 60 min. There was Helium 5.0 used as the carrier gas with flow rate of 1 mL.min$^{-1}$. The injection volume was 1 µL (EO sample was diluted in pentane), while the split/splitless injector temperature was set at 280 °C. The investigated sample with split ratio at 40.8 : 1 was injected in the split mode. Electron-impact mass spectrometric data (EI-MS; 70 eV) were acquired in scan mode over the m/z range 35–550. MS ion source and MS quadrupole temperatures were 230 °C and 150 °C, respectively. The acquisition of data started after the solvent delay time of 3 min. GC-FID analyses were performed on Agilent 6890N gas chromatograph coupled to FID detector. Column (HP-5MS) and chromatographic conditions were the same as for GC-MS. The temperature of the FID detector was set at 300 °C. The individual volatile constituents of injected essential oil samples were identified according to their retention indices (Adams, 2007) and were compared with the reference spectra (Wiley and NIST databases). The retention indices were experimentally determined by the standard method described in (Van Den Dool & Kratz, 1963) which included retention times of n-alkanes (C6-C34), injected under the same chromatographic conditions. The per-centages of the identified compounds (amounts higher than 0.1%) were derived from their GC peak areas.

### 2.4 Antioxidant activity of *S. officinalis* essential oil

The free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical as described in Kačániová et al. (2020).

### 2.5 Antimicrobial activity with disk diffusion method

Antimicrobial activity of *S. officinalis* EO was determined using the disc diffusion method. Bacteria were aerobically cultivated on Tryptone soya agar (TSA, Oxoid, Basingstoke, UK) at 37 °C for 24 h. An inoculum with an optical density of 0.5 McFarland standard (corresponded to 1.5 × 10$^{8}$ CFU.mL$^{-1}$) was prepared and an amount of 100 µL was used for Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK) inoculation. Clean discs with 6 mm diameter were saturated with 10 µL of *S. officinalis* EO and placed on the agar. Bacteria were incubated aerobically at 37 °C for 24 h. Each test was repeated 3 times.

### 2.6 Minimum inhibitory concentrations (MIC)

Bacteria were aerobically cultured for 24 h in a Mueller Hinton Broth (MHB, Oxoid, Basingstoke, UK) at 37 °C. The 50 µl of microbial suspension with optical density 0.5 McFarland standard was applied to a 96-well microtiter plate. 100 µL of MHB with *S. officinalis* EO in concentrations from 400 µL.mL$^{-1}$ to 0.2 µL.mL$^{-1}$, prepared with serial dilution, was added to sample. The contents of the wells were mixed by pipetting. MHB and EO were used as a negative control, and MHB with inoculum was used as a positive control of maximal growth. The MIC of biofilms was measured after 24 with use of a crystal

violet. Suspension with non-attached cells was discarded and wells were washed with distilled water three times, dried at room temperature, stained with crystal violet (200 µL 0.1% (w/v)) for 15 min, and repeatedly washed and dried. Stained biofilms were resolubilized with 200 µL of 33% acetic acid (Hassan et al., 2011). Absorbance was measured at 570 nm (Glomax spectrophotometer, Promega Inc., Madison, WI, USA). Each test had three replications.

### 2.7 Analysis of differences in biofilm development with MALDI-TOF MS Biotyper

The various phases of biofilm development were evaluated with MALDI-TOF MS Biotyper. The goal was to monitor changes in the structure of the biofilm on glass and wooden surfaces after treatment with *S. officinalis* EO. Experimental and control samples were prepared in 50 mL polypropylene tubes with 20 mL of MHB, a glass slide and a wooden toothpick. The experimental groups contained MHB enriched with 0.5% *S. officinalis* EO and inoculated samples were incubated at 37 °C on a slope 45° shaker at 170 rpm. Biofilm and planktonic cell samples were collected on days 3, 5, 7, 9, 12, and 14. Subsequently, the analysis of developmental phases and molecular differences of biofilms and dendrogram was performed with MALDI-TOF MS Biotyper (Kačániová et al., 2020).

### 2.8 *In situ* antimicrobial analysis on apples, carrots, and potatoes

The antimicrobial analysis *in situ* was tested in the vapor phase on biofilm-forming bacteria *B. subtilis* and *S. maltophilia*. Warm MHA was poured into 60 mm Petri dishes (PD) and the lid. Sliced carrot, apple and potatoe fruits (0.5 mm) were placed on agar. Inoculum was prepared as described previously. *S. officinalis* EO was diluted twice in ethyl acetate to obtain concentration of 500, 250, 125, and 62.5 µL.L$^{-1}$ and used for sterile filter paper inoculation. The filter paper was placed in for 1 min to evaporate the remaining ethyl acetate, sealed and incubated for 7 days at 37 °C and for fungi at 25 °C for 14 days. The growth assessment was performed as in the *in situ* antimicrobial activity method. *In situ* bacterial growth was determined using stereological methods. In this concept, the volume density (*Vv*) of bacterial colonies was firstly estimated 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). The volume density of bacterial colonies was consequently calculated as follows:

$$Vv\ (\%) = P/p \qquad (1)$$

The antibacterial activity of EO was defined as percentage of bacterial growth inhibition (*BGI*):

$$BGI = [(C - T)/C] \times 100 \qquad (2)$$

where:
C – bacterial growth (expressed as *Vv*) in the control group; *T* – in the treatment group. The negative results represented the growth stimulation

### 2.9 Statistical data evaluation

Microsoft-Excel® software was used for data processing. Results of the MIC value (concentration that caused 50% and 90% inhibition in bacterial growth) were determined by logit analysis.

## Results and discussion

### 3.1 Chemical composition of *Salvia officinalis* EO

**Table 1** Chemical composition of essential oil from *Salvia officinalis*

| No | RI* | Compound** | %*** |
|---|---|---|---|
| **1** | 926 | α-thujene | 0.1 |
| **2** | 938 | α-pinene | 3.1 |
| **3** | 948 | camphene | 2.0 |
| **4** | 977 | sabinene | 0.1 |
| **5** | 980 | β-pinene | 1.9 |
| **6** | 992 | β-myrcene | 0.6 |
| **7** | 1016 | α-terpinene | 0.1 |
| **8** | 1023 | *p*-cymene | 1.7 |
| **9** | 1028 | α-limonene | 2.1 |
| **10** | 1033 | 1,8-cineole | 12.1 |
| **11** | 1047 | (*E*)-b-ocimene | tr |
| **12** | 1060 | ɤ-terpinene | 0.4 |
| **13** | 1016 | α-terpinene | 1.1 |
| **14** | 1101 | α-thujone | 24.6 |
| **15** | 1114 | β-thujone | 5.4 |
| **16** | 1148 | camphor | 20.6 |
| **17** | 1170 | borneol | 3.6 |
| **18** | 1178 | 4-terpinenol | 0.6 |
| **19** | 1189 | α-terpineol | 0.7 |
| **20** | 1255 | linalool acetate | 0.2 |
| **21** | 1286 | bornyl acetate | 1.9 |
| **22** | 1289 | *trans*-sabinyl acetate | 0.1 |
| **26** | 1302 | carvacrol | 0.1 |
| **27** | 1379 | α-copaene | tr |
| **28** | 1422 | (*E*)-caryophyllene | 5.2 |

**Continuation of table 1**

| No | RI* | Compound** | %*** |
|---|---|---|---|
| **29** | 1440 | α-guaiene | tr |
| **30** | 1443 | aromadendrene | 0.2 |
| **31** | 1456 | α-humulene | 5.8 |
| **32** | 1485 | α-amorphene | tr |
| **33** | 1490 | β-selinene | 0.2 |
| **34** | 1492 | α-selinene | tr |
| **35** | 1498 | ledene | 0.5 |
| **36** | 1583 | caryophyllene oxide | 0.7 |
| **37** | 1593 | viridiflorol | 3.4 |
| | Total | | 99.1 |

* values of retention indices on HP-5MS column; ** identified compounds; *** tr – compounds identified in amounts less than 0.1%

There was used gas chromatography/mass spectrometry (GC/MS) and gas chromatography (GC-FID) of *S. officinalis* EO detected for detection of α-thujone 24.6%, camphor 20.6%, 1,8-cineole 12.1%, and α-humulene 5.8% as the major compounds (Table 1). In different study of Damyanova et al. (2016) the main compounds of *Salvia officinalis* essential oil were as follows: α-thujone (26.68%), (E)-β-caryophyllene (7.47%), 1,8-cineole (7.19%), α-humulene (6.11%), β-pinene (5.44%), β-thujone (5.35%), camphor (4.84%), allo-aromadendrene (4.55%), borneol (3.69%), and α-pinene (3.58%).

### 3.2 Antioxidant and antimicrobial activity, minimum inhibitory concentrations (MIC)

The antioxidant activity of *S. officinalis* EO measured by the DPPH method was determined at 30.7 ±1.3% of inhibition that corresponds to 171.93 ±1.90 TEAC. The antioxidant activity of essential oils depends on part of plant, solvent and chosen technique of extraction as it was found out for other Salvia species (Ozcan & Al Juhaimi, 2011). Our results are in line with the above mentioned data. The highest antimicrobial activity was found against *B. subtilis* by the use of both methods (Table 2). Studies on antimicrobial properties of *Salvia officinalis* EO revealed its activity against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Shigella flexneri*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella* spp., *Bacillus subtilis* and *Aspergillus niger* (Miladinovic & Miladinovic, 2000; Rota et al., 2004). Oliviera et al. (2018) set MICs 50 and 90 of *Salvia officinalis* essential oil for *C. albicans* at 6.25 and 6.25, respectively 12.5 μg.mL$^{-1}$, for *C. tropicalis* 12.5, resp. 25 μg.mL$^{-1}$, for *C. glabrata* 6.25 resp. 12.5 μg.mL$^{-1}$ and for *S. aureus* 5.28 resp. 10.56 μg.mL$^{-1}$. Tafi et al. (2020) established a minimum bactericidal concentration of the essential oil of 5.185 μg.mL$^{-1}$. Tadi et al. (2020) established MICs of 50 and 90 for *S. aureus* and *S. typhimurium* 15.62 and 31.14 μg.mL$^{-1}$, and 19.5 and 39 μg.mL$^{-1}$, respectively. Pavić et al. (2019) determined the minimum inhibitory concentrations for *B. subtilis* 15.6 μL.mL$^{-1}$, for *S. aureus* 62.5 μL.mL$^{-1}$ and for *P. aeruginosa* 10.82 ±0.02 μL.mL$^{-1}$.

### 3.3 Antibiofilm activity of *S. officinalis* EO

Mass spectra analysis of *B. subtilis* biofilm showed the similarity of the experimental spectra and the control planktonic spectrum on the 3$^{rd}$ day of the experiment (Fig. 1A) which indicated that bacterial biofilm developed equally due to the protein production. The changes in mass spectra on 5$^{th}$ day were more visible on biofilm formed on wood than one on glass surface (Fig. 3B). The changes in the mass spectra were observed in biofilm on both surfaces from 7$^{th}$ day in comparison to the control planktonic cells (Fig. 3C–F). The changes in protein profile of *B. subtilis* biofilm treated with *S. officinalis* EO was analysed. However, the effect of *S. officinalis* EO on protein production can be confirmed in biofilm forming bacteria *B. subtilis* compared to untreated control cells. The dendrogram constructed according to mass spectra also confirmed the similarity of young biofilms with planktonic cells and control cells. The distance of MSP growth was during the experiment progression which indicates the differences in protein production caused by infl uence of *S. officinalis* EO addition.

The difference between the mass spectra of biofilms on glass and wooden surface and control sample occurred from the 5$^{th}$ day (Fig. 1B–F). There were visible changes in protein profile of biofilm treated with *S. officinalis* EO. It seems that *S. officinalis* EO influences the homeostasis of bacterial biofilm formed on the wooden and the glass surface. The dendrogram was constructed as a visualization of mass spectra for determination of some similarities of biofilm structure regarding to the distance

**Table 2** Antibacterial activity of *S. officinalis*

| Microorganism | Zone inhibition (mm) | Activity of EO | MIC 50 (μL.mL$^{-1}$) | MIC 90 (μL.mL$^{-1}$) | Activity of EO |
|---|---|---|---|---|---|
| *Stenotrophomonas maltophilia* | 16.47 ±0.58 | *** | 0.39 | 0.78 | *** |
| *Bacillus subtilis* | 22.43 ±0.58 | *** | 0.20 | 0.39 | *** |

*** very strong inhibitory activity

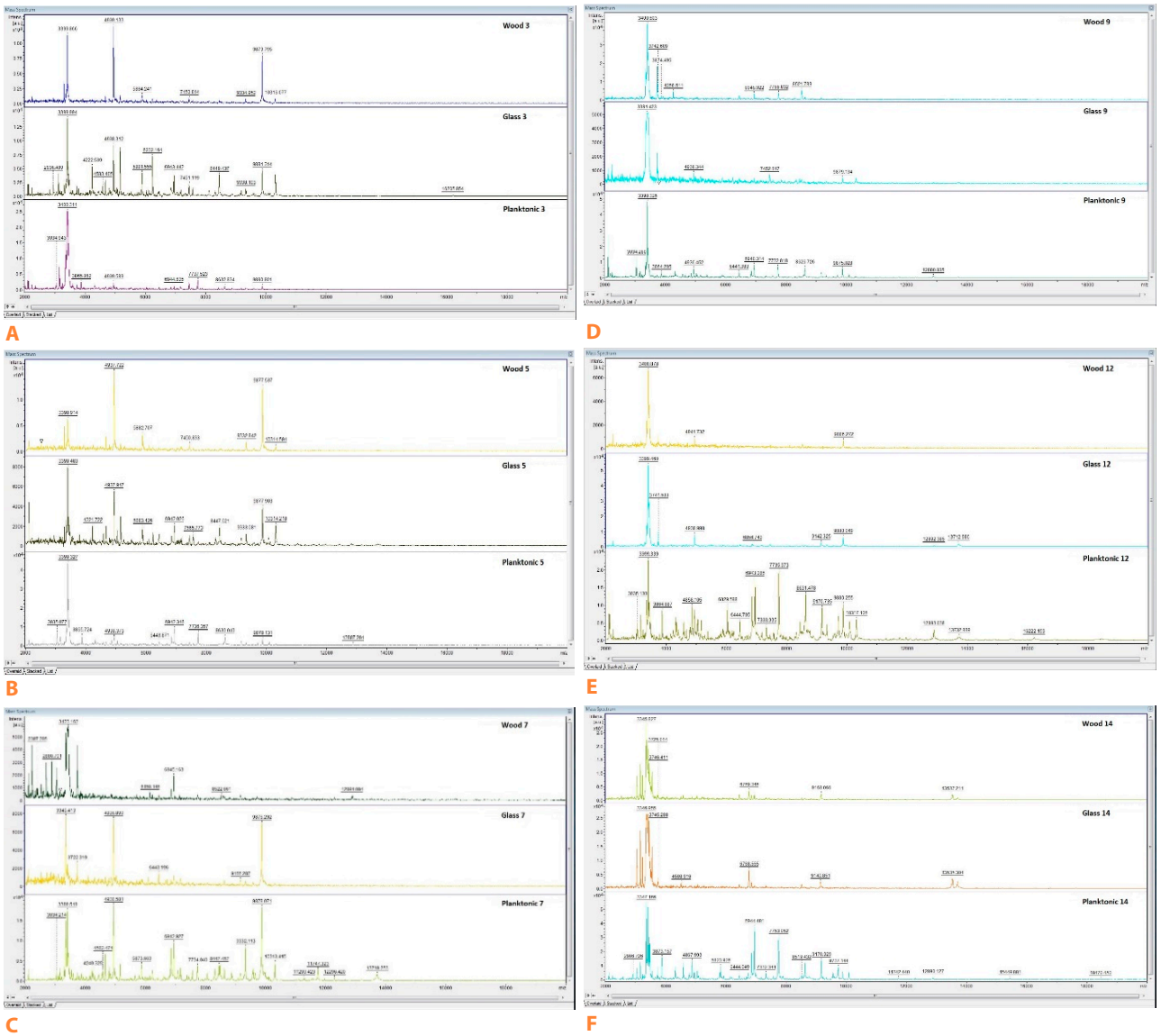


**Figure 1** Representative MALDI-TOF mass spectra of *B. subtilis*
A – 3rd day; B – 5th day; C – 7th day; D – 9th day; E - 12th day; F – 14th day

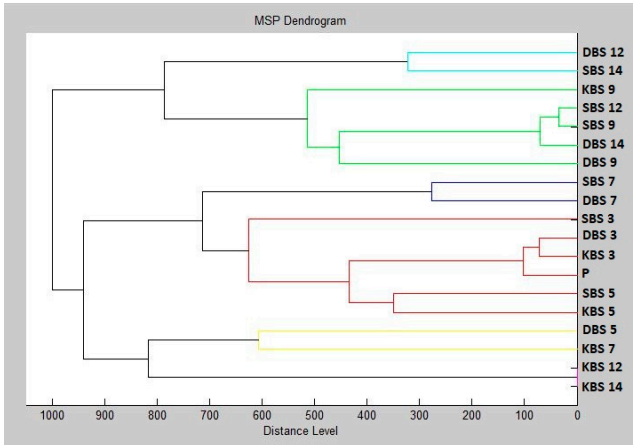


**Figure 2** Dendrogram of *B. subtilis* generated using MSPs for planktonic cells and the control
BS – *B. subtilis*; K – control; S – glass; D – wood; P – planktonic cells

of MSP. It can be stated from the constructed dendrogram (Fig. 2), that the planktonic stage (P) had the shortest distance together with control groups and with young biofilms from the 3rd day when it grew on the wood and the glass (SSM3, DSM3). The similarity in protein profile of the control groups was confirmed by short distances of MSP (Fig. 3). The young biofilms and control planktonic cells also had short distances of MSP which corresponds with mass spectra. The distance of MSP experimental groups increased gradually with days. Mass spectra prepared on the 12th and 14th day of the experiment had the longest distance of MSP which indicates the changes in bacterial biofilm protein profile of *S. maltophilia* (Fig. 4). The use of MALDI-TOF for detection of degradation of biofilm has been previously less reported. Kırmusaoğlu (2019) described various methods for biofilm detection

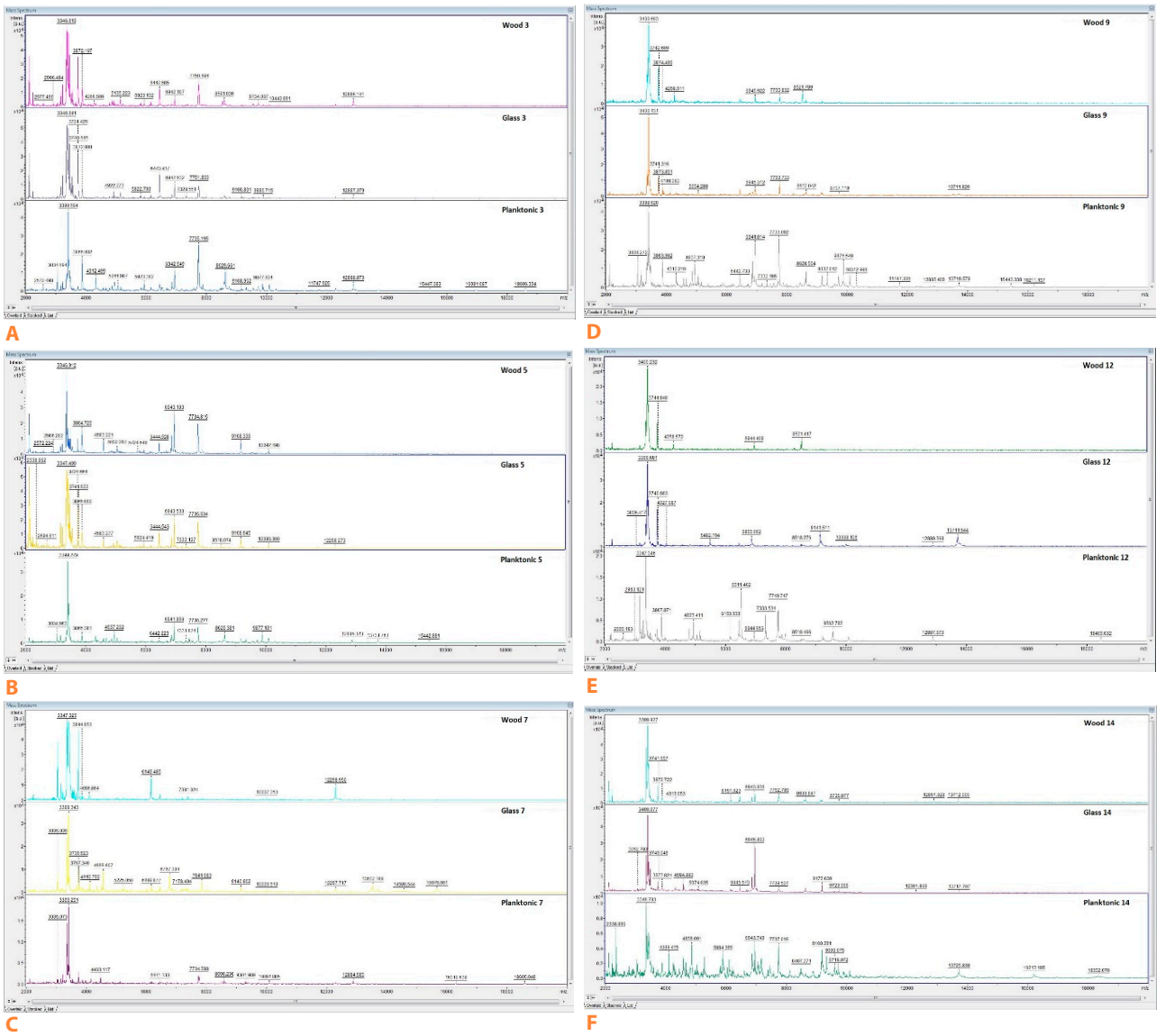


**Figure 3** MALDI-TOF mass spectra of *S. maltophilia* biofilm during development
A – 3rd day; B – 5th day; C – 7th day; D – 9th day; E – 12th day; F – 14th day

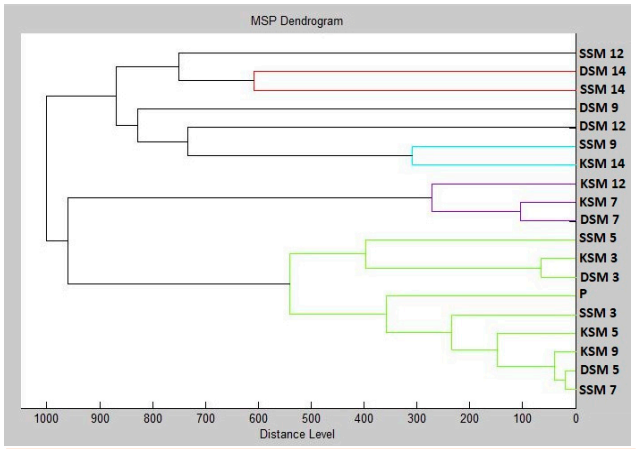


**Figure 4** Dendrogram of *S. maltophilia* generated using MSPs of planktonic cells and control
SM – *S. maltophilia*; K – control; S – glass; D – wood; P – planktonic cells

and stated that the mass spectrometry is a less common but a very suitable method for biofilm research. Stîngu et al. (2008) described the accuracy of identification biofilm forming bacteria by MALDI-TOF MS compared to 16S rRNA sequencing and confirmed that MALDI-TOF MS can distinguish the differences in the mass spectra of closely related biofilms.

### 3.4 *In situ* antimicrobial analysis on apples, carrots, and potatoes

The growth of *B. subtilis* was inhibited on apples, carrots, and potatoes as it is shown in Table 3. The highest inhibition was 78.45% at 125 $\mu L.L^{-1}$ on apple and the lowest of 1.89% at 125 $\mu L.L^{-1}$ on carrot. The inhibition of *S. maltophilia* was recorded on potato at 125 $\mu L.L^{-1}$ concentrations with the inhibition rate of 51.16% and the

**Table 3** Results of *in situ* analysis of antibacterial activity of the vapor phase of *S. officinalis* essential oil on apples, carrots, and potatoes

| **Bacteria strains** | **MBI (%) – apple *Salvia* (μL.L$^{-1}$)** | | | |
|---|---|---|---|---|
| | **62.5** | **125** | **250** | **500** |
| ***B. subtilis*** | 14.17 | 78.45 | 5.45 | -21.45 |
| ***S. maltophilia*** | 12.60 | -13.33 | -5.37 | -17.01 |
| | **62.5** | **125** | **250** | **500** |
| ***B. subtilis*** | 65.44 | 1.89 | -3.85 | 72.60 |
| ***S. maltophilia*** | 14.40 | 14.59 | 15.97 | 8.50 |
| | **62.5** | **125** | **250** | **500** |
| ***B. subtilis*** | 11.18 | 54.18 | 9.24 | 30.79 |
| ***S. maltophilia*** | 20.60 | 51.16 | 26.73 | 10.25 |

lowest of 8.50% at 500 μL.L$^{-1}$ (Table 3). *Salvia officinalis* EO was evaluated in the liquid and vapor phases against *Haemophilus influenza*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Streptococcus pneumonia*. Moreover, the MIC of the essential oil in the vapor phase against *Streptococcus pneumonia* was around 690 μL.L$^{-1}$ for bulk oil (Moghimi et al., 2017).

## 4 Conclusions

The results of our study show that the major component of *Salvia officinalis* essential oils was α-thujone, camphor, 1,8-cineole, and α-humulene. The antioxidant activity was evaluated as the medium high. *S. officinalis* EO had very good antimicrobial effects as well as antibiofilm effects observed on various surfaces and detected by MALDI-TOF MS Biotyper. MALDI-TOF MS Biotyper was a suitable method for evaluating phases of biofilms development. *S. officinalis* EO demonstrated inhibitory activity on microorganisms in a food model in the vapor phase.

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**Research Article**

# Selection indices for fruit yield improvement in elite cucumber (*Cucumis sativus* L.) cultivars

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Cucumber (*Cucumis sativus* L.) is an important vegetable crop, rich in vitamins and minerals and eaten fresh as a dessert. Its fruit yield is relatively low, though could be improved through knowledge of character association with it and selection of desirable materials for improvement programmes. Fifteen cultivars of Cucumber were evaluated at two locations (Abeokuta and Ibadan), South West, Nigeria in a randomized complete block design with three replicates in order to determine heritability, correlation, direct and indirect effects of characters on fruit yield. Data collected on agro-morphological characters were subjected to analysis of variance, estimates of heritability, correlation, and path analysis. Significant variations ($p$ <0.05) were observed in the cultivars. High heritability estimates (>90%) was observed for fruit length at both locations. A significant phenotypic and genotypic correlation was observed between fruit yield and fruit weight. Number of days to 50% flowering and fruit width could also be selected directly for improvement of fruit yield in cucumber.

**Keywords:** cucumber, selection indices, heritability, correlation, path analysis

## 1 Introduction

Cucumber (*Cucumis sativus* L) is an important crop in Nigeria (Nweke et al.,2013) and it's a useful ingredient in pharmaceuticals (Kumar et al., 2010). Crop improvement through selection depends on fruit yield and inter-relationships of characters that contributes to yield (Pal et al., 2017). Yield is polygenic character, which is determined by the inter-relationship of other characters. The extent of variability is determined by coefficients of variances (Johnson et al., 1955). High genotypic coefficients of variation are indicative of the potential for selection (Burton & DeVane, 1953). Heritability is an indication of the ease of transmission of characters from parent to offspring, while genetic advance estimates actual gain expected under selection (Ogunniyan & Olakojo, 2015). High heritability and genetic gain are indicative of additive gene action and response to selection (Singh & Rai, 1981). Correlation coefficients were used to determine pair-wise comparison between characters and yield while path analysis was used to partition correlation coefficients into direct and indirect effects, showing individual contribution of a character and how it contributes to yield. The objectives of this study were to determine the heritability and inter relationship of characters in cucumber will serve as selection indices for its improvement.

## 2 Materials and methods

### 2.1 Planting materials, field evaluation and data collection

The study was conducted at the Teaching and Research Farms, Federal University of Agriculture Abeokuta, Ogun State Nigeria (Latitude 7.2° N, Longitude 3.4° E) AS location 1 and the Vegetable Research Field of National Horticultural Research Institute (NIHORT) Idi-Ishin, Ibadan, Oyo State Nigeria (Latitude 7.4° N, Longitude 3.8° E) AS Location 2. Fifteen cultivars of cucumber were used in the experiments and arranged in a randomized complete block design with 3 replicates. Each cultivar was planted in a 2-row plot of 5 × 1m using an inter-row spacing of 0.5 m and intra-row spacing of 1 m. Two seeds were sown per hole and later thinned to one seedling per stand. Observations were made on number of days

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to emergence, number of days to 50% flowering, leaf length (cm), leaf width (cm), leaf area ($cm^2$), vine length (cm), fruit length (cm), fruit width (cm), number of fruits, number of days to maturity, fruit weight (g) and fruit yield (ton).

### 2.2 Data analysis

Data were subjected to analysis of variance using Statistical Analysis System (SAS), (2000) (ver. 8.1) software. Phenotypic, genotypic, and environmental variances were estimated according to Prasad et al. (1981). Broad sense heritability and genetic advance were calculated according to Johnson et al. (1955) and Allard (1960). Phenotypic and genotypic coefficients of variation were estimated with the formula of Johnson et al. (1955). Genotypic, phenotypic, and environmental correlations were computed according to Miller et al. (1958) and path coefficient analysis estimated as outlined by Dewey and Lu (1959).

## 3 Results and discussion

The combined analysis of variance (Table 1) revealed a significant ($p$ <0.05) interaction between cultivar × location effect for all characters except for fruit width, number of fruits, number of days to maturity and fruit yield. The effect of genotype X environment interaction and this implies that selection for such characters based on phenotypic performance will not be reliable. Hence, selection and recommendations for such characters should be location specific. High heritability estimates (Table 2) for number of days to germination, fruit length and fruit weight in both locations indicates the lesser role the environment plays in the phenotypic expression of these characters; hence selection for them is likely to be effective as also reported by Dubey et al. (2013) and Kumar et al. (2013). High estimate of genetic advance (GA) observed in fruit length and fruit weight in both locations and fruit yield in Abeokuta are indications of prospects for effective selection. High genotypic coefficient of variation (GCV) along with high heritability and genetic advance provides a better index for selection of genotypes as reported by Johnson et al. (1955). Hence, moderate to high GCV, heritability and genetic advance observed in number of days to germination, leaf area, fruit length, fruit weight and fruit yield could be attributed to additive gene action, which makes selection for them simple and reliable. Significant phenotypic correlations for most characters with fruit yield were recorded except for number of days to germination and number of fruits in both locations (Table 3). The leaves and fruit parameters showed a significant genotypic correlation with fruit yield in both locations (Table 4). The highly significant and positive phenotypic and genotypic correlations observed in fruit yield with fruit length, fruit width, number of days to maturity, fruit weight in both location implies that both characters can be improved simultaneously in a selection programme. These findings were similar to the earlier reports of Ullah et al. (2012) and Golabadi et al. (2013). The direct and indirect effect of characters on fruit yield in cucumber in both locations (Table 5) revealed that the highest direct effect was recorded for leaf length (0.87). However, leaf area showed a highest negative direct effect (-0.87).

**Table 1** Combined analysis of variance for agronomic and yield characters evaluated in fifteen cucumber cultivars in both locations

| Source | df | DTE | DTF | LL | LWD | LA | VL |
|---|---|---|---|---|---|---|---|
| **Block/location** | 4 | 0.49* | 10.48 | 3.76** | 4.51* | 750.77* | 1195.91** |
| **Location** | 1 | 4.01** | 132.01** | 8.71** | 19.19** | 3193.66** | 10113.67** |
| **Cultivar** | 14 | 4.40** | 27.37** | 5.01** | 8.69** | 1446.21** | 847.14** |
| **Location × cultivar** | 14 | 0.13 | 11.27** | 2.15** | 5.76** | 958.34** | 484.26** |
| **Error** | 56 | 0.19 | 3.85 | 0.83 | 1.43 | 237.77 | 159.49 |

| Source | FL | FWD | NoF | DTM | FWT | FYLD |
|---|---|---|---|---|---|---|
| **Block/location** | 4.13 | 1.87 | 37.26** | 38.21 | 5529.28** | 1034.71* |
| **Location** | 1.48 | 3.44 | 108.90** | 149.51** | 25934.58** | 240.36 |
| **Cultivar** | 191.41** | 12.32** | 12.04 | 47.64** | 18601.00** | 2676.02** |
| **Location × cultivar** | 6.98** | 1.91 | 8.50 | 30.06 | 1466.07* | 581.22 |
| **Error** | 2.65 | 1.41 | 7.97 | 20.01 | 654.71 | 316.53 |

** significant at $P \leq 0.01$, * significant at $P \leq 0.05$, DTE – number of days to emergence, DTF – number of days to 50% flowering, LL – leaf length (cm), LWD – leaf width (cm), LA – leaf area ($cm^2$), VL – vine length (cm), FL – fruit length (cm), FWD – fruit width (cm), NoF – number of fruits, DTM – number of days to maturity, FWT – fruit weight (g), FYLD – fruit yield ($tons.ha^{-1}$)

**Table 2** General mean estimates of variance components, broad sense heritability and genetic advance in both locations for fifteen cucumber varieties

| Character | Location | Mean | PV | GV | PCV | GCV | Hb | GA |
|---|---|---|---|---|---|---|---|---|
| **DTE** | 1 | 4.36 | 0.72 | 0.67 | 19.48 | 18.84 | 93.49 | 37.52 |
| | 2 | 4.78 | 0.79 | 0.71 | 18.57 | 17.60 | 89.81 | 34.37 |
| **LL** | 1 | 9.24 | 0.95 | 0.82 | 10.54 | 9.82 | 86.78 | 18.84 |
| | 2 | 9.86 | 1.44 | 1.01 | 12.16 | 10.21 | 70.48 | 17.66 |
| **LWD** | 1 | 12.21 | 2.49 | 2.31 | 12.91 | 12.46 | 93.12 | 24.77 |
| | 2 | 13.13 | 2.33 | 1.55 | 11.62 | 9.48 | 66.47 | 15.92 |
| **LA** | 1 | 103.20 | 413.62 | 385.16 | 19.71 | 19.02 | 93.12 | 37.80 |
| | 2 | 115.12 | 387.90 | 257.85 | 17.11 | 13.95 | 66.47 | 23.43 |
| **VL** | 1 | 110.49 | 372.43 | 293.09 | 17.47 | 15.50 | 78.70 | 28.32 |
| | 2 | 89.28 | 71.37 | 44.38 | 9.46 | 7.46 | 62.19 | 12.12 |
| **DTF** | 1 | 34.56 | 7.46 | 5.94 | 7.9 | 7.05 | 79.67 | 12.97 |
| | 2 | 32.13 | 5.43 | 4.38 | 7.25 | 6.51 | 80.66 | 12.05 |
| **FL** | 1 | 17.03 | 24.37 | 22.95 | 28.99 | 28.13 | 94.17 | 56.23 |
| | 2 | 17.29 | 41.75 | 41.41 | 37.37 | 37.22 | 99.17 | 76.36 |
| **FWD** | 1 | 14.43 | 1.89 | 1.30 | 9.51 | 7.91 | 69.08 | 13.54 |
| | 2 | 14.83 | 2.86 | 2.50 | 11.4 | 10.67 | 87.46 | 20.55 |
| **NoF** | 1 | 15.42 | 3.77 | 0.20 | 12.59 | 2.93 | 5.43 | 1.41 |
| | 2 | 13.22 | 3.28 | 1.73 | 13.7 | 9.96 | 52.88 | 14.92 |
| **DTM** | 1 | 61.64 | 15.15 | 8.50 | 6.31 | 4.73 | 56.08 | 7.30 |
| | 2 | 59.07 | 10.75 | 4.06 | 5.55 | 3.41 | 37.80 | 4.32 |
| **FWT** | 1 | 168.44 | 4470.70 | 4277.14 | 39.7 | 38.83 | 95.67 | 78.23 |
| | 2 | 202.39 | 2218.32 | 1975.40 | 23.27 | 21.96 | 89.05 | 42.69 |
| **FYLD** | 1 | 5029.34 | 754145.71 | 645903.97 | 34.53 | 31.96 | 85.65 | 60.93 |
| | 2 | 5226.22 | 230826.93 | 147634.13 | 18.39 | 14.70 | 63.96 | 24.22 |

1 – Abeokuta, 2 – Ibadan, PV – phenotypic variance, GV – genotypic variance, PCV – phenotypic coefficient of variation(%), GCV – genotypic coefficient of variation(%), Hb – broad sense heritability (%), GA – genetic advance as % of the mean, DTE – number of days to emergence, DTF – number of days to 50% flowering, LL – leaf length (cm), LWD – leaf width (cm), LA – leaf area ($cm^2$), VL – vine length (cm), FL – fruit length (cm), FWD – fruit width (cm), NoF – number of fruits, DTM – number of days to maturity, FWT – fruit weight (g), FYLD – fruit yield ($ton.ha^{-1}$)

**Table 3** Phenotypic correlation coefficients among the characters of cucumber cultivars evaluated in both locations

| Character | Location | LL | LW | LA | VL | DTF | FL | FW | NoF | DTM | FW | FYLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DTE** | 1 | 0.12 | 0.17 | 0.17 | -0.44** | 0.17 | -0.15 | -0.24 | -0.42** | 0.65** | 0.02 | -0.10 |
| | 2 | -0.21 | -0.32* | -0.32* | -0.05 | 0.43** | 0.00 | 0.01 | -0.46** | -0.09 | 0.10 | -0.21 |
| **LL** | 1 | | 0.95** | 0.95** | 0.30* | 0.04 | -0.51** | -0.60** | 0.32* | -0.39** | -0.80** | -0.76** |
| | 2 | | 0.95** | 0.95** | 0.53** | 0.15 | -0.30* | -0.43** | -0.34* | -0.03 | -0.34* | -0.61** |
| **LW** | 1 | | | 1.00** | 0.21 | -0.18 | -0.57** | -0.63** | 0.18 | -0.37* | -0.82** | -0.84** |
| | 2 | | | 1.00** | 0.52** | 0.10 | -0.35* | -0.40** | -0.23 | -0.10 | -0.43** | -0.63** |
| **LA** | 1 | | | | 0.21 | -0.18 | -0.57** | -0.63** | 0.18 | -0.37* | -0.82** | -0.84** |
| | 2 | | | | 0.52** | 0.10 | -0.35* | -0.40** | -0.23 | -0.10 | -0.43** | -0.63** |
| **VL** | 1 | | | | | 0.35* | -0.01 | 0.18 | 0.59** | -0.30* | -0.27 | -0.07 |
| | 2 | | | | | 0.12 | -0.27 | -0.15 | -0.14 | -0.19 | -0.24 | -0.33* |
| **DTF** | 1 | | | | | | 0.38** | 0.00 | 0.26 | 0.31* | 0.19 | 0.33* |
| | 2 | | | | | | -0.22 | -0.18 | 0.07 | -0.15 | -0.25 | - 0.18 |
| **FL** | 1 | | | | | | | 0.51** | 0.07 | 0.15 | 0.75** | 0.81** |
| | 2 | | | | | | | 0.79** | -0.33* | 0.79** | 0.90** | 0.76** |
| **FWD** | 1 | | | | | | | | 0.07 | 0.21 | 0.67** | 0.75** |
| | 2 | | | | | | | | -0.13 | 0.48** | 0.82** | 0.82** |
| **NoF** | 1 | | | | | | | | | -0.51** | -0.37* | -0.07 |
| | 2 | | | | | | | | | -0.34* | -0.47** | 0.20 |
| **DTM** | 1 | | | | | | | | | | 0.51** | 0.40** |
| | 2 | | | | | | | | | | 0.66** | 0.48** |
| **FWT** | 1 | | | | | | | | | | | 0.94** |
| | 2 | | | | | | | | | | | 0.77** |

*,** significant at 5% and 1% level of probability, respectively, 1 – Abeokuta, 2 – Ibadan, DTE – number of days to emergence, DTF – number of days to 50% flowering, LL – leaf length (cm), LWD – leaf width (cm), LA – leaf area ($cm^2$), VL – vine length (cm), FL – fruit length (cm), FWD – fruit width (cm), NoF – number of fruits, DTM – number of days to maturity, FWT – fruit weight (g), FYLD – fruit yield (ton $ha^{-1}$)

**Table 4** Genotypic correlation coefficients among the characters of cucumber varieties evaluated in both locations

| Character | Location | LL | LW | LA | VL | DTF | FL | FWD | NoF | DTM | FWT | FYLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DTE** | 1 | 0.14 | 0.22 | 0.22 | -0.50** | 0.20 | -0.16 | -0.32* | -1.77** | 0.92** | 0.02 | -0.11 |
| | 2 | -0.32* | -0.45** | -0.45** | -0.04 | 0.48** | -0.01 | 0.00 | -0.79** | -0.21 | 0.16 | -0.25 |
| **LL** | 1 | | 0.99** | 0.99** | 0.29* | 0.10 | -0.58** | -0.86** | 0.85** | -0.48** | -0.86** | -0.92** |
| | 2 | | 0.98** | 0.98** | 0.62** | 0.17 | -0.37* | -0.61** | -0.70** | 0.04 | -0.44** | -0.98** |
| **LWD** | 1 | | | 1.00** | 0.19 | -0.19 | -0.63** | -0.81** | 0.53** | -0.45** | -0.87** | -0.97** |
| | 2 | | | 1.00** | 0.65** | 0.09 | -0.44** | -0.59** | -0.52** | 0.03 | -0.55** | -1.03** |
| **LA** | 1 | | | | 0.19 | -0.19 | -0.63** | -0.81** | 0.53** | -0.45** | -0.87** | -0.97** |
| | 2 | | | | 0.65** | 0.09 | -0.44** | -0.59** | -0.52** | 0.03 | -0.55** | -1.03** |
| **VL** | 1 | | | | | 0.52** | -0.05 | 0.11 | 2.68** | -0.43** | -0.31* | -0.10 |
| | 2 | | | | | 0.14 | -0.35* | -0.23 | -0.21 | -0.52** | -0.35* | -0.51** |
| **DTF** | 1 | | | | | | 0.48** | 0.13 | 1.27** | 0.46** | 0.19 | 0.40** |
| | 2 | | | | | | -0.25 | -0.21 | 0.07 | -0.11 | -0.20 | -0.14 |
| **FL** | 1 | | | | | | | 0.53** | -0.20 | 0.28 | 0.81** | 0.85** |
| | 2 | | | | | | | 0.83** | -0.47** | 1.30** | 0.96** | 0.95** |
| **FWD** | 1 | | | | | | | | -0.63** | 0.50** | 0.87** | 0.88** |
| | 2 | | | | | | | | -0.22 | 0.96** | 0.96** | 1.14** |
| **NoF** | 1 | | | | | | | | | -2.08** | -1.30** | -1.82** |
| | 2 | | | | | | | | | -0.78** | -0.61** | -0.11 |
| **DTM** | 1 | | | | | | | | | | 0.71** | 0.70** |
| | 2 | | | | | | | | | | 0.99** | 0.74** |
| **FWT** | 1 | | | | | | | | | | | 1.05** |
| | 2 | | | | | | | | | | | 0.87** |

**, * significant at 5% and 1% level of probability, respectively, 1 – Abeokuta, 2 – Ibadan, DTE – number of days to emergence, DTF – number of days to 50% flowering, LL – leaf length (cm), LWD – leaf width (cm), LA – leaf area ($cm^2$), VL – vine length (cm), FL – fruit length (cm), FWD – fruit width (cm), NoF – number of fruits, DTM – number of days to maturity, FWT – fruit weight (g), FYLD – fruit yield ($ton/ha^{-1}$)

**Table 5** Direct and indirect effect of some characters on fruit yield in cucumber in both locations

| Characters | Location | Direct effect | LL | LW | LA | VL | DTF | FL | FW | DTM | FW | Genotypic correlation coefficients |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LL | 1 | 0.87 | | 0.00 | -0.86 | -0.15 | 0.04 | 0.01 | -0.70 | 0.15 | -0.27 | -0.92** |
| | 2 | -2.73 | | 0.01 | 1.50 | 0.29 | 0.05 | -0.40 | 0.35 | 0.01 | -0.05 | -0.98** |
| LWD | 1 | 0.00 | 0.87 | | -0.87 | -0.10 | -0.08 | 0.01 | -0.66 | 0.14 | -0.28 | -0.97** |
| | 2 | 0.01 | -2.69 | | 1.52 | 0.31 | 0.03 | -0.48 | 0.34 | 0.00 | -0.07 | -1.03** |
| LA | 1 | -0.87 | 0.87 | 0.00 | | -0.10 | -0.08 | 0.01 | -0.66 | 0.14 | -0.28 | -0.97** |
| | 2 | 1.52 | -2.67 | 0.01 | | 0.31 | 0.03 | -0.48 | 0.34 | 0.00 | -0.07 | -1.03** |
| VL | 1 | -0.53 | 0.25 | 0.00 | -0.17 | | 0.22 | 0.00 | 0.09 | 0.14 | -0.10 | -0.1 |
| | 2 | 0.47 | -1.69 | 0.00 | 1.00 | | 0.04 | -0.38 | 0.13 | -0.09 | -0.04 | -0.51** |
| DTF | 1 | 0.41 | 0.09 | -0.00 | 0.16 | -0.28 | | -0.01 | 0.11 | -0.15 | 0.06 | 0.40** |
| | 2 | 0.30 | -0.46 | 0.00 | 0.14 | 0.07 | | -0.27 | 0.12 | -0.01 | -0.02 | -0.14 |
| FL | 1 | -0.02 | -0.51 | -0.00 | 0.55 | 0.03 | 0.20 | | 0.43 | -0.09 | 0.26 | 0.85** |
| | 2 | 1.09 | 1.00 | -0.00 | -0.67 | -0.16 | -0.08 | | -0.48 | 0.15 | 0.12 | 0.95** |
| FWD | 1 | 0.82 | -0.74 | -0.00 | 0.70 | -0.06 | 0.05 | -0.01 | | -0.16 | 0.28 | 0.88** |
| | 2 | -0.58 | 1.65 | -0.00 | -0.89 | -0.11 | -0.06 | 0.91 | | 0.11 | 0.12 | 1.14** |
| DTM | 1 | -0.32 | -0.42 | -0.00 | 0.39 | 0.23 | 0.19 | -0.00 | 0.41 | | 0.23 | 0.70** |
| | 2 | 0.11 | -0.12 | 0.00 | 0.05 | -0.25 | -0.03 | 1.41 | -0.56 | | 0.12 | 0.74** |
| FWT | 1 | 0.32 | -0.74 | -0.00 | 0.76 | 0.17 | 0.08 | -0.01 | 0.71 | -0.23 | | 1.05** |
| | 2 | 0.12 | 1.21 | -0.00 | -0.84 | -0.16 | -0.06 | 1.04 | -0.55 | 0.11 | | 0.87** |

*, ** significant at 5% and 1% level of probability, respectively, location 1 – Abeokuta, location 2 – Ibadan, residual effect location 1 = 0.27; location 2 = 0.63, DTF – number of days to 50% flowering, LL – leaf length (cm), LWD – leaf width (cm), LA – leaf area ($cm^2$), VL – vine length (cm), FL – fruit length (cm), FWD – fruit width (cm), DTM – number of days to maturity, FWT – fruit weight (g)

Number of days to 50% flowering, fruit width and fruit weight all had positive direct contributions to fruit yield suggesting that these characters can be used for direct selection to improve fruit yield in cucumber cultivars in Abeokuta (Location 1). Despite the strong positive correlation of fruit yield with number of days to maturity and fruit length, their direct effect on fruit yield was negative. This shows the ineffectiveness of selection based only on phenotypic correlation alone. The residual effect of (0.27) Abeokuta and (0.63) Ibadan implies that 73% and 37% of the total variation in fruit yield has been determined. It further portrays the occurrence of some factors not considered in this study contributed to fruit yield in cucumber.

## 4 Conclusion

This study revealed significant variation in the cucumber cultivars evaluated in both locations. Characters such as number of days to germination, leaf area, fruit length, fruit weight and fruit yield could be rewarding in the selection for improved yield. Also, high heritability estimates of number of days to germination, fruit length and fruit weight in both locations shows the reliability of selection of these traits for yield improvement. Fruit length, fruit width, fruit weight and number of days to maturity in both locations could be improved simultaneously for fruit yield. Also, direct selection for number of days to 50% flowering, fruit width and fruit weight would serve as good indices for improvement of fruit yield in cucumber.

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**Research Article**

# Date palm compost versus peat and perlite: a comparative study on germination and plant development of muskmelon and tomato

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This research was carried out in the experimental site of the Arid and Oasian cultures Laboratory of the Institute of Arid Regions, Medenine, Tunisia. It aims at studying the effects of compost on seed germination patterns, seedling growth, and plant development of muskmelon and tomato under greenhouse conditions. Three growth media were used: compost of date palm wastes and two reference media (peat and perlite). The results showed that compost presented a promising threshold of both maturity and stability, which is related to its neutral pH, C : N ratio, greater humic acid vs fulvic acid, and low values of chlorophyll-type compounds. Seeds of muskmelon and tomato germinated at varying liquid compost extract concentrations and muskmelon reached higher germination index values even at the pure extract solution (100%). Moreover, seeds of both species germinated relatively faster in peat than in compost and an overall delay in germination was observed, with a more pronounced reduction on tomato germination percentage. Produced seedlings have attained a similar vigour index among media ($p$ <0.05). Compost of date palm was more suitable for muskmelon stem elongation and leaf-enlarging capacity than perlite. However, the gustative quality of fruits was not significantly affected by the medium-types. Thus, it is concluded the promoising effect of compost of date palm as potting medium and substrate in soilless culture under greenhouse conditions unless a pertinent choice of cultures.

**Keywords:** compost, stability, maturity, germination, greenhouse, soilless culture

## 1 Introduction

Worldwide, the increase in food requirements has stimulated agrochemicals inputs (Dayo-Olagbende et al., 2018). In order to permit a sustainable crop production system, a great interest was given to organic agriculture (Olojugba & Opeyemi, 2020). It is a production system excluding the use of synthetic products (Angadi et al., 2017) yet emphasizing the adoption of eco-friendly practices such as composting (Gastol et al., 2011; Islam et al., 2017).

Composting process is considered as a promising technique of toxic compounds' conversion into innocuous end products instead of their landfill input, dumping, and/or incineration (Lazcano et al., 2009). Therefore, it combines the recovery of valuable resources with environmental protection (Islam et al., 2017; Neher et al., 2015). Several studies affirmed that compost's amendment enhances soil properties (Ch'ng et al., 2014; Trupiano et al., 2017) and contributes to preventing plants from diseases (Khan et al., 2017). Besides, it can be used as potting media in nursery (Unal, 2015) and in soilless culture system (Neher et al., 2015). The principal requirements for the compost to be safely used are a suitable threshold of both stability and maturity which imply, respectively, stable organic matter content and absence of phytotoxic compounds, virulent pathogens, or viable seeds (Bernal et al., 1998; Neher et al., 2015).

In southern Tunisia, date palm (*Phoenix dactylifera* L.) is one of the most cultivated trees producing huge amounts of waste. This waste is largely managed by composting especially due to the fact that this region suffers from continuous soil degradation. Under greenhouses, this constraint is alleviated, among others, by soilless culture relying on the use of sandy desert and/or perlite. While the first medium is too heavy and subsides as it gets older, perlite is expensive and presents problems of management in post-production. Thus, exploration of relatively inexpensive, locally available and more

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environmentally friendly substitutes such as compost based on date palm waste is of great interest (Haddad, 2007), particularly under greenhouses.

Limited information is available on the integrated use of organic media under greenhouses heated by geothermal water, the present study was undertaken to determine the response of tomato and muskmelon to compost. For this purpose, potting experiments were performed to determine: (i) how the compost of date palm affects seed germination patterns, seedling growth, and fruiting quality. (ii) if there are any metabolically related changes in response to soilless cultivation by using compost. Thus, muskmelon and tomato seeds were germinated at varying compost extract concentrations and plants grown from seeds in three medium types including compost and two reference ones (peat and perlite), and germination requirements, morpho-physiological parameters and biochemical traits were analysed.

## 2 Material and methods

The experiment was carried out at the research site of the Arid and Oasian Cultures Laboratory of the Institute of Arid Regions, Medenine (Eastern-South of Tunisia).

### 2.1 Substrates

The research employed commercial compost of date palm (CP) obtained from the association of Chenini oases' protection. It is produced in a specific composting process referring to the method of Bouhouach et al. (2009). Briefly, compost was prepared by mixing dry wastes of date palm and ovine manure.

Peat and perlite were employed as reference media.

### 2.2 Compost analyses

pH and electrical conductivity (EC) of compost were measured, respectively, by potentiometry (pH meter Eutech Instruments) and a conductivity meter (Cond 510, XS Instruments) on compost: water suspension. For total nitrogen determination, a modified Kjeldahl method was used. Total organic carbon was measured according to the Colorimetry method (ISO 14235). Available phosphorus was determined calorimetrically in sulpho-molybdic acid system. The content of Na, K, Ca and Mg were determined referring to Haddad (2007). Both polyphenol, lignin, humic and fulvic acids contents were measured as reported by Radhouani et al. (2012), the humification index was determined as indicated by Zbytniewski and Buszewski (2005), and the decomposition of chlorophyll-type compounds was estimated by the assay of light absorption of acetone extracts of compost.

Physical characterization consisted of determination of the moisture content, bulk density, particle density, water retention capacity, and total space as the procedures described by Verdonck and Gabriëls (1992). The enumeration of total, fungal and bacterial flora was realized as reported by Radhouani et al. (2012).

The extraction of the micro-organisms was carried out on 5 g of compost mixed with 45 ml of 0.1 M buffer phosphate and 0.05% Tween 80. Cultivable total microflora, actinomycetes, and the fungal microflora were analysed on standard plate LPGA, Pochon and Tardieux and malt's extract. Calculations were done in triplicate by performing quantitative determinations based on colony forming units (CFU). All results were expressed as log CFU.g$^{-1}$ DW.

### 2.3 Experimental design and layout

Compost phytotoxicity was assessed through the germination index (GI%) of muskmelon (*Cucumis melo*) and tomato (*Solanum lycopersicum*) seeds. Six different solutions were used: sterile deionized water, as control solution, and solutions containing 25, 50, 75 and 100% extract of compost. Solutions were added to Petri dishes containing 5 sterile seeds. Germination percentage and seedlings' root length were recorded after 5 days of incubation according to Tiquia and Tam (1998). This index was assessed as follows:

$$GI\% = (GsLs)/(GcLc) \times 100 \quad (1)$$

where:
*Gs* and *Ls* – are, respectively, seed germination and root elongation (mm) for the samples; *Gc* and *Lc* –the corresponding values for controls (Trupiano et al., 2017). The test was repeated in triplicate.

Seeds were sown into cell plug trays containing peat and compost. The treatments were laid out in a completely randomized design. Germination was performed in an air-conditioned room at the temperature of 27 ±1 °C and the relative humidity of 90-95%. The number of germinated seeds was recorded 5, 10, 15 and 20 days after sowing (Tiquia & Tam, 1998). The cumulative percentage of germination (%) was determined. The mean germination time (MGT) was calculated referring to the procedure of Alvarado et al. (1987). The needed time to get 50% germination ($T_{50}$) was calculated according to Farooq et al. (2005). Damping off disease incidence of infected seedlings was calculated as described by Rahim et al. (2014).

Twenty days after sowing, the number of leaves of seedlings was counted; shoot height and root lengths

were measured, mutual shoot and root's dry matter were weighted, and their ratio was calculated. The vigour index was determined according to the International Seed Testing Association (1996).

For soilless culture experience, one-month-old tomato seedlings (cv. Romana) were transplanted into plastic pots (5 L) filled with compost and perlite. Plants were conducted in glasshouse under a controlled water regime, temperatures ranging between 12 and 25 °C, and natural day length corresponding to winter-spring season. For muskmelon, growth media were placed in white plastic containers with volume of 33 L and U shape. They were conducted under plastic greenhouses. The nutrient solution was formulated according to the chemical composition of irrigation water, norms of fertilization of each culture, and stage of development. It is of open system's type.

Plant morphological data were collected weekly by measuring stem height (cm) and girth (cm) 80 days after transplantation; surface leaf area ($cm^2$), the rate of dry matter and specific leaf area (SLA) were calculated 30 days after transplantation. At the end of culture, plants were uprooted, and dry weights of roots were measured. Days preceding maturity of the first fruit were counted. The average weight of fruits was determined, too. Gustative quality of produced fruits was evaluated via measurement of pH, EC, IR, acidity, and the IR/acidity ratio.

### 2.4 Statistical analysis

The collected data were subjected to analysis of variance (ANOVA) using Statistica for Windows, version 9. The Duncan's Multiple Range Test (DMRT) was carried out to determine if significant differences ($p$ <0.05) occurred between individual treatments.

## 3 Results and discussion

### 3.1 Characterization of date's palm compost

The finding of neutral pH of compost was similar to those of Forster et al. (1993), Ofosu-Budu et al. (2010), and Unal (2015). The content of nitrogen of 1.13% aligned with works of Francou (2003) who reported content oscillating between 1 and 4%. The C : N value of 15.23 reflected a net mineralization referring to Ch'ng et al. (2014). In previous studies, better values fluctuated between 10 and 30 (Francou, 2003). Moreover, Abdel-Razzak et al. (2018) have reported that composts with a C : N ratio ranging from 12 : 1 to 25 : 1 are ideal for nursery plant production. This N limitation seems to result in decomposing less of the easily available C by thermophilic bacteria and leaving more organic matter for fungal decomposition during the curing phase. Thus, a predominance of fungi flora with several 61 $10^6$CFU.$g^{-1}$ DM has characterized the pile of compost (Table 1).

Synthetic and transformational activities of these microorganisms result in production of 1.23 $10^{-3}$ g.$g^{-1}$ DM of phenolic compounds and 4.93 unit of lignin. This attribution was indicated by Gill and Al-Shankiti

**Table 1** Chemical, physical, and biological properties of both compost of date palm (CP) and peat (P)

| Parameter | | CP | P |
|---|---|---|---|
| **pH** | | 7.53 | 6.74 |
| **EC (dS.$m^{-1}$)** | | 4.83 | 1.22 |
| **OM (%)** | | 32.84 | 61.85 |
| **N (%)** | | 1.13 | 0.60 |
| **C : N** | | 15.23 | 26.92 |
| **Mineral composition (% DM)** | Na | 0.52 | 0.18 |
| | K | 0.46 | 0.53 |
| | Ca + Mg | 3.23 | 2.87 |
| | Cl | 0.16 | 0.04 |
| **Phenolic compounds (g.$g^{-1}$ DM)** | | 1.23 $10^{-3}$ a | 0.094 $10^{-3}$ b |
| **A665** | | 0.027 | – |
| **Lignin** | | 4.93 | – |
| **Humification (% DM)** | HA | 9.41 | 14.57 |
| | FA | 7.1 | 9.3 |
| | HI | 6.98 | 9.11 |
| **H (% DM)** | | 31.42 | 62.87 |
| **Porosity (% DM)** | | 46.83 | 84.24 |
| **Bulk density (g.$m^{-1}$)** | | 0.42 $10^{-2}$ | 0.26 $10^{-2}$ |
| **Real density (g.$m^{-1}$)** | | 0.79 $10^{-2}$ | 1.65 $10^{-2}$ |
| **Retention of water (l.$l^{-1}$)** | | 0.35 | 0.43 |
| **Air capacity (% V/V)** | | 19 | 35.00 |
| **Total flora ($10^{10}$ CFU.$g^{-1}$ DM)** | | 101 | – |
| **Fungi ($10^6$ CFU.$g^{-1}$DM)** | | 61 | – |
| **Bacteria ($10^3$ CFU.$g^{-1}$ DM)** | | 47 | – |

EC – electrical conductivity, OM – organic matter, HA – humic acid, FA – fulvic aid, HI – humification index

(2015). Referring to Zbytniewski and Buszewski (2005), higher content of humic acid (9.41% DM) in comparison with this of fulvic one (7.1% DM) is in favour of compost's maturity. In addition, humification index greater than 5 (6.98) reflected a complete compost maturation as reported by Radhouani et al. (2012) for compost of green wastes. These authors have reported that low index of decomposition of chlorophyllous compounds, as in the case of the studied compost, may provide a good level of maturity, too.

### 3.2 Germination on compost's extract

The germination index, GI, declined significantly ($F$ = 163.58***) for both species as the compost extract concentrations intensified (Fig. 1). This negative correlation was confirmed by $R^2$ of 0.97 for both species. This effect was found by Abdel-Razzak et al. (2018) for tomato, cucumber, and summer squash when adding higher quantities of tomato waste compost to potting media.

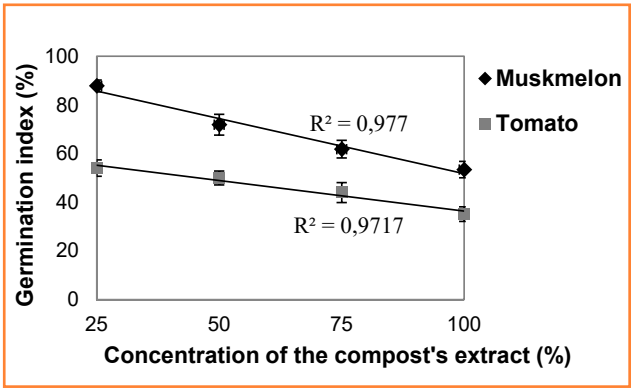


**Figure 1** Changes in germination index of muskmelon and tomato at varying compost water extract concentrations (25, 50, 75 or 100%)
Data represent mean ±SE, $n$ = 5

The results were in agreement with studies of Sanchez-Monedero et al. (1999) when using sewage sludge, poultry manure, pig slurry, olive mill wastewater, city refuse and the lingo-cellulosic cotton waste, maize straw and sweet sorghum bagasse composts' extracts. This reduction seems to be the most drastic one for tomato attaining a value of 35.15% with the solution of pure compost's extract. This selective effect was reported by Bernal et al. (1998). Abdel-Razzak et al. (2018) have indicated that it might be a direct consequence of genus-related genotypic effects when comparing cucumber and summer squash tomato's germination.

Zucconi et al. (1985) have reported that a germination index lesser than 50% reflects a lack of maturity of compost. Thus, biological evaluation may provide an acceptable threshold of maturity of compost showing stable chemical and microbiological characteristics with the potential to be used in agriculture without the risk of toxicity and even the lower value recorded for the tomato. This finding can be attributed to the more effective suppression of seed germination and radical elongation of seeds of dicotyledonous species such as tomato by the water compost extract and its sensitivity to the higher electrical conductivity (Lazcano et al., 2009; Ofosu-Budu et al., 2010).

### 3.3 Direct germination in compost

Among the treatments, the quickest germination was recorded for muskmelon sown in peat (Fig. 2).

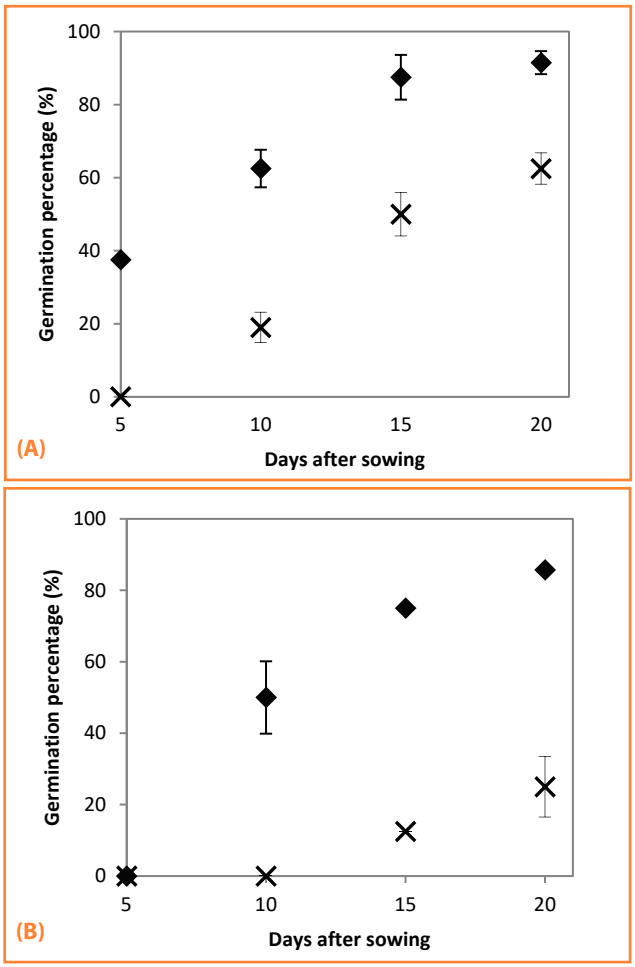


**Figure 2** Changes in germination percentage of (A) muskmelon and (B) tomato sown in compost (CP) or peat (P) after 5, 10, 15 and 20 days of sowing
Data represent mean ±SE, $n$ = 5

The delay of germination on compost was recorded by Abdel-Razzak et al. (2018) for tomato, hot pepper, cucumber, and summer squash with higher proportion of tomato waste compost. The lower performance of compost in terms of cumulative germination percentage

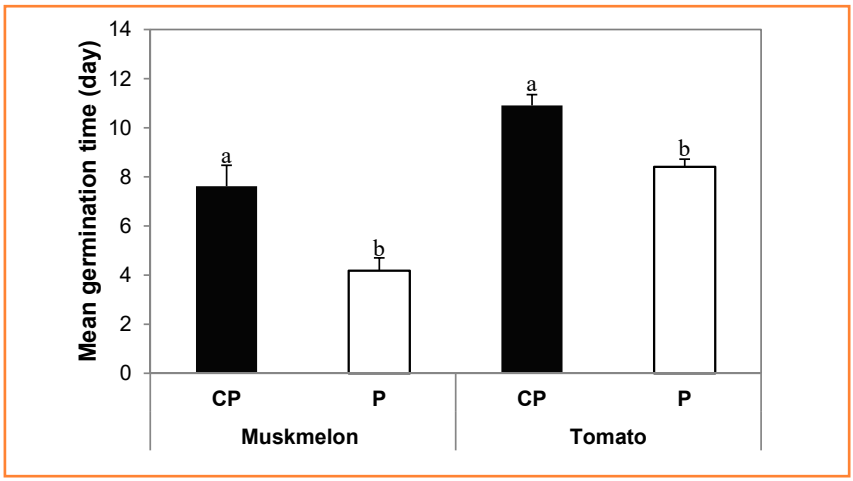


**Figure 3** Changes in mean germination time (MGT, days) of seeds of muskmelon and tomato sown in compost (C) or in peat (P)
Data represent mean ±SE, $n = 5$
For each species, different letters above bars are significantly different at $P < 0.05$ according to Duncan test

was observed by Herrera et al. (2008), too, on tomato sown in municipal solid waste compost. This attitude resulted from the delay on germination's stimulation as justifies the mean germination time (Fig. 3) and $T_{50}$ values which were of 12.59 and 6.97 days for compost of date palms and peat, respectively ($F$ = 8.74*).

The damping infected seedlings were of 4.1 and 6.2% for muskmelon and tomato, respectively. The delay of seed germination in compost as compared to peat is in accordance with works of Herrera et al. (2008) and Zaller (2007) for municipal solid wastes compost and vermi-compost, respectively. Faster stimulation of the germination's process in peat might stem from its promising total porosity (Table 1) that ensures better water-holding capacity (Abdel-Razzak et al., 2018) and aeration for seeds (Cai et al., 2010; Martin & Brathwaite, 2012).

However, the delay of germination in compost can be attributed to its higher electrical conductivity as explained by Abdel-Razzak et al. (2018) when adding great proportion of tomato waste compost to potting media. Cai et al. (2010), Herrera et al. (2008) and Medina et al. (2009) have confirmed the relationship. Indeed, Unal (2015) reported a suitable value between 2 and 4 $dS.m^{-1}$. The alleviation of this effect can be attributed to the content of $Ca^{2+}$ + $Mg^{2+}$ and humic compounds which help seedlings to tolerate salt stress (Cai et al., 2010) and increase cell membrane permeability, cell division, and cellular enlargement (Gill & Al-Shankiti, 2015), respectively.

### 3.4 Seedlings' growth

Referring to Table 2 showing the data recorded on seedlings' growth as affected by potting medium, it seems that peat displayed significantly ($p$ <0.001) higher shoot/root ratio. This effect corroborated works of Keeling et al. (1994); Lazcano et al. (2009); Martin & Brathwaite (2012), Unal (2015) and Zaller (2007) for compost of digested slurry of cow manure, vermicompost, cow manure compost, compost of spent mushroom and this of refuse derived, respectively. In contrast, Medina et al. (2009) have noticed lower aerial biomass of tomato and pepper cultivated in peat with respect to those grown in spent mushroom substrate. Ghehsareh et al. (2011) have reported similar effects of white peat and wood fibre substrate for tomato.

Peat has stimulated the elongation of roots by 1.5 and two times for muskmelon and tomato, respectively, in comparison with compost (Table 2). This result contrasted with the work of Unal (2015) on tomato seedlings grown on peat and spent mushroom's compost. This author explained difference between potting media by their acidity specifying that high pH values can impair this development, while Ch'ng et al. (2014), working on maize plants, attributed medium's effect to

**Table 2** Changes in some characteristics of tomato and muskmelon seedlings as affected by potting media

| Species | Muskmelon | | Tomato | |
|---|---|---|---|---|
| **Substrate** | **CP** | **P** | **CP** | **P** |
| **Height (m)** | 0.30 ±0.006a | 0.27 ±0.001b | 0.29 ±0.01a | 0.24 ±0.009b |
| **Shoot dry weight (g)** | 0.23 ±0.002a | 0.21 ±0.001b | 0.21 ±0.002a | 0.19 ±0.001b |
| **Shoot/Root DW ratio (%)** | 2.92 ±0.034b | 4.07 ±0.098a | 2.02 ±0.76b | 3.38 ±0.91a |
| **Roots' length (m)** | 0.06 ±0.001b | 0.08 ±0.008a | 0.05 ±0.002b | 0.09 ±0.002a |
| **Vigour index (%)** | 28.56 ±2.18a | 26.26 ±1.11a | 19.39 ±3.94a | 21.67 ±1.78a |

For each species, means within a row followed by the same letter are not significantly different at the $P = 0.05$ level for the substrate according to the Duncan test. Data represent mean ±SE, $n = 5$

its porosity. In opposition, compost was more suitable for stem elongation, and dry matter's accumulation at the aerial part was more important at compost. Produced muskmelon and tomato seedlings on the two potting media showed statistically similar vigour index ($p$ <0.05) (Table 2).

### 3.5 Compost's impact in soilless culture system

80 days after transplantation, the compost of date palms was more suitable for muskmelon's stem elongation in correlation with findings of Afriyie et al. (2017) for radish. For tomato, superiority was in favour of perlite (Fig. 4A) in contrast to results of Borji et al. (2010) and Ghulam et al. (2002) who found similar effects of compost of date palm and this of pine bark, respectively, with respect to perlite. Angadi et al. (2017) have noticed longer tomato plants when using organic manure.

For both species, the growth substrate did not result in significant ($P$ = 0.05) effect in plant stem girth (Fig. 4B). This trend was obtained by Borji et al. (2010) on tomato, whereas Ghulam et al. (2002) have noted that tomato plants grown in pine bark's compost reached higher diameter values than those grown in perlite.

In the same context, the results of higher leaf surface area noted on compost for the two horticultural species (Table 3) were noted in previous works of Ghulam et al. (2002) for tomato by comparing compost of pine bark and perlite. Whilst no significant difference was observed for muskmelon's leaf dry mass among media, in tomato, this parameter was higher in perlite (30.22 g) than compost (21.94 g).

Furthermore, the leaves of muskmelon were thicker (low SLA) on compost (1.28 $m^2.g^{-1}$) than those in perlite (153.74 $cm^2.g^{-1}$). Ch'ng et al. (2014) explained this superiority, for compost and biochar, by their richness on functional groups (COOH, phenolic, alcoholic OH and C = O) which served as exchange sites for the crops nutrients. In contrast, leaves of tomato showed higher SLA in compost (142.2 $cm^2.g^{-1}$) than in perlite (111.33 $cm^2.g^{-1}$) (Table 4). Lower surface area seems to be the cause of thick leaves as a positive correlation between them was established by Herrera et al. (2008). Both species showed higher root dry mass when grown in compost than in perlite (data not shown).

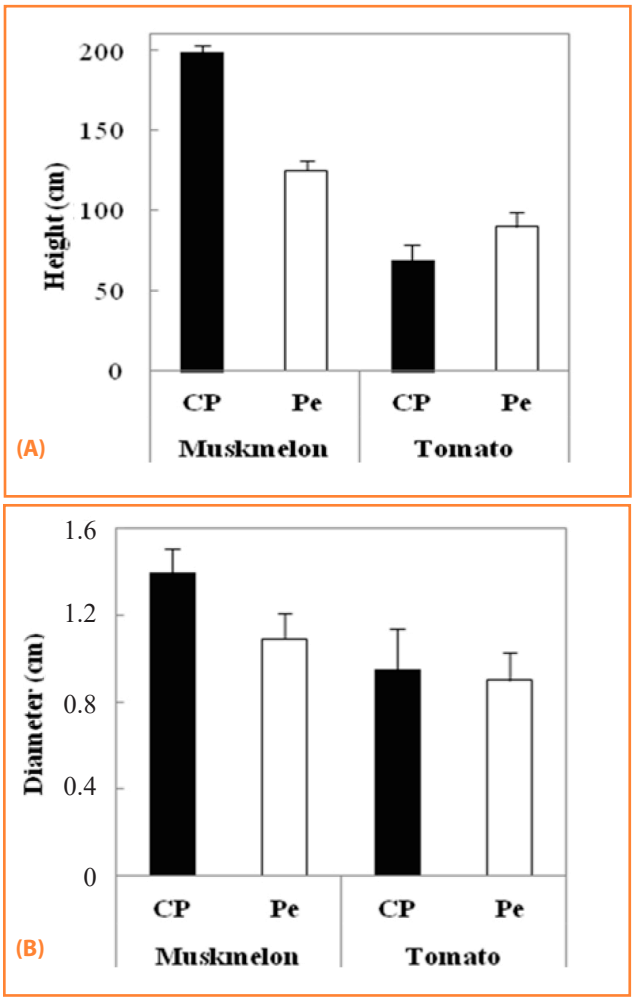


**Figure 4** Changes in (A) height (cm) and (B) diameter (m) of muskmelon and tomato plants grown in compost (CP) or in perlite (Pe)
Data represent mean ± SE, $n$ = 5
Different letters indicate significant differences between substrates at $P$ <0.05 according to the Duncan test

Maturity of muskmelon fruits was three-days earlier in compost than in perlite. This pattern was also marked for tomato. Similar results were found by Tzortzakis and

**Table 3** Changes in leaf parameters of muskmelon and tomato plants grown in compost of date palms (CP) and perlite (Pe)

| Species | Muskmelon | | Tomato | |
|---|---|---|---|---|
| **Substrate** | **CP** | **Pe** | **CP** | **Pe** |
| **Leaf area ($m^2$)** | 0.41 ±0.008a | 0.31 ±0.005b | 0.35 ±0.002a | 0.24 ±0.029b |
| **Dry mass (%)** | 16.68 ±0.08a | 14.51 ±0.04a | 21.94 ±2.03b | 30.22 ±1.43a |
| **Specific leaf area ($m^2.g^{-1}$)** | 1.28 ±0.01b | 1.53 ±0.02a | 1.42 ±0.01a | 1.11 ±0.03b |

For each species, means within a row followed by the same letter are not significantly different at the P = 0.05 level for the substrate according to the Duncan test. Data represent mean ±SE, $n$ = 5

**Table 4** Effect of media on gustative quality of muskmelon and tomato fruits.

| Parameter | Muskmelon | | Tomato | |
|---|---|---|---|---|
| | CP | Pe | CP | Pe |
| **pH** | 6.98 ±0.08a | 6.66 ±0.02a | 4.16 ±0.31a | 4.31 ±0.17a |
| **EC (dS.m$^{-1}$)** | 6.94 ±0.32a | 7.18 ±0.4a | 7.16 ±0.33a | 7.27 ±0.22a |
| **RI (° Brix)** | 11.8 ±0.65a | 10.1 ±0.8a | 7 ±0.01a | 7.8 ±0.03a |
| **Acidity (%)** | 1.17 ±0.13a | 1.4 ±0.02a | 13.62 ±0,31a | 14.01a |
| **RI/acidity** | 10.08 ±0.05a | 7.21 ±0.021b | 3 ±0.13a | 3 ±0.13a |

For each species, means within a row followed by the same letter are not significantly different at the $P = 0.05$ level for the substrate according to the Duncan test. Data represent mean ± SE, $n = 5$

Economakis (2005) who noted that plants grew faster in organic media as compared to inorganic ones. In contrast, Olle et al. (2012) reported that cucumber plants developed quicker in perlite or rockwool as compared to coconut fibre.

It should be noted that the heaviest fruits were produced by plants grown in compost and it was even more significant in case of muskmelon. A similar result was obtained by Tzortzakis and Economakis (2005) with compost of maize waste. In contrast, Borji et al. (2010) and Ghehsareh et al. (2011) concluded a significant superiority of perlite with respect to compost of date palms. Such superiority of organic media in comparison to inorganic one was reported by Olle et al. (2012) for seven tomato cultivars grown in coir straw in comparison to rockwool hence it was invalidated by Gąstol et al. (2011) when comparing organic and conventional crops. Higher effect of compost could be explained by the correlation between the fruit size and its degree of hydration (Ghehsareh et al., 2011). Indeed, it was shown that compost enhanced the nutrients availability (Olojugba & Opeyemi, 2020) and improved the plant capability of more nutrients' uptake from the surrounding soil (Olle et al., 2012). These effects were indicated for radish, tomato, and maize, respectively, by Afriyie et al. (2017) and Khan et al. (2017). In the same framework, Islam et al. (2017) have affirmed higher fruits weight of tomato grown in compost and vermin-compost regarding inorganic fertilizers. Indeed, soil fertility affects the movement and uptake of nutrients by roots, and their utilization within plants (Ch'ng et al., 2014).

The data related to qualitative attributes display significant variation among treatments. The greater refractrometric index of tomato fruits produced in perlite was in agreement with works of Borji et al. (2010) but in opposition to results reported by Ghehsareh et al. (2011) who found higher soluble solids of tomato fruits grown in cocopeat in relation to those cultivated in perlite. Gastol et al. (2011) found higher effect of organic media for pear, blackcurrant, beetroot, and celery, and a lower effect for apple and carrot in regarding to conventional ones. For both crops, fruit taste was not affected by the type of growth medium (Table 4). On the contrary, Olle et al. (2012) reported that vegetables grown in organic media could be tastier than those grown in inorganic ones.

## 4 Conclusion

Positive effects of the only adoption of date palm compost on the germination and plant development were noted in the current research. These effects were similar to or better than those of the used conventional media: peat and perlite. Our results confirmed the beneficial use of both solid and liquid forms of compost for horticultural crops such as muskmelon and tomato.

Thus, considering low cost and great availability of date-palm compost in the south of Tunisia, it appears that it can be employed under geothermal greenhouses. However, further studies and research on alleviation of its salinity should be prepared in order to enhance its effectiveness and avoid environmental risks.

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**Research Article**

# Transpiration and water use efficiency of maize in different soil moisture conditions

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Globally, agriculture accounts for 80–90% of the fresh water used by humans, and in many crop production systems; this water use is unsustainable. Irrigation of large areas of field and horticultural crops is impossible. Studies of the impact of drought on important field and horticultural crops are necessary to estimate dimensions of adaptation and mitigation measures to climate change. For this purpose, maize was monitored as a model crop in this study. In a three-year experiment (i) using the sap flow measurement method, the transpiration of maize was evaluated during flowering and grain filling, (ii) water use efficiency (WUE) was evaluated in four soil moisture conditions. The intensity of transpiration was closely correlated with the values of global radiation and vapor pressure deficit. However, soil water content was a major factor influencing transpiration under drought stress. The transpiration decreased when water content in the soil reached 28% of available water holding capacity (AWHC), but the yield of corn cobs decreased only under stress of 25% AWHC. Thus, the yield reacted less sensitively to lower water availability than transpiration. WUE increased with decreasing transpiration. Statistically significantly higher WUE was already observed at a water content of 42% AWHC, however, a higher WUE did not lead to a higher yield of corn cobs.

**Keywords:** sap flow, corn, WUE, drought, yield

## 1 Introduction

Currently, drought is the most significant environmental abiotic stress worldwide. The significance of drought increases with the time of its effect during the vegetation period and with its occurrence in the critical phases of the plants development. Canopy monitoring of meteorological elements is crucial for precise description of microclimatic conditions in the stand and their influence on plant physiological processes. Outcomes of microclimate monitoring provide valuable data for growth, phytopathology, yield and irrigation models, and wide range of other applications.

Changes in amount and distribution of precipitation can be expected in the future. As Spáčilová et al. (2014) proved in conditions of the Czech Republic, the number of days without precipitation may increase from the current 79.9 days to 141.6 days in the period 2071–2100. However, no significant decrease in the amount of precipitation per year is expected. The authors also predict an increase in the average sum of active temperatures above 10 °C for the Czech Republic from the current 2,717 °C to 3,732 °C. The predicted changes increase the evapotranspiration demands of the environment to which the stands will be exposed. A similar increase in the frequency of drought can be expected in the surrounding countries in the Central Europe.

For most plant species, the sufficient water supply in the soil is 45–75% of available water holding capacity (AWHC) of the soil. Nevertheless, it is different according to plant species and growth phase of the plant, whereas in the case of maize, the critical periods are flowering of both male and female inflorescences and the beginning of grain filling in terms of negative impact of water scarcity on grain yield (Çakir, 2004). The limit of transpiration sensitivity to the availability of soil water can also be influenced by a genotype (Klimešová et al., 2020).

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Transpiration intensity can be considered as an indicator of plant water status if the sap flow measurement is considered a suitable method for determining whole plant transpiration (Escalona et al., 2000) and drought stress monitoring (Gavloski et al., 1992). For practical purposes, determining the intensity of transpiration using the sap flow can be used to verify the dimensioning of irrigation.

The aim was (i) to study the response of maize plants to different levels of water supply, (ii) to determine the amount of water in the soil with an impact on transpiration, yield of corn cobs, harvest index, and water use efficiency (WUE).

## 2 Material and methods

The pot experiments were conducted in three years in „field" conditions in terms of natural day length, solar radiation, air speed, air temperature and humidity (Table 1), but with controlled irrigation. The observed part of the vegetation period (BBCH 61–87; (Meier, 1997)) was divided into periods according to the course of sap flow values, phenology of plants, and meteorological conditions.

The plants were maintained under four different irrigation regimes beginning at phase BBCH 50:

- condition A, the control, involved 80% of the AWHC,
- condition B, mild stress, at 42% AWHC,
- condition C, severe stress, at 28% AWHC,
- condition D, very severe stress, at 25% AWHC.

The experiment was conducted using the maize (*Zea mays* L.) genotype „2087", recommended as a drought-tolerant genotype. Six maize plants were planted in each container with a volume of 200 dm3 and dimensions of 0.73 × 0.54 × 0.51 m. Gleyic fluvisol with 49–58% of fine particles (<0.01 mm) was used as a substrate. Field water capacity of soil was 43.0 vol%, wilting point 20.8 vol%.

The cob yield and harvest index (HI) as well as the biomass yield and dry matter yield of whole plants were evaluated for all of the plants in each experimental treatment at the stage of full maturity (BBCH 89). The harvest index was calculated by dividing the dry weight of the cobs by the dry weight of the entire plant. The water-use efficiency of cob yield (WUEc) was calculated based on the amount of water consumed by the plant in a generative period (BBCH 61–87) and the cob yield: WUEc = dry matter yield of cobs per plant/sum of water transpired by the plant.

Transpiration was monitored through continuous measurements of xylem sap flow by EMS62 sap flow system (EMS Brno, Czech Republic), which uses the "stem heat balance" method (Kučera et al., 1977). The sap flow values ($g.h^{-1}.plant^{-1}$) were provided at 10-min intervals. Only diurnal sap flow values were included for the analyses. Two plants from each condition were measured between BBCH 61 and BBCH 87.

The relative air humidity (%), air temperature (°C) and global solar radiation ($W.m^{-2}$) were measured at 10-min intervals using Minikin sensors (EMS Brno, Czech Republic). The soil moisture content (%) was measured at 15-min intervals using VIRRIB automatic electromagnetic sensors (AMET Velké Bílovice, Czech Republic).

The experimental data were statistically analyzed using STATISTICA12 software (StatSoft Inc., Tulsa, USA). The analyses performed included variance analysis and the consequent testing using the Tukey's HSD 95% confidence test.

## 3 Results and discussion

### 3.1 Intensity of transpiration in different moisture conditions

Drought stress was manifested by a decrease in transpiration, which was significant during the flowering period and at the beginning of grain filling (BBCH 65–71), i.e. 1–2 weeks of stress. At this stage, the differences in transpiration between variants were the greatest.

The evaluation of the average sap flow of maize plants over the entire vegetation period in three years confirmed a statistically significant reduction of sap flow under the influence of drought stress (Fig. 1). Soil water content at 42% AWHC (condition B) did not cause a statistically

**Table 1** Average daily values of global solar radiation and vapor pressure deficit in the bright part of the day in three years

| Period | Growth stage (BBCH) | Days after sowing | Vapor pressure deficit (kPa) | | | Global solar radiation ($W.m^{-2}$) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2012 | 2013 | 2014 | 2012 | 2013 | 2014 |
| I | 61–65 | 99 | 2.37 | 1.81 | 1.25 | 474 | 403 | 404 |
| II | 65–80 | 108 | 2.06 | 1.10 | 0.63 | 396 | 389 | 313 |
| III | 80–83 | 125 | 1.51 | 0.66 | 0.53 | 311 | 329 | 326 |
| IV | 83–87 | 144 | × | 0.31 | 1.05 | × | 274 | 425 |

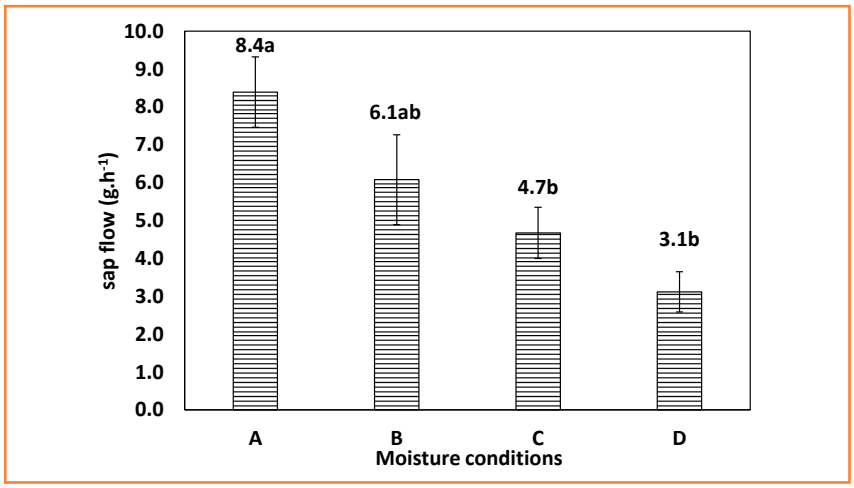


**Figure 1** Sap flow of maize (g.h$^{-1}$.plant$^{-1}$). Average value for evaluated phases – BBCH 61–87
Data from a three-year observation. Statistically significant different means ($p \leq 0.05$) are indicated by different letters

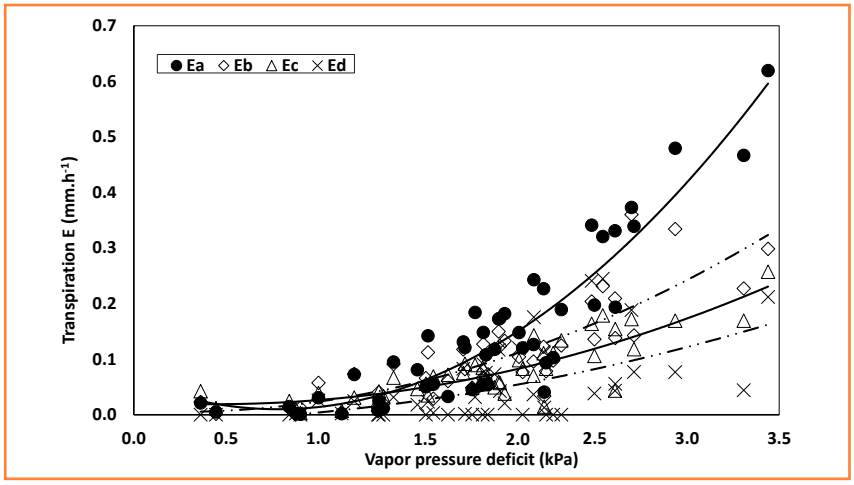


**Figure 2** Relationship between vapor pressure deficit (kPa) and transpiration (mm.h$^{-1}$) in a daily step in four moisture conditions
A – Ea: $R^2 = 0.857$, B – Eb: $R^2 = 0.708$, C – Ec: $R^2 = 0.733$, D – Ed: $R^2 = 0.318$, year 2012

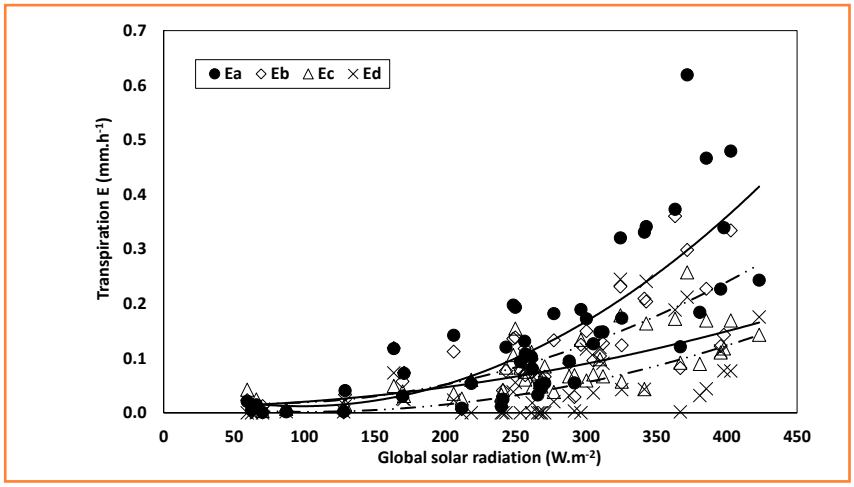


**Figure 3** Relationship between global solar radiation (W.m$^{-2}$) and transpiration (mm.h$^{-1}$) in a daily step in four moisture conditions
A – Ea: $R^2 = 0.606$, B – Eb: $R^2 = 0.577$, C – Ec: $R^2 = 0.540$, D – Ed: $R^2 = 0.327$, year 2012

significant decrease of transpiration compared to the control (condition A). The soil water content at 25–28% AWHC (conditions C and D) reduced the transpiration of plants by 44–63% compared to the control. The limit of AWHC, which affects the intensity of transpiration, varies greatly. Vamerali et al. (2003) defined the soil AWHC limit 20% and 60% at which there was a significant reduction of transpiration depending on a genotype. Significant genotypic differences of maize in response to soil moisture availability were also observed in the experiment of Klimešová et al. (2020). The AWHC limit for statistically significant reduction of transpiration compared to control was 25–30% AWHC for a stress-tolerant genotype, 40% AWHC for a stress-sensitive genotype. Wu et al. (2011) observed a statistically significant decrease in transpiration of maize already when the soil AWHC was reduced to 80% AWHC. In the study of Matejka et al. (2005), the calculated evapotranspiration of maize stand was affected when the AWHC decreased to 58%.

In all three years, a significant dependence of transpiration on meteorological elements was observed, namely on global radiation and vapor pressure deficit (Fig. 2 and Fig. 3). This dependence was very close in case of well-watered plants (A) or under mild stress of drought (B). The dependence weakened with advancing plant senescence or under stronger drought stress (conditions C and D). This fully corresponds to the results of Cai et al. (2020), who showed a decrease of transpiration under drought stress in the reproductive phase of maize and its independence from the course of photosynthetically active radiation values. At high vapor pressure deficit values under conditions of sufficient water supply (A), no reduction in transpiration was observed due to the closure of stomata. The effect of

water shortage was mainly reflected in the decrease of absolute transpiration values already in conditions of mild evapotranspiration requirements in the morning.

### 3.2 Cob yield and water use efficiency

Yield parameters of maize were influenced by the year, the intensity of stress, and also the interaction of year with intensity of stress (Table 2).

The year 2012 differed from 2013 and 2014 in high values of dry weight of biomass and stem. The weight of the cobs was statistically significantly higher in 2012 and 2014 compared to 2013. The comparable cob yield in these years is surprising, because the intensity of transpiration in 2014 was statistically significantly lower by 41%. The highest values of harvest index (HI = 0.34) were found, as well as higher values of water use efficiency (WUEc) in 2014. On the contrary, in 2013, when the lowest weight of cobs, weight of biomass dry matter and the lowest harvest index were achieved, the intensity of transpiration was highest.

Different irrigation regime affected some yield parameters. The effect of water shortage on dry weight of total biomass or stem biomass was not proven. This indicates a limited effect of drought stress in generative phase of growth.

As the availability of water in soil decreased, the weight of cobs and HI value decreased. Statistically significant differences were found between condition D (25% AWHC) and conditions A (80% AWHC) and B (42% AWHC). Statistically significant decrease in transpiration compared to the control was confirmed in conditions of severe (C) and very severe stress (D). The yield thus reacted less sensitively than transpiration to lower intensity of watering. Wu et al. (2011) point to the fact that dry weight of maize is less sensitive to different degrees of water scarcity compared to transpiration. They also compared cumulative values of maize transpiration over the entire growing season with amount of dry matter formed and found that at higher transpiration, the decrease in cumulative transpiration (from the elongation phase to ripeness) has no effect on dry matter reduction. Klocke et al. (2004) found that watering lower by 40% compared to the control caused only a 16% decrease in yield. Limited watering thus increases the efficiency of water use

**Table 2** Effect of year, variants, and interactions of variant and year on yield parameters of maize (average value). Statistically significant different means ($p \leq 0.05$) are indicated by different letters

| Year × condition | | Dry matter yield (g.plant$^{-1}$) | Cob yield (g.plant$^{-1}$) | Dry matter yield of stover (g.plant$^{-1}$) | Harvest index |
|---|---|---|---|---|---|
| 2012 | A | 128 bc | 40.9 b | 87.3 cd | 0.31 abc |
| | B | 127 bc | 44.3 b | 82.3 bcd | 0.34 bc |
| | C | 132 c | 44.8 b | 87.5 cd | 0.34 bc |
| | D | 103 abc | 4.1 a | 99.2 d | 0.04 a |
| 2013 | A | 69 a | 15.2 ab | 53.9 ab | 0.22 abc |
| | B | 75 a | 21.1 ab | 53.7 b | 0.28 ab |
| | C | 76 a | 12.2 ab | 63.9 abc | 0.15 ab |
| | D | 71 a | 13.4 ab | 57.3 abc | 0.17 ab |
| 2014 | A | 89 abc | 34.5 ab | 54.9 ab | 0.38 bc |
| | B | 89 abc | 41.6 b | 47.5 a | 0.46 c |
| | C | 85 ab | 29.8 ab | 49.0 a | 0.30 abc |
| | D | 64 a | 18.3 ab | 45.6 a | 0.22 abc |
| **Year** | | | | | |
| 2012 | | 123 b | 33.6 b | 89.1 b | 0.26 ab |
| 2013 | | 73 a | 15.5 a | 57.2 a | 0.20 a |
| 2014 | | 82 a | 31.1 b | 49.3 a | 0.34 b |
| **Condition** | | | | | |
| A | | 96 a | 30.2 b | 65.4 a | 0.30 b |
| B | | 97 a | 35.7 b | 61.2 a | 0.36 b |
| C | | 98 a | 28.9 b | 66.8 a | 0.26 ab |
| D | | 79 a | 11.9 a | 67.3 a | 0.14 a |

(WUE) by plants due to a greater reduction in water use compared to yield (Djaman & Irmak, 2012).

Water use efficiency for cobs (WUEc) was influenced by the year. The reason for different values of WUEc could be the course of global radiation values and especially the vapor pressure deficit. In 2014, by 60% lower values of vapor pressure deficit were found compared to 2012, which supported higher WUEc. Lower VPD may be associated with higher WUE values for maize (Tallec et al., 2013). Lower WUEc was repeatedly observed in conditions without drought stress, in the control. It increased with the strength of stress (conditions B, C), but decreased again under severe drought stress (condition D). Thus, with the amount of consumed water, the efficiency of its use decreased. Moderate drought stress limits transpiration due to partial stomata closing, while the assimilation of the $CO_2$ continues, which basically leads to WUE increase (Chaves et al., 2010). Limited watering thus increases WUE due to a greater reduction in water consumption than in yield (Djaman & Irmak, 2012). However, under severe stress, growth is already significantly reduced, so the WUE value decreases (Farooq et al., 2009).

## 4 Conclusions

Soil water content was the main factor influencing the transpiration under the influence of medium to severe water shortage. Yield responded less sensitively to lower watering intensity than the transpiration. WUEc decreased with increasing amount of water consumed. The limit of soil water content, at which there was a decrease in transpiration and yield, was at the level of severe drought stress (25% of AWHC). Prediction of maize biomass yield during dry periods is quite problematic. The relationship between the amount of maize biomass and moisture conditions is not definitely linear.

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**Review paper**

# Sustainable food systems and healthy diets: the case of mediterranean diet

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Sustainability should be an imperative in everyone's lifestyle in order to achieve an equilibrium between humans and ecosystem for the wellbeing of current and future generations. Sustainable food systems and healthy diets are main key-players to achieve sustainable planet and lifestyle and at the same time to be in line with the Sustainable Development Goals (Agenda of 17 global goals set by the United Nations General Assembly in 2015 to achieve better and sustainable future for all). Such food systems offer not only a way towards ending the hunger, but also enable healthy nations and less environmental pollution. A good representative of a sustainable food system is the Mediterranean diet that is affordable and accessible even in the regions far from the Mediterranean basin. Raw or minimally cooked plant-based food products flavoured by different herbs and spices are the foundation of this diet packed with powerful nutrients, vitamins, and minerals, enriched with healthy fats from extra virgin olive oil. The Mediterranean lifestyle provides many health and wellbeing benefits for humans. Authors believe that adhering to it leads to healthy nations and a sustainable world with less hunger.

**Keywords:** mediterranean lifestyle; sustainable food systems; healthy diets, sustainable development goals

## 1 Introduction

Today, natural cycles are significantly disturbed by the humanity's impact on the planet. Degrading or losing vital ecosystem services can negatively impact human security, health, and biodiversity. Limited natural resources, like land and water, as well as the Earth's capacity to absorb the increased pollution, such as carbon dioxide emissions, methane, etc are alarming global challenges. On the other hand, the increasing population is greatly dependent on the goods and services that the Earth's system provides (food, water, energy). A suddenly increased population, especially in urban areas with strong economic growth, has resulted in a rising demand for natural resources and if these resources continue to be spent in uncontrolled ways, then serious consequences on public health and environment might happen. Although economic growth and global urbanisation have improved the human wellbeing, the demand for natural resources has put increased pressure on the environment and it leads to climate changes and global warming of the planet (Global Sustainable Development Report, 2019; Lucas & Wilting, 2018; Röös et al., 2017; Vanham et al., 2019). The traditional diets cannot satisfy the whole population on the Earth, they have been replaced by many unhealthy diets comprising ultra-processed industrial foods, meat products, fast and ready-to-eat food choices rich in salt, preservatives, refined sugars, and unhealthy trans fats (Monteiro et al., 2013; Seto & Ramankutty, 2016).

The search for healthy diets that are beneficial for both, the humans and the ecosystem, has not been always easy. Different aspects of sustainability, health, environment, culture, economy, society are important and should be taken in consideration when adopting a healthy diet. Sustainable food systems are essential to nourish a projected global population of ~10 billion by 2050. Overweight and obesity and their associated diet-related non-communicable diseases (NCDs) are

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contributing to 4 million deaths globally (HLPE, 2017, 2020; Fanzo, 2019; Mozaffarian, 2016; Swinburn et al., 2019). Malnutrition is costly for the health of individuals, their wellbeing and productivity. It also has high socio-economic costs for societies in all regions of the world. Poor diets are a major contributory factor to the rising prevalence of malnutrition in all its forms. Moreover, unhealthy diets and malnutrition are among the top ten risk factors contributing to the global burden of disease (Burlingame & Dernini, 2019).

Today's food systems are far from being sustainable and there is "a short time period" for action. Not only are dietary risk factors and malnutrition in all its forms the leading contributors to the global forms of diseases, but also food systems are not operating within the planetary boundaries; they are the big contributors to irreversible environmental breakdown degradation and damage of natural resources and biodiversity (HLPE, 2017; Lawrence et al., 2019; Meybeck & Gitz, 2017). A wide range of multilateral environmental agreements has been proposed in the past few decades. All of them set targets for sustainable human development; the most notable one is the 2030 Agenda of UN for Sustainable Development. It sets out a long-term global vision for sustainable development; the 17 Sustainable Development Goals (SDGs) are focussed to achieve a prosperous, socially inclusive, stable economic growth and environmentally sustainable future for humanity, young generations, and the whole planet (Fanzo, 2018; Global Sustainable Development Report, 2019; Johnston et al., 2014; Lucas & Wilting, 2018; Meybeck & Gitz, 2017).

In this context, many novel, sustainable and alternative diets are proposed; they offer substantial health benefits, and if widely adopted, they will result in reduced impact on climate changes, reduction in global agricultural greenhouse gas emissions, reduced land clearing and species extinctions, as well as they would help in prevention of diet-related chronic NCDs and diseases related to a polluted planet. There are many multisectoral strategies which are recommended to achieve transformation of food systems, such as producing more nutritious food not just more food, sustainable food production, proper usage of land, water, oceans, stopping land clearing, reduce food losses and food waste, etc. (Alexander et al., 2016; Katz, 2019; Lawrence et al., 2019). Mediterranean Diet (MD) is a good representative of a sustainable food system that is mainly plant-based healthy diet (Trajkovska Petkoska & Trajkovska-Broach, 2020; Trajkovska Petkoska & Trajkovska-Broach, 2021).

**Prerequisite for a sustainable future: Living in a harmony with the environment**

The term "sustainability" refers to the ability to maintain a certain standard of human living without causing environmental damage or any other harming of the nature. It should be understood that it benefits the human health and well-being, has socio-economic benefits and contributes to environmental integrity (Baroni et al., 2018; Bornkessel et al., 2019; Chen et al., 2019; Monsivais et al., 2012). The 17 SDGs cover three sustainability pillars: environmental, social, and economical. Social sustainability refers to the improvement of living conditions for both current and future generations, while economic sustainability is defined as the ability of the economy to support and maintain economic growth, but at the same time efficient exploitation of natural resources. Hence, socio-economic sustainability could be understood as the ability to ensure economic growth without undermining humans' interests, i.e., to meet the humans' needs without destroying the nature and the environmental system around (Berry, 2019; Egal & Berry, 2020; Meyer & Reguant-Closa, 2017; Skvarciany et al., 2020; Tarsitano et al., 2019).

Agriculture is one of the main producers of emissions of greenhouse gases (GHGs), in particular methane ($CH_4$) and nitrous oxide ($N_2O$), which are responsible for global warming. Other parts of the food system chain contribute to carbon dioxide ($CO_2$) emissions from the use of fossil fuels in food processing, transportation, retailing, storage, and preparation (Joyce et al., 2014; Meyer & Reguant-Closa, 2017; Serra-Majem & Medina, 2015; Springmann et al., 2017; Tom et al., 2016). Currently, food systems are responsible for a significant share (20–35%) of GHG emissions, and are a major driver of land conversion, deforestation, and loss of biodiversity. Agriculture alone accounts for ~70% of global freshwater withdrawals and causes water pollution; it also occupies more than 30% of all potentially cultivatable land. Moreover, the livestock consume around two-thirds of all the land dedicated to agriculture and contribute to about a half of the farming-related GHGs (Aleksandrowicz et al., 2016; Egal & Berry, 2020; Garnett, 2011; Hallström et al., 2015; Röös et al., 2017).

Food production is also a big player of environmental pressures and depletion of natural resources, related to climate change, water usage, pollution, and toxic emissions (Röös et al., 2017; Serra-Majem & Medina, 2015). In general, food systems include: i) the activities related to the production, processing, distribution, preparation, and consumption of food, and ii) the outcomes of these activities – contribution to the food security, such as food availability, food access and

food use (Berry, 2019). In this context, the current food systems as major drivers of environmental pressures are far from sustainable. According to FAO (2017), global food demand is projected to increase by 60% towards 2050 compared to 2007, driven by changing consumption patterns and population growth. By 2050, there is an assumption that dietary trends would be a major contributor to an estimated 80% increase in global agricultural GHGs from food production and global land clearing (Aleksandrowicz et al., 2016; Egal & Berry, 2020; Hoekstra et al., 2014; Knorr et al., 2020; Meybeck & Gitz, 2017).

Why the current food systems are not sustainable? There are many factors, which negatively influence the vital pillars of sustainability; in most of the cases, food production uses increasingly unsustainable amounts of natural resources (land, water, energy) with harmful impacts on ecology, economy, and society. Most of the food productions lead to environmental pollution (GHGs, pesticides) with huge impacts on biodiversity and human health. The access to food is not equal worldwide; there is hunger and malnutrition in some parts and excessive food waste, over-consumption and obesity in other parts of the world. Thus, it seems that food consumption instead of having positive impacts on human health, has significant negative impacts on vital pillars. It will not improve if nations and authorities worldwide do not take suitable sustainable actions; the population is growing each year and more and more food will be needed in the future (Aiking & de Boer, 2018; Bhargava, 2019; Fanzo, 2018; HLPE, 2020; Jones et al., 2016; Röös et al., 2015).

Sustainable food systems (SFS) take into account the environmental needs along the entire food system chain, from production to consumption, incorporating social, health, and economic concerns. The vision of SFS is a world where the earth can produce enough nutritious, safe, affordable food to feed the population, while preserving the biodiversity and ecological needs of the planet now and for the future generations. Such systems ensure food security and nutrition and accommodate most of the 17 SDGs; they are beneficial for every citizen and country, good for the whole planet (Berry, 2019; Burlingame & Dernini, 2019; Fanzo, 2019; Knorr et al., 2020; Meybeck & Gitz, 2017; Trajkovska Petkoska & Trajkovska-Broach, 2020; Von Koerber et al., 2017).

On the other hand, the idea of sustainable diet started by Gussow & Clancy (1986) when they claimed that promoting food sustainability and ecological harmony were essential to promoting a healthy diet for the individuals, and later this concept was further defined by FAO and many others (Aboussaleh et al., 2017; Auestad & Fulgoni III, 2015; Burlingame & Dernini, 2019; Fanzo, 2019; Johnston et al., 2014; Meybeck & Gitz, 2017; Pradyumna, 2018; Röös et al., 2015; Springmann et al., 2018; Germaniet al., 2014). According to Berry (2019), the healthy diet is not just a list of "do's" and "don'ts", but rather should be a pleasurable, social and tasty experience, which should be instilled in children from a young age as one of the Proverbs 22:6 says: "Train up a child in the way he should go: and when he is old, he will not depart from it"; so the earlier the young generations are taught and practiced, the more likely they are to take root and persist to healthy habits (Berry, 2019; Jakobovich et al., 2019).

In the global nutrition policy sphere, the term "malnutrition" no longer refers only to undernutrition, such as wasting, stunting, underweight or deficiencies in vitamins or minerals. Malnutrition in all its forms includes obesity, as well as dietary factors that increase the risk of NCDs, such as heart disease, stroke, diabetes, and certain types of cancers; they are a major cause of disability and death in all countries (Baroni et al., 2018; FAO & WHO (2019); HLPE, 2019, 2020; Swinburn et al., 2019). Many studies now point to synergies between healthy diets and reduced environmental pressures, leading to the notion of sustainable diets, for healthy lives and healthy ecosystems. Not all food-secure diets are sustainable, but all sustainable diets should be food-secure (Berry, 2019; Jones et al., 2016). The Intergovernmental Panel on Climate Change (IPCC) has also recognized that "Consumption of healthy and sustainable diets presents major opportunities for reducing GHG emissions from food systems and improving health outcomes." A good representative of a sustainable healthy diet is the Mediterranean diet.

## Mediterranean way of living: A sustainable food pattern and a healthy lifestyle

Territorial diets have been linked to specific geographies, but over time they integrated other influences through the migration of people, mixing of cultures, exchange of material goods and foods, etc. They are linked not only to the landscape that characterize agriculture and the economy, but also to particular ecologies, historical and cultural contents, as well as social resources including institutions, knowledge, traditional practices, recipes, and rituals (Barre et al., 2018; Dernini & Berry, 2016; FAO & WHO, 2019; UNESCO, 2010).

The Mediterranean Diet (MD) and the New Nordic Diet (NND) are plant-based diets with little to moderate amounts of animal-sourced foods (FAO & WHO (2019)) amongst the other territorial diets. There is a lot of scientific evidence for MD for being nutrient-packed diet with benefits on the overall wellbeing and health along with its economic and socio-cultural benefits (Baroni et al.,

2018; Cena & Calder, 2020; Davis et al., 2015; Donini et al., 2016; González et al., 2019; Roman et al., 2019; Tarsitano et al., 2019; Trichopoulou et al., 2014). It has emerged as a science-backed diet mainly plant-based to prevent a good physical and mental health, reduce the risks for severe diseases, such as NCDs, and promote healthy aging and longevity (Dinu et al., 2017). The MD is a highly diversified heritage, in which food cultures and systems vary from country to country in the Mediterranean basin. In the Mediterranean, there is a widespread awareness of the social, cultural, economic and health aspects of food, and this is shared by all Mediterranean people in countries bordering the Mediterranean Sea; adherence to this diet is easy and its transferability to non-Mediterranean regions has been proven (Aboussaleh et al., 2017; Antonopoulou et al., 2020; Baroni et al., 2018; Benhammou et al., 2016; Bonaccio et al., 2012; Dernini & Berry, 2016; Hidalgo-Mora et al., 2020; Peng et al., 2018a; Peng et al., 2018b; Serra-Majem et al., 2003–2004; Trichopoulou et al., 2014).

In general, MD emphasizes daily use of whole-grains, fruits, and vegetables, not only cultivated products, but also wild species, spices, and herbs, thus sustaining them together with the local, indigenous, and traditional knowledge about their use and food preparation. In addition, a variety of legumes, nuts, and seeds are consumed in abundance along with moderate amounts of dairy products and proteins from animal sources (Serra-Majem & Medina, 2015; Siotos et al., 2019; Trajkovska Petkoska & Trajkovska-Broach, 2021). Fig. 1 presents MD selected typical foods with their nutrients. Extra virgin olive oil is one of the crucial ingredients of the MD and consumed in large quantities (25–40% dietary fats of the total calories), not only for its inherent

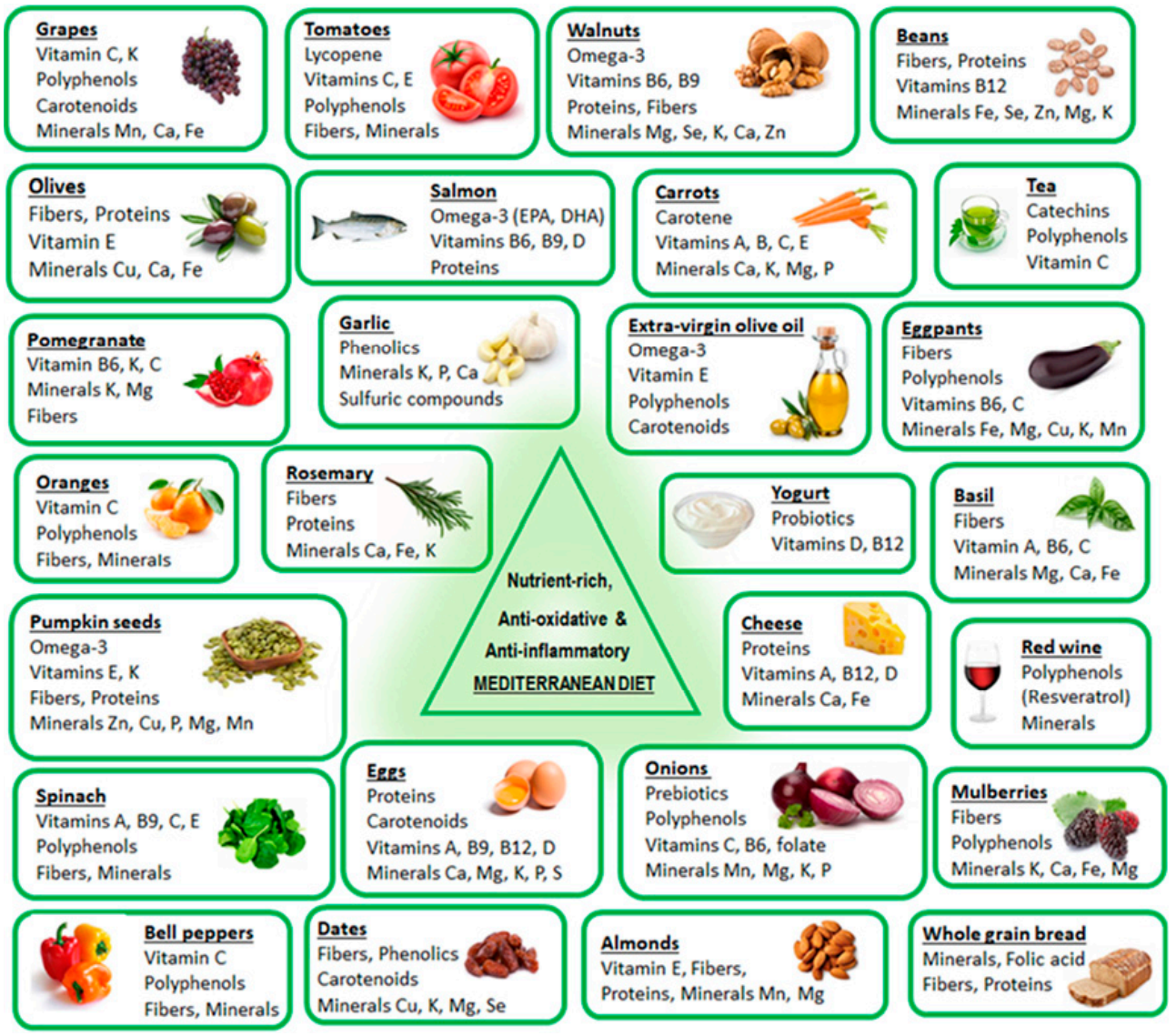


**Figure 1** Selected typical foods in the MD are mainly of plant-based origin (MD Inspired™)

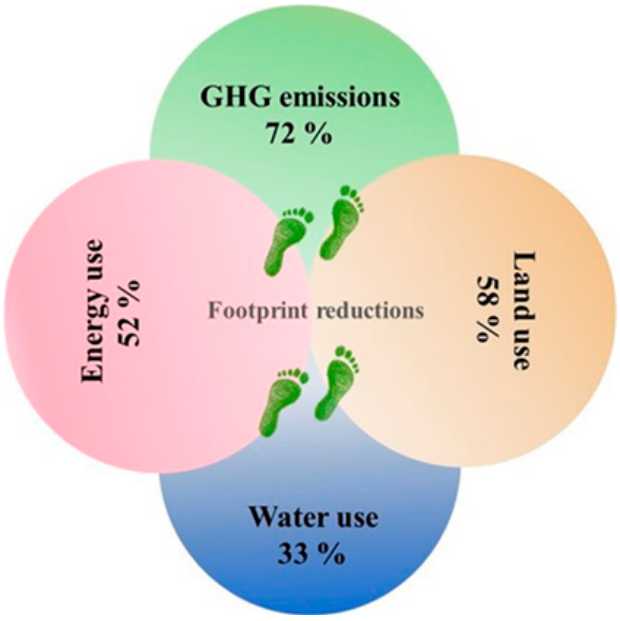


**Figure 2** Estimated reductions in the environmental footprints by adopting MD: reductions in GHG emissions and the consumption of energy, land and water use

nutritional effects, but also the cumulative benefits of the foods typically prepared in it (e.g. vegetables), which have been proven to have anti-thrombotic, anti-inflammatory and antioxidative properties (Muralidharan et al., 2019; Ramírez-Anaya et al., 2015). Contrary to MD, the nutritional guidelines in the Western countries limit the total fat intake to less than 30% (even less than 20%) of total calories, which mainly originates from animal sources (Blomhoff et al., 2006; Bower et al., 2016; Mazzocchi et al., 2019; Serra-Majem et al., 2003–2004; Trajkovska Petkoska & Trajkovska-Broach, 2021). MD is not just a dietary pattern; it is more than that – it is a lifestyle; the social aspects – conviviality, preparing and eating the food together with others, the sense of community, play a big role in the lives of people adhering to the Mediterranean lifestyle.

MD as a plant-based diet has been associated with a reduced risk of chronic diseases, cancers, dementia, Alzheimer's disease and has also been linked to longevity of the people adhered to it. A range of bioactive compounds, phytochemicals, found in fruits and vegetables has been reported for their protective health benefits and reduction of the risk for developing NCDs, which are attributed to chronic inflammation and oxidative stress. For example, the dietary polyphenols in MD are known to be immunomodulatory and anti-inflammatory in reducing the risk of cardiovascular disease, neurological diseases, and cancer (Ramirez-Anaya et al., 2015; Dinu et al., 2017; Childs et al., 2019; Gonzalez et al., 2019; Roman et al., 2019). MD has also been proven to improve gut microbiome diversity, which is due to consumption of various plant-derived foods (fruit, vegetables, nuts, seeds, whole grains) in abundance as well as healthy fats from extra virgin olive oil. The gut microbiome is further regulated by live microbes (probiotics) originating from the regular consumption of fermented milk products (yoghurt, curds, cheeses) or fermented vegetables (Mazzocchi et al., 2019; Muralidharan et al., 2019; Roman et al., 2019; Trichopoulou et al., 2014). In addition, Mediterranean dietary pattern affects not only the human health and wellbeing, but also the natural resources and the ecosystem. A Spanish study compared the MD to the modern diets and showed that adherence to the MD would significantly reduce all environmental footprints – GHG emissions, land, water and energy use. On the other hand, the adherence to Western dietary patterns implies an increase in all these descriptors somewhere between 12% and 72% (Fig. 2) (Sáez-Almendros et al., 2013).

## Final thoughts: Is it worth adhering to MD?

While for decades the focus in food science has been primarily on increasing productivity and achieving lower prices at higher volumes of energy-dense foods, the future of sustainable production should be focused on quality and tendency to achieve healthier nutrient-dense foods originating from sustainable production systems. Nutrition policy should prioritize food-based dietary targets, public communication of trusted science,


**Figure 3** MD positively affects the human health and wellbeing, society, economy, and environment, all in line with the SDGs

investment, and cultural strategies to create sustainable food systems across the regions, nations and worldwide (Irza et al., 2019; Mozaffarian et al., 2010; Mozaffarian & Forouhi, 2018; Mozaffarian et al., 2018; Nelson et al., 2016; Poore & Nemecek, 2018). Shifting dietary habits towards the healthy ones, especially between younger generation from early age and on, presents a significant challenge for cultural, ecological, and economic reasons, and will require actions from different entities such as governments, businesses, and individuals that go beyond information and education programmes.

Today, we are facing multiple recommendations from professionals toward healthy diet choices emphasizing plant-based diets vs. animal-based foods; promoting better and healthier way of life associated with lesser environmental impact. A perfect representative of such lifestyle is the MD; it emphasizes consumption of vegetables, fruits, nuts, legumes and seeds, virgin olive oil, fermented products, fish and lean meat rich with vitamins, minerals, probiotics, dietary fibres and other phytonutrients, which in combination with the physical activities and the socializing aspects of this lifestyle contribute to the overall wellbeing and health. Simply, MD is not a dietary pattern only, but a way of living with a positive impact on the health, society, economy, and environment. It seems to be the best compromise between the need to reduce the environmental impact of food consumption and still maintain a healthy food consumption behaviour, biodiversity, and food security. The growing body of scientific evidence has shown the significant health and wellbeing benefits, positive societal and economic impacts, and low environmental footprints of MD (Fig. 3) (Trajkovska Petkoska & Trajkovska-Broach, 2020; Trajkovska Petkoska & Trajkovska-Broach, 2021). As Dernini et al. (2015, 2016) have stated "A well fed nation is a healthy nation; it is a sustainable and productive nation", we all could benefit from the Mediterranean lifestyle (Conrad et al., 2018; Dernini et al., 2017; Dernini & Berry, 2015; Galanakis, 2020; Martínez-Gonzálezet al., 2017; Mantzioris & Villani, 2019; Moore et al., 2018; Muscogiuri et al., 2020; Pairotti et al., 2015).

Knowing the history of MD as a diet of mainly poor people which is no longer considered as such, we strongly believe that adhering to the Mediterranean lifestyle could lead to healthy nations and a sustainable world with less hunger.

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**Research Article**

# Antioxidant capacity of wild-growing bilberry, elderberry, and strawberry fruits

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Chemical properties (*L*-ascorbic acid and total sugars content, pH, titratable acidity, and dry solid content), phenolic compounds (total phenolics, tannins, flavonoids, anthocyanins, and flavan-3-ols) and antioxidant capacity were measured in ripe fruits of wild-growing strawberry, bilberry, and elderberry from eastern Serbia. All three selected fruits are rich sources of nutraceuticals: vitamin C, sugars, and different classes of phenolic compounds and their extracts expressed high antioxidant activity. Elderberry fruits possess highest concentration of all measured biomolecules.

**Keywords:** antioxidant activity, berry, fruits, phenolic compounds

## 1 Introduction

The antioxidant potential of phytochemicals in health maintenance has been increasingly recognized in recent years. Sufficient evidence has shown that free radicals play an important role in most major health problems such as cancer, cardiovascular disease, and degenerative diseases associated with aging (Zhang et al., 2015). Polyphenols are especially important antioxidants because of their high redox potentials allowing them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Kasote et al., 2015).

Red fruits, including different berries, are characterized by high amounts of bioactive molecules and rich sources of natural antioxidants: phenolic acids, tannins, carotenoids, vitamin A, C, E, folic acid, and minerals such as calcium, selenium, and zinc. These chemical compounds are secondary metabolites that prevent the fruit from environmental factors that could induce oxidation processes, such as air, oxygen, light, attacks of phytopatogens and herbivorous animals. Phenolic antioxidants interfere with the oxidation process as free radical terminators and sometimes also as metal chelators (Manganaris et al., 2013; Oanacea et al., 2015, Hidalgo & Almajano, 2017; Šapčanin et al., 2017).

Strawberry (*Fragaria* sp.) is a genus of flowering plant in the rose family, Rosaceae, commonly known as strawberry for their edible fruits. There are more than 20 described species and many hybrids and cultivars. While primarily valued for their taste and flavour, strawberries also have potential health benefits. Strawberries are high in the vitamins and mineral content, and they are also rich in different phenolic molecules, including anthocyanins, hydrolyzable tannins and phenolic acids (Liston et al., 2014). Vaccinium species: bilberries (*Vaccinium myrtillus* L.) and blueberries (*Vaccinium corymbosum* L.), have been proven to have high phenolic content and strong antioxidant potential (Bunea et al., 2011, Kevers et al., 2014). Bilberries and blueberries have multiple biological and health-promoting effects, including anticarcinogenic, anti-inflammatory, and antimicrobial effects (Johnson & Arjmandi, 2013; Salamon et al., 2021). *Sambucus nigra* L. is a species from the Adoxaceae family, known as a low growing tree. Elderberry is recognized for its therapeutic properties such as antidiabetic and antiviral effects, diuretic properties, and prevention of atherosclerosis, cardiovascular diseases, and cancer (Młynarczyk et al., 2018).

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In the present study, the chemical composition, content of phenolic compounds, and antioxidant capacity of the three wild edible berry fruits from eastern Serbia region were assessed by different assays. An assessment of the correlation between the antioxidant activity of the fruits and the phenolic components contents was performed.

## 2 Material and methods

### 2.1 Plant material

Fresh fruits of wild-growing strawberries, bilberries, and elderberries were picked up manually at full maturity in a forested area near the town of Žagubica, Homolje Carpathian region, eastern Serbia. The samples were kept on ice during transportation to laboratory, divided into two subsamples for chemical and biochemical analysis, and stored. For all spectrophotometric analysis, the ThermoScientific Evolution 220 UV-visible spectrophotometer was used.

### 2.2 Chemical composition

Dry solid content of fruits was analysed by the evaporation method. Fresh fruits of known weights were placed in glass dishes and dried in laboratory oven at 80 °C until constant weight (Bradley, 2010). pH and titratable acidity were measured by methods described by Sadler and Murphy (2010). Results for titratable acidity were expressed as g citric acid 100 $g^{-1}$ fruit. Total sugar (carbohydrate) content was estimated by the phenol-sulfuric acid method (BeMiller, 2010). The standard curve was constructed using different concentrations of glucose, and the results were expressed as mg glucose equivalents (GE) per gram of fruit (mg.GE.$g^{-1}$). Content of vitamin C (ascorbic acid, AA) in fresh wild-growing strawberries, bilberries, and elderberries was determined by the 2,4-dinitrophenylhydrazine (DNP) method as described by Al-Ani et al. (2007). Calibration curve was conducted by solutions with different concentration of ascorbic acid and results were expressed as mg of ascorbic acid equivalent per g of fresh fruit (mg.AAE.$g^{-1}$).

### 2.3 Phenolic compounds

One gram of edible parts of each fruit was homogenized and extracted in 10 ml of 70% (v/v) methanol overnight. The extracts were filtered and centrifuged at 8000 rpm for 15 minutes. Extracts were kept in refrigerator and used for further biochemical analysis.

The content of total phenols (TP) was determined using a Folin-Ciocalteu reagent (Nagavani & Raghava Rao, 2010). The diluted Folin-Ciocalte solution was mixed with 20 µl of the extracts. After five minutes 400 µl of 20% $Na_2CO_3$ solution was added. A series of standard dilution of gallic acid was used to construct the calibration curve. After 60 min, the absorbance at $\lambda$ = 730 nm was read on a spectrophotometer. The TP content of the extracts tested was expressed as mg of gallic acid (GA) equivalents per g of fresh fruit weight (mg of GAE.$g^{-1}$ FW). All samples were prepared and analysed in triplicate. The content of total tannins was determined by the same method as content of total phenolics after the removal of tannins by their adsorption on an insoluble PVPP (polyvinylpolypyrrolidone) matrix. The calculated values were subtracted from the TP content. The total flavonoid (TF) content in the obtained fruit samples was measured by the aluminium chloride spectrophotometric assay by the method described by Saha et al. (2013). Total flavonoid content was determined from the regression equation of the quercetin calibration curve and expressed as mg quercetin equivalent (QE) gram of fresh weight of selected fruits (mg of QE $g^{-1}$ FW). The content of total anthocyanins (TA) in the methanol extracts of three selected fruits was determined spectrophotometrically by measuring the difference in the absorbance between solutions of pH 1.0 and pH 4.5 at absorption on 510 and 700 nm (Giusti & Wrolstad, 2001). Values are expressed as mg cyaniding-3-glucoside (C3G) equivalents per gram of fresh fruit weight (mg C3GE.$g^{-1}$ FW). The content of flavan-3-ols in selected fresh fruits was measured by the vanillin assay using the daily prepared working solution of 4% vanillin in methanol (Laličić-Petronijević et al., 2016). The results were expressed in mg of (+)-catechin equivalents (CE) per g of fresh fruits weight (mg of CE.$g^{-1}$ FW).

### 2.4 Antioxidant capacity

Scavenging of free radicals was tested using a DPPH (2,2-diphenyl-1-picrylhydrazyl) acetone solution (Lai & Lim, 2011). The ferric-reducing antioxidant power (FRAP) assay was carried out according to the procedure described by Valentão et al. (2002). The ABTS (2,2'azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt) assay was based on a method described by Miller et al. (1993). A reducing power assay (total reduction capacity-TRC) was performed by the method of Saha et al. (2013). The standard curve for antioxidant tests (DPPH, FRAP, ABTS and TRC) was plotted using trolox solution and the results were expressed as mg trolox equivalents (TE) per g of the fresh plant material (mg TE.$g^{-1}$ FW). NBT (nitro blue tetrazolium) test or superoxide dismutase-mimetic (SOD-mimetic) activity was assayed according to the slightly modified method of Kalaskar and Surana (2014) by measuring fruit extracts ability to inhibit photochemical reduction of NBT. The reaction mixture contained 1 mL of 50 mM potassium phosphate buffer (pH 7.8) with dissolved NBT, EDTA (ethylenediaminetetraacetic acid),

L-methionine, riboflavin and 20 µL of the fruits' extract. It was kept under a fluorescent lamp for 10 min, and then the absorbance was read at 560 nm. One unit of the SOD activity was defined as the content of enzymes required to inhibit reduction of NBT by 50%. The activity of the extracts was expressed as IU SOD.g$^{-1}$ fresh plant material. NO generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci et al. (1994). The reaction mixture containing 3 mL SNP phosphate buffered saline (pH 7.3), with or without 40 µL of fruit extract, was incubated at 25 °C for 180 min in front of a visible light source. The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO.) which was assayed at 30 min intervals by mixing incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylenediaminedihydro chloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediaminedihydro chloride was measured at 546 nm. The nitrite generated in the presence or absence of the fruit extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations.

### 2.5 Statistical analysis

Results were expressed as a mean value of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by comparison of means by the Duncan's multiple range test ($P$ <0.05) calculated using STATISTICA for Windows version 13.2 (StatSoft, Tulsa, OK, USA). Stepwise multiple regression analyses were used to determine correlation among variables.

## 3 Results and discussion

### 3.1 Chemical composition

Berries contain a wide variety of primary and secondary biomolecules that may help protect cellular systems from oxidative damage and lower the risk of chronic diseases (Skrovankova et al., 2015). The content of dry solids, total sugars, pH values, titratable acidity, and ascorbic acid content of elderberry, strawberry and bilberry fruits are shown in Table 1. Dry solid content of three selected wild-growing fruits ranged from 10.83% for bilberry up to 22.27% for strawberry and 23.60% for elderberry. The pH of the bilberry pulp was 3.12, which was lower than the pH of strawberry (3.57) and elderberry (3.84). These results are in agreement with findings of other researchers who investigated properties of elderberry (Vujanović et al., 2020; Zhou et al., 2020) and bilberry (Bernal et al., 2014; Colak et al., 2016; Celik et al., 2018). Titratable acidity of all three investigated fruits was within the same range (1.22–1.28% citric acid) and is in good agreement with literature data (Vulić et al., 2008; Özgen et al., 2010; Colak et al., 2016; Celik et al., 2018). Sugar content in fruits is an important parameter. Consumers mostly prefer fruits with higher sugar content. There were no statistically significant differences in sugar content between all three selected fruits from this experiment. Total sugar concentration ranged from 5.17 g.100 g$^{-1}$ (elderberry) to 5.70 g.100 g$^{-1}$ (bilberry) and 6.59 g.100 g$^{-1}$ (strawberry). Similar findings are reported by other authors. For elderberry, Vujanović et al. (2020) reported 3.74 g of total sugars per 100 g of fruits, Elez Garofulić et al. (2012) reported 6.91 g.100 g$^{-1}$, while Vulić et al. (2008) measured 8.88 g.100 g$^{-1}$ in their samples. In plants, *L*-ascorbic acid (vitamin C) has several functions: as an enzyme cofactor, a radical scavenger, and a donor/acceptor in electron transport either in the plasma membrane or in the chloroplasts. In humans and animals, vitamin C functions as a cofactor in the enzymatic hydroxylation, preventing curvy and protects against different diseases. Currently, most of the daily intake of *L*-ascorbic acid for humans comes from fruits and vegetables (Fenech et al., 2019; Doseděl et al., 2021). The highest content of vitamin C was measured in elderberry fruits (0.30 mg AAE g$^{-1}$), while concentrations of vitamin C in strawberry and bilberry were 0.20 mg AAE g$^{-1}$ and 0.19 mg.AAE.g$^{-1}$, respectively. Literature data agrees with our findings (Vulić et al., 2008;

**Table 1** Chemical composition of wild-growing strawberry, bilberry, and elderberry fruits

| | Fruit | | |
|---|---|---|---|
| | **strawberry** | **bilberry** | **elderberry** |
| **Dry solid (%)** | 22.27 ±0.36$^{a}$ | 10.83 ±0.20$^{b}$ | 23.60 ±0.69$^{a}$ |
| **pH** | 3.57 ±0.01$^{a}$ | 3.12 ±0.01$^{b}$ | 3.84 ±0.03$^{a}$ |
| **Titratable acidity (% of citric acid)** | 1.26 ±0.04$^{a}$ | 1.28 ±0.06$^{a}$ | 1.22 ±0.06$^{a}$ |
| **Ascorbic acid (mg.g$^{-1}$)** | 0.20 ±0.02$^{b}$ | 0.19 ±0.00$^{b}$ | 0.30 ±0.03$^{a}$ |
| **Total sugars (g 100.g$^{-1}$)** | 6.59 ±0.46$^{a}$ | 5.70 ±1.56$^{a}$ | 5.17 ±2.43$^{a}$ |

The data are presented mean values ± standard error; a–b values without same superscript within each row differ significantly ($P$ <0.05); 1%

Milivojevic et al., 2012; Poiana et al., 2012; Bernal et al., 2014).

### 3.2 Phenolic compounds

Results of the determination of the main phenolic compounds (TP, TT, TF, TA and flavan 3-ols) of wild-growing strawberry, bilberry and elderberry are shown in Table 2. Different parameters, such as fruit species, cultivar, maturity stage, harvesting time, postharvest conditions, extraction procedure and others affect the chemical and biochemical composition of fruits (Prvulović et al., 2016). Role of phenolics in fruits are numerous: they actively inhibit or stimulate physiological processes, represent defending system against pathogens and stress, and contribute to some of the quality properties of fruits (aroma, flavors, color, and astringency) (Sulusoglu, 2014). Significant differences in TP content among selected fruit species were recorded (Table 2). Elderberry contained the highest concentration of TP (17.40 mg.GAE.g$^{-1}$ FW), followed by strawberry (14.33 mg GAE.g'1 FW) and bilberry fruits (11.14 mg.GAE g$^{-1}$ FW). The content of measured TP in our experiment was higher compared to literature data for elderberry (Özgen et al., 2010; Elez Garofulić et al., 2012; Vujanović et al., 2020; Zhou et al., 2020), strawberry (Wang & Lewers, 2007; Peñarrieta et al., 2009; Dyduch-Siemińska et al., 2015) and bilberry (Bunea et al., 2011; Milivojevic et al., 2012; Poiana et al., 2012; Bernal et al., 2014; Celik et al., 2018). Some researchers detected higher amount of TP in elderberry fruits compared to results obtained in our experiment (Tumbas et al., 2010; Colak et al., 2016).

Tannins are compounds with a relatively high molecular weight and could be divided into two groups: condensed and hydrolysable tannins. Condensed tannins (proanthocyanidins) are found in abundance in fruits and fruit products and are partly responsible for astringency and color of fruits (Skrovankova et al., 2015). Strawberry and elderberry fruits contained similar quantity of TT (13.41 mg.GAE.g$^{-1}$ FW and 13.24 mg GAE.g'1 FW, respectively), while bilberry fruits had lower amounts of these compounds (9.93 mg.GAE.g$^{-1}$ FW). Few researchers detected lower amount of TT in elderberry fruits compared to results of our research (ElezGarofulić et al., 2012; Najda et al., 2014; Dyduch-Siemińska et al., 2015; Vujanović et al., 2020).

Flavonoids has a variety of biological activities in plants: color and aroma of flowers and fruits, to attract pollinators, protect plants from different biotic and abiotic stresses and act as UV filters, detoxifying agents, signal molecules, allopathic compounds, phytoalexins, and antimicrobial defensive components. In addition, flavonoids have roles against frost hardiness, drought resistance and play a role in plant heat acclimatization and freezing tolerance (Panche et al., 2016). Significant differences of TF content among observed fruit species were confirmed. The highest levels of TF were found in elderberry fruits (8.09 mg.QE.g$^{-1}$ FW) followed by bilberry (2.53 mg.QE.g$^{-1}$ FW) fruits. The lowest levels of TF were measured in strawberry (0.081 mg.QE.g$^{-1}$ FW).

Anthocyanins are water-soluble phenolic compounds that serve as plant pigments responsible for red, purple or blue color of many plant organs. Seventeen anthocyanidins could be found in nature, whereas only six of them are present in most fruits: malvidin, pelargonidin, peonidin, petunidin, delphinidin and cyanidin. Anthocyanins are antioxidants that play a very important role in reducing risks of different human degenerative diseases (Hidalgo & Almajano, 2017). Elderberry showed the greatest total anthocyanins (TA) content of all three fruits (1.5.37 mg.C3G.g$^{-1}$ FW), followed by bilberry (3.95 mg.C3G.g$^{-1}$ FW) and the lowest TA content were found in strawberry fruits (0.29 mg C3G g$^{-1}$ FW). The present TA content values for elderberry (Özgen et al., 2010; Elez Garofulić et al., 2012; Zhou et al., 2020), bilberry (Bunea et al., 2011; Poiana et al., 2012; Bernal et al., 2014; Celik et al., 2018) and strawberry (Wang & Lewers, 2007; Dyduch-Siemińska et al., 2015) are in agreement with other studies.

Flavan-3-ols is one of the groups of plant phenolics that are distributed widely in plants and are synthesized via

**Table 2** Phenolic compounds of wild-growing strawberry, bilberry and elderberry fruits

| | Fruit | | |
|---|---|---|---|
| | **strawberry** | **bilberry** | **elderberry** |
| **Total phenolics (mg of GAE.g$^{-1}$ of FW)** | 14.33 ±0.66$^{b}$ | 11.14 ±0.70$^{c}$ | 17.40 ±0.85$^{a}$ |
| **Total tannins (mg of GAE.g$^{-1}$ of FW)** | 13.41 ±0.16$^{a}$ | 9.93 ±0.16$^{b}$ | 13.24 ±1.34$^{a}$ |
| **Total flavonoids (mg of QE.g$^{-1}$ of FW)** | 0.81 ±0.24$^{c}$ | 2.53 ±0.07$^{b}$ | 8.09 ±0.56$^{a}$ |
| **Total anthocyanins (mg of C3G.g$^{-1}$ of FW)** | 0.29 ±0.05$^{c}$ | 3.95 ±0.05$^{b}$ | 5.37 ±0.17$^{a}$ |
| **Flavan 3-ol (mg of CE.g$^{-1}$ of FW)** | 0.005 ±0.001$^{c}$ | 0.17 ±0.01$^{a}$ | 0.03 ±0.10$^{b}$ |

The data are presented mean values ± standard error; a–c values without same superscript within each row differ significantly ($P$ <0.05)

the phenylpropanoid and flavonoid pathways. Flavan-3-ols also have diverse biological activities, including protection against herbivorous and phytopatogens, and possess strong allelopathic activity (Enomoto et al., 2020). From the results shown in Table 2 it can be seen that bilberry fruits possess more flavan-3-ols (0.170 mg.CE.g$^{-1}$ FW) compared to fruits of elderberry (0.030 mg.CE.g$^{-1}$ FW) and strawberry (0.005 mg.CE.g$^{-1}$ FW).

### 3.3 Antioxidant capacity

Within the present study, the antioxidant capacity of three selected fruits was not uniform and depends on assay performed (Table 3). To date, antioxidant activities of plant extracts are measured using a panel of assays whereby each assay has its own advantages and limitations. It is not sufficient to use one *in vitro* assay to claim antioxidant capacity of an extract (Sadeer et al., 2020). Elderberry extract expressed the strongest antioxidant activity in FRAP, ABTS and NO assays, strawberry extract in ABTS, DPPH, TAC and NBT assays, while bilberry extract performed the highest antioxidant capacity in TRC and NBT assays, compared with other two fruits' extracts. After comparing with literature data, it is obvious that all three fruits possess high antioxidant properties in comparison to many other fruits, crops, and vegetables. Results of our study are in agreement with previous works on strawberry (Wang & Lewers, 2007; Peñarrieta et al., 2009; Najda et al., 2014), elderberry (Elez Garofulić et al., 2012; Vujanović et al., 2020) and bilberry (Bunea et al., 2011; Milivojevic et al., 2012; Poiana et al., 2012; Bernal et al., 2014; Colak et al., 2016; Celik et al., 2018).

**Table 3** Antioxidant capacity of wild-growing strawberry, bilberry, and elderberry fruits

| | Fruit | | |
|---|---|---|---|
| **Antioxidant tests** | **strawberry** | **bilberry** | **elderberry** |
| **FRAP (mg TE.g$^{-1}$ FW)** | 40.55 ±6.03$^{a}$ | 32.54 ±3.68$^{b}$ | 51.46 ±2.40$^{a}$ |
| **ABTS (mg TE.g$^{-1}$ FW)** | 36.23 ±2.35$^{a}$ | 25.89 ±1.98$^{b}$ | 36.98 ±0.65$^{a}$ |
| **DPPH (mg TE.g$^{-1}$ FW)** | 163.13 ±6.46$^{a}$ | 92.51 ±6.44$^{c}$ | 125.03 ±9.59$^{b}$ |
| **TAC (mg TE.g$^{-1}$ FW)** | 195.96 ±2.60$^{a}$ | 102.20 ±11.81$^{b}$ | 71.52 ±0.83$^{c}$ |
| **TRC (mg TE.g$^{-1}$ FW)** | 98.20 ±2.06$^{b}$ | 108.01 ±0.86$^{a}$ | 98.60 ±1.16$^{b}$ |
| **NBT (IU SOD.g$^{-1}$ FW)** | 256.05 ±11.11$^{a}$ | 196.82 ±6.64$^{b}$ | 151.70 ±5.50$^{c}$ |
| **NO inhibition (% of inhibition of NO radicals)** | 78.30 ±3.65$^{b}$ | 96.36 ±0.23$^{a}$ | 95.79 ±3.68$^{a}$ |

The data are presented via mean values ± standard error; a–c values without the same superscript within each row differ significantly ($P$ <0.05)

**Table 4** Statistical analysis

| **Total phenolics** | **TAC** | **FRAP** | **ABTS** | **DPPH** | **NBT** | **TRC** | **NO inhibition** |
|---|---|---|---|---|---|---|---|
| **Correlation coefficient (*r*)** | -0.2074 | 0.8925* | 0.8793* | 0.4625 | -0.4114 | -0.8335* | -0.0815 |
| **Coefficient of determination ($r^2$)** | 0.043 | 0.800 | 0.773 | 0.214 | 0.169 | 0.695 | 0.007 |
| **Total tannins** | | | | | | | |
| **Correlation coefficient (*r*)** | 0.2901 | 0.7164* | 0.8702* | 0.8226* | 0.0994 | -0.8607* | -0.4456 |
| **Coefficient of determination ($r^2$)** | 0.084 | 0.513 | 0.757 | 0.677 | 0.010 | 0.741 | 0.196 |
| **Total flavonoids** | | | | | | | |
| **Correlation coefficient (*r*)** | -0.828* | 0.716* | 0.341 | -0.2676 | -0.9139* | -0.2536 | 0.6395 |
| **Coefficient of determination ($r^2$)** | 0.686 | 0.513 | 0.116 | 0.072 | 0.835 | 0.064 | 0.409 |
| **Total anthocyanins** | | | | | | | |
| **Correlation coefficient (*r*)** | -0.994* | 0.3286 | -0.1793 | -0.7136* | -0.972* | 0.2698 | 0.9074* |
| **Coefficient of determination ($r^2$)** | 0.988 | 0.108 | 0.032 | 0.509 | 0.945 | 0.728 | 0.823 |
| **Flavan 3-ol** | | | | | | | |
| **Correlation coefficient (*r*)** | -0.9615* | 0.4772 | 0.012 | -0.566 | -0.9872* | 0.0821 | 0.8272* |
| **Coefficient of determination ($r^2$)** | 0.925 | 0.228 | 0.000 | 0.320 | 0.974 | 0.007 | 0.684 |

* values marked with asterisk are statistically significant ($P$ >0.05)

### 3.4 Statistical analysis

Statistical correlation between different phenolic compounds and applied antioxidant assays are presented in Table 4. TP and TT revealed positive correlation with FRAP (0.8925 and 0.7164, respectively) and ABTS (0.8793 and 0.8702, respectively) assays, TF with FRAP assay (0.7160) while TA and flavan-3-ols expressed positive correlation only with NO radical scavenging test (0.9074 and 0.8272, respectively). TAC and NBT assays did not perform positive correlation with any of measured groups of phenolic compounds. It could be assumed that antioxidant capacity of extracts of selected fruits does not depend on phenolic compounds present only. Some other class of extracted molecules could play significant role in antioxidant activity of extracts.

## 4 Conclusions

In this work, the chemical properties, phenolic compounds and antioxidant capacity of wild growing strawberry, bilberry and elderberry from eastern Serbia were determined. All three fruits are high in ascorbic acid, sugars, and phenolics content and possess strong antioxidant activity. The elderberry fruits had the highest ascorbic acid and TT, TF and TA content. Antioxidant capacity of fruits appears to be largely influenced by TP and TT contents while ability of inhibition of NO radicals depends on the content of TA and flavan-3-ols in samples.

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**Research Article**

# Performance and character contributions to variability in okra (*Abelmoschus esculentus* L. Moench) genotypes

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Okra is an important vegetable crop, but its optimal production is constrained by a myriad of problems including pests, poor agronomic practices, and improper varietal identification among others. A study was carried out to determine the field performance and contribution of agronomic characters to overall variation in eighteen okra genotypes over two locations, Teaching and Research Farms of the Federal University of Agriculture, Abeokuta, Nigeria and Rehoboth Farms Limited, Moniya, Ibadan, Nigeria. The experiments were laid down in a randomized complete block design with three replicates and data were collected on number of days to emergence, number of days to 50% flowering, number of branches per plant, pod length, pod width, number of pods per plant, plant height, pod weight, number of seeds per pod, number of ridges per pod, 100 seed weight, seed, and pod yield. The data were subjected to analysis of variance, heritability in the broad sense, principal component analysis as well as the single linkage cluster analysis. Results revealed significant ($p$ <0.05) variation in the genotypes and high heritability estimates for most of the characters. Number of branches per plant, plant height, number of pods per plant accounted for the highest contributor to variations in the accessions while clustering analysis revealed genotypes; NGB00303, NGB00342 and NGB00346 were distant from all genotypes making them useful materials for hybridization studies.

**Keywords:** okra, heritability, pca, dendrogram

## 1 Introduction

Okra, *Abelmoschus esculentus* [L.] Moench is an important vegetable crop in West Africa, and it contributes 60% share of fresh vegetables export (Shete, 2000) and rich in essential amino acids and dietary fibre (NAP, 2006). Selection of okra plants with wide adaptability and yield potential plays a crucial role in breeding programmes (Balai et al., 2014). The choice of breeding methods is conditioned by the variability inherent in available germplasms and the target environment where they are being selected. Wray and Visscher, 2008 reported that estimation of heritability, gives an insight of variance components responsible for transmission of traits. Also, the use of numerical tools such single linkage cluster analysis (SLCA) and principal component analysis (PCA) has been valuable for classifying large number of accessions of agronomic importance (Badenes et al., 2000). The PCA allows visual differentiation among entries and identify possible associations by providing a two-dimensional scatter plot consisting of individual entries. PCA saves time and resources thereby improving the selection responses in breeding programs. The objectives of this study were to determine the performance, heritability of characters and their contribution to and relationships between the genotypes.

## 2 Materials and methods

### 2.1 Planting materials, field evaluations and data collection

Eighteen okra accessions were sourced from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. Field evaluations were carried out in the early wet season (average temperature of 26 °C, rainfall 175.26 mm, humidity of 87% and sunshine 12 hrs 26 minutes). The experiments were laid out in a Randomized Complete Block Design (RCBD) with three replicates in single row plots at the Teaching and

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Research Farms of the Federal University of Agriculture, Abeokuta, Nigeria (Latititude 7° 15′ N and longitude 3° 25 ′E) and Rehoboth Farms Limited, Moniya, Ibadan, Oyo State, Nigeria (Latitude 3° 56′ N and Longitude 3° 11′ E). Planting was done on 5 m long row with ant inter and intra row spacing of 0.75 m and 0.45 m, respectively. Weed control was done when due and NPK 16 : 16 : 16 fertilizer was applied at 60 kg.ha$^{-1}$. Insect pests were controlled using cypermethrin at 80 g active ingredient per ha fortnightly till harvest. Data were collected on number of days to emergence, number of days to 50% flowering, number of branches per plant, pod length (cm), pod width (mm), number of pods per plant, plant height (cm), pod weight (g), number of seeds per pod, number of ridges per pod, seed weight (g), 100 seed weight (g), seed yield (kg.ha$^{-1}$) and pod (kg.ha$^{-1}$).

### 2.2 Data analysis

Data collected were subjected to analysis of variance using SAS Release 8.0 (SAS Institute, 1999) and means were separated using Duncan's multiple range test. Variance components and heritability estimates were computed, while principal component analysis (PCA) and single linkage cluster analysis (SLCA) were used to determine the character contribution and variation pattern in the accessions, respectively.

## 3 Results and discussion

Genetic variation is an important index for yield improvement in crop plant. The genotypes performed differently with respect to characters evaluated except for pod weight and 100 seed weight, while significant genotype × interaction were observed for number of days to emergence, number of branches, plant height, number of pods per plant, pod width and pod yield (Table 1). This shows the environment effect on these characters over locations, which implies that selection for these characters based on phenotypic performance, will not be reliable as they are not stable and responsive to the environment. Accession NGB 00327 germinated earlier than all other accessions. Earliness to flowering is an important factor in selection of high yielding varieties and it was observed that NBG 00298 was the earliest flowering genotype (Table 2). Also, the high yield of NGB 00316 will make is a good material for hybridization studies. The difference between the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (PCV) for the characters (Table 3), suggests the role environment plays in expression of phenotypic characters. Generally, the higher heritability estimates for all characters with moderate for pod weight and 100 seed weight shows that these characters were less amenable by the environment. Therefore, selecting for these characters is likely to be effective. Murtadha

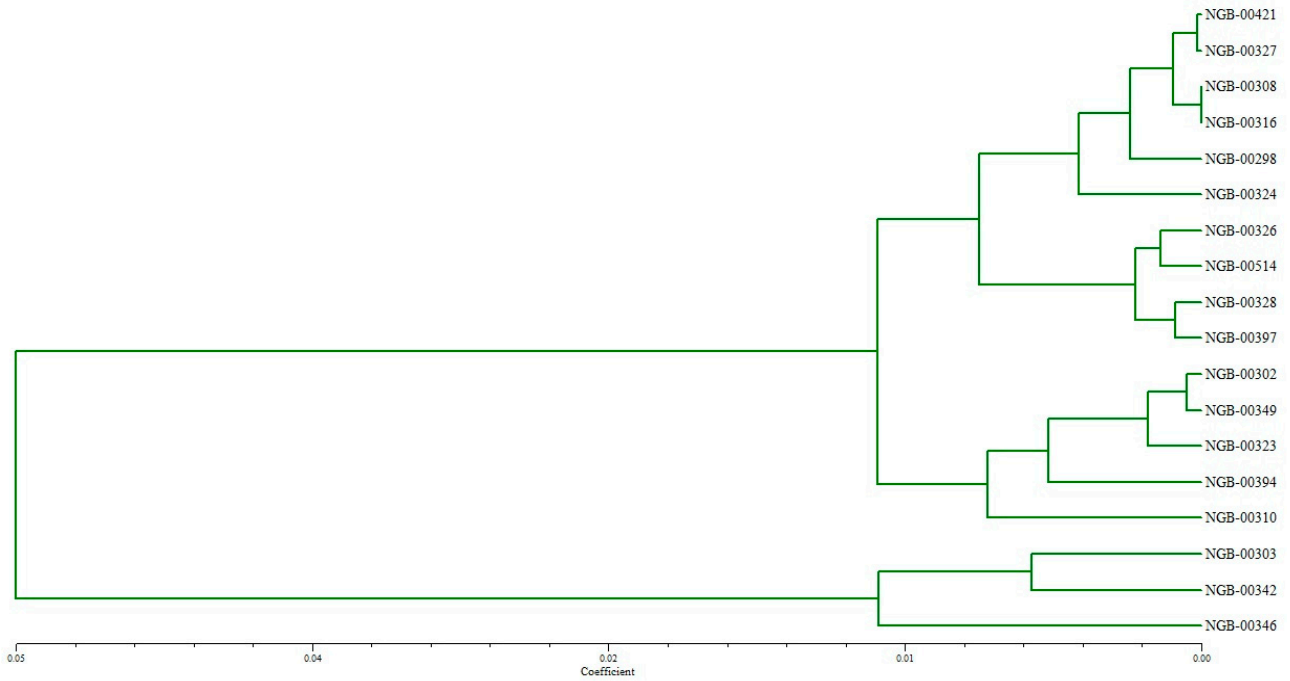


**Figure 1** Dendrogram showing similarity coefficients of the okra genotypes evaluated

**Table 1** Pooled analysis of variance of characters evaluated for eighteen okra genotypes

| Source of variation | *df* | Number of days to emergence | Number of days to flowering | Number of branches.plant$^{-1}$ | Plant – height (cm) | Number of pods.plant$^{-1}$ | Pod length (cm) | Pod width (mm) | Pod weight (g) | Number of seed.pod$^{-1}$ | Number of ridges.pod$^{-1}$ | 100 seed weight (g) | Seed yield (kg.ha$^{-1}$) | Pod yield (kg.ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Block** | 2 | 0.20 | 0.75 | 3.17 | 1384.07 | 63.37* | 4.89 | 13.54 | 87.46 | 434.07 | 0.30 | 5.27* | 29561.71 | 72584.47 |
| **Genotype** | 17 | 0.77** | 10.35** | 21.20** | 5718.46** | 51.44** | 19.65** | 94.98** | 68.27 | 2159.76** | 8.04** | 1.22 | 52387.69** | 196841.06** |
| **Location** | 1 | 16.47** | 0.27 | 224.20** | 72763.11** | 403.10** | 3.13 | 11.03 | 12.52 | 101.45 | 1.02 | 0.38 | 687649.51** | 3146530.91** |
| **Gen*Loc** | 17 | 0.47* | 6.68 | 14.12** | 1389.69** | 21.97* | 2.92 | 23.54** | 56.09 | 166.91 | 0.49 | 1.900 | 24910.07 | 78905.71* |
| **Error** | 69 | 0.22 | 4.68 | 3.15 | 578.87 | 11.71 | 1.85 | 10.40 | 61.83 | 313.92 | 0.61 | 1.12 | 16855.44 | 43307.13 |

**Table 2** Mean performance of the eighteen genotypes of okra for seed yield and seed related characters

| Genotype | Number of days to emergence | Number of days to flowering | Number of branches.plant$^{-1}$ | Plant height (cm) | Number of pods.plant$^{-1}$ | Pod length (cm) | Pod width (mm) | Pod weight (g) | Number of seeds.pod$^{-1}$ | Number of ridges.pod$^{-1}$ | 100 seed weight (g) | Seed yield (kg.ha$^{-1}$) | Pod yield (kg.ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **NGB 00421** | 6.53bc | 72.50a-c | 5.29c-e | 158.23a-d | 9.87bc | 11.76c | 25.15e | 8.11b | 69.78d-f | 7.22f | 5.73a | 267.64a-f | 592.77a-c |
| **NGB 00298** | 6.83bc | 70.50a | 5.55d-f | 175.29a | 8.12b-d | 10.94cd | 32.47ab | 13.08ab | 139.15a | 9.33ab | 5.36a | 371.71ab | 717.49ab |
| **NGB 00302** | 6.58bc | 73.00 a-c | 4.65b-e | 138.93b-e | 5.27c-e | 10.14c-e | 29.52a-d | 11.13b | 96.07bc | 8.52a-e | 6.42a | 197.80b-f | 426.12c-f |
| **NGB 00303** | 7.08c | 74.83c | 3.24a-d | 112.45e-g | 3.33e | 16.08a | 30.44 a-d | 11.79b | 97.55bc | 9.38a | 6.30a | 105.73f | 223.62ef |
| **NGB 00308** | 6.96bc | 71.83 a-c | 5.89sf | 187.33a | 10.67ab | 10.79 cd | 30.16 a-d | 9.55b | 86.50b-e | 8.30b-e | 6.07a | 352.32a-c | 728.51ab |
| **NGB 00310** | 7.02bc | 74.83c | 2.68ab | 95.25g | 4.45de | 10.77 cd | 29.72 a-d | 8.98b | 89.30b-d | 8.48 a-e | 5.59a | 116.79f | 271.25de |
| **NGB 00316** | 6.99bc | 71.50ab | 7.59fg | 189.48a | 14.28a | 11.15 cd | 32.04a-c | 11.17b | 91.03 b-d | 8.83a-c | 5.77a | 391.51a | 809.87a |
| **NGB 00323** | 6.79bc | 73.67bc | 3.70a-e | 103.81fg | 5.23 c-e | 10.58 c-e | 26.71de | 21.56a | 91.32 b-d | 7.90c-f | 4.94a | 154.38ef | 335.58c-f |
| **NGB 00324** | 6.54bc | 71.50ab | 4.57 b-e | 135.74c-e | 9.13 b-d | 13.56b | 18.33f | 5.00b | 48.43f | 5.28g | 4.81a | 178.35c-f | 448.89b-f |
| **NGB 00326** | 6.89bc | 71.33ab | 5.84ef | 173.56a | 6.11b-e | 10.06c-e | 31.37 a-c | 10.01b | 76.10c-e | 8.22 c-f | 5.56a | 301.44a-e | 541.48a-d |
| **NGB 00327** | 5.49a | 72.00 a-c | 8.54g | 169.03ab | 8.82 b-d | 8.79e | 33.21a | 10.12b | 83.93 c-e | 8.39 a-e | 5.86a | 264.16a-f | 597.66a-c |
| **NGB 00328** | 6.99bc | 72.00 a-c | 8.19g | 165.26a-c | 10.59ab | 10.20 c-e | 30.48 a-d | 9.97b | 77.70 c-e | 7.48ef | 6.03a | 341.67a-d | 530.73b-d |
| **NGB 00342** | 6.56bc | 73.00 a-c | 2.09a | 118.42 e-g | 4.73de | 10.27 c-e | 28.05b-e | 9.51b | 90.38 b-d | 8.55 a-e | 6.42a | 99.74f | 174.49f |
| **NGB 00346** | 6.83bc | 74.17bc | 2.95a-c | 129.18d-f | 3.35e | 9.32de | 31.91 a-c | 9.45b | 93.87 b-d | 8.20 c-f | 5.94a | 167.88d-f | 264.49d-f |
| **NGB 00349** | 6.80bc | 73.83 a-c | 3.13a-d | 131.07d-f | 6.28 b-e | 11.00 cd | 30.27a-d | 11.11b | 109.17b | 8.74a-d | 5.63a | 221.49a-f | 463.50b-e |
| **NGB 00514** | 6.75bc | 72.67 a-c | 3.97a-e | 139.06 b-e | 8.59 b-d | 13.96b | 20.44f | 6.62b | 63.48ef | 5.15g | 5.36a | 230.82a-f | 386.21c-f |
| **NGB 00394** | 6.83bc | 74.67c | 4.89 b-e | 88.90g | 5.73 c-e | 9.57de | 27.77c-e | 8.60b | 91.97 b-d | 7.48ef | 6.07a | 205.06b-f | 344.46c-f |
| **NGB 00397** | 6.37b | 73.80bc | 3.49a-e | 140.36 b-e | 6.11 b-e | 10.04 c-e | 28.43b-e | 9.15b | 96.58bc | 7.72d-f | 5.76a | 310.97a-e | 472.68b-e |

Means followed by the same alphabet along columns are not statistically different from one another

**Table 3** General mean, estimate of variance components and broad sense heritability of the characters evaluated in the okra genotypes

| Character | Mean | Phenotypic variance | Genotypic variance | Phenotypic coefficient of variation | Genotypic coefficient of variation | Heritability (%) |
|---|---|---|---|---|---|---|
| **Number of days to emergence** | 6.72 | 0.77 | 0.70 | 13.06 | 12.43 | 91 |
| **Number of days to flowering** | 72.90 | 10.35 | 8.78 | 4.41 | 4.07 | 85 |
| **Number of branches.plant$^{-1}$** | 4.80 | 21.20 | 20.15 | 95.84 | 93.43 | 95 |
| **Plant height (cm)** | 142.00 | 5718.46 | 5525.50 | 53.26 | 52.35 | 97 |
| **Number of pods.plant$^{-1}$** | 7.26 | 51.44 | 47.53 | 98.84 | 95.01 | 92 |
| **Pod length (cm)** | 11.06 | 19.65 | 19.03 | 40.07 | 39.43 | 97 |
| **Pod width (mm)** | 28.69 | 94.98 | 91.51 | 33.97 | 33.34 | 96 |
| **Pod weight (g)** | 10.30 | 68.27 | 47.66 | 80.22 | 67.03 | 70 |
| **Number of seeds.pod$^{-1}$** | 88.34 | 2159.76 | 2055.13 | 52.61 | 51.32 | 95 |
| **Number of ridges.pod$^{-1}$** | 7.96 | 8.04 | 7.83 | 35.63 | 35.17 | 97 |
| **100 seed weight (g)** | 5.76 | 1.22 | 0.84 | 19.14 | 15.93 | 69 |
| **Seed yield (kg.ha$^{-1}$)** | 236.36 | 52387.69 | 46769.21 | 96.84 | 91.50 | 89 |
| **Pod yield (kg.ha$^{-1}$)** | 462.06 | 196841.06 | 182405.35 | 96.02 | 92.43 | 93 |





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**Table 4** Character loading, eigen values and variance components characters evaluated in both locations

| Character | Component | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| **Number of days to emergence** | 0.41 | 0.03 | 0.66 | 0.02 |
| **Number of days to flowering** | -0.50 | 0.22 | 0.52 | -0.03 |
| **Number of branches.plant$^{-1}$** | 0.80 | -0.17 | -0.03 | -0.07 |
| **Plant height (cm)** | 0.87 | -0.11 | 0.06 | -0.10 |
| **Number of pods.plant$^{-1}$** | 0.81 | -0.31 | 0.02 | 0.11 |
| **Pod length (m)** | -0.14 | -0.42 | 0.59 | 0.23 |
| **Pod width (mm)** | 0.29 | 0.77 | -0.10 | 0.05 |
| **Pod weight (g)** | 0.07 | 0.30 | -0.22 | 0.60 |
| **Number of seeds.pod$^{-1}$** | 0.21 | 0.79 | 0.13 | 0.24 |
| **Number of ridges.pod$^{-1}$** | 0.21 | 0.81 | 0.08 | 0.19 |
| **100 seed weight (g)** | 0.09 | 0.35 | 0.02 | -0.70 |
| **Seed yield (kg.ha$^{-1}$)** | 0.87 | -0.15 | 0.01 | -0.01 |
| **Pod yield (kg.ha$^{-1}$)** | 0.93 | -0.10 | 0.00 | 0.04 |
| **Eigen value** | 4.33 | 3.06 | 1.15 | 1.10 |
| **Variance (%)** | 30.94 | 21.87 | 8.23 | 7.82 |
| **Cumulative variance (%)** | 30.94 | 52.807 | 61.041 | 68.86 |

et al. (2000) suggested that a trait with high GCV and heritability will be a good predictor of pod yield. The high heritability and GCV observed for number of branches per plant, number of pods per plant, total seed yield and total pod yield could be attributed to additive gene action thus making selection for them simple. The lower GCV indicated for number of days to emergence and flowering, and 100 seed weight indicated them less amenable to improvement by selection. Ariyo (1989) reported the need to breed for specific environments because the response of most characters to environment was non-linear. Several investigators also studied heritability of different traits in okra crop which support the present findings. Singh et al. (2006) reported high heritability for days to flowering and pod weight. Indurani and Veeragavathatham (2005) and Singh et al. (2006) also reported moderate to high heritability for plant height. The first three principal components were the most important in discrimination accessions as reported by Clifford and Stephenson (1975). From this study, four of the 13 principal components had eigen values greater than one and accounted for 30.90%, 21.87%, 8.23% and 7.82% of the total variation, respectively and 68.86% cumulatively (Table 4). The first principal component was loaded largely with number of branches per plant, plant height, number of pods per plant, seed, and pod yield.

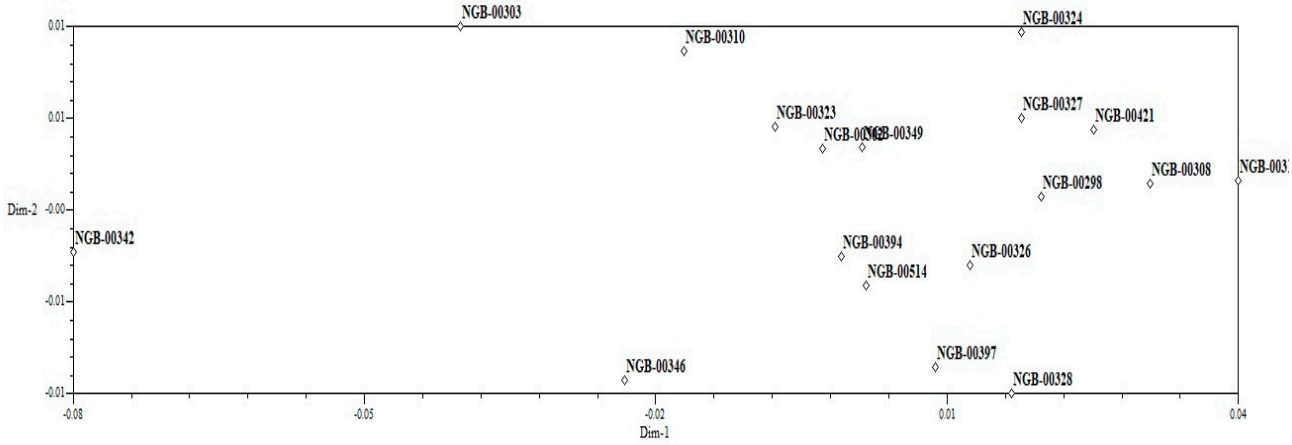


**Figure 2** Two dimensions plots of the configurations of the okra genotypes

While the second principal component axis comprised pod width, number of seeds per pods and number of ridges per pod. The eigen values for axis one was high (4.33) and low (1.10) for axis four. The contributions pod and seed characters were relatively high in the principal axes 1 and 2. This agrees with the report of Ariyo and Odulaja (1991) and Ogunbodede (1997). Clustering of the accessions also suggest a close relationship between them. Aliyu and Fawole (2000) also demonstrated the importance of cluster analysis based on similarity coefficients. The genotypes were grouped into two major groups: I and II. Group I had only three genotypes which were late flowering, had fewer number of pods per plant and low yielding (Fig. 1). Genotypes NGB00303, NGB00342 and NGB00346 were the most diverse as also revealed in Fig. 2. Which makes them a useful source for hybridization with members of the other group in okra improvement programme.

## 4 Conclusion

This study revealed significant variability in the genotypes and characters such as plant height, number of branches and number of pods were useful in discriminating the okra genotypes. High heritability estimates were observed for pod related parameters which makes them amenable for selection. Also, distinctions of genotypes: NGB00303, NGB00342 and NGB00346 from all other genotypes will make them useful for hybridization studies.

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**Research Article**

# The impact of apple preparation on the content of chlorpyrifos pesticide residues in the final products

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The apples of Red Delicious are round fruits that have red colour when ripe according to which they are named. They can be eaten fresh, but also can be processed into a variety of processed products including apple juice. In order to grow or obtain better yield of a good quality apples, pesticides are usually used to protect apple trees, but they can adversely affect human health, therefore, some precautions should be taken when these chemicals are used as food contact materials. This study summarizes the presence of chlorpyrifos pesticide residues in apples that are prepared by different methods including mechanical treatments, fresh, washed, peeled as well as heat treatment of apples when prepared into an apple juice. For this purpose, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used for residue extraction in apple samples after different methods of preparation, and their analyses were performed by liquid chromatography-mass spectrometry (LC-MS/MS). The concentration of the pesticide chlorpyrifos in different samples of apples was in the range of less than 0.0005 $mg.kg^{-1}$ to 0.00348 $mg.kg^{-1}$. This study provides a conclusion that all samples of apples are safe for consumption while the peeling method and heat treatment are the most efficient in reduction of chlorpyrifos content in the final products.

**Keywords:** apple, chlorpyrifos, apple juice, processing factor, food safety

## 1 Introduction

Pesticides are chemicals usually used to protect plants from diseases and pests in the period before and after harvesting the fruits and vegetables, in order to reduce their impact on the quantity and quality of plants (Singh et al., 2014; Lozowicka et al., 2015; Suárez-Jacoboa et al., 2017). They could be used in the cultivation of apples as well, but their concentration should be restricted by national/regional laws in order not to overcome the maximum level; otherwise it will cause adverse effects on human health and environment (Simon et al., 2011). According to Badr et al. (2019), pesticides have consequent long-term effects on the national income, the ecosystem, and public health. High concentrations of pesticide residues (active substances (a.s), metabolites or decomposition products), which exceed the maximum allowed limits (Official Journal of the European Union, 2005; Koch et al., 2017; Official Newspaper of the Republic of Macedonia, 2018), can accumulate in human body and have a negative impact on human health; therefore, it is necessary to monitor their concentration in the fruits (Sabarwal et al., 2018). In many countries, monitoring of pesticide residues in fruits and vegetables is one of the most important procedures to reduce potential health hazards. In this context, according to Mebdou et al. (2017), in good agricultural practice (GAP), pesticide residues in food items should not exceed the maximum residue limits (MRLs) and detected concentrations of pesticide residues should be within the prescribed values. The continuous usage of organophosphorus pesticides increases the possibility of these pesticides to be found in horticultures and thus affecting their safety and quality; this is the reason for the commitment of public health and food safety institutions (Quintero et al., 2008). Organophosphate pesticides are toxic to insects and mammals, including humans; they have the ability to affect the central nervous system by inhibiting some important enzymes such as acetylcholine. Consuming unsafe food that is a source of toxic substances (pesticides and their metabolites), increases exposure to pesticides

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as a potential health risk (Sharma et al., 2010; Drouillet-Pinard et al., 2011). In general, the risk of pesticides is related to their accumulation in the food chain which can lead to human exposure to increased levels of pesticides in food (Badr et al., 2019; Jankuloska et al., 2020), and the main health effects are associated conditions such as cancer, birth defects, neurological and endocrine disorders, and reproductive changes (Khan et al., 2020). Chlorpyrifos is a representative of toxic organophosphate insecticides, the second most detected pesticide in water and food (Mackay et al., 2014); it has been detected as the most common pesticide found in apples (Badr et al., 2019). In general, chlorpyrifos is applied for protection of pome fruits (apples and pears) against San Jose scale, Rosy apple aphid, Pandemispyrusana, Obliquebanded leafroller, Climbing cutworms, and American plum borer. The widespread use of chlorpyrifos in agriculture and its persistence in the environment have raised public safety awareness and concerns; therefore, novel technologies are proposed that will overcome pollution and toxicity problems with this chemical (John & Shaike, 2015). According to the Official Journal of the European Union (2020)b, this pesticide has shown negative effects on eventual genotoxic and neurological effects in child development. Due to health issues caused by this pesticide, the EU countries have forbidden the import of fruits that contain this pesticide as well as the use of it (deadline of adjustment is set in the Official Journal of the European Union (2020)a, Official Journal of the European Union (2020)b; and Official Journal of the European Union (2021)). Among different food categories, fruits and vegetables are recognized as a group that could contain higher levels of pesticide residues compared to other food groups, because they are mainly consumed as raw (Stachniuk et al., 2017). Apples can be eaten fresh, but also can be processed into an apple juice, apple compote, apple cider vinegar, jam or other products (Hancock et al., 2008), but also can be prepared as apple pulp used as a raw material to produce other products (Paz et al., 2017).

There are several varieties of apples cultivated in R.N. Macedonia; but in the region of Resen, the most often cultivated variety is Red Delicious. This variety is very lush, diploid, and usually flowers late. The fruits are round, large, usually ripen in months of September/ October (Icka& Damo, 2014). The apple juice is the second most consumed fruit juice worldwide (Rupasinghe & Thilakarathna, 2016) and its consumption has many positive effects on human health due to its nutritional value and bioactive components, such as antioxidant and antimicrobial activity (Paz et al., 2015). Various scientific data have showed that mechanical processing of the fruit removes some of the present pesticide residues, while heat treatment of the fruits further reduces their amount depending on the nature of the pesticide and the nature of fruits/vegetables (Chavarri et al., 2005; Štěpán et al., 2005; Balinova et al., 2006; Kaushik et al., 2009; Ling et al., 2011; Satpathy et al., 2012). Therefore, it is expected that in apples, by washing, peeling and processing them into apple juice, the amount of pesticide residues will be reduced (Keikotlhaile et al., 2009). Dasika et al. (2012) have presented the level of chlorpyrifos in apples before or after washing step with warm water or salt water, but chlorpyrifos was still present in certain amounts. Washing the fruits and vegetables allowed decreasing the concentration of endosulfan (54.24%), imidacloprid (59.24%), diafentiuron (20.96%) and emamectin benzoate (9.09%), while washing with detergent followed by sun drying reduced the concentration of pesticide residues by up to 95% of the peel (Satpathy et al., 2012; Sheikh et al., 2015; Yang et al., 2017).

In this study, samples of fresh apples of Red Delicious variety as well as in its processed forms – washed, peeled and converted into apple juice – were examined in terms of the presence of pesticide residues. The pesticide was extracted by using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method (Anastassiades et al., 2003; Bruzzoniti et al., 2014). The main advantages of the QuEChERS method over the traditional sampling methods include recoveries for a high number of pesticides, good accuracy and precision, reducing the volume of used organic solvent, preparation of a high number of samples for short time and ease of implementation. Modern instrumental methods, including chromatographic techniques, are commonly used to analyze pesticides. More particularly, liquid chromatography is the most widely used separation technique including analysis of pesticides in fruits (Radišićet al., 2009).

## 2 Materials and methods

Detection of the pesticide chlorpyrifos in apples of the Red Delicious variety and its processed forms is monitored in this study. The samples of apples were taken from two locations in the region of Resen, i.e. location 1 (Evla) and location 2 (Kriveni), after their harvest (October 2020). Table 1 presents the samples used in this study. Samples 1 and 2 are unwashed apples, while samples 3 and 4 are washed (washed with cold tap water at 12 °C for 30 s). Samples 5 and 6 are samples that were initially washed and then peeled off with a sharp knife (the peel thickness was 1 ±0.3 mm). Peeled apples were homogenized and then analyzed for the purpose of this study. Samples 7 and 8 represent juice prepared of collected apples for both locations (preparation occurred at in-house conditions according to the traditional recipe). Fig. 1 shows a diagram for apple juice preparation.

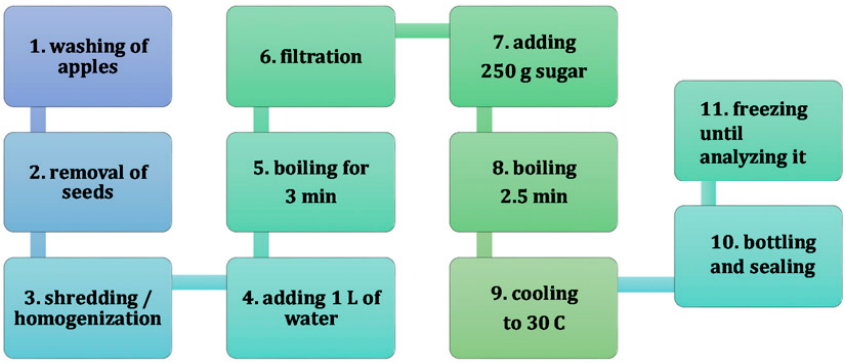


**Figure 1** Steps for apple juice preparation

Extraction of chlorpyrifos pesticide residues was performed using the QuEChERS method according to the standard MKS EN 15662:2018 LC-MS/MS. The QuEChERS method involves liquid extraction of pesticide residues from fruits using a solvent acetonitrile (Anastassiades et al., 2003; Bruzzoniti et al., 2014). This method consists of using gas or liquid chromatography (Standardization Institute of the Republic of Macedonia, 2011). The procedure for extracting residues was as follows: 10 ±0.1 g homogenized fruit sample was weighed in a tube of 50 mL, then was added 10 mL acetonitrile and the sample was extracted with ultra turax for 2 min at 4,000 rpm. Then a mixture of 4 g of magnesium sulfate anhydride ($MgSO_4$), 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate hexahydrate were added to the extract. The sample was vigorously mixed by hand for 1 min and centrifuged at 4,000 rpm for 10 min. A 6 mL extract was transferred to a 15 mL plastic tube which already contained 150 mg PSA (primary secondary amine) and 900 mg $MgSO_4$. The mixture was mixed vigorously by hand or vortex for 30 s and centrifuge at 3,000 rpm for 5 min. When necessary, the extract was filtered into an autosampler vial through a 0.45 µm filter; then 0.1% formic acid was added and applied to the UPLC-MS/MS system. The dilution factor was 1. The samples were analyzed by liquid chromatography (Waters, UPLC-MS/MS), mass spectrometer with triple quadrupole (XEVO TQ-S micro, Waters), analytical column (Acquity UPLC BEH C18 1.7 µm, 2.1 × 100 mm, Waters), mobile phase A – LC/MS water with 0.1% formic acid and 5 mM ammonium formate, mobile phase B – methanol with 0.1% formic acid and 5 mM ammonium formate. Processing factors (PF) were calculated for all transformation steps by a ratio between the pesticide residue concentration ($mg.kg^{-1}$) in the processed commodity and the pesticide residue concentration ($mg.kg^{-1}$) in the raw, non-processed commodity (Scholz et al., 2018; El-Sayedet al., 2021).

**Table 1** Designation of apple samples used for this study

| Sample number | Type of processing | Location |
|---|---|---|
| **1** | unwashed apples | Evla |
| **2** | unwashed apples | Kriveni |
| **3** | washed apples | Evla |
| **4** | washed apples | Kriveni |
| **5** | peeled apples | Evla |
| **6** | peeled apples | Kriveni |
| **7** | apple juice | Evla |
| **8** | apple juice | Kriveni |

## 3 Results and discussion

The analysis of the pesticide chlorpyrifos was performed in 8 samples; fresh (unwashed), washed and peeled apples as well as apple juice were analyzed. The results are presented in Table 2.

From the results it can be seen that the highest concentration of the pesticide chlorpyrifos was observed in the fresh (unwashed) apples (samples 1 and 2). Namely, in sample 2, chlorpyrifos is detected at a concentration of 0.00348 $mg.kg^{-1}$, while in sample 1 it is 0.002895 $mg.kg^{-1}$. The concentration of chlorpyrifos in samples 3 and 4 was decreased compared to samples 1 and 2; sample 3 showed 0.001048 $mg.kg^{-1}$, that is 63.8% less than in sample 1, while the concentration of chlorpyrifos in sample 4 was 0.001419 $mg.kg^{-1}$ or 59.2% less than in sample 2. Peeled samples of apples showed further decrease in pesticide concentration; namely, in samples 5 and 6 there were detected <0.0005 $mg.kg^{-1}$ of chlorpyrifos. [Since the range of detection of the instrument for the analysis is 0.0005 $mg.kg^{-1}$, it could be concluded that the pesticide residues are less than this limit or even no presence of it in the tested samples]. The amount of present chlorpyrifos in sample 5 was decreased by 82.7% compared to sample 1, while in sample 6, the amount of chlorpyrifos was decreased up to <0.0005 $mg.kg^{-1}$, i.e. was decreased by 85.6% compared to sample 1. The concentration

**Table 2** Presence of chlorpyrifos in various apple samples

| Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Chlorpyrifos (mg.kg$^{-1}$) | 0.002895 | 0.00348 | 0.001048 | 0.001419 | <0.0005 | <0.0005 | <0.0005 | <0.0005 |

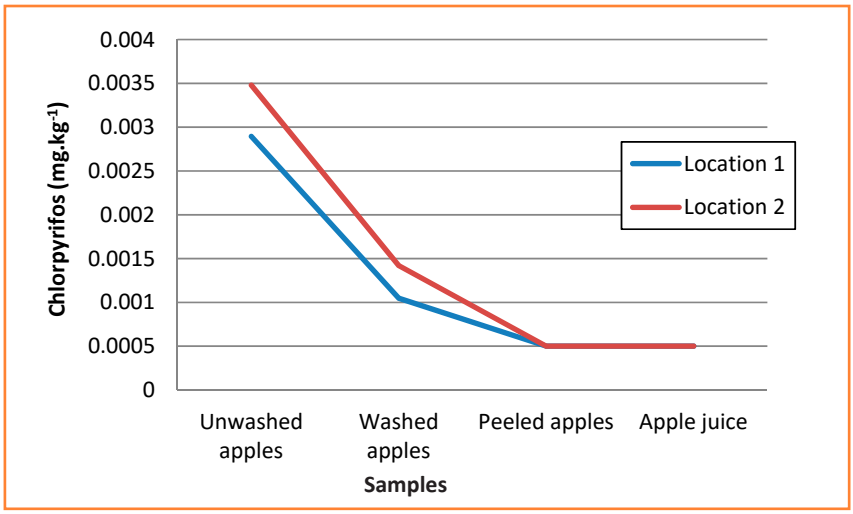


**Figure 2** Effect of apple processing on the concentration of chlorpyrifos (mg chlorpyrifos.kg$^{-1}$ sample) in all tested samples from locations 1 and 2

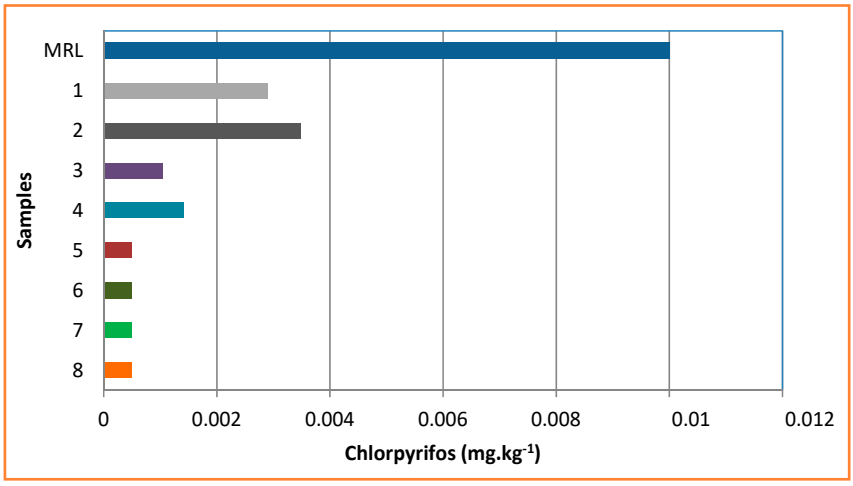


**Figure 3** Concentration of chlorpyrifos (mg chlorpyrifos.kg$^{-1}$ sample) in all tested samples compared to MRL

of present chlorpyrifos in sample 5 was decreased compared to sample 3 to <0.0005 mg.kg$^{-1}$, or was decreased by 52.3%, and in sample 6, the amount of the pesticide decreased to <0.0005 mg.kg$^{-1}$, or the concentration was decreased by 64.7% in relation to sample 4. The concentration of the pesticide chlorpyrifos in apple juice from both locations 1 and 2 was less than <0.0005 mg.kg$^{-1}$. In sample 7, the pesticide concentration was decreased by 82.7% compared to sample 1, and in sample 8, the amount of present pesticide, compared to sample 2, was decreased by 85.6%. The concentration of the pesticide chlorpyrifos in sample 7 was decreased by 52.3% compared to sample 3. The concentration of pesticide in sample 8 was decreased by 64.7% compared to sample 4. These results are in a good agreement with the results of the research conducted by Ling et al. (2011) and Sheikh et al. (2015). Fig. 2 shows the decrease in chlorpyrifos concentration in the analyzed samples.

Processing factors (PF) were calculated after each heat or mechanical treatment of the examined samples and the values are as follows: for sample 3 it is 0.41 while for sample 4 it is 0.36 while PF for peeled and heat treated apples (samples 5to 8) is 0.14. According to Bonnechère et al. (2012) and Scholz (2018), if a PF is lower than 1, it indicates the reduction of a pesticide, while if higher than 1, it indicates a concentration in regulatory practice, regardless of changes in volume or weight for the processed food (due to dilution, removal or degradation). According to the Official Journal of the European Union (2020)a and the Official Newspaper of the Republic of Macedonia (2018), the maximum residue levels (MRL) for chlorpyrifos in apples is 0.01 mg.kg$^{-1}$. Fig. 3 shows the concentration of the pesticide chlorpyrifos in all analyzed samples compared to maximum residue level.

Fresh (unwashed) apples from location 1 have the chlorpyrifos concentration that is 3.45 times lower than MRL, while unwashed apples from location 2 have the chlorpyrifos concentration of 2.87 times lower than MRL. Washed apples from locations 1 and 2 have the chlorpyrifos concentration lower by 9.54 and 7.04 times than MRL, respectively. Peeled apples from both locations as well as apple juice were found to contain less than 0.0005 mg.kg$^{-1}$ of chlorpyrifos that is 20 or more times less than MRL.

In all tested samples, the concentration of the pesticide chlorpyrifos are within the MRL which means that apples/apple products are safe for consumption. These observations are in good agreement with other studies conducted (Dömötörová et al., 2006; Kovalczuk et al., 2008; Dasika et al., 2012; Yang et al., 2017; Rahman et al., 2021).

## 4 Conclusions

In this study, the presence of pesticide chlorpyrifos was analyzed in fresh and processed apples. Namely, the influence of the mechanical and thermal treatment of the apples of the Red Delicious variety on the concentration of the pesticide was monitored. Chlorpyrifos was detected in all samples before any treatment, but in an allowed limit concentration. After processing of the apples, the concentration of chlorpyrifos decreases to a value that is in the detection range of the instrumental technique, from which one can conclude that any processing method of apples has an impact on the content of chlorpyrifos. The greatest decrease in the concentration of chlorpyrifos was observed during the heat treatment of the apples, i.e. in the prepared apple juice. Also, the mechanical preparation of apple samples affects the reduction of the pesticide content while the washing of the apples has the least impact. Additional research is needed on this topic or by expanding the analysis to other types of pesticides used for protection on apple trees.

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**Research Article**

# Assessment of genetic variability among accessions of okra (*Abelmoschus esculentus* L. Moench)

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Okra yields are low in West and Central Africa and factors including narrow genetic base of available germplasm have been implicated. An assessment of genetic variability among germplasm from various sources and knowledge of trait contributions to these variations is crucial to the success of okra breeding program. Eighteen okra accessions were evaluated during the 2020 cropping season in Nigeria to assess the genotypes for genetic diversity, group the accessions into clusters and identify traits that chiefly accounted for the variations among the genotypes. Data obtained were subjected to analysis of variance (ANOVA), metroglyph and principal component analyses (PCA). ANOVA revealed significant accession mean squares for majority of the measured traits. Metroglyph analysis grouped the accessions into four clusters with members of Cluster IV generally high-yielding, early-maturing and short genotypes. NGB00353 and NGB00356 that were among genotypes with high total index scores were members of Cluster IV. The first four principal components (PCs) accounted for 80% of the total observed variation. PC1 with the highest discriminatory power was loaded with days to budding, internode length, pod yield.plot$^{-1}$ and the number of pods.plant$^{-1}$. The variation within and between clusters could be explored in okra breeding program.

**Keywords:** clusters, early-maturing, high yield, okra germplasm, variations

## 1 Introduction

Okra is one of the most important fruit vegetable crops and a source of calorie for human consumption (Nwangburuka et al., 2012). It is a multipurpose vegetable due to the various uses of the fresh leaves and immature fruits which are consumed as vegetables, salads, soup and stew (Rashwan, 2011). Though okra worldwide production and total area of cultivation area has increased over the years, low yields are still being recorded on farmers' fields particularly in West Africa (Olayiwola et al., 2015). Ahiakpa et al. (2013) identified lack of adapted cultivars, narrow genetic base of germplasm collections as well as disease and pest incidence to be responsible for the relatively lower yields in the sub-region. Kumar et al. (2010) and Alake et al. (2012) advocated for the need to develop new and superior genotypes to replace existing older and low-yielding genotypes. Plant breeders select and develop superior genotypes from existing variability within the germplasm. Omonhinmin and Osarawu (2005) reported high genetic variability within *Abelmoschus* spp. However, Kumar et al. (2010) noted that much of the variability was yet to be explored due to the relatively lower breeding activities on okra. A detailed understanding of the magnitude and pattern of genetic variability is required for the improvement of any crop. Plant breeders have thus employed several multivariate techniques including Metroglyph and Principal Component Analyses (PCA) to profile the variability within the germplasm of different crops (Aremu, 2011; Nwangburuka et al., 2011; Osarawu et al., 2013). Anderson (1960) proposed Metroglyph analysis, a technique that reveals patterns of morphological variation in crop species by reducing the complex inter-relationship among accessions into a pictorial scatter

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diagram showing clusters based on relatedness and genotype superiority based on trait profiles. Olayiwola et al. (2015) concluded that information from metroglyph analysis could guide breeders on the choice of parents for hybridization. PCA partitions total variation into components and identifies trait(s) that chiefly account(s) for the observed variability among genotypes (Bhusal et al., 2016). The current study aimed to determine the relationships among the evaluated okra accessions and identify trait(s) responsible for the variations among the genotypes.

## 2 Materials and methods

### 2.1 Experimental site and planting materials

The study was carried out at the Teaching and Research Farm of College of Agricultural Sciences, Yewa Campus (7° 12′ N; 3° 3′ E), Olabisi Onabanjo University. Eighteen (18) accessions of okra were sourced from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan.

### 2.2 Field preparation, layout and evaluation

The land was cleared, ploughed and harrowed. The experiment was laid out in a Randomized Complete Block Design and was replicated thrice. Each of the accessions was sown on a single-row plot that was 4.5 m long. The inter-row spacing was 0.45 m while within-row spacing was 0.45 m giving a total of eleven plants per plot. Two seeds were planted per hole but were later thinned to one per stand. All recommended agronomic practices required for optimum performance of the crop were followed.

### 2.3 Data collection and analysis

Observations were made on days to budding, days to 50% Flowering, plant height at flowering, internode length (cm), number of branches/plant, days to maturity, plant height (cm), number of pods, pod width (cm), pod length (cm), pod weight (kg) and pod yield.plot$^{-1}$ (kg)

The data were subjected to Analysis of Variance (ANOVA) and significantly different means were separated by Duncan multiple range test (DMRT) on SAS Institute Inc. (2001). Furthermore, data were subjected to Metroglyph analysis (Anderson, 1960) as earlier described by Olayiwola et al. (2015) and Adeniji et al. (2020). Briefly, coefficient of variation (CV) was determined for each of the traits. Two traits with the highest CV were used as coordinates (*Y*- and *X*-axes) on the scatter diagram and the point of intersection was the marker (glyph) for each accession. The other traits were then represented by rays at different points on the glyph. Rays for a particular trait maintained similar positions on each glyph. The range of values for each trait was grouped into three classes with each class having an associated index score. The index score determined the length of the rays and total sum of the scores represented the value or worth of the evaluated accessions for the measured traits. Based on positions on the scatter diagram the accessions were grouped into clusters. Data were also subjected to principal component analysis to identify the traits that mostly accounted for the total variation observed.

## 3 Results and discussion

The significant accession mean squares for most of the measured characters (Table 1) underscored the large genetic divergence among the okra accessions (Nwangburuka et al., 2012). This variability could thus be explored to develop superior genotypes. The highest coefficients of variation (CVs) were associated with pod yield and number of pods.plant$^{-1}$ (Table 2). The two traits thus the *Y* and *X* axis of the metroglyph scatter diagram (Adeniji et al., 2020). NGB00355 was the earliest to flower and was significantly different from NGB00314 and three other accessions (Table 3). This implies that NGB00355 could be a potential source of alleles for earliness in okra breeding programme (Olayiwola et al., 2015). NGB00644 had the tallest plants and could therefore be targeted for tallness in a breeding scheme. NGB00353 had the highest yield among the accessions and could thus be considered

**Table 1** Analysis of variance of nine characters measured on eighteen accessions of okra

| Sources of variation | *Df* | Days to 50% flowering | Plant height | Plant height at flowering | Days to budding | Days to podding | Number of branches.plant$^{-1}$ | Internode length | Number of pods.plant$^{-1}$ | Pod length | Pod width | 1000-seed weight | Pod yield.plot$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Rep** | 2 | 73.11 | 3,687.22** | 2,107.39 | 58.68 | 21.85 | 1.19 | 18.21 | 40.07 | 73.58 | 146.51 | 0.99 | 16,673.65 |
| **Accession** | 17 | 133.78 | 3,907.88** | 1,507.14 | 80.11 | 150.72** | 3.09 | 7.65* | 360.86** | 83.63 | 146.83 | 6.71 | 83, 119.04** |
| **Error** | 34 | 78.81 | 429.12 | 842.68 | 64.88 | 53.98 | 1.72 | 3.21 | 87.07 | 83.16 | 134.89 | 5.48 | 22,260.72 |

*.**: significant at $P \leq 0.05$ and 0.01, respectively

**Table 2** Range, mean, coefficient of variation in percentage (CV (%)), index scores and position of characters on the glyph

| | Range | Mean | CV (%) | Index scores | | | Positions |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | |
| **Days to 50% flowering** | 56.00-73.93 | 64.19 | 8.84 | 56.00–63.08 | 63.09–70.17 | ≤73.93 | ↑ |
| **Plant height** | 66.07–176.17 | 113.57 | 30.98 | 66.07–102.76 | 102.76–139.46 | ≤176.17 | ↓ |
| **Plant height at flowering** | 49.99–120.77 | 80.00 | 27.23 | 49.99–73.57 | 73.57–97.19 | ≤120.77 | ↗ |
| **Days to budding** | 47.17–64.10 | 55.19 | 8.75 | 47.17–52.81 | 52.81–58.45 | ≤64.10 | ↖ |
| **Days to podding** | 56.53–78.33 | 67.71 | 9.47 | 56.53–63.43 | 63.43–70.88 | ≤78.33 | ← |
| **Number of branches.plant$^{-1}$** | 4.83–8.17 | 6.25 | 15.76 | 4.83–5.93 | 5.94–7.05 | ≤8.17 | → |
| **Internode length** | 4.17–9.50 | 6.95 | 22.33 | 4.17–5.94 | 5.95–7.71 | ≤9.50 | ↘ |
| **Number of pods.plant$^{-1}$** | 3.67–39.00 | 16.17 | 65.94 | 3.67–15.44 | 15.45–27.22 | ≤39.00 | *y*-axis |
| **Pod length** | 6.40–29.67 | 8.83 | 58.09 | 6.40–14.15 | 14.16–21.90 | ≤29.67 | ↙ |
| **Pod width** | 8.23–11.90 | 11.73 | 57.96 | 8.23–9.44 | 9.45–10.67 | ≤11.90 | ↙ |
| **1000-seed weight** | 12.63–18.90 | 15.4 | 9.45 | 12.63–14.71 | 14.72–16.80 | ≤18.90 | ← |
| **Pod yield.plot$^{-1}$** | 56.00–520. 83 | 256.55 | 63.05 | 56.00–210.93 | 210.94–365.89 | ≤520.83 | *x*-axis |





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**Table 3** Mean performance of the evaluated 18 okra accessions with their index scores for each character in parenthesis

| Accession | Days to 50% flowering | Plant height | Plant height at flowering | Days to budding | Days to podding | Number of branches.plant$^{-1}$ | Internode length | Number of pods.plant$^{-1}$ | Pod length | Pod width | 1000-seed weight | Pod yield.plot$^{-1}$ | Total index score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **NGB00314** | 72.27$^{a}$(-3) | 155.03$^{ab}$(2) | 104.93$^{ab}$(3) | 61.27$^{ab}$(-3) | 77.70$^{a}$(-3) | 6.67$^{a-d}$(2) | 9.50$^{a}$(3) | 11.33$^{c-e}$(1) | 7.90$^{b}$(1) | 39.50$^{a}$(1) | 17.17$^{a-c}$(3) | 188.9$^{b-e}$ (1) | 8 |
| **NGB00342** | 60.33$^{a-d}$(-1) | 102.57$^{c}$-f(2) | 96.20$^{ab}$(3) | 56.00$^{ab}$(-2) | 75.65$^{ab}$(-3) | 5.00$^{d}$(2) | 7.67$^{a-d}$(1) | 4.0$^{e}$(1) | 7.50$^{b}$(3) | 10.70$^{b}$(1) | 14.00$^{bc}$(1) | 56.0$^{e}$(1) | 9 |
| **NGB00380** | 73.93$^{a}$ (-3) | 174.50$^{a}$(3) | 120.77$^{a}$(3) | 64.10$^{a}$(-3) | 78.33$^{a}$(-3) | 7.33$^{a-d}$(3) | 9.50$^{a}$(1) | 3.67$^{e}$(1) | 8.40$^{b}$(1) | 10.30$^{b}$(2) | 15.03$^{a-c}$(2) | 68.9$^{e}$(1) | 8 |
| **NGB00387** | 73.60$^{a}$ (-3) | 97.27 $^{c}$-f (1) | 105.50$^{ab}$(3) | 60.67$^{ab}$(-3) | 75.75$^{ab}$(-3) | 5.75$^{a-d}$(1) | 7.57$^{a-d}$(2) | 4.50$^{e}$(1) | 7.10$^{b}$(1) | 10.75$^{b}$(3) | 15.15$^{a-c}$(2) | 68.1$^{e}$(1) | 6 |
| **NGB00302** | 72.77$^{a}$ (-3) | 121.40$^{bc}$(2) | 119.27$^{a}$(3) | 62.83$^{ab}$(-3) | 75.50$^{ab}$(-3) | 6.17$^{a-d}$(2) | 8.50$^{a-c}$(3) | 7.33$^{de}$(1) | 6.40$^{b}$(1) | 8.23$^{b}$(1) | 12.63$^{c}$(1) | 119.7$^{de}$(1) | 6 |
| **NGB00373** | 68.70 $^{a-d}$ (-2) | 87.57 $^{c}$-f (1) | 76.30$^{ab}$(2) | 55.20$^{ab}$(-2) | 69.17$^{a-c}$(-2) | 8.17$^{a}$(3) | 5.50$^{b-d}$(1) | 24.67$^{a-d}$(2) | 6.87$^{b}$(1) | 9.20$^{b}$(1) | 15.57$^{a-c}$(2) | 373.9$^{a-d}$(3) | 10 |
| **NGB00297** | 67.37 $^{a-d}$ (-2) | 66.07f(1) | 52.77$^{b}$(1) | 55.267$^{ab}$(-2) | 66.03$^{a-c}$(-2) | 6.67$^{a-d}$(2) | 4.17$^{d}$(1) | 21.33$^{a-e}$(2) | 7.17$^{b}$(1) | 11.90$^{b}$(3) | 16.43$^{a-c}$(2) | 319.6$^{a-e}$(2) | 9 |
| **NGB00298** | 60.75 $^{a-d}$ (-1) | 164.00$^{a}$(3) | 67.50$^{ab}$(1) | 58.50$^{ab}$(-3) | 66.75$^{a-c}$(-2) | 5.50$^{b-d}$(1) | 9.0$^{ab}$(3) | 4.0$^{e}$(1) | 7.50$^{b}$(1) | 11.00$^{b}$(3) | 14.00$^{bc}$(1) | 72.0$^{e}$(1) | 9 |
| **NGB00421** | 65.67 $^{a-d}$ (-2) | 108.93$^{c-e}$(2) | 61.27$^{b}$(1) | 54.20$^{ab}$(-3) | 67.93$^{a-c}$(-2) | 5.33$^{cd}$(1) | 6.167$^{a-d}$(2) | 9.67$^{c-e}$(1) | 10.50$^{b}$(1) | 10.33$^{b}$(2) | 15.83$^{a-c}$(2) | 151.0$^{de}$(1) | 6 |
| **NGB00323** | 62.43 $^{a-d}$ (-1) | 76.43$^{d}$-f(1) | 53.07$^{b}$(1) | 51.70$^{ab}$(-1) | 68.93$^{abc}$(-2) | 6.17$^{a-d}$(2) | 5.50$^{b-d}$(1) | 24.00$^{a-d}$(2) | 7.40$^{b}$(1) | 11.20$^{b}$(3) | 13.73$^{bc}$(1) | 326.1$^{a-e}$(2) | 10 |
| **NGB00356** | 61.60 $^{a-d}$ (-1) | 88.70$^{c}$-f(1) | 73.07(1) | 47.37$^{b}$(-1) | 63.33$^{bc}$(-1) | 8.00$^{ab}$(3) | 5.33$^{cd}$(1) | 30.67$^{ab}$(3) | 7.40$^{b}$(1) | 9.27$^{b}$(1) | 15.47$^{a-c}$(2) | 462.5$^{a-c}$(3) | 13 |
| **NGB00499** | 61.60 $^{a-d}$ (-1) | 88.70$^{c}$-f(1) | 92.22$^{ab}$(2) | 52.00$^{ab}$(-1) | 63.27$^{bc}$(-1) | 4.83$^{d}$(1) | 6.67$^{a-d}$(2) | 25.67$^{a-c}$(2) | 6.90$^{b}$(1) | 11.53$^{b}$(3) | 14.77$^{a-c}$(2) | 378.2$^{a-d}$(3) | 14 |
| **NGB00644** | 61.27$^{a-d}$(-1) | 176.17$^{a}$(3) | 68.57$^{ab}$(1) | 54.67$^{ab}$(-2) | 64.33$^{a-c}$(-2) | 7.75$^{a-c}$(3) | 8.33$^{a-c}$(3) | 5.3$^{e}$(1) | 8.33$^{b}$(1) | 10.00$^{b}$(2) | 17.57$^{ab}$(3) | 91.8$^{de}$(1) | 13 |
| **NGB00353** | 60.80$^{a-d}$(-1) | 97.47$^{c}$-f(1) | 70.97$^{ab}$(1) | 52.50$^{ab}$(-1) | 67.23$^{a-c}$(-2) | 6.167$^{a-d}$(2) | 6.33$^{a-d}$(2) | 26.33$^{a-c}$(2) | 6.90$^{b}$(1) | 9.50$^{b}$(2) | 18.90$^{a}$(3) | 520.8$^{a}$(3) | 13 |
| **NGB00412** | 60.33$^{a-d}$(-1) | 72.77$^{e}$f(1) | 49.99$^{b}$(1) | 52.50$^{ab}$(-1) | 62.57$^{bc}$(-1) | 5.33$^{cd}$(1) | 5.17$^{cd}$(1) | 24.367$^{a-d}$(2) | 7.27$^{b}$(1) | 9.33$^{b}$(1) | 15.53$^{a-c}$(2) | 478.3$^{ab}$(3) | 10 |
| **NGB00477** | 59.43$^{b-d}$(-1) | 93.37$^{c}$-f(1) | 93.30$^{ab}$(2) | 50.33$^{ab}$(-1) | 61.57$^{bc}$(-1) | 6.167$^{a-d}$(2) | 6.00$^{a-d}$(2) | 9.67$^{c-e}$(1) | 29.67$^{a}$(3) | 9.60$^{b}$(2) | 15.40$^{a-c}$(2) | 197.4$^{b-e}$(1) | 13 |
| **NGB00396** | 56.63$^{cd}$(-1) | 114.63$^{cd}$(2) | 67.27$^{ab}$(1) | 47.167$^{b}$(-1) | 56.53$^{c}$(-1) | 5.33$^{cd}$(1) | 6.33$^{abcd}$(1) | 39.00$^{a}$(2) | 8.53$^{b}$(1) | 9.10$^{b}$(1) | 14.17$^{bc}$(2) | 505.2$^{a}$(3) | 11 |
| **NGB00355** | 56.00$^{d}$(-1) | 158.73$^{a}$(3) | 67.15$^{ab}$(1) | 52.75$^{ab}$(-1) | 58.15$^{c}$(-1) | 6.167$^{a-d}$(2) | 7.87$^{a-c}$(3) | 15.50$^{b-e}$(1) | 7.25$^{b}$(1) | 9.70$^{b}$(2) | 15.85-$^{c}$(2) | 239.5$^{a-e}$(2) | 14 |

Means with different letters are significantly different from one another at $p \leq 0.05$ using DMRT. Negative index score indicates preference for lower values for the corresponding traits

**Table 4** Trait loading, eigen value, proportion of variance explained, and cumulative variance obtained from principal component analysis

| Character | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| **Days to 50% flowering** | 0.36 | 0.11 | -0.38 | 0.08 |
| **Plant height** | 0.28 | 0.02 | 0.53 | -0.34 |
| **Plant height at flowering** | 0.33 | -0.03 | -0.14 | 0.26 |
| **Days to budding** | 0.40 | -0.02 | -0.18 | -0.08 |
| **Days to podding** | 0.25 | 0.39 | -0.30 | 0.24 |
| **Number of branches** | 0.03 | 0.51 | 0.18 | 0.22 |
| **Internode spacing** | 0.38 | -0.04 | 0.26 | -0.27 |
| **Number of pods.plant$^{-1}$** | -0.37 | 0.21 | -0.18 | -0.15 |
| **Pod length** | -0.04 | -0.22 | 0.34 | 0.77 |
| **Pod width** | 0.18 | 0.38 | 0.02 | -0.05 |
| **1000-seed weight** | -0.06 | 0.52 | 0.40 | 0.06 |
| **Pod yield** | -0.37 | 0.26 | -0.14 | -0.09 |
| **Eigenvalue** | 5.26 | 1.82 | 1.41 | 1.12 |
| **Proportion of variance explained** | 0.44 | 0.15 | 0.12 | 0.09 |
| **Cumulative variance** | 0.44 | 0.59 | 0.71 | 0.80 |

as a source of high-yielding genotypes. The superiority or desirability of a genotype is more reliably determined if considered across several traits rather than a single trait (Yan & Fregeau-Reid, 2018). In our study, the total index scores reflect the genotypic values of the accessions across all traits of interest. NGB00355 and NGB00499 had the joint highest index score and were jointly followed by NGB00477, NGB00353, NGB00356 and NGB00644. Among the accessions with top index scores NGB00353 and NGB00356 combined earliness to flowering with high-yield thus indicating the desirability of the genotypes among the evaluated accessions. Though the accessions with the highest index scores (NGB00355 and NGB00499) also flowered early, they only had low to moderate pod yield. This re-emphasized the need to allot appropriate weights to plant characters in the development of selection index. However, the accessions with the lowest total index scores NGB00302, NGB00387 and NGB00421 were medium- to late-maturing and had low yields. This underscored the relative ability of the technique to discriminate among accessions based on field performance. Number of pods.plant$^{-1}$ and that had the highest CVs were used as the X and Y coordinates in plotting the metroglyph (Fig. 1). NGB00380 and NGB00396 exhibited the largest divergence implying that the genetic differences between the genotypes could be explored for heterotic breeding (Ranpise et al., 2018). The metroglyph classified the accessions into four clusters and thus indicated that the technique captured the variations among the accessions (Khalid et al., 2018). Cluster I had nine members that were generally low-yielding but an admixtures of tall and short accessions as well as genotypes. Two of the nine members of this group were among the accessions with top total index scores across all measured traits. The within group variations could be explored either by selection or by developing composites with improved attributes that could arise from transgressive segregation (Shujaat et al., 2014). Cluster II had one member that was tall, early-maturing and relatively than members of Cluster I. The four members of Cluster III were evenly divided between low- and medium-yielding as well as early- and medium-maturing genotypes. The within group variation could also be explored in okra improvement programme. Cluster IV had four members that were generally high-yielding, early-maturing and short genotypes. Members of this group were generally associated with high total index scores indicating their potential as sources of favourable alleles for the development of quality pure lines and elite populations. The differences between groups could also be explored to develop superior hybrids and composites that could be used to broaden the genetic base of adapted germplasm. The principal component analysis which detailed the trait contributions to the variation among the accessions was presented in Table 4. The first four principal components (PCs) had significant discriminatory powers based on the eigen values (>1) and accounted for 80% of the total variation. PC1 with the highest discriminatory power explained more than half of the total variation that was accounted for by the four PCs. The next components, PC2, PC3 and PC4, when combined explained less than

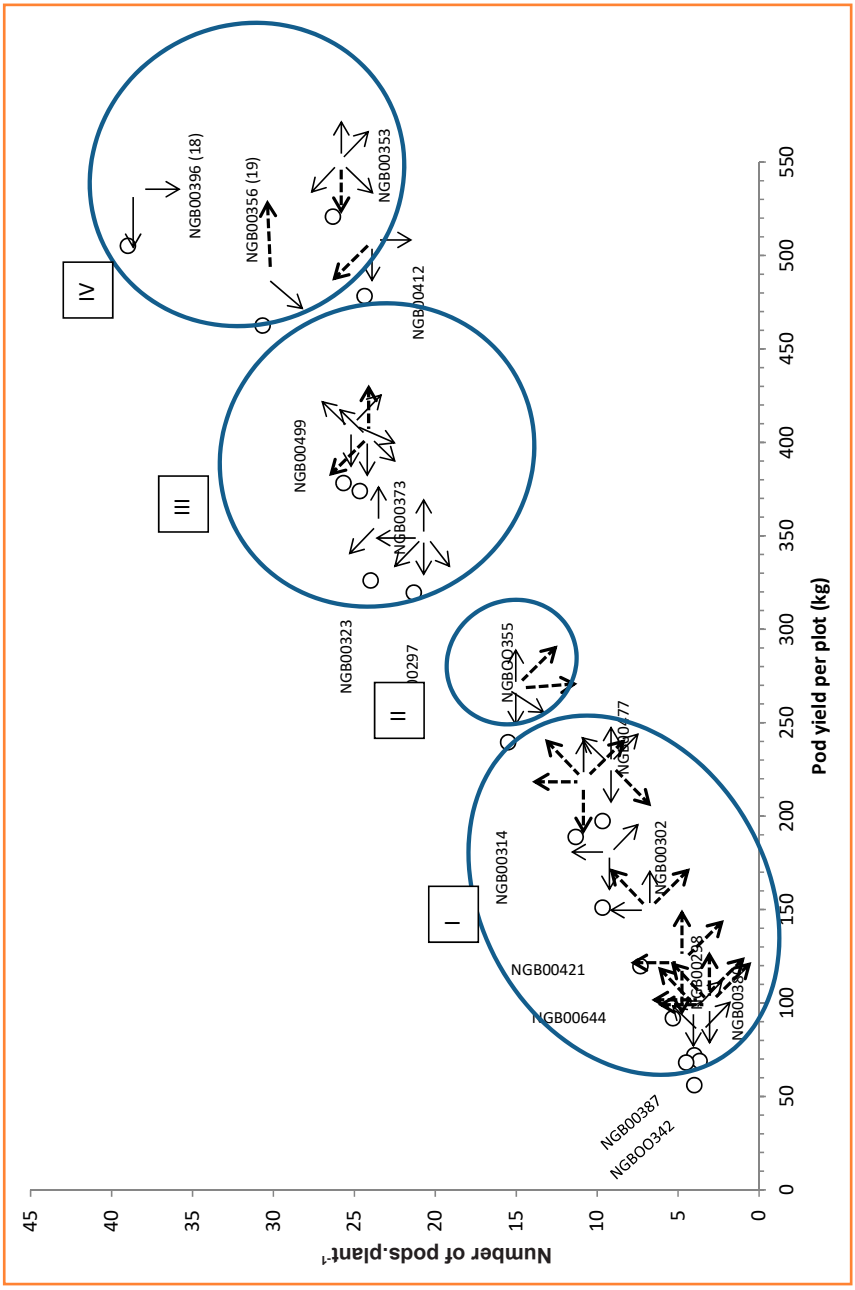


**Figure 1** Scatter diagram from Metroglyph analysis showing the classification of the eighteen genotypes into four clusters

half of the total variation captured by the four components. This implies that loadings on the other three components must be considered with caution. PC1 was loaded with days to budding, internode length, pod yield.plot$^{-1}$ and the number of pods.plant$^{-1}$. This implied that these traits chiefly contributed to the variations observed among the accessions and therefore could be targeted for developing selection index in okra breeding program.

## 4 Conclusion

There was substantial genetic variation among the eighteen accessions of okra for the measured characters. Some accessions combined earliness to maturity with high-yielding potential and would be valuable sources of favourable alleles for okra improvement. Accessions classified into clusters based on phenotypic similarities and variation within and between clusters could be explored through selection or heterosis in okra breeding program. Four traits were identified to have contributed substantially to the observed variation among the genotypes and could be considered in the development of efficient selection index.

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**Research Article**

# Biological activity of essential oil from *Foeniculum vulgare*

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*Foeniculum vulgare* Mill. is a medicinal plant, used as a flavouring agent. The essential oil from *F. vulgare* has potential antimicrobial and insecticidal effects, and can be used in food industry in order to protect the food resources and food products against microbial and pest's contamination. The aim of the research was to characterize the volatile components of *F. vulgare* essential oil by Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography (GC-FID) and to observe the antimicrobial activity by disk diffusion method and in vapour phase. Also, insecticidal activity of the vapour phase of the essential oil of *F. vulgare* was detected. We found that major components of the essential oil from *F. vulgare* were *trans*-anethole (73.6%), fenchone (6.0%), and limonene (5.7%). Antimicrobial activity on gram-positive, gram-negative, and yeasts was weak in liquid phase, but vapour phase showed stronger activity against *B. subtilis* at the concentration 250 µL.L$^{-1}$ (98.65% of bacterial growth inhibition). Vapour phase of essential oil was effective against insects, where 25% concentration had 80% lethality.

**Keywords:** *Foeniculum vulgare*, antimicrobial activity, essential oil, vapour phase

## 1 Introduction

*Foeniculum vulgare* Mill., commonly called the fennel, is a perennial plant that belongs to carrot family Apiaceae. The medicinal plant with yellow flowers, aromatic seeds and white fruits is widely used as a flavouring agent of foods and beverages for its characteristic aroma (Rather et al., 2016). Fennel is used in traditional medicine for many years due to beneficial antioxidant, anti-inflammatory and analgesic effects (Choi & Hwang, 2004). The fennel is full of phytochemicals, especially volatile compounds extracted to essential oils (EOs) (Badgujar et al., 2014). *Foeniculum vulgare* EOs have hepatoprotective and antidiabetic properties on *in vivo* models (Abou et al., 2011; Özbek et al., 2003). With the high concentration of *trans*-anethole, EOs from *F. vulgare* possess a strong antioxidant activity (Shahat et al., 2011). The antimicrobial activity against gram-positive, gram-negative, and fungi was observed, thus volatile compounds of fennel EO can be used in food preservation (Diao et al., 2014; Mimica-Dukić et al., 2003). Insecticidal activity against *Trogoderma granarium* (Ghanem et al., 2014), *Brevicoryne brassiceae* (Lucca et al., 2015), *Acyrthosiphon pisum*, and *Myzus persicae* (Digilio et al., 2008) was also observed.

Nowadays, the use of natural substances over the artificial ones has been increasing in order to preserve food resources against pathogens, both microscopic ones and pests. Essential oils have various positive biological effects that can be applicable in food preservation. Due to the volatility of the substances, essential oils can be applied without the direct contact with a food resource, thus addition of natural food preserver will not change the chemical or sensory properties of the foods.

The aim of this study was to characterize the essential oil from *F. vulgare* Mill var. dulce from a Slovak company to obtain the chemical composition of the EO from

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*F. vulgare* and to observe the activity of the gas phase against pathogenic microorganisms on the carrot used as a food model. Also, the insecticidal activity against the *Pyrrhocoris apterus* in the gas phase was detected.

## 2 Material and methods

### 2.1 Essential oil

EO prepared from *Foeniculum vulgare* Mill var. dulce was purchased from Hanus, s.r.o. (Nitra, Slovakia) and was extracted by steam distillation of dried fruits. It was stored in the dark at 4 °C throughout the analyses.

### 2.2 Tested microorganisms

Microorganisms (*Bacillus subtilis* CCM 1999, *Pseudomonas aeruginosa* CCM 3955, *Yersinia enterocolitica* CCM 7204, *Staphylococcus aureus* subsp. *aureus* CCM 8223, *Enterococcus faecalis* CCM 4224, *Salmonella enteritidis* subsp. *enteritidis* CCM 4420, *Candida krusei* CCM 8271, *Candida albicans* CCM 8261, *Candida tropicalis* CCM 8223, and *Candida glabrata* CCM 8270) were obtained from the Czech collection of microorganisms. The biofilm-forming bacteria *Bacillus subtilis* and *Stenotrophomonas maltophilia* were obtained from the dairy industry and identified with 16S rRNA sequencing and MALDI-TOF MS Biotyper.

### 2.3 Chemical characterization of essential oil by gas chromatography/mass spectrometry (GC/MS) and gas chromatography (GC-FID)

GC/MS analysis of the EO sample was performed using Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA). A HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used. The temperature program was set from 60 °C to 150 °C (increasing rate 3 °C.min$^{-1}$) and 150 °C to 280 °C (increasing rate 5 °C.min$^{-1}$). The total running time was 60 min. Helium 5.0 was used as the carrier gas with the flow rate of 1 mL.min$^{-1}$. The injection volume was 1 µL (the EO sample was diluted in pentane), while the split/splitless injector temperature was set at 280 °C. The sample was injected in the split mode with the 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. MS ion source temperatures was 230 °C and MS quadrupole temperature was 150 °C. Acquisition of data started after solvent delay time of 3 min. GC-FID analyses were performed on Agilent 6890N gas chromatograph coupled to FID detector. Column (HP-5MS) and chromatographic conditions were the same as for GC/MS. The temperature of the FID detector was set at 300 °C.

The volatile constituents of samples were identified according to their retention indices (Adams, 2007) and they were compared with the reference spectra (Wiley and NIST databases). The retention indices were experimentally determined by a standard method described by (van Den Dool & Dec Kratz, 1963) and 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 the GC peak areas.

### 2.4 Disk diffusion method

The antimicrobial activity of *F. vulgare* EO was determined using the disk diffusion method. Cultivation of microorganisms was performed aerobically for 24 h on Tryptone soy agar (TSA). The bacteria were incubated at 37 °C and the yeasts at 25 °C. The inoculum was prepared to an optical density of 0.5 McFarland (1.5 × 10$^{8}$ CFU. mL$^{-1}$). 100 µL of inoculum was spread on Petri dishes (PD) with Mueller Hinton agar. A sterile 6 mm diameter paper blank disks were placed on the PD and, subsequently, 10 µL of 100% *F. vulgare* EO was applied. The prepared PDs was incubated aerobically for 24 h, the bacteria at 37 °C and the yeast at 25 °C. The criteria for detecting inhibitory activity were: inhibition zone diameter below 5 mm – very weak inhibitory activity, above 5 mm – weak inhibitory activity, above 10 mm – medium inhibition and above 15 mm – very strong inhibitory activity. Each test was repeated three times. The measurement was performed in triplicate, the mean and the standard deviation was calculated.

### 2.5 Antimicrobial activity of the vapour phase of the Essential oil

The *in situ* antimicrobial effect of *F. vulgare* EO against biofilm-forming microorganisms *B. subtilis* and *S. maltophilia* was evaluated on carrots. The carrots were cut into 5 mm slices, washed with distilled water, and left to dry for 15 minutes at room temperature. A thin layer of Mueller Hinton agar (MHA) was poured into 60 mm diameter PDs and their lids. The individual carrot slices were placed on solidified MHA. A bacterial inoculum with an optical density 0.5 McFarland (1.5 × 10$^{8}$ CFU.mL$^{-1}$) was prepared. The inoculum was applied by three stabs into the carrot slice. *F. vulgare* essential oil was diluted in 100% ethyl acetate to concentrations 500, 250, 125, and 62.5 µL.L$^{-1}$. A circle of sterile 55 mm diameter filter paper was placed on the solidified MHA in the lid and 100 µL of the appropriate concentration of essential oil was applied on the filter paper. 100 µL of ethyl acetate was used as a negative control. The ethyl acetate was evaporated during the 1 minute and, subsequently, the PDs were hermetically sealed. The samples were incubated at 37 °C for 7 days.

The antimicrobial effect of *F. vulgare* EO was evaluated using a stereological method. The bulk density (Vv) of the bacterial colonies was estimated using ImageJ software. The *Vv* was calculated using the formula *Vv* (%) = *P*/*p* × 100, where *P* represents the stereological grid points where the bacterial colonies were grown, and p represents all the stereological grid points on the substrate. The volume density of the colonies was the percentage inhibition of *F. vulgare* EO calculated using the formula BGI = [(*C* – *T*)/*C*] × 100, where *C* is the bulk density of the bacterial colonies in the control group and *T* is the bulk density of the colonies in the treated samples.

### 2.6 Vapour phase insecticidal activity

The insecticidal activity of *F. vulgare* EO was tested using *Pyrrhocoris apterus*. The EO was diluted in 0.1% polysorbate solution. The concentrations of 25, 12.5, and 6.25% were tested. 0.1% polysorbate was used as a negative control. 30 individuals of *P. apterus* were placed in 90 mm PD with vents. A circle of sterile filter paper was placed into the PD lid. 100 µL of the appropriate concentration of EO was applied to the filter paper and the plates were sealed with parafilm. *P. apterus* were exposed to EO vapours for 24 h at room temperature. After exposure, live and dead subjects were counted, and the percentage of insecticidal activity was calculated.

## 3 Results and discussion

### 3.1 Chemical composition of the EO

The GC/MS and GC-FID analyses showed (Table 1) that the major component of *F. vulgare* EO was *trans*-anethole with 73.6%, fenchone with 6.0%, and limonene with 5.7%. Trans-anethole is the major component of the EOs from *F. vulgare*, and fenchone, estragole, and limonene have been also reported as main components of essential oils derived from the seeds of *F. vulgare* (Belabdelli et al., 2020; Diao et al., 2014; El-Nasr et al., 2013). The content of the individual components of the essential oil can be influenced by geographical and environmental factors (Piccaglia & Marotti, 2001) Also, the extraction method can affect the chemical composition of the essential oil (Bagherifard et al., 2014).

**Table 1** Chemical composition of *F. vulgare* EO

| No | RI | Identified compounds | (%) |
|---|---|---|---|
| **1** | 938 | α-pinene | 4.4 |
| **2** | 980 | β-pinene | 0.4 |
| **3** | 992 | β-myrcene | 0.5 |
| **4** | 1004 | α-phellandrene | 1.7 |
| **5** | 1023 | *p*-cymene | 0.3 |
| **6** | 1028 | α-limonene | 5.7 |
| **7** | 1030 | β-phellandrene | 1.7 |
| **8** | 1060 | γ-terpinene | 0.2 |
| **9** | 1085 | fenchone | 6.0 |
| **10** | 1195 | methyl chavicol | 4.2 |
| **11** | 1252 | *p*-anisaldehyde | 0.6 |
| **12** | 1284 | *trans*-anethole | 73.6 |
| **Total** | | | 99.3 |

RI – values of retention indices on HP-5MS column; compounds identified in amounts higher than 0.1%

### 3.2 Antimicrobial activity

The results of the disk diffusion method are shown in Table 2. Only very weak to weak inhibitory activity was observed for all tested microorganisms. Among the all tested microorganisms, the inhibition of *B. subtilis* was the most pronounced one.

Garzoli et al. (2018) reported that due to the increased content of estragole, limonene and fenchone, the essential oil of *F. vulgare* has an inhibitory effect on the growth of the genus *Candida*. In our study, despite the presence of these components in the tested essential oil, we did not observe any significant antimicrobial activity against the genus *Candida*. Alzoreky and Nakahara (2003) found in their work that the extracts obtained from dried seeds of *F. vulgare* show weak antimicrobial activity. Differences in the composition and percentage of active ingredients in EO (Bozin et al., 2006), species, subspecies or diversity of plants, geographical locations, methods of collection, drying and extraction in the production of essential oils can greatly affect the antimicrobial

**Table 2** Disk diffusion method in the *F. vulgare* EO against G+, G- bacteria, and yeasts (inhibition zones in mm)

| Microorganism | Zone inhibition |
|---|---|
| ***Bacillus subtilis*** | 7.78 ±1.39 |
| ***Pseudomonas aeruginosa*** | 3.00 ±0.71 |
| ***Enterococcus faecalis*** | 4.67 ±0.87 |
| ***Yersinia enterocolitica*** | 4.67 ±0.87 |
| ***Salmonella enteritidis*** | 3.33 ±0.71 |
| ***Staphylococcus aureus*** | 3.56 ±1.01 |
| ***Candida glabrata*** | 5.00 ±0.87 |
| ***Candida krusei*** | 5.33 ±0.87 |
| ***Candida albicans*** | 5.89 ±1.27 |
| ***Candida tropicalis*** | 5.22 ±1.48 |
| ***Stenotrophomonas maltophilia* biofilm** | 3.56 ±1.01 |
| ***Bacillus subtilis* biofilm** | 7.89 ±0.93 |

**Table 3** Inhibitory activity of vapour phase of *F. vulgare* EO on carrot samples

| Bacterial growth inhibition (%) | | | | |
|---|---|---|---|---|
| **Microorganisms** | **concentration (µL.L$^{-1}$)** | | | |
| | **62.5** | **125** | **250** | **500** |
| ***Stenotrophomonas maltophilia*** | 2.15 | 23.98 | 5.21 | 14.44 |
| ***Bacillus subtilis*** | 0.29 | 1.48 | 98.65 | 100.00 |

**Table 4** insecticidal activity of vapour phase of *F. vulgare* EO against *P. apterus*

| **Concentration (%)** | **Number of living individuals** | **Number of dead individuals** | **Insecticidal activity (%)** |
|---|---|---|---|
| **25** | 6 | 24 | 80 |
| **12.5** | 12 | 18 | 60 |
| **6.25** | 18 | 12 | 40 |
| **Control group** | 30 | 0 | 0 |

properties of the essential oil (Burt, 2004; Sarac & Ugur, 2008).

### 3.3 Antimicrobial activity in vapour phase

The results of the antibacterial activity of the gas phase of *F. vulgare* essential oil on carrots are summarized in Table 3. *S. maltophilia* was maximally inhibited at 125 µL.L$^{-1}$ by 23.98%. The lowest inhibition was found at the concentration of 62.5 µL.L$^{-1}$. *B. subtilis* was inhibited by the concentration of 250 µL.L$^{-1}$ by 98.65% and at 500 µL.L$^{-1}$ the inhibition was at 100%. As with *S. maltophilia*, the lowest inhibition at 62.5 µL.L$^{-1}$ was only 0.29%.

The vapour phase method makes it possible to monitor the antimicrobial activity of exclusively volatile components of the essential oil. The vapour state can increase the antimicrobial effect at lower concentrations compared to contact antimicrobial activity of the liquid EO (Ács et al., 2018). Dorman and Deans (2000), Garzoli et al. (2021), and Nedorostova et al. (2009) confirmed the higher effectiveness of antimicrobial effects of the vapour phase than liquid phase of essential oils.

### 3.4 Insecticidal activity

The results of the insecticidal activity of the vapour phase of *F. vulgare* EO against *P. apterus* are shown in Table 4. All concentrations tested showed insecticidal activity. It was most pronounced at a concentration of 25% where we recorded the killing of 80% of individuals. At the concentration of 12.5%, we observed the killing of 60% of individuals, and at the concentration of 6.25%, 40% of individuals were killed.

Effective insecticidal activity against three types of insects was observed by Pavela et al. (2016). The insecticidal effect of the essential oil depends mainly on the substances that the essential oil contains (Bakkali et al., 2008). *F. vulgare* EOs can be used as insect control agents and can be useful in managing field plant populations (Kim et al., 2002).

## 4 Conclusions

The essential oil from *F. vulgare* showed good antimicrobial and insecticidal activity due to its volatile compounds. The major component was *trans*-anethole in quantity 73.6%. Fenchone, α-limonene, methyl chavicol, and α-pinene were also present. The vapour phase effectively inhibited the growth of the *B. subtilis*. Also, insecticide activity was observed against *P. apterus*. EO from *F. vulgare* has potential in use as natural supplement in food industry, due to ability to inhibit pathogenic microorganisms. Also, EOs can be potentially used on food resources and industrial crops, due to insecticide activity. Use of volatile compounds present in essential oils can be also advantage, because the sensory properties of the product will not be changed.

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