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African Journal of **Plant Science**

July 2022
ISSN 1996-0824
DOI: 10.5897/AJPS
www.academicjournals.org

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Vol. 16(6), pp. 111-124, July 2022
DOI: 10.5897/AJPS2022.2246
Article Number: 02A407C69431
ISSN 1996-0824

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African Journal of Plant Science

Full Length Research Paper

Identification and relative quantitative comparison of compounds in bottle gourds [*Lagenaria siceraria* (Mol.) Standl.]

Zhaohui Zhang[1], Xuan Du[2], Na Liu[2] and Chunhong Chen[1]*

[1]Shanghai Academy of Agricultural Sciences, Zhuanghang Comprehensive Experiment Station, Shanghai, 201403, China.
[2]The Protected Horticulture Institute, Shanghai Academy of Agricultural Sciences, Shanghai Key Laboratory of Protected Horticultural Technology, Shanghai, 201403, China.

Received 25 March, 2022; Accepted 30 June, 2022

To study the diversity and cultivar-specific of phytochemicals in bottle gourd, Gas chromatography-mass spectrometry (GC-MS)-based untargeted metabolomics method was used to analyze the metabolic profiles of two groups of bottle gourd cultivars exhibiting different tastes and flavors: fresh and slightly bitter (BBGs) / fragrant and sweet (SBG). A total of 155 metabolites (16 differential metabolites and 139 non-differential metabolites) were identified/annotated. The relative contents of 16 differential metabolites in BBGs are all higher than that in SBGs. The differential metabolites profiles of the two different groups were distinguished using principal component analysis (PCA), and the main differential metabolic pathways between the BBG and the SBG included those relating to plant nutrition and energy metabolism. This study provides new insights into the differences in metabolite profiles among bottle gourds with different tastes and flavors.

Key words: Gas chromatography-mass spectrometry (GC-MS), metabolomics, bottle gourd, taste, flavor.

INTRODUCTION

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] (2n = 2x = 22), also known as long calabash, is an annual cucurbit crop, and its tender fruit is cooked as a vegetable because of its unique flavor (Wu et al., 2017). The bottle gourd contains essential nutrients, such as protein, vitamin, pectin, fibers, etc (Zhang et al., 2020). It is planted in Asia and Africa, as well as it is used in crafts, decorations, and medicinal (Xu et al., 2011). It can be used as the rootstock of other Cucurbitaceae, such as watermelon (Sari, 2003).

The fruits of different gourd varieties have different shapes, sizes and flavors (Wu et al., 2017; Zhang et al., 2020). These phenotypic differences are caused by gene transcription regulation (Zhang et al., 2020), and differences at gene level led to differences at metabolic level. There are few studies on the differences of metabolism of different gourd fruits.

Sugars, amino acids (Kader et al., 1978), organic acids

*Corresponding author. E-mail: Chenchunhong2021@126.com, yyliuna@163.com.

Zhang Zhaohui and Du Xuan contributed equally to this article.

(Robert and Franco, 2018), vitamins (Kafkas et al., 2006) components of plant organisms, but also the ratio of their contents to their contents affects the taste and flavor of edible parts of plants, such as fruits (Hobson and Bedford, 2015). The tastes that human beings can perceive are sweet, sour, bitter, salty and delicious. Generally speaking, the taste of the fruit is caused by its organic components. And traditionally, sugar content determines the sweetness of the fruit. However, with the continuous discovery of new organic compounds and technological progress, sugar is not the only substance with a sweet taste. For example, thaumatin is a kind of protein with a sweeter taste than sucrose (Healey et al., 2017). In addition, the content of organic acids and their derivatives in fruit pulp has important dietary considerations, which affect the taste of fruit and the applicability of processing into various fruit products in some cases. It can be seen that whether the fruit is sweet or sour has a great relationship with the proportion of sweet and sour substances in the fruit. The taste of tomato was the first concern and a balanced sugar-acid ratio was preferred in cherry tomatoes (Hobson and Bedford, 2015). Cucurbita pepo contains the toxic tetracyclic triterpenoid compound cucurbitacin, which is the cause of bitterness and toxicity (Verma and Jaiswal, 2015). The factors affecting fruit umami are free amino acids, nucleotides (monophosphates of inosinate or guanylate, inosine 5′-monophosphate, and guanosine-5′monophosphate), short peptides and organic acids (Kurihara, 2009; Wu et al., 2017). Previous studies have found that, free glutamate (Glu) was found to have the most significant association with umami taste, suggesting that free Glu was the main umami-conferring ingredient of bottle gourd (Wu et al., 2017).

Although the above-mentioned research shows that free glucose is an important factor affecting the fresh taste of bottle gourd fruit, there are many factors involved in the taste of the fruit, and the metabolomics analysis of bottle gourd with different tastes can reveal the metabolic reasons for this difference. However, there is no report on the metabolism of bottle gourd fruit taste. Therefore, in order to find out the difference of metabolism between two kinds of bottle gourd fruits with different tastes and provide a theoretical basis for quality breeding of bottle gourd, we collected and analyzed the metabonomic information of slightly bitter bottle gourd (BBG) and sweet bottle gourd (SBG).

MATERIALS AND METHODS

Plant materials and chemicals

Five bottle gourd cultivars were cultivated in a randomized field plot according to standard agricultural practices in a field at the Zhuanghang comprehensive experimental station (E 121°28, N 30°57′) of the Shanghai Academy of Agriculture Sciences in 2019. Two bitter-tasted bottle gourd cv. BG27 and BG32, and three sweet-tasted bottle gourd cv. BG33, BG56, and BG58 were cultivated. They were sowed in March and planted in April. The photon flux density was range 650~850 $W.m^{-2}$. The temperature and relative humidity of the cultivation environment were about 10~25°C and 50~70%, respectively. The fruits were selected 14 days after simultaneous pollination, having a length of 20-35 cm and a diameter of 4.2-5.8 cm. Three marketable immature fruits were harvested for each cultivar and homogenized with equal weight to form a sample. All samples were stored at −80°C until analysis.

Chromatographic grade methanol and chloroform were purchased from Merck Chemicals (Germany). All of the chemicals, such as pyridine, methoxyamine hydrochloride, and N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), and the reference standards used in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-2-Chlorophenylalanine at 3.1 mg ml^{-1} in water was prepared and used as internal standards (IS).

Sample preparation and extraction for metabolomic analysis

An aliquot of 1 g bottle gourd sample and 10 ml methanol-water (7:3 v/v) were mixed for metabolite extraction at 60 Hz for 180 s on a tissuelyzer. Further, the mixtures were ultrasonically extracted for 40 min and stored at 4°C for 24 h. After centrifugation at 12,000 × g for 10 min, 400 µl of the supernatant was spiked with 4 µl of the IS, L-2-chlorophenylalanine, and dried completely in a vacuum concentrator. An aliquot of 80 µl methoxyamine hydrochloride (15 mg/ml in pyridine) was added to the residue and incubated at 37°C for 90 min for methoxyamination. Subsequently, the sample was trimethylsilylated by adding 80 µl BSTFA (with 1% TMCS) and incubated at 70°C for 60 min. The derivatized samples were cooled to room temperature before being analyzed (Jaeger, 2008).

GC/MS conditions

For each cultivar, three biological replicates were independently analyzed. In total, 15 samples were randomly analyzed to reduce analysis bias. Under the adjusted initial pressure, all samples were analyzed in randomized order by a 7890B gas chromatography coupled with a 5977B mass spectrometer (Agilent Inc., CA, USA). A DB-5ms capillary column (30 m ×250 µm inner diameter, 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) was used to separate compounds. The injector port was heated to 280°C and injections (1 µl) were performed with a split ratio of 5:1. Helium (purity > 99.999%) was used as the carrier gas at a constant flow of approximately 1 ml/min. The column temperature was held at 60°C for 1 min, then increased to 300°C at 5°C/min, and held for 11 minutes. The total run time was 60 min. The temperatures of the transfer line, ion source and quadruple were maintained at 280, 230 and 150°C, respectively. Electron impact ionization mass spectra were recorded with an ionization energy of 70 eV and EM voltage of 970 V. Mass spectra were scanned from 33 to 600 amu in total ion chromatogram mode after a solvent delay of 6.5 min (Weckwerth et al., 2004).

Data processing and statistical analysis

Metabolite identification was performed by the National Institute of Standards and Technology (NIST) mass spectral library (2017) in MSD ChemStation (version E.02.02.1431; Agilent Inc., CA, USA). The raw data acquired by Agilent GC/MS were imported into ChromaTOF (version 4.50.8.0; Leco Corporation, MI, USA) in NetCDF format. ChromaTOF could automatically compute baseline, find peaks above a signal to noise (S/N) of 100:1, deconvolute (identify overlapped peaks), integrate using specific masses, and align the same compound in different samples. The resulting

Figure 1. Five bottle gourd cultivars.
Source: Authors

three-dimensional dataset comprised sample information, peak retention time, and peak intensity. Some artificial peaks generated by noise, column bleed, and by-products in the silylation procedure were removed manually from the dataset. The resulting data were normalized to the area of the IS (L-2-chlorophenylalanine) for further statistical analysis. L-2-chlorophenylalanine was also utilized to assess process variability during sample preparation and data processing (Weckwerth et al., 2004).

For differential metabolites, we usually screen through VIP, such as screening out variables with VIP greater than 1.5, then calculating p-value, further screening out variables less than 0.05, and then observing these folds change was 2.0. Metabolites have been used for principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) by R (www.r-project.org/) to study metabolite cultivars-specific accumulation according to the described (Wang et al., 2018).

To further illustrate the biological significance associated with bitter/sweet taste, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to link differential metabolites to metabolic pathways in the SBGs compared with those in BBGs. Enrichment P-values were computed from a hypergeometric distribution. A p-value < 0.01 was selected to reduce the false discovery rate. The software used for drawing and data analysis was GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Differences in morphologies among the fruits of the five bottle gourd cultivars

These bottle-gourd cultivars were divided into two groups based on their taste and flavor. One group had two members, BG27 and BG32, which tasted lightly bitter (BBGs). The other had three ones, BG33, BG56, and BG58, which tasted thick sweet (SBGs). Though the two groups of bottle gourd cultivars were planted simultaneously and grown in the same field and under the same conditions, not only the taste of the fruits was distinct, but the shape and peel color also appeared different. The shape of BG27 was like Corbel. BG32 had a round body with a long neck. The shape of BG33 was like a long gun. Both BG56 and BG58 were like Cylinders. The peel color of the five bottle gourd cultivars belonged to a different degree of green (Figure 1).

Metabolic profiling

As is mentioned above, a few differences in tastes and shape between the two groups of bottle gourd cultivars existed. While as we all know the soluble solids content and acid-base titration method, which were referred to rough judgments, sometimes cannot exactly respond to the real ones. To make sure the differences in total contents and also in composition, we applied a newly developed GC–MS-based widely untargeted metabolomics method (German et al., 2005). A total of 155 metabolites (16 differential metabolites and 139 non-differential metabolites) were identified/annotated (Table 1; Supplementary S1). The results of all detected metabolites are shown in an Excel file (Supplementary S1), which indicates the differences in the metabolite levels of the five cultivars. To gain more insight into the metabolic differences between BBG and SBG, differential metabolite screening was performed among all 155 metabolites identified/annotated according to the fold-change and the variables determined to be important in the projection (VIP) scores. A fold-change score ≥2 or ≤0.5 among the metabolites with a VIP value>1 was used as an identification criterion. The screening results have been illustrated using volcano plots (Figure 2A). The overall situation of relative expression of 16 differential metabolites is shown by heat map visualization (Figure 2B).

We detect 6 different metabolites of saccharides,

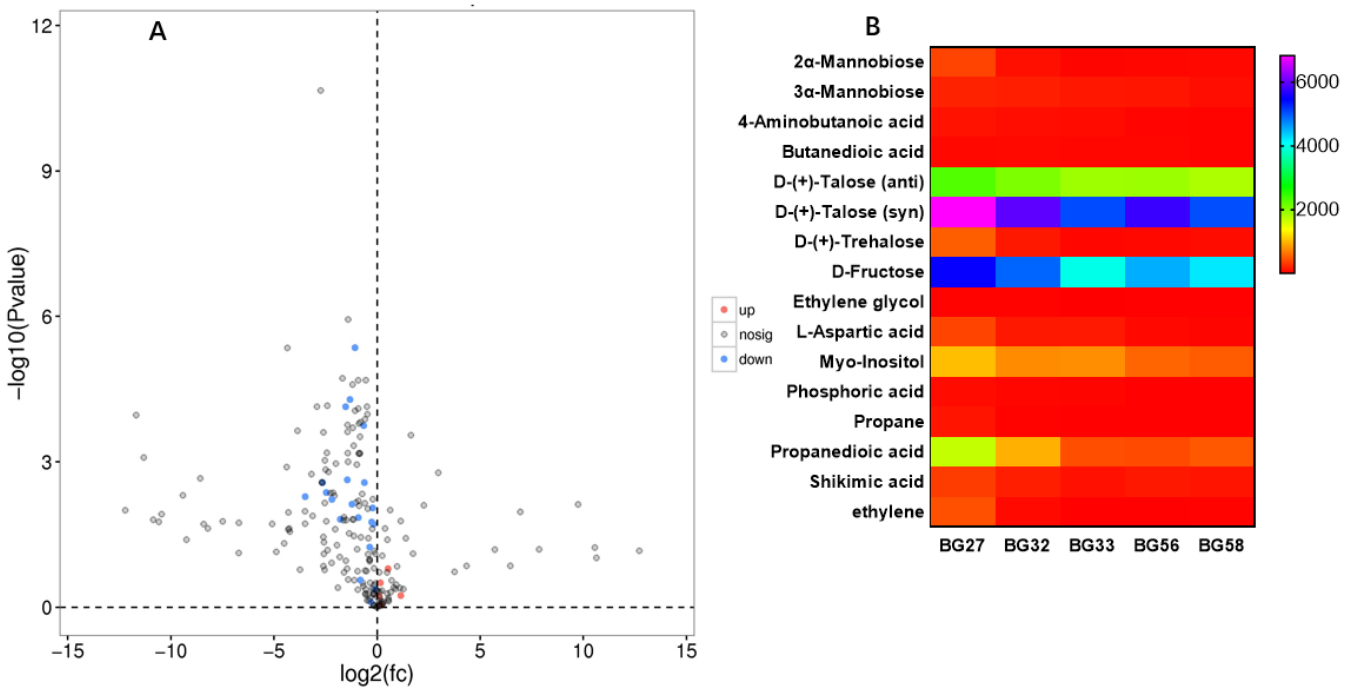

Figure 2. The volcano plot (A) shows the differential metabolite expression levels between BBGs and SBGs. Blue dots represent downregulated differentially expressed metabolites; red spots represented upregulated differentially expressed metabolites; and gray represented non-differentially expressed metabolites. Heat map visualization (B) shows the overall situation of relative expression of 16 differential metabolites.
Source: Authors

namely, 2α- mannobiose, 3α-mannobiose, D-(+)-talose (anti), D-(+)-talose (syn), D-(+)-trehalose, D-fructose (Figure 3A–F). The MNB content of two kinds of group BBGs is obviously higher than that of SBGs group (Figure 3A and B). The contents of D-tal(anti) and D-tal (syn) in a gourd with different taste and flavor are distinct, and their content in BBGs are higher than SBGs (Figure 3C and D). The content of trehalose in BBGs is higher than SBGs (Figure 3E). In these battle gourds of this study, in BBGs, the relative content of D-fru is 5176 and 5037, respectively, whereas in SBGs, it is 4252, 4693, and 4335 (Table 1, Figure 3F).

In this research, there are two kinds of amino acids that are different in the metabolism of two groups of gourds with different flavors, namely 4-aminobutanoic acid and L-aspartic acid. The relative content of 4-aminobutanoic acid or L-Asp in BBGs is significantly more than the group SBGs (Figure 4A and B).

In this study, it was found that there were significant differences in the contents of butanedioic acid, phosphoric acid, propanedioic acid and shikimic acid in two different tastes and flavors of bottle gourd (Figure 5A-D). The content of these four acids in BBG cultivars is higher than that in SBG cultivars (Figure 5A-D).

In addition to the above-mentioned metabolites, there are significant differences in the content of various bottle gourds between the two groups, and there are also four metabolites, namely *myo*-Inositol, ethylene, ethylene glycol and propane. Similarly, BBGs have significantly larger levels of these four substances than SBGs (Figure 6A-D).

Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) reveal differences in the metabolite profiles

In the PCA plot (Figure 7A), the duplicate samples were grouped together, indicating that the duplicate samples had similar metabolic profiles and that the entire analysis was stable and repeatable. Pairwise comparisons are carried out between BBG and SBG to identify the metabolites that are responsible for the taste differences. PLS-DA models are used to generate pairwise comparisons of the metabolite contributions. The predictability (Q2) and goodness of fit (R2X, R2Y) of the PLS-DA models were observed in the comparison between BBG and SBG (Q2=0.767, R2X=0.723, R2Y=0.981; Figure 7B).

Differential metabolic pathways between BBG and SBG

To obtain detailed pathway information, all metabolites

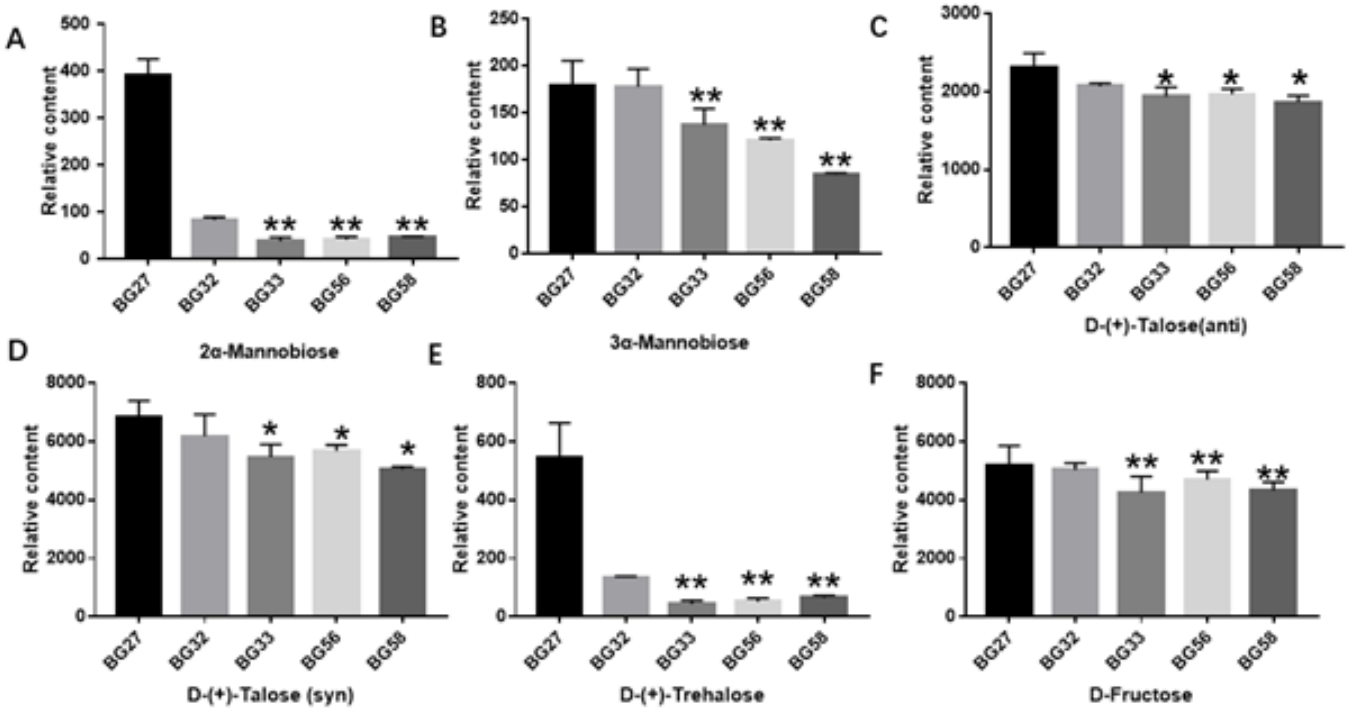

Figure 3. Saccharides content in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements. * and ** indicate a significant difference from that of BG27 at $p < 0.05$ and $p < 0.01$, respectively, by the Dunnett's test.
Source: Authors

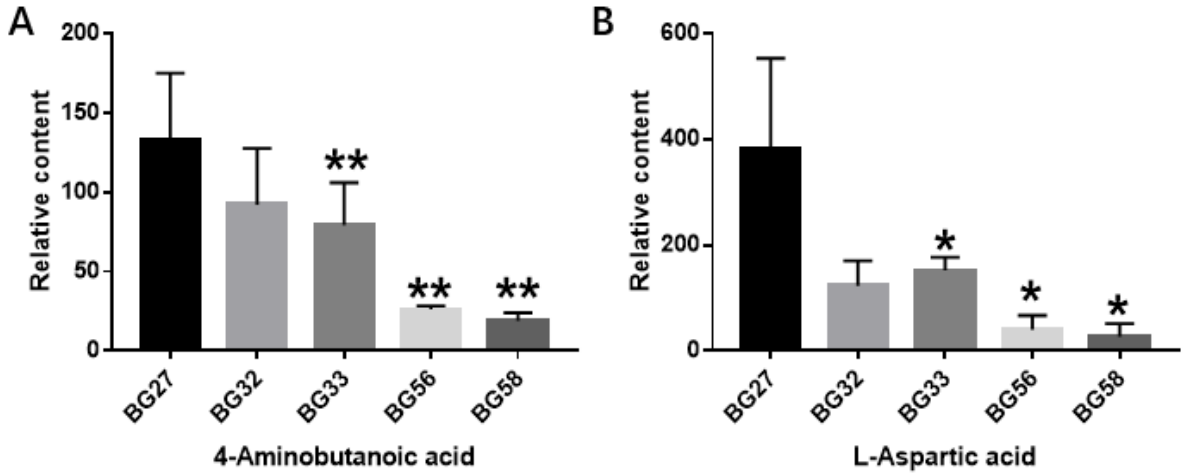

Figure 4. Amino acids content in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements. * and ** indicate a significant difference from that of BG27 at $p < 0.05$ and $p < 0.01$, respectively, by the Dunnett's test.
Source: Authors

and the differential metabolites were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) (Figure 8A and B). In the mapping of KEGG, there were 12 different metabolites involved in metabolisms, 1 involved in genetic information processing and 1 involved in environmental information processing (Figure 8A). The differential metabolites were mainly involved in beta-alanine metabolism, indole alkaloid biosynthesis, galactose metabolism, ect (Figure 8B).

Table 1. The relative content of 24 differential metabolites.

Compounds	The relative content														
	BG27			BG32			BG33			BG56			BG58		
2-αMannobiose	459.87	352.78	363.16	87.38	91.00	73.70	32.42	55.09	28.14	50.26	44.90	30.92	47.34	47.05	49.27
3-αMannobiose	200.69	186.40	148.10	198.84	172.34	162.70	156.40	131.58	123.52	123.09	118.84	120.39	84.18	82.79	86.01
4-Aminobutanoic acid	181.59	109.49	107.54	133.09	73.87	69.59	110.05	60.91	67.38	27.37	23.73	27.72	13.04	21.09	22.60
Butanedioic acid	52.94	49.10	42.59	123.90	56.07	55.39	38.38	37.90	29.60	45.74	39.86	39.58	19.05	28.10	25.49
D-(+)-Talose (anti)	2632.77	2300.85	1993.33	2134.59	2021.71	2060.50	2166.18	1754.75	1894.00	2105.26	1900.43	1890.99	1761.29	2029.89	1807.46
D-(+)-Talose (syn)	7807.93	6821.00	5884.16	5030.32	7568.70	5960.85	6328.20	5023.23	5057.73	5979.91	5299.36	5760.43	4924.17	5206.41	5036.75
D-(+)-Trehalose	677.82	448.57	508.57	132.17	139.13	136.96	41.22	57.68	35.15	65.88	48.78	41.60	58.44	69.41	71.15
D-Fructose	5640.66	5483.04	4405.97	5300.11	4915.11	4897.13	4886.84	3969.96	3903.63	5037.39	4518.13	4525.44	4148.26	4649.78	4209.55
Ethylene glycol	12.94	24.95	22.21	24.03	23.21	24.80	10.76	9.37	9.72	12.14	10.94	10.56	9.80	10.91	10.53
L-Aspartic acid	559.20	370.52	220.61	164.57	132.99	72.69	134.96	181.02	141.14	9.93	60.20	51.33	35.85	0.00	46.32
Myo-Inositol	1279.23	1005.94	1024.71	906.18	757.21	681.04	849.35	770.92	689.59	641.46	548.95	547.97	467.68	524.90	493.87
Oxalic acid	71.85	66.80	66.44	129.19	108.93	114.91	63.32	74.12	73.01	75.13	65.83	72.50	72.18	91.65	67.18
Phosphoric acid	74.15	45.08	63.60	56.06	42.03	38.87	34.72	32.06	21.90	20.32	18.38	18.03	14.55	15.43	18.34
Propane	129.42	86.12	112.60	23.26	26.66	23.45	8.93	18.78	10.16	15.29	13.94	10.66	18.62	16.91	18.57
Propanedioic acid	1995.95	1175.56	1681.84	1022.72	945.90	914.86	512.12	401.78	422.33	462.35	399.81	381.46	405.02	558.61	475.54
Shikimic acid	578.15	244.78	325.48	163.80	164.65	246.22	162.64	81.80	93.43	141.68	128.51	136.30	109.35	112.45	145.47
Ethylene	434.32	347.13	432.77	74.59	54.23	45.20	17.64	26.94	14.05	24.84	20.02	14.63	21.05	24.90	20.79

Source: Authors

DISCUSSION

Bottle gourd fruit varies in shape, size and flavor (Wu et al., 2017; Zhang et al., 2020). Previous studies have revealed the root causes of these differences at the genome and transcriptome levels (Konan et al., 2020). However, there is no research on the difference of metabonomics of gourd with different tastes. The metabolites of different gourd fruits are different. The metabolism of fruits with different tastes was different. GC-MS-based untargeted metabolomics of two groups of bottle gourd accessions yielded 16 differential metabolites. In previous reports, sugars were the main factor affecting sweetness (Cameron, 2011). The sweetness of the saccharides differs. Furthermore, the sweetness of some saccharides varies with the solution concentration, while the sweetness of others does not (Cameron, 2011). Mannobiose, composed of D-mannose units, is a nondigestible disaccharide and rich in Cucurbitaceae crops. MNB can modulate intestinal and systemic immune responses in mice and prevent LPS-induced immune suppression as well as directly stimulate innate immune mechanisms *in vitro* as a TLR4 agonist (Kovacs-Nolan et al., 2013). Therefore, the difference in MNB contained in bottle gourds with different tastes will play a certain role in cultivating bottle gourds with high MNB content. D-talose is a variety of rare monosaccharides, different from the exception of isomers such as D-glucose and D-fructose, which exist in great abundance and showed considerable growth inhibition upon the nematode (Sakoguchi et al., 2016). Trehalose is a nonreducing disaccharide with two glucose units linked in an α, α-1,1 configuration, widespread in plant kingdoms and its phosphorylation forms play a vital role in the integration of plant metabolism and development (Richards et al., 2002). Evidence showed that exogenously-supplied trehalose can make winter wheat better cope with heat stress (Luo et al., 2018). D-fru is one of the most common and also the sweetest monosaccharide, but it is not the substance with the highest

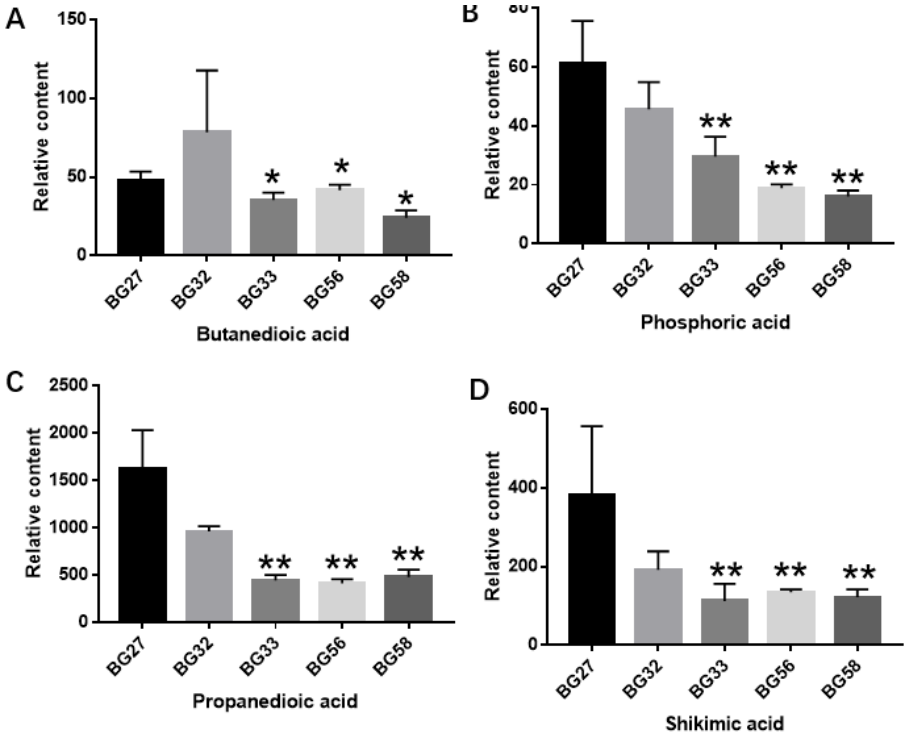

Figure 5. Organic acids and inorganic acidscontent in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements. * and ** indicate a significant difference from that of BG27 at $p < 0.05$ and $p < 0.01$, respectively, by the Dunnett's test.
Source: Authors

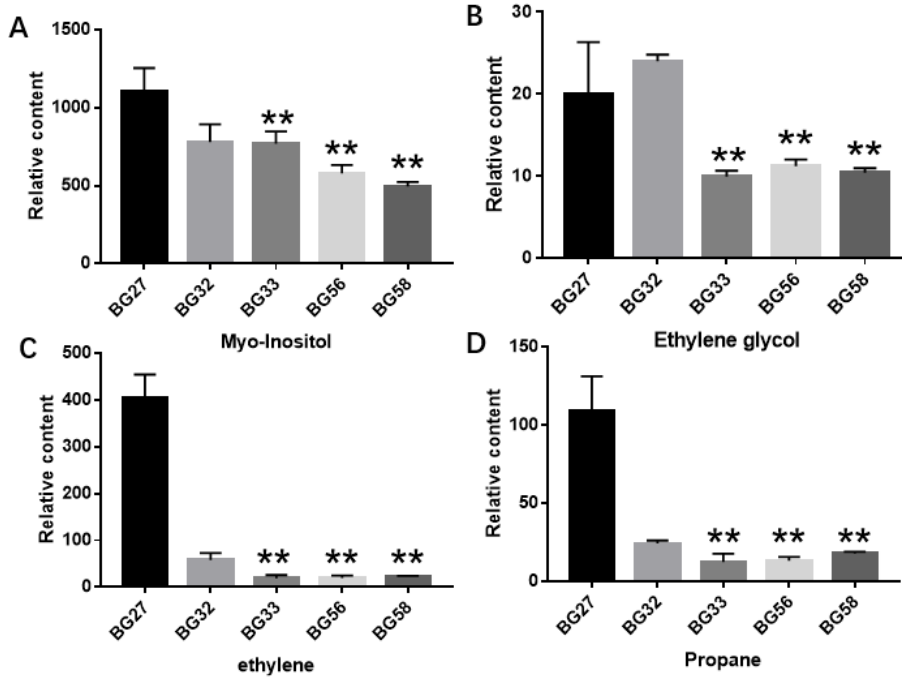

Figure 6. Other metabolites in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements ** indicate a significant difference from that of BG27 at $p< 0.01$, by the Dunnett's test.
Source: Authors

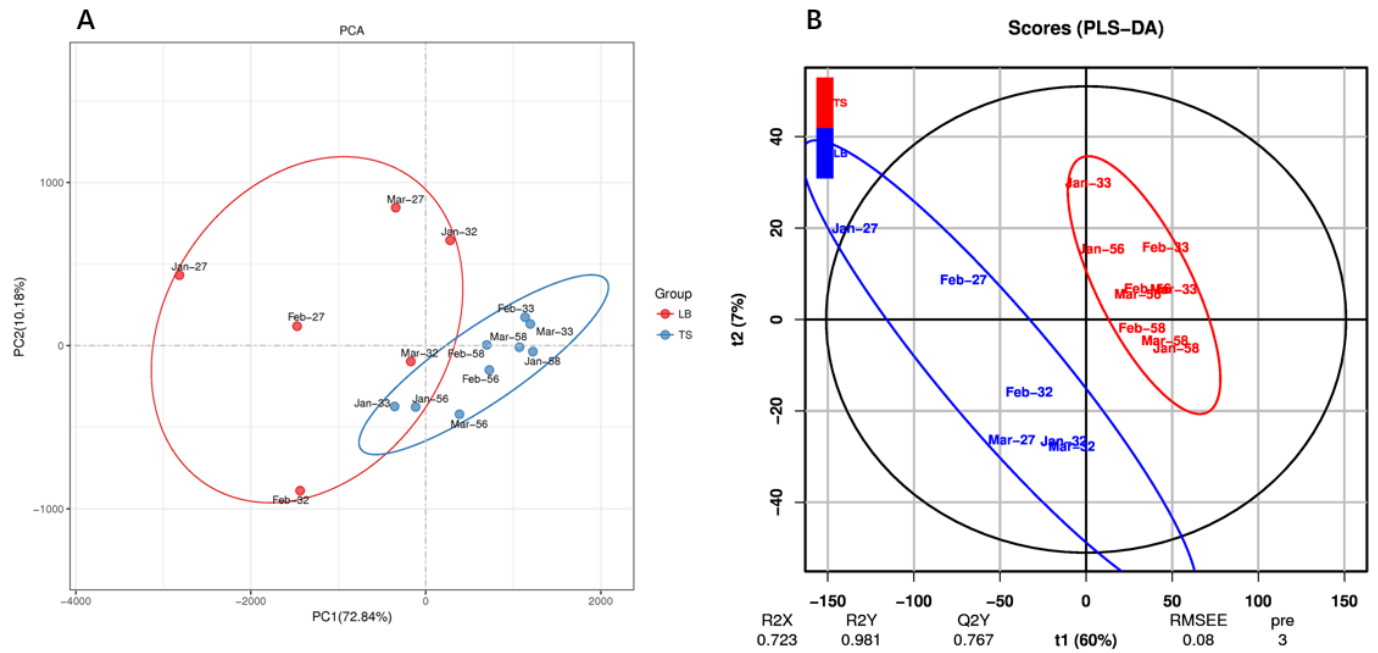

Figure 7. PCA and PLS-DA of the relative differences in secondary metabolites in BBGs and SBGs cultivars. BG27: Jan-27, Feb-27, Mar-7; BG32: Jan-32, Feb-32, Mar-32; BG33: Jan-33, Feb-33, Mar-33; BG56: Jan-56, Feb-56, Mar-56; BG58: Jan-58, Feb-58, Mar-58.
Source: Authors

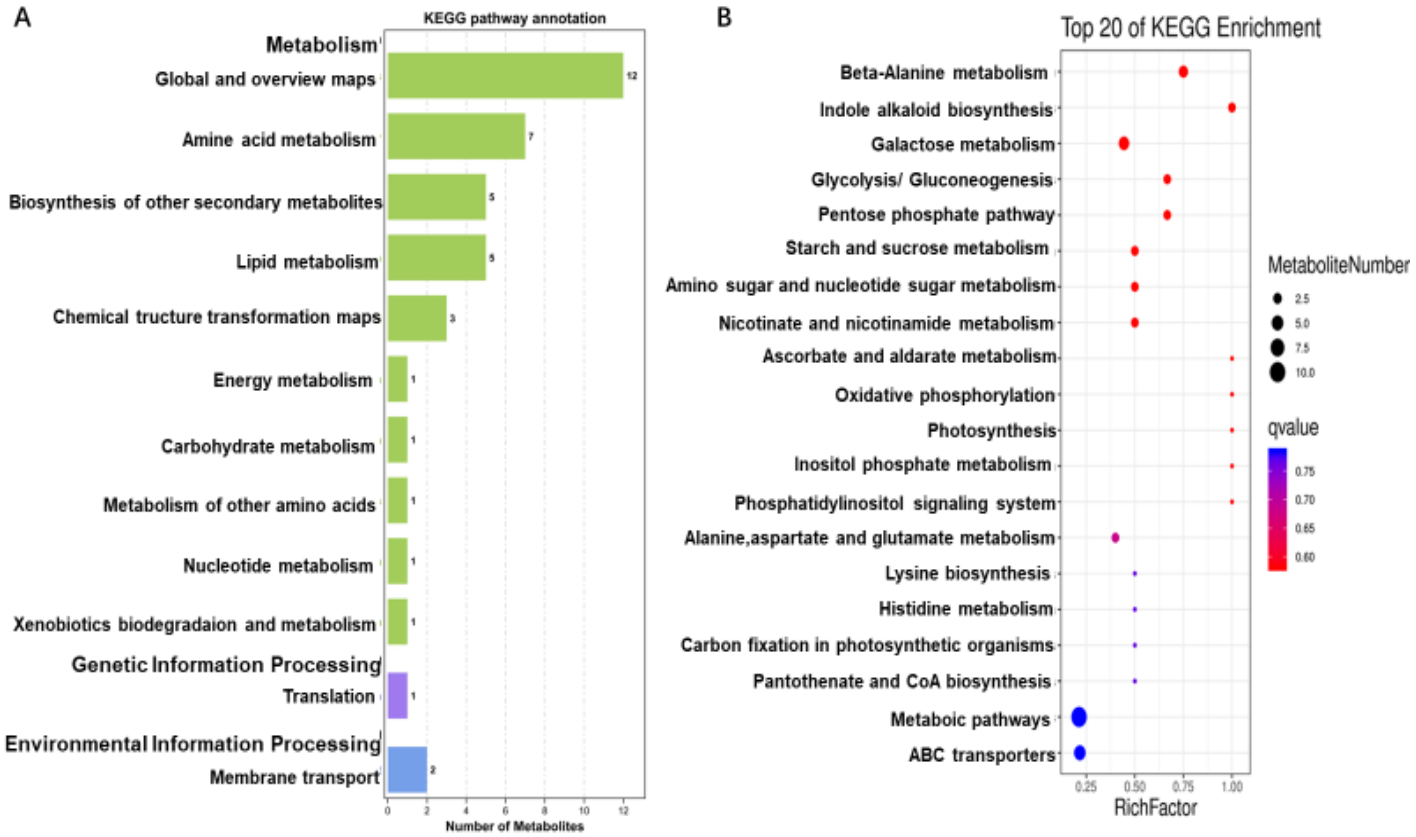

Figure 8. Enrichment of the differential metabolites to distinct KEGG pathways.
Source: Authors

sweetness in nature. For example, protein thaumatin, first isolated from the katemfe fruit, *Thaumatococcus daniellii*, is 1600 times sweeter than sucrose on a weight basis (Wel and Loeve, 1972). Therefore, it is probably not only sugar that determines the strong sweet taste of the gourd.

4-Aminobutanoic acid was also known as the name of gamma-Aminobutyric acid (GABA), was a non-proteinogenic amino acid and was first isolated from potato tubers in 1949 (Ramesh et al., 2017). In plants, GABA acts as a signal substance to regulate plant growth, development and response to stress. In mammals, its action has been mainly described in the nervous system, where GABA receptors regulate brain function and development. L-aspartic acid is one of the 20 types of amino acids that make up a protein's structural unit (Akcay et al., 2012). Free amino acids have been found as the main umami ingredients in many vegetables, especially free glutamate and free aspartate are the two main umami ingredients (Zhang et al., 2013). Previous data has shown that free glutamic acid is the major factor affecting umami taste in bottle gourds (Wu et al., 2017). However, our data found that there were significant differences in aspartic acid metabolism, but there was no significant difference in glutamic acid content in different flavors of bottle gourd.

Besides sugars and amino acids, sour ingredients have an important influence on the flavor of fruits. Whether the taste of the fruit is sour or sweet depends on the ratio of sweet substances to sour substances. Shikimic acid is widely found in higher plants, with high content in the fruit of Illicium verum, which has an anti-tumor effect and is also an important synthetic raw material of Tamiflu, an anti-influenza drug (Coleman, 2005). Myo-Inositol belongs to B vitamins and is the precursor of various biological functional compounds containing inositol phosphate and lipid. Inositol and phosphatidylinositol play a key role in many metabolic pathways, and if affected, they will have adverse effects on human health (Benvenga and Antonelli, 2016). Ethylene is a plant hormone that can regulate fruit ripening. Ethylene glycol and Propane also exist in the flesh of bottle gourd, but their unique roles are not clear.

Multivariate statistics are used to further assess the differences in metabolic profiles among the two gourd groups. PCA is one of the most widely used chemometric tools to reveal the internal structure of several variables by a few principal components and extract and rationalize information from any multivariate description of a biological system (Park et al., 2013). The results showed that the samples from the two cultivars were grouped into two areas in the plot, indicating that different flavors had relatively distinct metabolic profiles. Especially, the dots of a gourd with sweet taste are more concentrated than the dots of a gourd with slightly bitter taste, which indicates that the metabolic regulation leading to the sweet taste of the gourd is more consistent, while the metabolic regulation related to bitter taste is more complex. The plot suggests that the differences in metabolic profiles detected in our study correlated with fruit flavors. In the PLS-DA models, BBGs are clearly separated from SBGs, indicating major distinctions in the metabolic profiles between the different fruit tastes of bottle guards. For BG32 and SBGs, there was some overlap in PCA (Figure 7A), but a clear difference was obtained in the PLS-DA score plots (Figure 7B), indicating that the BG32 with a slightly bitter taste could be grouped from the guard with a sweet taste.

The roles of carbohydrates, and their metabolism, in plants have a vast influence on both plant growth and development. During the storage of coffee beans in the pod, the change of flavor is related to the metabolism of sugars and free amino acids in coffee beans (Hinneh et al., 2018). Previous evidence has found that, free glutamate content was a key factor conferring umami taste in the bottle gourd (Wu et al., 2017).

Conclusion

However, in the study, we found that there were differences in metabolic substances and metabolic pathways between the two different tastes of bottle gourd, which lay the foundation to discover the metabolic architecture of the sweety/bitter taste in bottle gourd and facilitate the breeding of new varieties with better taste and quality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

This research was funded by the Shanghai Science and Technology Agriculture Promotion Project (2022-02-08-00-12-F01105). Thanks to the Instrumental analysis center, Shanghai Jiao Tong University for its instrument service.Thanks to the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences for providing the germplasm materials for the bottle gourd. Thanks to Guangzhou Gene Denovo Biotechnology Co., Ltd. for its Omicsmart cloud analysis platform.

REFERENCES

Akcay N, Bor M, Karabudak T, Ozdemir F, Turkan I (2012). Contribution of Gamma amino butyric acid (GABA) to salt stress responses of Nicotiana sylvestris CMSII mutant and wild type plants. Journal of Plant Physiology 169(5):452-458.

Benvenga S, Antonelli A (2016). Inositol(s) in thyroid function, growth and autoimmunity. Reviews in Endocrine and Metabolic Disorders 17(4):471-484.

Cameron AT (2011). The Relative Sweetnesses of Certain Susgars, Mixtures of Sugars, and Glycerol. Canadian Journal of Research 22(3):45-63.

Coleman WF (2005). Shikimic Acid. Journal of Chemical Education 82:656.
German JB, Watkins SM, Fay LB (2005). Metabolomics in Practice: Emerging Knowledge to Guide Future Dietetic Advice toward Individualized Health. Journal of the American Dietetic Association 105:1425-1432.
Healey RD, Lebhar H, Hornung S, Thordarson P,Marquis CP (2017). An improved process for the production of highly purified recombinant thaumatin tagged-variants. Food Chemistry 237:825-832.
Hinneh M, Semanhyia E, Van de Walle D, De Winne A, Tzompa-Sosa DA, Scalone GL, De Meulenaer B, Messens K, Van Durme J, Afoakwa EO, De Cooman L (2018). Assessing the influence of pod storage on sugar and free amino acid profiles and the implications on some Maillard reaction related flavor volatiles in Forastero cocoa beans. Food Research International 111:607-620.
Hobson GE, Bedford L (2015). The composition of cherry tomatoes and its relation to consumer acceptability. Journal of Horticultural Science 64(3):321-329.
Jaeger FH (2008). Simplified Plant Sample Preparation for use in Gas Chromatography-Mass Spectrometry (GC-MS) Based Metabolomic Profiling and Targeted Analyte Quantitation. Available at: https://repository.lib.ncsu.edu/handle/1840.16/2684
Kader AA, Stevens MA, Albright M,Morris LL (1978). Amino acid composition and flavor of fresh market tomatoes as influenced by fruit ripeness when harvested. Journal American Society for Horticultural Science 103(4):541-544.
Kafkas E, Koşar M, Türemiş N, Başer KHC (2006). Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. Food Chemistry 97(4):732-736.
Konan JA, Guyot R, Koffi KK, Vroh-Bi I,Zoro AIB (2020). Molecular confirmation of varietal status in bottle gourd (*Lagenaria siceraria*) using genotyping-by-sequencing. Genome 63(11):535-545.
Kovacs-Nolan J, Kanatani H, Nakamura A, Ibuki M,Mine Y (2013). beta-1,4-mannobiose stimulates innate immune responses and induces TLR4-dependent activation of mouse macrophages but reduces severity of inflammation during endotoxemia in mice. Journal of Nutrition 143(3):384-391.
Kurihara K (2009). Glutamate: from discovery as a food flavor to role as a basic taste (umami). American Journal of Clinical Nutrition 90(3):719S-722S.
Luo Y, Wang W, Fan YZ, Gao YM, Wang D (2018). Exogenously-Supplied Trehalose Provides Better Protection for D1 Protein in Winter Wheat under Heat Stress. Russian Journal of Plant Physiology 65(1):115-122.
Park S-Y, Lim S-H, Ha S-H, Yeo Y, Park WT, Kwon DY, Park SU,Kim JK (2013). Metabolite Profiling Approach Reveals the Interface of Primary and Secondary Metabolism in Colored Cauliflowers (*Brassica oleracea* L. ssp. botrytis). Journal of Agricultural and Food Chemistry 61(28):6999-7007.
Ramesh SA, Tyerman SD, Gilliham M, Xu B (2017). γ-Aminobutyric acid (GABA) signalling in plants. Cellular and Molecular Life Sciences 74(9):1577-1603.
Richards AB, Krakowka S, Dexter LB, Schmid H, Wolterbeek APM, Waalkens-Berendsen DH, Shigoyuki A,Kurimoto M (2002). Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. Food and Chemical Toxicology 40(7):871-898.
Robert PW, Franco F (2018) Organic Acids in Fruits: Metabolism, Functions and Contents. John Wiley & Sons, Inc, Perugia.
Sakoguchi H, Yoshihara A, Izumori K, Sato M (2016). Screening of biologically active monosaccharides: growth inhibitory effects of D-allose, D-talose, and L-idose against the nematode Caenorhabditis elegans. Bioscience,Biotechnology and Biochemistry 80(6):1058-1061.
Sari HY (2003). Effect of different rootstock on plant growth, yield and quality of watermelon. Animal Production Science 43(10):1269-1274.
Verma A, Jaiswal S (2015). Bottle gourd (*Lagenaria siceraria*) juice poisoning. World Journal of Emergency Medicine 6(4):308.
Wang A, Li R, Ren L, Gao X, Zhang Y, Ma Z, Ma D,Luo Y (2018). A comparative metabolomics study of flavonoids in sweet potato with different flesh colors (*Ipomoea batatas* (L.) Lam). Food Chemistry 260:124-134.
Weckwerth W, Wenzel K, Fiehn O (2004). Process for the integrated extraction, identification and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks. Proteomics 4(1):78-83.
Wel HVD, Loeve K (1972). Isolation and Characterization of Thaumatin I and II, the Sweet -Tasting Proteins from Thaumatococcus daniellii Benth. European Journal of Biochemistry 31(2):221-225.
Wu X, Xu P, Wu X, Wang B, Lu Z,Li G (2017). Genome-wide association analysis of free glutamate content, a key factor conferring umami taste in the bottle gourd [*Lagenaria siceraria* (Mol.) Standl.]. Scientia Horticulturae 225:795-801.
Xu P, Wu X, Jie L, Wang B, Li G (2011). Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. BMC Genomics 12:467.
Zhang H, Tan J, Zhang M, Huang S, Chen X (2020). Comparative Transcriptomic Analysis of Two Bottle Gourd Accessions Differing in Fruit Size. Genes 11(4):359.
Zhang Y, Venkitasamy C, Pan Z, Wang W (2013). Recent developments on umami ingredients of edible mushrooms – A review. Trends in Food Science and Technology 33(2):78-92.

Supplementary Table S1

Table S1 A list of 155 compounds identified in bottle gourd flesh

Compounds	The Relative Content															control_mean	case_mean	log2_FC(case_mean/control_mean)	Pvalue	fdr	vip
	BG27			BG32			BG33			BG56			BG58								
1,2,3-Butanetriol	2.55	1.91	1.80	1.05	0.93	1.03	1.62	1.48	1.23	0.00	0.59	0.00	0.00	0.00	0.00	1.54	0.55	(1.50)	0.02	0.04	0.18
1,2-Butanediol	0.00	33.42	39.08	0.00	6.96	7.89	0.00	6.76	0.00	6.42	5.74	4.59	4.54	4.67	4.66	14.56	4.15	(1.81)	0.09	0.15	0.63
1,2-Ethenediol	5.52	6.25	5.00	5.74	5.11	4.96	4.44	3.45	3.28	4.28	4.18	3.66	2.93	3.52	3.75	5.43	3.72	(0.55)	0.00	0.00	0.30
1-Monopalmitin	0.38	0.14	0.21	0.29	0.09	0.15	0.17	0.10	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.21	0.04	(2.50)	0.00	0.01	0.08
1-O-trans-p-Coumaroylglycerol	0.00	0.00	0.00	1.22	2.46	2.63	0.00	0.67	0.33	0.00	0.41	0.00	2.79	2.99	2.66	1.05	1.09	0.06	0.95	0.96	0.23
1H-Indole-2,3-dione	8.30	5.83	7.45	4.05	0.00	0.00	1.77	3.08	2.01	0.00	0.00	0.00	0.00	1.27	1.30	4.27	1.05	(2.03)	0.02	0.05	0.27
2'-Hydroxy-6'-methyl-3,4-methylenedioxychalcone	5.31	4.54	0.00	1.18	0.00	0.00	0.00	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.84	0.08	(4.51)	0.05	0.09	0.24
2,3,4-Trihydroxybutyric acid	4.78	3.99	3.52	3.91	2.69	2.75	2.49	3.12	2.21	2.29	1.88	1.72	1.14	1.37	1.23	3.61	1.94	(0.89)	0.00	0.00	0.25
methylphosphonofluoridate	0.28	0.00	0.00	0.39	0.26	0.42	0.00	0.39	0.27	0.22	0.20	0.00	0.21	0.28	0.66	0.23	0.25	0.13	0.83	0.88	0.05
2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa	1.52	0.00	0.00	3.13	2.18	2.00	0.00	0.00	2.07	0.00	0.89	0.00	0.00	1.71	4.25	1.47	0.99	(0.57)	0.52	0.61	0.22
2,4-Dimethyl-3-pentanol	4.40	3.68	3.14	0.00	0.00	0.00	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.87	0.10	(4.26)	0.02	0.05	0.22
benzonitrile	4.30	3.76	4.43	0.77	1.39	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.55	0.00	(11.32)	0.00	0.00	0.28
2-αMannobiose	459.87	352.78	363.16	87.38	91.00	73.70	32.42	55.09	28.14	50.26	44.90	30.92	47.34	47.05	49.27	237.98	42.82	(2.47)	0.00	0.02	2.33
2-Butene-1,4-diol	0.93	0.89	0.85	0.96	0.91	0.90	0.79	0.68	0.50	0.81	0.60	0.68	0.52	0.71	0.62	0.91	0.66	(0.47)	0.00	0.00	0.11
2-Butenedioic acid	6.41	3.86	2.67	14.53	4.49	3.50	3.87	3.04	2.65	2.42	2.20	2.13	1.36	1.41	1.68	5.91	2.31	(1.36)	0.03	0.06	0.46
propane	1.48	1.38	1.26	1.29	1.30	1.44	1.25	1.24	1.15	1.38	1.28	1.26	1.26	1.55	1.32	1.36	1.30	(0.06)	0.32	0.42	0.04
2-Hydroxychalcone	3.07	0.00	2.57	5.00	2.62	3.32	0.00	1.20	3.09	1.70	0.00	0.00	0.00	2.59	7.04	2.76	1.74	(0.67)	0.36	0.48	0.28
2-O-Glycerol-d-galactopyranoside	17.25	7.25	11.19	8.05	3.31	4.50	7.75	1.85	3.01	1.77	1.47	1.20	0.61	0.00	0.00	8.59	1.96	(2.13)	0.00	0.02	0.42
3,5-Dihydroxyflavone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.22	0.00	0.33	0.00	0.00	0.09	6.46	0.14	0.22	0.08
3-αMannobiose	14.92	10.40	9.46	6.32	5.88	5.43	6.57	6.46	4.87	4.84	4.42	3.99	3.79	4.12	3.83	8.74	4.77	(0.87)	0.01	0.03	0.34
3-Amino-2-piperidone	2.82	1.71	0.00	0.00	0.00	0.00	0.00	1.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76	0.13	(2.54)	0.17	0.25	0.18
3-Methyl-1,2-bis(trimethylsilyl)butane	14.22	11.64	12.13	11.29	14.95	13.07	2.08	2.33	1.58	1.89	2.68	2.54	1.51	1.11	1.70	12.89	1.94	(2.73)	0.00	0.00	0.91
3-Oxaoct-4-en-2-imine	116.01	106.64	112.96	130.14	123.90	144.49	132.60	137.93	126.75	130.72	112.61	120.55	135.43	155.87	131.77	122.36	131.58	0.10	0.19	0.27	0.39
3-Pyridinol	7.37	7.77	7.60	7.86	7.09	7.77	7.60	6.70	6.69	8.10	6.83	7.32	7.34	8.71	7.45	7.57	7.42	(0.03)	0.60	0.67	0.07
4-Aminobutanoic acid	181.59	109.49	107.54	133.09	73.87	69.59	110.05	60.91	67.38	27.37	23.73	27.72	13.04	21.09	22.60	112.53	41.54	(1.44)	0.00	0.01	1.49
4-Ethoxy-7-methoxycoumarin	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.22	0.25	0.00	0.00	0.00	0.05	0.08	0.55	0.71	0.78	0.04
4-Hydroxybenzyl alcohol	3.09	2.08	2.92	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.40	0.00	(10.45)	0.01	0.03	0.20
4-Methyl-1,2-bis(trimethylsilyloxy)pentane	0.98	0.58	0.71	0.66	0.52	0.40	0.40	0.45	0.31	0.21	0.21	0.41	0.00	0.18	0.00	0.64	0.24	(1.41)	0.00	0.01	0.12
5-Iodouridine	0.00	9.64	7.60	6.33	5.51	0.00	5.41	4.55	3.95	0.00	4.55	0.00	4.17	0.00	0.00	4.85	2.51	(0.95)	0.18	0.26	0.42
7-Diethylaminocoumarin	1.41	1.33	1.86	0.73	0.49	0.31	0.15	0.47	0.14	0.29	0.35	0.00	0.21	0.00	0.18	1.02	0.20	(2.36)	0.00	0.01	0.17
7-Hydroxy-4-(methoxymethyl)coumarin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.08	0.05	0.00	0.00	0.00	0.00	0.00	0.02	4.34	0.14	0.22	0.03
7-Nitroindazole	4.89	0.00	2.86	2.21	2.24	2.01	0.00	0.00	1.84	1.83	0.00	0.00	0.00	0.00	0.00	2.37	0.41	(2.54)	0.01	0.02	0.25
D-Galactofuranoside	25.41	21.30	16.86	17.41	17.84	0.00	18.37	13.66	14.00	16.09	14.38	13.83	11.84	15.28	13.31	16.47	14.53	(0.18)	0.52	0.61	0.56
D-Glucopyranuronic acid	22.61	17.85	14.31	13.91	11.02	7.87	7.79	7.98	5.78	6.44	5.33	4.64	3.56	3.63	3.54	14.59	5.41	(1.43)	0.00	0.00	0.55
Gentiobiose	7.09	4.14	0.00	0.00	0.28	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	0.02	(6.71)	0.08	0.13	0.30
Linolenic acid	3.58	2.44	4.09	5.05	2.87	2.96	3.76	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.50	0.65	(2.43)	0.00	0.00	0.41
Adenosine	7.28	4.60	5.41	8.29	4.88	4.52	3.37	4.41	2.84	1.96	2.03	1.57	0.98	1.17	1.06	5.83	2.16	(1.43)	0.00	0.00	0.47
Alanine	3.41	3.41	3.38	2.09	2.43	2.45	2.44	1.95	1.40	1.62	2.75	0.00	1.79	0.69	0.00	2.86	1.40	(1.03)	0.01	0.02	0.22
Allyldimethyl(prop-1-ynyl)silane	1.41	0.00	1.06	2.46	1.75	0.00	0.00	1.40	1.57	1.03	1.02	0.80	0.00	0.00	3.96	1.11	1.09	(0.03)	0.97	0.97	0.07
Arabinitol	6.53	4.83	6.12	6.17	5.12	4.58	3.82	4.67	3.54	2.98	2.83	2.50	1.92	2.27	2.02	5.56	2.95	(0.91)	0.00	0.00	0.38
Arabinofuranose	0.00	7.52	6.79	9.28	7.52	6.05	3.31	3.28	2.41	4.14	4.24	0.00	3.47	0.00	3.57	6.19	2.71	(1.19)	0.02	0.04	0.72
Asparagine	10.87	5.81	33.23	2.72	0.00	0.00	7.34	18.91	15.10	0.00	0.00	0.00	0.00	0.00	0.00	8.77	4.59	(0.93)	0.43	0.53	0.21
Boric acid	2.67	1.80	1.61	0.00	1.00	1.14	4.44	1.43	2.43	3.41	1.54	1.73	3.85	3.69	4.77	1.37	3.03	1.15	0.02	0.04	0.45

Supplementary Table S1. Cont'd

D-Butanal	7.01	6.07	5.51	5.37	3.49	3.66	4.89	2.93	3.03	3.11	2.05	2.15	1.48	1.84	2.12	5.19	2.62	(0.98)	0.00	0.01	0.28
L-Butanal	2.75	2.43	1.94	0.00	1.39	1.38	1.30	0.60	0.83	1.62	1.38	1.38	0.94	1.13	1.25	1.65	1.16	(0.51)	0.18	0.26	0.18
Butanedioic acid	52.94	49.10	42.59	123.90	56.07	55.39	38.38	37.90	29.60	45.74	39.86	39.58	19.05	28.10	25.49	63.33	33.74	(0.91)	0.01	0.04	1.54
Butanoic acid	6.14	4.48	3.35	0.41	0.00	0.00	1.88	1.47	1.33	2.07	1.83	1.44	1.08	1.08	1.13	2.40	1.48	(0.69)	0.31	0.42	0.34
Cadaverine	0.42	0.33	0.00	0.00	0.29	0.33	0.00	0.00	0.48	0.48	0.43	0.00	0.47	0.53	0.00	0.23	0.27	0.22	0.76	0.82	0.05
Caffeic acid	1.74	1.44	1.43	0.81	0.98	0.98	1.81	1.14	1.18	1.61	1.35	1.27	0.82	1.65	1.35	1.23	1.35	0.14	0.49	0.58	0.22
Cephalothin	0.00	86.32	0.00	87.81	78.60	77.66	0.00	0.00	70.42	0.00	0.00	0.00	69.75	69.47	72.78	55.06	31.38	(0.81)	0.28	0.38	2.00
Chizo-Inositol	0.00	1.26	1.19	1.81	0.00	0.00	0.79	1.14	1.03	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.33	(1.11)	0.28	0.38	0.18
Citric acid	33.23	21.74	27.21	19.46	15.69	16.99	15.19	12.83	9.69	10.51	9.20	8.69	7.12	8.41	7.44	22.38	9.90	(1.18)	0.00	0.00	0.65
Cyclohexanol	0.33	0.00	0.00	0.47	0.25	0.39	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.34	0.62	0.24	0.16	(0.55)	0.55	0.63	0.07
D-(+)-Talofuranose	3.06	2.38	2.11	1.62	0.00	0.00	0.00	2.01	1.56	0.00	0.00	0.00	0.00	0.00	0.00	1.53	0.40	(1.95)	0.05	0.10	0.16
D-(+)-Talose	2632.77	2300.85	1993.33	2134.59	2021.71	2060.50	2166.18	1754.75	1894.00	2105.26	1900.43	1890.99	1761.29	2029.89	1807.46	2190.62	1923.36	(0.19)	0.02	0.04	2.98
D-(+)-Talose	7807.93	6821.00	5884.16	5030.32	7568.70	5960.85	6328.20	5023.23	5057.73	5979.91	5299.36	5760.43	4924.17	5206.41	5036.75	6512.16	5401.80	(0.27)	0.02	0.04	5.51
D-(+)-Trehalose	677.82	448.57	508.57	132.17	139.13	136.96	41.22	57.68	35.15	65.88	48.78	41.60	58.44	69.41	71.15	340.54	54.37	(2.65)	0.00	0.01	2.87
D-(-)-Fructose	2.49	2.74	2.01	12.35	0.59	6.82	0.80	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.50	0.23	(4.30)	0.01	0.03	0.68
D-(-)-Ribofuranose	206.66	190.94	159.09	94.18	103.90	110.26	198.52	125.38	140.83	162.80	155.67	149.16	141.28	151.44	161.56	144.17	154.07	0.10	0.59	0.66	2.25
D-Allose	9.66	10.40	8.73	11.76	9.52	10.42	10.41	9.33	8.57	10.56	8.88	8.40	8.35	9.13	8.44	10.08	9.12	(0.14)	0.07	0.12	0.21
D-Arabinose	74.58	60.15	44.75	87.30	75.65	71.54	75.13	55.14	56.78	90.77	73.56	73.61	40.24	46.22	47.37	69.00	62.09	(0.15)	0.43	0.53	0.34
D-Fructose	5640.66	5483.04	4405.97	5300.11	4915.11	4897.13	4886.84	3969.96	3903.63	5037.39	4518.13	4525.44	4148.26	4649.78	4209.55	5107.00	4427.66	(0.21)	0.01	0.03	4.15
D-Galactose	4.77	13.16	2.40	6.96	10.49	20.45	4.05	5.64	3.71	8.08	6.56	0.00	5.63	4.71	6.33	9.71	4.97	(0.97)	0.06	0.12	0.69
D-Glucitol	3.01	3.04	2.41	3.84	3.34	2.47	1.76	1.84	1.82	1.92	1.52	1.83	1.29	0.97	1.45	3.02	1.60	(0.92)	0.00	0.00	0.33
D-Glucose	0.00	0.00	274.44	0.00	0.00	116.61	0.00	100.26	0.00	94.71	0.00	90.53	0.00	99.92	91.22	65.17	52.96	(0.30)	0.78	0.82	1.14
D-Mannose	11.40	11.48	9.43	0.00	6.71	0.00	25.51	15.45	16.94	5.30	5.73	13.57	7.57	3.09	2.37	6.50	10.61	0.71	0.28	0.38	0.73
D-Ribose	3.03	3.83	1.77	3.03	3.41	4.50	3.02	2.93	1.98	2.71	1.61	1.68	2.23	3.27	3.43	3.26	2.54	(0.36)	0.10	0.17	0.19
D-Threitol	3.93	3.01	3.11	3.28	2.71	2.41	2.44	2.57	2.09	1.65	1.44	1.35	1.13	1.69	1.23	3.07	1.73	(0.83)	0.00	0.00	0.24
D-Xylose	0.00	1.38	0.76	0.00	0.00	0.00	0.00	0.64	0.42	0.00	0.57	0.00	0.80	0.00	0.00	0.36	0.27	(0.40)	0.72	0.79	0.06
DL-Homocysteine	0.00	0.00	0.00	2.65	0.63	0.00	0.92	2.25	0.94	0.59	0.31	0.28	0.37	0.41	0.00	0.55	0.68	0.30	0.78	0.82	0.14
DL-Phenylalanine, TMS derivative	1.41	3.45	15.58	11.48	0.00	0.00	18.58	14.24	11.55	5.15	5.05	4.20	5.49	0.00	3.05	5.32	7.48	0.49	0.52	0.61	0.21
Decanedioic acid	0.29	0.00	0.41	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	(7.49)	0.02	0.04	0.08
Demexiptiline	3.38	1.83	1.83	0.00	0.00	0.00	1.16	0.96	0.94	0.00	0.45	0.00	0.34	0.00	0.00	1.17	0.43	(1.45)	0.16	0.24	0.20
Disiloxane	12.44	6.40	6.92	0.00	2.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.74	0.00	(12.21)	0.01	0.03	0.38
Dopamine	0.30	0.89	0.92	0.00	1.42	0.00	0.00	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.59	0.05	(3.49)	0.02	0.04	0.14
Erythritol	7.98	6.41	5.77	3.89	3.29	3.38	4.73	4.60	3.54	4.86	4.00	3.74	3.43	3.60	3.34	5.12	3.98	(0.36)	0.11	0.18	0.26
Ethanesulfonic acid	0.53	0.39	0.43	1.08	0.44	0.57	0.57	0.85	0.58	0.48	0.30	0.32	0.34	0.30	0.33	0.57	0.45	(0.34)	0.31	0.42	0.11
Ethanolamine	28.58	18.14	23.64	34.62	17.70	15.87	22.95	17.40	15.91	9.64	12.90	10.72	6.52	6.40	8.60	23.09	12.34	(0.90)	0.01	0.02	0.67
Ethyl D-glucopyranoside	4.39	4.13	3.21	5.65	4.07	4.58	4.25	5.24	3.68	4.98	3.37	3.52	3.45	4.11	2.81	4.34	3.93	(0.14)	0.35	0.46	0.13
Ethylene glycol	12.94	24.95	22.21	24.03	23.21	24.80	10.76	9.37	9.72	12.14	10.94	10.56	9.80	10.91	10.53	22.02	10.53	(1.07)	0.00	0.00	1.16
Fructofuranoside	4.70	2.97	3.09	3.54	3.51	3.22	2.07	1.46	1.40	1.49	1.46	1.22	0.95	0.87	0.97	3.50	1.32	(1.41)	0.00	0.00	0.33
Galactitol	8.29	7.27	6.29	6.82	5.93	7.10	7.08	6.84	5.49	6.60	5.48	5.11	5.58	5.84	5.14	6.95	5.90	(0.23)	0.02	0.05	0.17
Galactose oxime	21.70	18.22	8.72	14.11	13.82	10.82	20.94	10.44	12.53	13.74	16.16	14.95	8.27	8.28	13.08	14.57	13.15	(0.15)	0.54	0.63	0.48
Glipizide	4.92	4.15	4.79	14.67	1.76	3.61	9.49	45.35	10.75	4.92	2.34	2.07	7.67	9.51	2.23	5.65	10.48	0.89	0.42	0.53	0.41
Glucoheptonic acid	0.00	3.91	0.00	3.88	0.00	3.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.85	0.00	(10.86)	0.02	0.04	0.45
Glucopyranose	0.00	0.14	0.00	0.00	0.17	0.00	0.16	0.00	0.19	0.19	0.14	0.00	0.21	0.00	0.00	0.05	0.10	0.93	0.34	0.45	0.04
Glucose	189.14	158.38	143.57	91.27	93.78	104.40	180.87	114.74	139.14	164.57	142.53	136.77	133.96	158.85	143.54	130.09	146.11	0.17	0.31	0.42	2.36
Gly-Asp-Lys	3.15	6.19	0.00	0.00	0.00	0.00	0.00	0.00	12.46	0.00	0.00	0.00	0.00	0.00	0.00	1.56	1.38	(0.17)	0.93	0.95	0.15
Glyceric acid	55.70	37.11	38.70	39.29	29.00	32.45	27.59	21.36	17.20	25.55	20.50	21.21	15.84	21.06	18.96	38.71	21.03	(0.88)	0.00	0.00	0.77
Glycerol	69.37	44.64	52.05	51.73	34.70	35.14	38.82	29.96	30.09	25.86	23.84	22.80	21.73	24.46	22.12	47.94	26.63	(0.85)	0.00	0.00	0.82
Glyceryl-glycoside TMS ether	5.82	3.50	3.73	4.18	3.12	3.03	3.59	2.04	1.65	1.82	1.48	1.66	0.95	1.33	1.45	3.90	1.78	(1.13)	0.00	0.00	0.26
Glycine	24.64	20.91	22.92	21.88	14.52	13.37	23.31	28.00	19.92	24.19	27.45	25.34	19.41	22.17	20.86	19.71	23.40	0.25	0.09	0.14	0.68
Glycolic acid	28.76	16.49	19.14	3.66	3.00	1.58	3.00	2.05	2.00	2.28	1.92	2.08	1.98	2.62	1.84	12.11	2.20	(2.46)	0.02	0.04	0.56

Supplementary Table S1. Cont'd

Hydracrylic acid	4.28	4.31	3.58	4.25	3.55	3.60	3.10	3.04	2.32	2.25	2.80	3.05	2.97	3.23	2.41	3.93	2.80	(0.49)	0.00	0.00	0.24
Hydroxylamine	1.00	3.94	0.00	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.82	0.06	(3.74)	0.17	0.25	0.14
Ile-Trp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.10	0.13	0.12	0.00	0.00	0.00	0.05	5.71	0.06	0.12	0.05
Indoramin	0.00	0.00	1.17	1.51	0.00	0.00	0.00	0.48	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.07	(2.60)	0.14	0.22	0.19
Isocitric acid lactone	1.97	0.00	1.53	1.29	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.04	(4.31)	0.02	0.05	0.14
Kenpaullone	0.00	0.00	12.88	2.55	0.00	0.00	8.16	10.27	9.46	0.00	0.00	0.00	0.00	5.95	0.00	2.57	3.76	0.55	0.65	0.72	0.10
L-(+)-Threose	1.53	2.62	3.01	2.91	2.46	2.63	3.57	3.35	3.07	3.54	3.15	2.32	3.56	4.43	2.70	2.53	3.30	0.39	0.02	0.05	0.14
L-5-Oxoproline	354.46	347.75	609.11	550.95	264.70	210.44	847.06	1050.33	756.68	318.75	436.57	365.79	404.51	449.34	451.62	389.57	564.52	0.54	0.16	0.24	2.43
L-Alanine	52.94	45.41	30.31	40.35	28.06	30.96	32.10	31.99	26.12	23.77	14.08	17.20	13.08	21.33	27.53	38.00	23.02	(0.72)	0.00	0.02	0.68
L-Aspartic acid	17.62	17.19	25.50	21.48	14.00	13.46	15.00	12.69	12.05	5.55	5.04	3.49	3.27	2.07	1.95	18.21	6.79	(1.42)	0.00	0.00	0.83
L-Glutamic acid	1.63	1.33	1.43	0.88	1.18	0.00	0.00	1.32	0.00	0.00	0.55	0.38	0.55	0.00	0.34	1.08	0.35	(1.62)	0.02	0.04	0.15
L-Glutamine	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.39	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.06	0.13	0.94	0.96	0.07
L-Isoleucine	1.35	0.00	1.40	2.78	0.93	0.00	4.23	4.28	3.14	3.35	4.03	3.54	1.97	2.72	2.95	1.08	3.36	1.64	0.00	0.00	0.40
L-Lysine	18.27	14.57	21.57	16.57	8.65	8.14	24.01	21.65	17.29	13.45	16.95	17.40	10.28	9.65	12.92	14.63	15.95	0.13	0.63	0.70	0.42
L-Ornithine,	0.00	0.00	0.00	0.00	0.00	0.00	0.85	6.62	2.33	0.00	1.63	0.00	0.00	0.28	2.61	0.00	1.59	10.64	0.10	0.16	0.22
L-Phenylalanine	5.80	5.47	6.82	5.81	3.77	2.75	11.55	12.93	9.72	3.94	5.76	5.14	4.65	4.88	5.35	5.07	7.10	0.49	0.19	0.27	0.34
L-Rhamnose	4.71	3.69	3.01	3.18	2.49	2.38	2.07	1.77	1.53	1.77	1.52	1.31	1.20	1.35	1.41	3.24	1.55	(1.07)	0.00	0.00	0.24
L-Serine	32.29	23.94	31.73	52.80	18.80	12.09	46.51	53.76	38.18	24.22	37.89	31.11	19.33	26.56	25.56	28.61	33.68	0.24	0.45	0.55	0.43
L-Threonine	14.00	10.91	13.89	11.51	5.18	4.66	15.36	16.97	13.64	7.71	9.36	8.67	7.34	9.22	8.44	10.03	10.75	0.10	0.73	0.79	0.35
L-Threonine	0.00	0.17	0.08	0.00	0.17	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.02	(2.21)	0.12	0.19	0.04
L-Tyrosine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.63	16.37	0.00	11.64	13.98	0.00	6.74	12.72	0.07	0.12	0.55
L-Valine	7.18	6.02	5.24	5.18	3.93	3.84	3.05	3.80	3.20	4.97	2.75	4.15	3.25	5.68	4.24	5.23	3.90	(0.42)	0.04	0.07	0.19
L-Valine	3.53	3.51	3.56	5.24	2.40	2.06	7.17	6.63	5.56	5.03	5.81	5.39	3.59	3.93	4.17	3.38	5.25	0.63	0.01	0.03	0.36
Lactic Acid	32.79	69.89	26.51	17.46	13.43	22.19	33.20	17.02	18.42	22.18	19.27	22.41	16.27	16.49	17.11	30.38	20.26	(0.58)	0.18	0.26	0.53
Leu-Gly	0.00	0.00	1.23	3.76	1.16	0.00	4.16	4.27	3.20	2.62	3.28	2.80	0.00	1.95	1.94	1.02	2.69	1.39	0.04	0.07	0.23
Linoelaidic acid	2.19	1.87	2.50	3.43	2.14	2.44	3.36	1.64	1.49	1.23	1.06	0.00	0.00	0.00	1.11	2.43	1.10	(1.14)	0.02	0.04	0.27
Malic acid	2594.18	2371.89	1663.37	1928.18	1702.22	1647.61	2099.15	2077.36	1785.47	2103.90	1833.82	1781.87	1632.08	1739.73	1659.39	1984.58	1856.97	(0.10)	0.42	0.53	4.42
Maltose	17.23	7.92	13.48	10.70	4.79	4.73	6.12	2.66	2.21	3.45	1.24	1.43	0.72	1.38	2.86	9.81	2.45	(2.00)	0.00	0.01	0.47
Meperidine	0.00	0.22	0.00	0.40	0.44	0.42	0.00	0.47	0.27	0.29	0.35	0.00	0.00	0.25	0.00	0.25	0.18	(0.44)	0.53	0.61	0.12
Methyl galactoside	5.26	4.42	3.32	2.99	2.73	2.67	1.90	2.06	1.69	2.36	2.09	1.82	1.82	2.15	2.10	3.57	2.00	(0.84)	0.00	0.00	0.22
Myo-Inositol	67.73	55.66	61.48	16.36	14.39	11.60	5.14	9.61	4.69	6.50	5.57	4.01	5.71	6.37	5.98	37.87	5.95	(2.67)	0.00	0.01	0.95
Niflumic acid	0.00	1.14	1.02	0.00	0.00	0.00	0.71	1.34	0.19	0.00	0.47	0.00	0.19	0.00	0.00	0.36	0.32	(0.16)	0.89	0.92	0.07
Oxalic acid	71.85	66.80	66.44	129.19	108.93	114.91	63.32	74.12	73.01	75.13	65.83	72.50	72.18	91.65	67.18	93.02	72.77	(0.35)	0.06	0.11	1.75
Palmitic Acid	10.59	7.92	8.87	22.14	12.81	16.58	17.74	9.96	11.72	12.76	11.58	11.98	8.53	10.13	12.06	13.15	11.83	(0.15)	0.53	0.61	0.35
Pentanedioic acid	1.80	1.40	1.43	1.20	0.90	0.00	0.00	1.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.12	0.12	(3.18)	0.00	0.01	0.19
Pentasiloxane	16.09	17.54	13.71	13.14	12.29	12.05	13.71	11.13	11.61	14.23	12.83	13.71	10.59	14.49	12.42	14.14	12.75	(0.15)	0.16	0.24	0.26
Phosphoric acid	74.15	45.08	63.60	56.06	42.03	38.87	34.72	32.06	21.90	20.32	18.38	18.03	14.55	15.43	18.34	53.30	21.52	(1.31)	0.00	0.00	1.16
Pinacol	26.79	19.85	23.11	5.40	5.88	5.51	2.20	4.24	2.34	3.82	3.15	2.47	4.11	4.43	4.15	14.42	3.43	(2.07)	0.01	0.02	0.55
Propanal	1.13	1.19	1.17	0.00	0.33	0.00	0.00	0.17	0.00	0.15	0.00	0.16	0.18	0.00	0.00	0.64	0.07	(3.13)	0.01	0.04	0.12
Propane	129.42	86.12	112.60	23.26	26.66	23.45	8.93	18.78	10.16	15.29	13.94	10.66	18.62	16.91	18.57	66.92	14.65	(2.19)	0.01	0.02	1.21
Propanedioic acid	1995.95	1175.56	1681.84	1022.72	945.90	914.86	512.12	401.78	422.33	462.35	399.81	381.46	405.02	558.61	475.54	1289.47	446.56	(1.53)	0.00	0.00	5.61
Propanoic acid	0.97	0.52	0.35	0.23	0.00	0.00	0.35	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.34	0.06	(2.58)	0.05	0.09	0.11
Ribonic acid	3.00	2.00	2.47	1.74	1.33	1.88	1.75	1.68	1.15	1.59	1.44	1.23	1.21	1.02	1.26	2.07	1.37	(0.60)	0.01	0.02	0.14
Sedoheptulose	16.88	16.70	18.40	16.82	9.41	9.55	15.43	13.19	7.61	17.32	13.74	16.47	16.41	0.00	11.43	14.63	12.40	(0.24)	0.42	0.53	0.18
Shikimic acid	578.15	244.78	325.48	163.80	164.65	246.22	162.64	81.80	93.43	141.68	128.51	136.30	109.35	112.45	145.47	287.18	123.52	(1.22)	0.01	0.02	2.25
Silanol	106.09	87.31	84.97	85.58	67.90	62.43	82.68	69.12	65.34	48.51	47.04	42.90	57.06	66.19	62.63	82.38	60.16	(0.45)	0.01	0.03	0.76
Stearic acid	4.19	2.55	2.64	4.01	2.38	2.01	2.18	1.35	2.33	2.41	1.96	2.17	0.00	0.00	3.04	2.96	1.72	(0.79)	0.04	0.07	0.17
Sucrose	13.28	9.11	12.09	21.68	9.85	12.54	5.18	6.77	5.53	0.00	2.67	0.00	1.95	0.00	0.00	13.09	2.46	(2.41)	0.00	0.00	0.97
Talose	5.67	4.79	2.35	8.65	6.13	6.03	5.28	3.44	3.29	5.49	5.04	4.54	2.97	3.53	3.59	5.60	4.13	(0.44)	0.08	0.13	0.26
Tartaric acid	2.46	1.95	1.77	1.22	1.16	1.21	1.08	0.00	0.86	0.00	0.00	0.00	0.00	0.00	0.00	1.63	0.22	(2.92)	0.00	0.00	0.24
Terephthalic acid	5.85	2.59	2.32	1.89	1.81	1.59	1.96	1.52	1.80	1.36	0.00	1.29	0.00	0.00	0.00	2.68	0.88	(1.60)	0.01	0.04	0.23
Tetrasiloxane	5.66	5.35	6.11	5.54	5.20	7.17	6.37	5.31	6.22	7.40	6.74	8.81	9.26	12.28	11.86	5.84	8.25	0.50	0.04	0.08	0.36

Supplementary Table S1. Cont'd

ethylene	434.32	347.13	432.77	74.59	54.23	45.20	17.64	26.94	14.05	24.84	20.02	14.63	21.05	24.90	20.79	231.37	20.54	(3.49)	0.01	0.02	2.40
carbamate	20.06	18.78	17.15	19.34	18.03	20.32	17.27	16.17	15.96	18.75	17.14	18.90	17.85	19.13	18.52	18.95	17.74	(0.09)	0.08	0.13	0.18
Trisiloxane	4.37	3.83	3.58	3.95	3.54	3.62	3.70	3.56	3.45	4.06	3.28	3.57	3.33	4.61	3.26	3.81	3.65	(0.06)	0.44	0.54	0.10
Xylitol	8.97	6.64	8.11	10.28	6.82	0.00	6.11	4.17	5.25	4.58	4.62	0.00	3.33	0.00	0.00	6.80	3.12	(1.13)	0.03	0.07	0.31
Xylonic acid	8.99	7.68	7.36	6.97	5.85	5.62	5.52	5.51	4.20	4.49	3.81	3.76	2.92	3.33	2.94	7.08	4.05	(0.80)	0.00	0.00	0.34
Xylulose	45.35	41.82	28.17	40.60	36.36	37.58	30.86	24.29	22.52	30.47	27.08	25.22	21.42	25.53	22.10	38.31	25.50	(0.59)	0.00	0.00	0.73
d-Galactose	75.56	157.09	97.02	25.95	21.77	25.82	65.78	57.05	1045.43	31.72	25.20	38.43	26.70	26.98	23.37	67.20	148.96	1.15	0.57	0.65	1.17
d-Mannose	9.62	6.91	5.01	8.24	6.25	5.56	3.52	3.83	2.71	2.25	2.32	2.21	0.97	0.82	0.85	6.93	2.16	(1.68)	0.00	0.00	0.49
propanoic acid	10.41	8.03	6.69	8.55	6.40	5.17	8.79	8.78	6.52	5.12	5.08	4.22	5.68	3.99	4.46	7.54	5.85	(0.37)	0.10	0.17	0.23
trans-2-Hydroxycinnamic acid	2.84	1.19	1.88	1.65	0.80	0.77	1.00	0.55	0.00	0.44	0.38	0.00	0.00	0.00	0.00	1.52	0.26	(2.53)	0.00	0.01	0.20

Vol. 16(6), pp. 125-137, July 2022
DOI: 10.5897/AJPS2022.2244
Article Number: 3C8884C69441
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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African Journal of Plant Science

Full Length Research Paper

Effects of grain spawn and substrates on growth and yield of oyster mushroom grown under different cropping shelters

Modeste Hakizimana[1]*, Robert M. Gesimba[1] and Japhet M. Muthamia[2]

[1]Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536-20115, Nakuru, Kenya.
[2]Biological Sciences, Egerton University, P. O. Box 536-20115, Nakuru, Kenya.

Received 18 March, 2022; Accepted 13 May, 2022

Limited access to oyster mushroom (*Pleurotus Ostreatus*) substrates and the high cost of production are among the constraints affecting mushroom farming in Kenya. In an effort to solve the above problem, a study was carried out in Egerton University to determine the effect of grain spawn and substrates on growth and yield of oyster mushroom grown under different cropping shelters. The study was laid out as 8×2×3 factorial experiment in a completely randomized design (CRD), where 8 levels of substrates (wheat straw, kikuyu grass, uncomposted grevillea sawdust, corn cobs, and their combinations), 2 levels of grain spawn (popcorn, rice), and 3 levels of cropping shelters (mikeka, shade net and dark house) were evaluated on their effect on growth and yield of oyster mushroom. The results showed that the substrates and cereal grain spawn significantly affected the growth and yield of *Pleurotus ostreatus* grown under mikeka, shade net, and dark cropping shelters at P≤0.005. The total biological efficiency showed the highest yields in interaction of mikeka shelter × corn cobs × rice spawn with 109.1 g, respectively. The study recommends corn cobs with rice spawn grown under mikeka cropping shelter to be used for the production of oyster mushroom in Kenya.

Key words: Mushroom seeds, agriculture residuals, production structure, harvest.

INTRODUCTION

Commercial mushroom farming and enterprises in Kenya are relatively few compared to other places. However, the mushroom industry in the country is rapidly growing, and production cannot currently meet increasing local demand. The total production of mushrooms is 500 tons per year against the demand of 1200 tons annually (NAFIS, 2014). In Kenya, unlike previously when consumption was confined to rural communities, urban dwellers are increasingly consuming mushrooms (Ojwang, 2014). The increase in demand for edible mushrooms has resulted in the setting up of several mushroom units in different parts of the country. Mushroom cultivation has not been given a lot of importance and the sector is underdeveloped with only two exotic species (*Pleurotus ostreatus* and *Agaracus bisporus* grown for the hotel industry) (Waiganjo et al., 2008; Odendo et al., 2009; Onyango et al., 2011). Oyster mushroom (*P. ostreatus*) is the second largest

*Corresponding author. E-mail: modestehakim@gmail.com.

commercially produced mushroom in the world, second to *A. bisporus* (Sánchez, 2010; Pardo-Giménez et al., 2010). Among the white-rot fungi, oyster mushrooms of genus *Pleurotu*s are well-known for the conversion of lignocellulosic materials into fruiting bodies (Porselvi and Vijayakumar, 2019).

P. ostreatus are grown on organic substances termed as substrates, which are lignocellulosic material that supports the development, growth and fruiting of mushrooms (Anike et al., 2016). Mushroom spawn is the planting material or mycelium that serves as seed of a given substrate in mushroom cultivation (Stanley and Awi-Waadu, 2010). *P. ostreatus* is the best mushroom crop to cultivate in developing countries for many reasons. One of the reasons is that they are grown on agricultural residuals (Kumla et al., 2020). By-product residuals that are generated at harvesting time are normally disposed by burning and the smoke produced is usually an environmental nuisance. Therefore, by using the waste to cultivate mushrooms, the environment is conserved (Singh et al., 2020). Many agriculture wastes are lignocellulosic materials, so they could be a suitable substrate for solid-state fermentation processes required by oyster mushrooms to grow and produce edible fruiting bodies (Ritota and Manzi, 2019). Compared to other types of mushrooms, oyster mushroom (*Pleurotus* spp.) utilizes more varied kinds of substrate materials (Yang et al., 2016) to produce biomass of high market value. Transformation of those unused agricultural residuals into useful materials in mushroom farming is the one of the solutions to reduce the threat to the environment and public health, that are increasingly associated with alternative waste disposal methods, such as burning and other forms of environmentally destructive disposal of agricultural wastes. Lignocellulosic materials such as wood materials, sawdust, cereals straws, bagasse, papers, grasses and cotton seed hull, and uncomposted grevillea sawdust are used for mushroom cultivation (Tekeste et al., 2020; Tesfay et al., 2020; Baysal et al., 2007; Nongthombam et al., 2021l).

Mushroom farming can be a good source of employment as an agro-industrial activity (Thakur, 2020); and thus it can help as a source of income, employment. It also presents a good opportunity for small to middle-scale farmers, such as women and youth, in developing countries where the standard of living is very low (Amuneke et al., 2011). Mushroom farming for small farmers requires relatively little space; they can be stacked using shelf-like culture systems, other materials that can create moisture and low temperature like, mud, mikeka (a traditional mat made out of sisal fibres), and shade nets (black shade of 60% density). Therefore, this will lead to an increase in the economy of not only small-scale farmers but other weak sections of communities as well (Shah et al., 2004). Generally, *Pleurotus* spp. cultivation technology is very crucial in solving the problems of pollution of the environment, shortage of food and malnutrition, which are the challenges that human beings are still facing, due to the continued increase of climate change, and natural resource degradation and pollution all over the world (Oseni et al., 2012). This study aimed at investigating the effect of different sources of mushroom substrate on the growth and yield of oyster mushroom, including: cereal grain spawn (popcorn, rice); local substrates (popcorn cobs, uncomposted grevillea sawdust, kikuyu grass, wheat straw); and cropping shelters (mikeka, shade net and dark house).

MATERIALS AND METHODS

Experimental sites

This field-based research was conducted under three shelter structures between January 2021 and July 2021 at Egerton University in three experimental fields. The site lies between longitude 35° 35´ E and latitude 0° 23´ S, and at an altitude of 2238 m asl. The annual mean precipitation is 1000 mm and the annual mean temperature is 15.9°C. The site is situated in the agro-ecological zone III and has thick humic topsoil (mollic andosols) (Jaetzold et al., 2007). The site has high relative humidity and low temperature, which are suitable for oyster mushroom production.

Variety description

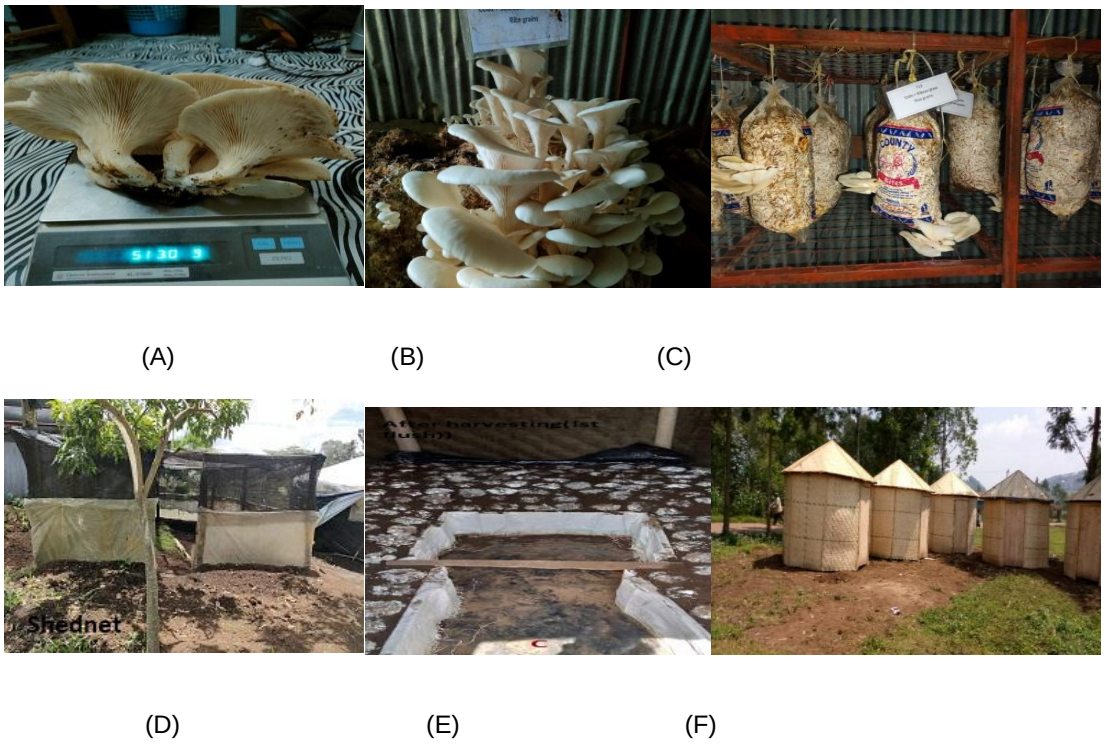
Oyster mushroom is scientifically classified in the Kingdom – Fungi, Phylum – Basidiomycota, Class – Agaricomycetes, Order - Agaricales, Family - Pleurotaceae, and Genus – *Pleurotus*, Species - *Pleurotus ostreatus* (Randive, 2012). The pearl oyster is a common mushroom prized for its edibility. Most edible mushrooms do well within a pH range of 3 to 7 at a temperature ranging from 20°C to 25°C (Randive, 2012). The ecological requirements of *P. ostreatus* vary at the various stages of the growing period. The optimal temperatures for growing mycelia and pin forming are between 20 to 30°C and 10 to 20°C, respectively. Substrate moisture should be from 60 to 75%, but it should be 80 to 95% during the fruiting because 80%, or substantially more, of the fruit body is water (Nadir et al., 2016).

Substrates preparation

The dry wheat straw, uncomposted grevillea sawdust, popcorn cobs, and lawn grass were cleaned and air-dried. The straws were chopped into pieces of 2 cm width and 4 cm length, according to the suggestions made by Kimenju et al. (2009). The organic residuals were then soaked in water for 3 days. The wastes were dried to minimize the moisture content to 75%. All substrates were emended with dry wheat bran (5% by weight) to increase the amount of nitrogen and some minerals, and 1.5% by weight dry calcium carbonate to adjust the pH of organic wastes (Zakil et al., 2022; Carrasco et al., 2018). The dried mixture of organic wastes was packed in polypropylene bags (12 × 22 cm), then they were tied with a rubber band, and each bag contained 1200 g of cultivation substrate. Pasteurization of substrates was carried out using hot steam at 80°C for 6 h within a metal barrel.

Spawning

Spawning was done under aseptic conditions (Laminar airflow



(A) (B) (C)

(D) (E) (F)

Figure 1. Images of mushroom fruiting bodies and related production operations. (A) weight of one cluster (B) Yields under shade net (C) Mushroom production house (D) Shade net structure (E) Mikeka after 1st flush (F) Mikeka structure.
Source: Authors

hood). The grain spawns (popcorn and rice) obtained for the first experiment (that is, tissue culture) were mixed in all substrates using 45 g of the total weight of the packet. After spawning, bags were kept in total darkness, and 9 small holes were pierced in the walls of the bags for aeration.

Spawn running

Room temperature, varying from 22 to 26°C, and relative humidity of 80 to 90% were maintained during the spawn run. Humidity was maintained by water spraying three times a day. After the completion of spawn run in the straw, the substrate became a compacted mass, which also stuck to the polypropylene bags. And after the complete spawn run in the bags, some of the bags were moved to an outdoor location under a semi-tunnel with soil in a shade net and mikeka shelters. Others were maintained in a dark mushroom house.

Fruiting management

Egerton university farm (named field three) was selected as a site where the ambient temperature can be manipulated to vary between 15 and 25°C, and the clay content of the soil at 40-cm depth is about 20%. Besides production houses for mushroom cultivation, shades were built using a net of 60% and others using mikeka, semi-tunnels were built inside the shade net. For Shade net, a trench of 2.5 m wide, 6.3 m long, and 0.4 m deep was dug and the trench was divided into 48 experimental units; whereas, for mikeka shade, a circular trench of 9-m circumference, 2.8 m in diameter and 0.4 m deep were built. Then, the floor was disinfected by treating with 2.0 kg of hydrated lime for preventing pests such as termites and moles. A portion of the top-soil (20 cm) was set aside for later use as a 'cover' over the mushroom substrate after digging the trench. The soil was also sterilized with hydrated lime (1 full wheelbarrow with 0.5 kg of lime). The plastic bags were removed from the substrate packs that were neatly placed vertically into the trench (3 per experimental unit) and the substrates were covered with disinfected soil to a depth of 15 mm. The plastic semi-tunnels were constructed over the filled trench and Bamboo was used to construct the semi-tunnel under the shade net. The trench containing the mushroom substrate was watered with 10 l of clean water per day and per square meter of trench for maintaining the moisture content of substrates. Then l closed the semi- tunnels using the clear tunnel and waited until the formation of the primordia (3-7 days); water was reduced to 10 l clean water within the entire trench. Once the fruit bodies (mushrooms) appeared, the amount of water applied was reduced to 5 l per day and per square meter within the entire trench. The semi-tunnels were opened between 10:30 and 11:00 am for 30 min every day for aeration; however, during one rain they were left closed for that day irrespective of the season. For the dark mushroom production house, 144 bags were used in that environment according to the 18 treatments with 3 replications, where each experimental unit had 3 bags hanging under shelves. For the initiation of pinheads and fruiting bodies, a temperature of 18-21°C and a relative humidity of 75-90% were maintained. Figure 1 shows the process of production under different structures.

Data collection

Data were collected on the following parameters:

Full colonization to pinhead formation [TFCP] (days)

After full colonization of bags, the formation of primordia were observed during every two days-intervals; and the number of days the bag took for the first primordia formation was observed and recorded.

Length of Stalks [LS] (cm)

Length was measured using a ruler (units for ruler is in cm ruler). Five fruiting bodies were randomly selected using a simple random technique. The lengths of the stalks were measured from the tip of the stalk to the base of the caps. This was done for each harvest.

Diameter of the cap [DC] (cm)

Diameter was measured using a thread; and its length was determined using a measuring rule. The thread was used to trace the diameter of the caps of the five randomly selected fruiting bodies, and the length of the thread that stretched across the diameter of the caps was measured on the tape ruler, and the value recorded. This was done for each harvest.

Average number of primordia per packet [ANP]

Number of primordia was measured by accounting for the total number of primordia and dividing by the number of packets.

Number of fruiting bodies per packet [NFB]

Only well-developed fruiting bodies per each packet were counted, while dry and pinheaded fruiting bodies were discarded. However, tiny fruiting bodies were included in the counting.

***The average weight of the individual fruiting body* [AWIF]**

The weight of each fruiting body was calculated by dividing the total weight of the fruiting bodies per packet by the total number of the fruiting bodies per packet.

***Biological yield* [BY]**

Biological yield per each harvest was measured by weighing the whole cluster of the fruiting body per each treatment and per each harvest, without removing the lower hard and dirty portion.

***Biological efficiency* [BE]**

Yield of mushroom per weight of substrate (on a dry weight basis) was calculated by the formula proposed by Chang et al. (1981) as follows:

$$\text{Biological efficiency (\%)} = 100 \times \frac{total\ biological\ weight(g)}{total\ weight\ substrate(g)}$$

***Economic yield* [EY]**

Economic yield per treatment was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

Statistical analyses

Normality of the data was examined using the Shapiro Wilk test to determine if the data was sufficiently distributed customarily to meet the assumptions of the statistical tests and the Probability Plot at 95% of the confident interval were conducted in SAS Software 9.4 M6 (SAS Institute inc, Cary, NC 2017) prior to analysis. Analysis of variance (ANOVA) was also performed for determining if there were significant differences among the grain spawn, substrates and cropping shelters ($P \leq 0.05$). Tukey grouping for differences in means and post ANOVA was used to observe the differences between the substrates. Pearson correlations were used for testing the relationship between growth and yield of oyster mushroom.

Statistical model

The following is the linear model:

$$Y_{ijkl} = \mu + R_k + G_i + E_j + EG_{ij} + S_k + GS_{ik} + ES_{jk} + EGS_{ijk} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = Overall observations, μ= Overall mean, Ei= Effect due to i^{th} environment level, $R_{k=}$ Effect due to ki^{th} replication level, S_k= Effect due to k^{th} substrate level, G_j= Effect due to j^{th} grain spawn levels, EG_{ij}= Interaction effect due to environment and grain, ES_{ik}= Interaction effect due to environment and substrate, GS_{jk}= Interaction effect due to grain and substrate, EGS_{ijk}= Interaction effect due to environment, grain, and substrate, ε_{ijkl}= Random error.

RESULTS

Effect of two cereal grain spawns on yield

The effect of grain spawn on growth and yield is presented in Table 1. Grain spawn significantly influenced yield ($P \leq 0.05$) for the variables TFCP, LS, NFB, ANP and BY4th (definition of abbreviations is seen in Table 1). Popcorn spawn (4.8 days) was better in TFCP than rice spawn (5.0 days) and were statistically significant from each other**.** Rice spawn (5.0cm) was better than popcorn spawn (4.8 cm) in influencing LS**.** Rice spawn highly influenced (11.8 cm) NFB of oyster mushroom compare to popcorn spawn (11.1 cm). The rice spawn highly affected (15.5) the ANP of mushroom while popcorn spawn affected less (14.7); whereas both cereal grain spawn were not significantly different from each other on DC, all flushes, TBY, BE and EY**.**

Effect of different substrates on yields

The effects of different substrates on oyster mushrooms are presented in Table 2. The results indicated that the substrates significantly influenced ($P \leq 0.05$) the TFCP, LS, DC, NFB, ANP, AWIF, BY1st, BY2nd, BY3rd, BY4th, TBY, BE and EC. Growth and yield for oyster mushrooms widely varied under different substrate levels. The highest TFCP (6.35 days) was obtained with S3; whereas the lowest (3.85 days) was observed under S3. Therefore, the TFCP Tukey grouping for means of substrates ($P \leq 0.05$) showed that S1, S2, S5 and S8; S3, S7, S1,

Table 1. Effect of two cereal grain spawns on yields.

	Means (%)												
	Days	cm					Grams						
Spawn	TFCP	LS	DC	NFB	ANP	AWIF	BY1st	BY2nd	BY3rd	BY4th	TBY	BE	EY
Rice	4.85[a]	5.51[a]	9.41	11.19[a]	14.73[a]	27.00	348.05[a]	312.65	201.34	101.38	963.42	68.82	907.54
Popcorn	5.08[b]	5.88[b]	9.58	11.86[b]	15.51[b]	27.55	362.39[b]	322.29	203.78	101.60	990.07	74.29	981.95
Std. Errors	0.06	0.05	0.07	0.10	0.09	0.22	6.05	5.37	4.90	2.14	12.95	2.66	37.29

TFCP: Time from full colonization to primordia initiation, LS: Length of the stalk, DC: Diameter of caps, NFB: Number of fruiting bodies, ANP: Average number of primordia initiation, AWIF: Average weight of individual fruiting bodies, BY1st: Biological yield of 1st flush., BY2nd: 2nd flush, BY3rd: 3rd flush, BY4th: 4th flush, TBY: Total biological yield, BE: Biological efficiency and EC: Economic yield. The means followed by the same letters are not significantly different using Tukeys' honest significant difference (HSD) test at 5% level of significance.
Source: Authors

Table 2. Effect of different substrates on yields.

	Means (%)												
	Days	cm					Grams						
Sub.	TFCP	LS	DC	NFB	ANP	AWIF	BY1st	BY2nd	BY3rd	BY4th	TBY	BE	EY
S1	4.00[d]	4.50[e]	8.26[d]	10.98[cd]	13.85	30.44	409.50	374.95	253.07	101.34	1138.86	81.35[bc]	1070.4[bc]
S2	5.32[bc]	4.44[ef]	7.52[e]	12.85[a]	16.83	20.97	269.03	232.46	160.75	79.70	741.93	53.00[de]	691.7[de]
S3	6.35[a]	4.04[f]	7.52[e]	10.39[d]	13.43	16.01	139.23	125.40	99.74	68.26	432.62	30.90[e]	390.7[e]
S4	3.85[d]	7.04[b]	11.15[b]	12.85[a]	17.32	34.34	536.83	473.19	295.71	125.43	1431.15	116.51[a]	1554.6[a]
S5	5.54[b]	5.07[d]	8.57[cd]	10.37[d]	14.00	23.17	251.44	210.94	123.62	77.20	663.19	47.37[de]	629.3[de]
S6	4.89[c]	5.27[d]	9.13[c]	10.98[cd]	14.57	27.55	342.64	306.04	162.40	105.76	916.85	65.49[cd]	873.4[cd]
S7	5.44[b]	6.62[c]	9.11[c]	11.68[bc]	15.15	32.09	430.61	390.87	244.82	126.98	1193.29	85.24[bc]	1126.3[bc]
S8	4.33[d]	8.59[a]	14.72[a]	12.09[ab]	15.80	33.62	462.50	425.90	280.38	127.27	1296.04	92.58[b]	1221.1[b]
SE	0.12	0.09	0.14	0.19	0.17	0.45	12.09	10.75	9.79	4.28	25.90	5.33	74.58

Sub: substrate, S1: wheat straw, S2: Pre-composted grevillea uncomposted grevillea sawdust, S3: kikuyu grasses, S4: popcorn cobs, S5: kikuyu grass+ uncomposted grevillea sawdust, S6: uncomposted grevillea sawdust+ popcorn cobs, S7: uncomposted grevillea sawdust+ popcorn cobs+ kikuyu grass, S8: uncomposted grevillea sawdust+ popcorn cobs+ wheat straw, Grain1: rice grain spawn, Grain 2: popcorn spawn. The means followed by the same letters are not significantly different using Tukeys' honest significant difference (HSD) test at 5% level of significance.
Source: Authors

and S6 were significantly different from each other; whereas S5, S7 and S2; S2, S6 and S8, S1, S4 were not significantly different from each other. The LS of oyster mushroom was highly influenced by S8 (8.5cm) followed by S4 (7.0 cm). And the least was S3 with 4.0 cm of LS; and substrates S8, S4, S7, S6, S1, and S3 showed significant differences from each other, while S5, S6 and S2, S3 did not show any significant difference from each other. The highest DC values of oyster mushroom caps were found in S8 substrate (14.7 cm), and the smallest was S3 with

Table 3. Different cropping shelters on oyster mushroom yields.

	Means												
	Days	cm					Grams						
Shelters	TFCP	LS	DC	NFB	ANP	AWIF	BY1st	BY2nd	BY3rd	BY4th	TBY	BE	EY
Mikeka	4.68[b]	7.31[a]	9.92[a]	12.04[a]	14.58	28.50	395.42	350.94	203.88	102.73	1052.96	75.21	998.55
Shade net	4.65[b]	6.76[b]	9.92[a]	11.80[a]	14.49	28.04	363.79	317.18	186.87	86.56	954.40	68.17	896.99
dark house	5.57[a]	3.02[c]	8.65[b]	10.74[b]	16.29	25.29	306.46	284.29	216.92	115.19	922.87	71.28	938.70
Stad. Errors	0.07	0.06	0.09	0.12	0.10	0.27	7.41	6.58	6.00	2.62	15.86	3.26	45.67

Where the abbreviations are: TFCP: Time from full colonization to primordia initiation, LS: Length of the stalk, DC: Diameter of caps, NFB: Number of fruiting bodies, ANP: Average number of primordia initiation, AWIF: Average weight of individual fruiting bodies, BY1st: Biological yield of 1st flush BY2nd: 2nd flush, BY3rd: 3rd flush, BY4th: 4th flush, TBY: Total biological yield, BE: Biological yield and EC: Economic yield. The means followed by the same letters are not significantly different using Tukeys' honest significant difference (HSD) test at 5% level of significance.
Source: Authors

7.5 cm of oyster DC. Moreover, S8, S4, S6, S1, and S2 were significantly different, while S6, S7; S5, S1 and S2, S3 were not significantly different from each other. The substrate S4 highly affected the NFB (12.8) while the less substrate was found with S5 of 10.3 fruiting bodies. Moreover, the results showed that S4, S7; S8, S6 and S8, S1 were statistically different from each other, while S4, S2; S8, S7; S6, S1 and S5, S3 were not significant. S4, like NFB, had the greatest influence on the average number of primordia with 17.3, followed by uncomposted grevillea sawdust with 16.8 fruiting bodies, while S3 had the least influence on the average number of primordia per cluster, with 13.4 primordia. Therefore, S4, S7, S5; S2, S8, S1 and S3, S7 were significantly different; whereas S4, S2; S8, S7; S7, S6 and S5, S3, S1 were not different from each other. The maximum and minimum AWIF were found in S4 (34.3 g) and S3 with 16g. Therefore, the mean separation showed that S4, S7, S6; S5, S2 and S3 were significantly different from each other; whereas S4, S8; S8, S7; S7, S1 were not significant from each other. The biological yields for all flushes showed that S4 mostly influenced the yields of oyster mushrooms unless on the 4th flush, where S8 mostly affected the yields; and S3 was the least in influencing the yields for all flushes. The biological yield of 1st flushes Tukey grouping for means of substrates showed that S4, S8, S6, S2 and S3 were significantly different; whereas S8, S7 and S1 were not. For the 2nd flush, S4, S8, S1, S2 and S3 were significantly different; whereas S2 and S3 were not. For the 3rd flush, S4, S6 and S3 were significantly different; whereas S6 and S2; S8 and S1 were not. For 4th flush, S8, S6 and S2; S7, S1and S5 were significantly different; whereas S8, S7 and S4; S6 and S1; S2, S5 and S3 were not. The TBY and BE for oyster mushroom showed the highest yields on S4 (143.1 and 116.5 g of yields, respectively); whereas S3 substrates was the least in influencing TBY and BE (432.6 and 30.9 g yields, respectively). Therefore, S4, S8, S6, S2 and S3 were significantly different; whereas S8, S7 and S1 were not for TBY, respectively. However, S4, S1 and S5 were significantly different from each other; whereas S7, S1 and S6; S2, S5 and S3 were not for BE. Substrate (S4) with 1554.6 g was the best in influencing the EC of oyster mushrooms; whereas the least was kikuyu grass with 390.7 g of yields. The mean separation indicated that S4, S8 and S3 were significantly different, while S7, S1, S6; S2, S5 and S3 were not significantly different from each other.

Effects of different cropping shelters on oyster mushroom yields

The effects of different cropping shelters are presented in Table 3. The findings showed that cropping shelters statistically affected (P≤ 0.005) the TFCP, LS, DC, NFB, ANP, AWIF, BY1st, BY2nd, BY3rd, BY4th, and TBY. Mikeka shelter structure took fewer days (4.6) for TFCP, followed by shade net shelter structure (4.67 days) and the least was dark house shelter (5.56 days); and both dark house shelters were significantly different from others. Mikeka shelter (7.3 cm) highly affected the LS of oyster mushroom; whereas dark house shelters (3.0 cm) affected less. Therefore, all shelters were significantly different from each other. The oyster mushroom produced under mikeka structure had a high DC (9.9 cm), while dark shelter structure produced small mushrooms (DC=8.6 cm). The dark house shelters were statistically significantly different from others. The production carried under mikeka shelter was the best in NFB (12.0); while the dark shelter (10.7) was the least and the dark house shelter was significantly different from others. Dark shelter mostly influenced the ANP (16.2) followed by mikeka structure(14.5), while the shade net structure (14.4) was less to influence the ANP and dark house shelter was significantly different from others. Mikeka shelter mostly contributed the highest yields (28.4g) of AWIF of oyster mushroom whereas dark shelter was the least with 25.2g and dark house shelter was significantly different from others**.** The TBY of oyster mushroom produced under mikeka shelter was the highest with 1052.9 of yields. The dark house shelter was significantly different from others, whereas none of them were

Table 4. Pearson correlation coefficients for vegetative growth, primordia initiation and average number of fruiting bodies for oyster mushroom.

Variable	Vegetative (mycelium)	Fruiting body
Fruiting body	0.384**	-
Primordia	0.277**	0.69.7***

, * significant at (P≤0.01) and (P≤0.001) respectively.
Source: Authors

different from each other for BE. All Cropping shelters (mikeka, shade net and dark house) were not significantly different from each other for EY.

Interaction of cropping shelters× substrates on oyster mushroom yields

The effect of different local cropping shelters× substrates on oyster mushroom yields are presented in Table 5**.** The findings showed that cropping shelters statistically affected (P≤0.005) the TFCP, LS, NFB, ANP and AWIF. The minimum and maximum TFCP were obtained in mikeka shelter × S4 (3.3 days) and dark house shelter (6.7 days). The interaction between mikeka shelter × S8 was the highest with 11.9 cm of stalk; whereas the lowest was found in the interaction of dark house× S1 with 2.7 cm of the stalk length. The maximum and minimum cap diameters were found in the interaction between mikeka and S8 with 1.9 cm and interaction between between dark shelter and S2 with 6.7 cm, respectively. The interaction of mikeka× S4 was the highest with 13.9 for fruiting bodies; whereas the lowest was observed in the interaction of dark house× S3 with 9.32 for oyster mushroom fruiting bodies. The highest and lowest ANP were obtained from the interaction of dark shelter× S2 (18.8) and interaction of shade net× S1 (12.8) for oyster mushroom primordia, respectively. The interaction of shade net × S4 substrate were the highest with 35.8g for fruiting bodies; and the lowest were found in the interaction of dark shelter× S3 with 115.4 g for oyster mushroom fruiting body. Based on the interaction, the maximum yields were observed in the interaction of dark shelter× S4 with 1501.3g for TBY and with 107.2g of BE; whereas the minimum yields were observed in the interaction of S3× dark shelter with 436.07g for TBY and 31.15g for BE**.** Both spawn were not significantly different from each other on TBY and BE. The highest and smallest EY of oyster mushroom were found on the interaction of mikeka shelter× S4 and mikeka shelter× S5 with 162.4 and 44.4g of yields, respectively.

Interaction of cropping shelters× grain spawn, substrates × grain spawn, substrates × shelters × grain spawn on oyster mushroom yields

The findings showed that the cropping shelters × grain spawn, substrates × grain spawn, and substrates × shelters × grain spawn had no statistically significant influence (P ≥ 0.005) on the TFCP, LS, DC, NFB, ANP, AWIF, BY1st, BY2nd, BY3rd, BY4th, TBY, BE, and EY.

Pearson correlation for vegetative growth (mycelium growth), primordia initiation and average number of fruiting bodies

The Pearson Correlation for vegetative growth (mycelium growth), primordia initiation and average number of fruiting bodies revealed that there was no strong positive correlations between mycelium growth and number of fruiting bodies (r = 0.38.**); between primordia initiation and mycelium growth (r = 0.27**); whereas there was a slightly strong correlation between primordia initiation and fruiting bodies (r = 0.69***), although all are statistically significant (P < 0.05) (Table 4).

DISCUSSION

Oyster mushroom growth response to spawn, substrates and cropping shelters

Pleurotus ostreatus is well known for its degradation ability of lignocellulosic residuals (Ritota and Manzi, 2019; Bellettini et al., 2019). Assessment of spawn use success is based on mushroom growth and yield. In this study, grain spawn, substrates and shelter structure treatments affected time from full colonization to primordia initiation, length of mushroom stalk, diameter of mushroom caps, the average number of primordia, average number of fruiting body, and average of individual weight of fruiting bodies. Musanze (2013) and Muswati et al. (2021) evaluated the suitability of locally available substrates for oyster mushroom and found that substrates had affected significantly the number of pinning, stalk length and caps diameter. The findings, however, were not in agreement with Tavarwisa et al. (2021) who reported that wheat straw demonstrated significantly (p≤ 0.05) higher mycelial colonization rate than uncomposted grevillea sawdust and maize cobs. Kimenju et al. (2009) performed the study on relative performance of *Pleurotus florida* on agro-industrial and agricultural substrate and found that popcorn cobs

Table 5. Interaction between cropping shelters and substrates on oyster mushroom yield.

Means														
		Days			cm			Grams						
Shelters	**Sub.**	**TFCP**	**LS**	**DC**	**NFB**	**ANP**	**AWIF**	**BY1st**	**BY2nd**	**BY3rd**	**BY4th**	**TBY**	**BE**	**EY**
Mikeka	S1	3.67	5.67^{ghi}	8.72	11.56^{bcdefghi}	13.22^{jk}	32.24^{abcde}	429.28	385.29	259.78	99.45^{cdefgh}	1173.81	83.85	1108.13
	S2	5.11	5.39^{hij}	7.89	12.72^{abcd}	15.72^{cdefg}	21.02^{i}	291.30	278.97	164.50	71.12^{fgh}	805.88	57.56	757.44
	S3	6.33	4.45^{kl}	7.89	11.06^{efghi}	13.06^{k}	16.53^{j}	178.34	155.52	134.61	76.05^{efgh}	544.51	38.90	503.16
	S4	3.33	9.50^{c}	11.67	13.95^{a}	17.28^{bc}	36.23^{a}	600.54	490.34	283.66	126.76^{abcd}	1501.30	107.23	1431.20
	S5	5.28	6.28^{ef}	8.89	10.45^{ij}	13.22^{jk}	24.16^{hi}	294.45	250.42	117.01	75.98^{efgh}	737.86	52.71	703.98
	S6	4.67	6.72^{e}	9.50	11.45^{cdefghi}	13.89^{hijk}	28.81^{efg}	374.45	362.37	165.20	114.39^{abcde}	1016.40	72.60	972.48
	S7	5.11	8.50^{d}	9.39	12.17^{bcdefgh}	14.67^{fghij}	34.18^{abc}	476.05	436.18	237.97	130.58^{abc}	1280.77	91.49	1218.87
	S8	3.94	11.95^{a}	15.45	13.00^{abc}	15.56^{defg}	34.83^{abc}	518.93	448.41	268.34	127.48^{abc}	1363.17	97.37	1293.12
Shade net	S1	3.67	5.11^{ijk}	8.72	10.89^{fghi}	12.89^{k}	31.74^{bcde}	431.24	399.56	252.27	87.77^{defgh}	1170.84	83.64	1103.62
	S2	5.06	4.84^{jkl}	7.89	12.56^{abcde}	15.89^{cdef}	20.66^{i}	265.37	224.30	139.81	62.52^{h}	692.01	49.43	641.54
	S3	6.00	4.28^{l}	7.89	11.22^{defghi}	13.00^{k}	16.46^{j}	137.56	134.65	76.60	61.69^{h}	410.50	29.32	368.40
	S4	3.56	8.89^{cd}	11.67	13.11^{abc}	16.50^{cde}	35.85^{ab}	555.73	485.29	287.80	104.64^{bcdefg}	1433.46	102.39	1353.91
	S5	5.17	5.72^{fgh}	8.89	10.61^{ghij}	13.67^{ijk}	24.09^{hi}	253.70	204.10	108.08	60.24^{h}	626.12	44.72	592.27
	S6	4.56	6.11^{efg}	9.50	11.22^{defghi}	14.28^{ghijk}	27.38^{fgh}	344.13	293.38	143.26	77.06^{efgh}	857.82	61.28	814.02
	S7	5.11	8.17^{d}	9.39	12.28^{abcdefg}	14.67^{fghij}	32.97^{abcd}	443.69	384.51	224.06	109.00^{abcdef}	1161.26	82.95	1093.86
	S8	4.06	11.00^{b}	15.45	12.50^{abcdef}	15.00^{efghi}	35.13^{ab}	478.88	411.66	263.12	129.53^{abc}	1283.18	91.66	1208.33
Darkhouse	S1	4.67	2.72^{m}	7.34	10.50^{hij}	15.45^{defgh}	27.33^{fgh}	367.99	340.01	247.16	116.78^{abcd}	1071.94	76.57	999.70
	S2	5.78	3.09^{m}	6.78	13.28^{ab}	18.89^{a}	21.24^{i}	250.42	194.12	177.93	105.45^{bcdefg}	727.91	52.00	676.28
	S3	6.72	3.41^{m}	6.78	8.89^{j}	14.22^{ghijk}	15.04^{j}	101.79	86.02	88.03	67.03^{gh}	342.86	24.49	300.66
	S4	4.67	2.72^{m}	10.11	11.50^{cdefghi}	18.17^{ab}	30.95^{cdef}	454.21	443.95	315.66	144.90^{a}	1358.71	139.91	1878.86
	S5	6.17	3.22^{m}	7.95	10.06^{ij}	15.11^{efghi}	21.26^{i}	206.15	178.31	145.76	95.37^{cdefgh}	625.59	44.69	591.84
	S6	5.45	2.98^{m}	8.39	10.28^{ij}	15.55^{defg}	26.45^{gh}	309.34	262.37	178.76	125.84^{abcd}	876.32	62.59	833.87
	S7	6.11	3.20^{m}	8.56	10.61^{ghij}	16.11^{cdef}	29.13^{defg}	372.11	351.93	272.44	141.38^{ab}	1137.84	81.28	1066.44
	S8	5.00	2.83^{m}	13.28	10.78^{ghij}	16.84^{bcd}	30.91^{cdef}	389.70	417.62	309.67	124.81^{abcd}	1241.79	88.70	1161.94
Stad.Erros		0.21	0.16	0.25	0.34	0.30	0.78	20.95	18.61	16.96	7.41	44.86	9.23	129.17

The means followed by the same letters are not significantly different using Tukeys' honest significant difference (HSD) test at 5% level of significance.
Source: Authors

influenced greatly stem circumference (49.0%), mushroom height 69% and cap diameter (16.6%) compared to the control (elephant grass). The findings were in agreement to the results from Hoa et al. (2015) who found that Substrates with 100% CC were the most suitable substrate

formulas for cultivation of oyster mushrooms *Pleurotus ostreatus* and PC in which they gave the highest values of cap diameter, stipe thickness, mushroom weight, yield, BE, protein, fibre, ash, mineral content (Ca, K and Mg) and short stipe length. Materials with high quality of lignin, hemicelluloses and cellulose contents make mycelia to remain vegetative for a long time, which results in vigorous growth, late pinning and fruit bodies formation (Kimenju et al., 20009) when compared to substrates with low content of carbohydrate, which influence the primordium and fruiting body formation. Hence, we can conclude that popcorn cobs and other combinations of popcorn cobs had poor nutritional value compared to kikuyu grass and saw dust.

Furthermore, other factors have been reported to influence the delay of pinning and fruiting bodies formation, such as high moisture content in substrate (Kimenju et al., 2009). This study also revealed that rice spawn performed better compared to popcorn spawn on growth and yield of *P. ostreatus*. The findings are in agreement with Hoa et al. (2015) and Jayachandran et al. (2017) who reported that Brown rice was found to be the most favorable for mycelium growth of two oyster mushroom species and they found that corn cobs and acacia saw dust were selected as favorable lignocellulosic substrate sources for mycelium growth, pinning, number of fruit body of both oyster mushrooms in their study for evaluating the effects of temperature and nutritional conditions on mycelium growth of two oyster Mushrooms (*P. ostreatus* and *Pleurotus cystidiosus*). The presence of the right proportion of α-cellulose, hemicellulose, pectin, and lignin in the popcorn cobs, wheat straws and their mixtures to other substrates were the probable cause of the higher rate of mycelium in the corn cobs substrate. Naraian et al. (2009) reported that the carbon and nitrogen ratio for 100% of corn cobs, 100% of saw dust, 50% of corn cobs+ 50% of saw dust were 34.5, 51.7 and 42.55, respectively; They concluded that mycelium growth and primordial development of *Pleurotus florida* were dependent on the lignocellulosic materials, especially the C/N ratio. These finding were in agreement to the results reported by Kim et al. (2010) that higher C/N ratio favoured the mycelium growth, and lower C/N ratio favoured the fruiting body growth. The capacity of mushrooms to grow on lignocellulosic substrates is related to the vigour of their mycelium (Kortei et al., 2014).

Pin-head formation (primordium initiation) was observed following the invasion of substrates by mycelia growth. In this study, the mixture of popcorn cobs and rice spawn under semi-controlled condition (mikeka) structure showed the shortest time to the primordia initiation with only 3±1 days after full colonization of substrates. In general, the results showed that the primordia were initiated in the range of 3 to 8 days after full colonization for all substrates. The time required for the formation of pin-heads is comparable with reports by other similar studies elsewhere; Girmay ET AL (2016) reported pin-head formation of oyster mushrooms cultivated in different substrates to be between 23 and 27 days from spawning, while Fan et al. (2000) reported it to be 20 to 23 days. On the other hand, the findings were in agreement to Shah et al. (2004) who found that pin-heads appeared in about 6 days. Such variations in mycelia growth rate, colonization and primordial initiation have been observed when mushroom species were grown on a range of substrates including grevillea sawdust, wheat straw, corn cobs, bagasse, and banana leaves (Vetayasuporn, 2006; Islam et al. 2009; Gizaw, 2015). These results differed with Iqbal et al. (2016) who reported 46 ± 3 days after spawn inoculation. Pinhead formation is closely related to temperature and humidity. Temperatures below 17°C directly delay the pinhead formation (Pathmashini et al., 2008). Mikeka and shade net structure favoured the pin formation because the soil used to mulch the complete colonization substrates in trenches under shelters maintained the moisture content for primordia initiation. However, Ananbeh and Almomany (2005) and Shah et al. (2004) working on wheat straw and wheat straw mixed with saw dust reported somewhat shorter periods of 31 ± 4 and 28 ± 1 days after inoculation, respectively. The time from the pinhead formation to the first harvest for *Pleurotus ostreatus* was around 4 ± 1 days for popcorn cobs and it combines with other substrates, being in agreement with those of Iqbal et al. (2005) who conducted similar research. Shah et al. (2004) reported 24 days for pinhead formation on uncomposted grevillea sawdust medium. The days for pinhead formation and days for flush (fruiting bodies) formation recorded in this study were longer than previous findings. This may probably be associated with the temperature and humidity.

Oyster mushroom yield (Total biological yield, biological efficiency, economic yield) response to spawn, substrates and cropping shelters

Grain spawn, substrate and cropping condition treatments also had an effect on average weight of the individual fruiting body, the number of fruiting body, total biological yield, biological efficiency and economic yield of *Pleurotus ostreatus*. The results showed that the two treatments of popcorn cobs × rice grain spawn and (popcorn cobs+ wheat straw+ saw dust) × rice grain spawn were the best performer in all three cropping environmental conditions (controlled, semi- controlled and uncontrolled conditions). These two treatments influenced significantly either the average weight of an individual fruiting body, number of fruiting bodies, total biological yield, biological efficiency and economic yield of oyster mushrooms. Though there was an increase of 24, 27 and 28% of total biological yield, biological efficiency and economic yield, respectively, over positive

control wheat straw under a controlled condition, this small ratio of carbon to nitrogen might have been responsible for the higher biological efficiency and economic yield of oyster mushroom as reported by kim et al. (2010) and Alborés et al. (2006) who revealed that higher C/N ratio favoured the mycelium growth, and lower C/N ratio favoured the fruiting body growth. The findings are in full agreement to Hoa et al. (2015) who reported that substrate formula of 100% of corn cobs gave the highest yield and biological yield compared to other substrates such as sawdust, banana leaves and wheat straw. The results of the study showed that the uncomposted grevillea sawdust substrates under Mikeka shelter condition decreased 27% below positive control wheat straw for total biological yield, biological efficiency and economic yield. The findings differ with Vetayasuporn (2006) who reported that grevillea sawdust gave the maximum mushroom yield (536.85 g per 1 kg substrate) and this yield was significantly different to those found from bagasse (360.84 g), peat of coconut husk (278.78 g) at a confidence level of 95% for the study of oyster mushroom cultivation on different cellulosic substrates.

Girmay et al. (2016) performed a study to evaluate the growth and yield performance of *P. ostreatus* on different substrates and found that the lowest biological and economic yield, as well as the lowest percentage of biological efficiency of oyster mushroom, was from uncomposted grevillea sawdust. The performance of oyster growth and yield in uncomposted grevillea sawdust substrate was minimal. Similarly, the biological efficiency (BE) also varied significantly among the different substrates used. Variable ranges of BE have been reported when different lignocellulosic materials were used as substrates for cultivation of oyster (Liang et al., 2009). This could be attributed to the fact that the lignocellulosic materials in uncomposted grevillea sawdust are generally low in protein content and thus insufficient for the cultivation of mushrooms (Obodai et al., 2003; Rambey et al., 2019). Therefore, grevillea sawdust substrate for mushroom production should undergo a period of composting to break down the cellulose and lignin components of the wood to release the essential materials for the establishment of mushroom mycelia. It may also require additional nitrogen, phosphate and potassium. Shah et al. (2004) in a comparative study on cultivation and yield performance of oyster mushroom on different substrates (wheat straw, leaves, saw dust) reported that as a substrate, saw dust showed best biological efficiency(64.69 %) followed by saw dust + leaves (62.9 %), wheat straw + leaves (57.85 %), wheat straw (44.72 %), sawdust + wheat straw (43.59 %) and leaves (21.05 %). The results showed that kikuyu grass alone and in combination and interaction were the least mainly in the interaction of popcorn spawn under controlled environment with 329 g, 23 % and 290 g for total biological yield, biological efficiency and economic yield, respectively. This was in agreement with Onyango et al. (2011) who reported that grass straw produced the least number of the fruiting body and least biological yield of 23%, while corn cobs and wheat straw had 67 and 40.8%, respectively. Onyango et al. (2011) reported that the wheat brans used to increase proteins in grass straw might alleviate the biological yields.

Kumari and Achal (2008) and Musanze (2013) conducted a study on effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* and found that small and tiny fruit bodies were found in case of lawn grass as substrate. Generally, the Growth, development, productivity and post-harvest quality of any crop largely depend on the interaction between the plant genetics and the environmental conditions under which they are grown (Rajasekar et al., 2013). Rajasekar et al (2013) reported that vegetables grown under shade net produced better yields than that under open fields; that may be the reason why the oyster mushroom under shade net and Mikeka structures expressed better yields than that of inside the house condition. The water holding capacity of substrates caused by top soils influenced the relative humidity inside the shelters and high yields of oyster mushroom, though, the dark house shelter condition indicated the least of yields due to the low substrates moisture contents.

Pearson correlation coefficiency analysis between vegetative growth, pin head formation and number of fruiting body

Fungi are similar to plants, but unlike plants, they lack chlorophyll; thus, fungi cannot carry out photosynthesis. For plants, vegetative growth determines the yields to be produced, contrary, for oyster mushrooms as fungi; there is no relationship between vegetative growth (mycelia growth), primordia initiation and a number of the fruiting body. The linear Pearson correlation coefficient analysis showed that, mycelium running rate or extension for mycelium was not correlated with the number of fruiting bodies produced. This means that, the higher mycelia growth for substrates the less the number of fruiting bodies and less number of primordia initiation. Contrary, the more primordia initiation, the more fruiting bodies were obtained. These results are supported by Bilal et al. (2014) who revealed that, materials with high-quality lignin and cellulose contents take a longer time to start pinning and initiate fruiting body as compared to the substrates with low contents of the lignin and cellulose. This may be the reason why kikuyu grass and wheat straw took only 2-3 weeks to be fully colonised. While corn cobs, saw dust and their mixtures took more than 3 weeks to complete the colonization. As compared to the substrates with low nutrition values, the substrates with high nutrition values take a short time for full colonization and ramification. This is because the mycelia remain

vegetative for a longer period hence the vigorous growth and late pinning. In turn, the highly colonized substrates exhibited low mycelia densities. The primordia initiation, number of fruiting bodies and average weight of individual fruiting body of oyster mushroom was not associated with a high rate of mycelia. The result was similar to the finding of Alborés et al. (2006) who reported that there was a positive correlation between the C/N ratio of substrate and mycelium growth rate but not correlated to primordia.

Comparison for four flushes under different cropping environment conditions

The yield per flush and percentage yield per flush for the first four flushes varied with the substrates. In all treatments, the yields were highest in the first flush, and then declined gradually in the second, the third and the fourth. According to the results presented in the Tables 1 to 6, the variation among the flushes is very high under all environmental conditions (controlled, semi-controlled and uncontrolled). The yields declined from the first flush to the second, ranging between 1-20%, from the 2nd flush to the 3rd flush, ranging 13-34%, while form 3rd flush to the 4th they were 34- 65%. The large variation found at 4th flush was due to the overuse of nutrients by the substrates. Among the cropping environmental conditions, the controlled condition was the best in diminishing the variation between flushes. The mixture of uncomposted grevillea sawdust + kikuyu grass + wheat straw substrate showed declines from the 2nd flush to the 3rd, and from 3rd to the 4th flush, with 54 and 35, respectively of variation under semi-controlled conditions (mikeka shelter); both conditions, semi-controlled and uncontrolled, used trenches and soils as casing to maintain moisture. The biological efficiency of substrates cultivated under mikeka and shade net were high at 1st and 2nd flushes, while the BE started to decline at 3rd and 4th flushes; this due to over degradation of carbohydrates in substrates by oyster mushroom. The study indicates that yields per flush decreased as the flushes advanced from first to fourth. This finding was slightly in agreement with Kimenju et al. (2009), who recorded 32.2 g per flush from first flush, second 17.1 g and third 5.5. He also gave corresponding percentages as 69.6, 23.6 and 6.8%. The overall yields for the three flushes were 4051 g, giving an average of 202.6 g per bag of 2 kg. This finding differs from Kivaisi et al (2003), who reported 4 to 6 flushes, with a yield of 643.4 g per bag when using varied substrates.

Oei (2005) reported that the yield is expected to be 20% of the weight of the wet substrate. Kimenju et al. (2009) and (Sharma et al., 2013) recorded only 3 flushes on wheat straw, sugar bagasse, and rice straw. Thus, the lack of nitrogen may be one of the factors affecting the overall yield values in uncomposted grevillea sawdust, kikuyu grass and their combinations cropping in different shelters. Corn cobs and the mixtures of other substrates also contain high amount of lignin. Low degradation of lignocellulosic substances of uncomposted grevillea sawdust and kikuyu grass by *P. ostreatus* might be another factor affecting the overall low yield values of oyster mushrooms.

Conclusion

Mushroom farming is a short duration, high yielder, which requires intensive care for better production. In Kenya, farmers normally use dark or semi-dark house shelters to grow oyster mushrooms and this may be challenging to small farmers due to the requirements for controlling the production in such environmental conditions. In the present study, popcorn cobs substrates spawned by rice grain spawn mulch with soil under Mikeka cropping shelter showed promising results in terms of biological and economic yields. Mikeka and shade-net cropping shelters showed a potential to influence higher yields than dark house shelters. Generally, these findings provide the easiest way to cultivate oyster mushrooms by using crop residuals rather than those normally used that pose environmental nuisances. This study recommends the use of rice grain spawn with corn cobs residuals as substrates under Mikeka cropping shelters to obtain better growth and yields.

ACKNOWLEDGMENTS

The authors are grateful for the funding by World Bank through Centre of Excellent for Sustainable Agriculture and Agribusiness Management (CESAAM) at Egerton University.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alborés S, Pianzzola MJ, Soubes M, Cerdeiras MP (2006). Biodegradation of agro industrial wastes by *Pleurotus spp.* for its use as ruminant feed. Electronic Journal of Biotechnology 9(3):4-9.

Amuneke EH, Dike KS, Ogbulie JN (2011). Cultivation of *Pleurotus ostreatus*: An edible mushroom from agro base waste products. Journal of Microbiology and Biotechnology Research 1(3):1-14.

Ananbeh KM, Almomany AR (2005). Production of oyster mushroom Pleurotus ostreatus on olive cake agro waste. Dirasat Agricultural Sciences 32(1):64-70.

Anike FN, Yusuf M, Isikhuemhen OS (2016). Co-substrating of peanut shells with cornstalks enhances biodegradation by Pleurotus ostreatus. Journal of Bioremediation and Biodegradation 7(1):1-7.

Baysal E, Yigitbasi ON, Colak M, Toker, H, Simsek H, Yilmaz F (2007). Cultivation of *Agaricus bisporus* on some compost formulas and locally available casing materials Part I, Wheat straw based compost formulas and locally available casing materials. African Journal of Biotechnology 6(19):2225-2230.

Bellettini MB, Fiords FA, Maieves HA, Teixeira GL, Ávila S, Hornung

PS, Ribani RH (2019). Factors affecting mushroom Pleurotus spp. Saudi Journal of Biological Sciences 26(4):633-646.
Bilal S, Mushtaq A, Moinuddin K (2014). Effect of different grains and alternate substrates on oyster mushroom (*Pleurotus ostreatus*) production. African Journal of Microbiology Research 8(14):1474-1479.
Carrasco J, Zied DC, Pardo JE, Preston GM, Pardo-Giménez A (2018). Supplementation in mushroom crops and its impact on yield and quality. AMB Express 8(1):1-9.
Chang ST, Lau OW, Cho KY (1981). The cultivation and nutritional value of *Pleurotus sajor-caju*. European Journal of Applied Microbiology and Biotechnology 12(1):58-62.
Fan L, Pandey A, Mohan R, Soccol CR (2000). Use of various coffee industry residues for the cultivation of Pleurotus ostreatus in solid state fermentation. Acta Biotechnologica 20(1):41-52.
Girmay Z, Gorems W, Birhanu G, Zewdie S (2016). Growth and yield performance of *Pleurotus ostreatus* (oyster mushroom) on different substrates. AMB Express 6(87):1-7.
Gizaw B (2015). Cultivation and yield performance of Pholiota nameko on different agro industrial wastes. Academia Journal of Food Research 3(3):032-042.
Hoa HT, Wang CL, Wang CH (2015). The effects of different substrates on the growth, yield, and nutritional composition of two Oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology 43(4):423-434.
Iqbal SM, Rauf CA, Sheik MI (2005). Yield performance of oyster mushroom on different substrates. International Journal of Agriculture and Biology 7:900-903.
Iqbal B, Khan I, Shan B, Naeem A, Ullah W, Khan N, Adnan M, Shah RA, Junaid K, Ahmed N, Iqbal M (2016). Substrates evaluation for quality, production and growth of oyster mushroom. Journal of Entomology and Zoology Studies 4(3):98-107.
Jaetzold R, Schimdt H, Hornetz B, Shisnaya C (2007). Farm management handbook of Kenya. Natural conditions and farm management information. Volume II B. Nairobi Kenya. pp. 379-413.
Jayachandran A, Nadesan S, Murugaiyan K (2017). Comparative study of different grains on spawn development of Pleurotus florida. International Journal of Science Invention Today 6(6):728-735.
Kim SS, Lee JS, Cho JY, Kim YE, Hong EK (2010). Effects of C/N ratio and trace elements on mycelial growth and exo-polysaccharide production of Tricholoma mistake. Biotechnology and Bioprocess Engineering 15(2):293-298.
Kortei NK, Odamtten GT, Appiah V, Obodai M, Adu-Gyamfi A, Annan T, Akonor PT, Annan SN, Acquah SA, Armah JO, Mills SW (2014). Microbiological quality assessment of gamma irradiated fresh and dried mushrooms (*Pleurotus ostreatus*) and determination of D10 values of *Bacillus cereus* in storage packs. European Journal of Biotechnology and Bioscience 2(1):28-34
Kimenju JW, Odero GOM, Mutitu EW, Wachira PM, Narla RD, Muiru WM (2009). Suitability of locally available substrates for oyster mushroom (*Pleurotus ostreatus*) cultivation in Kenya. Asian Journal of Plant Sciences 8(7):510-514.
Kivaisi AK, Magingo FSS, Mamiro B (2003). Performance of *Pleurotus flabellatus* on water hyacinth (*Eichhornia crassipes*) shoots at two different temperature and relative humidity regimes. Tanzania Journal of Science 29(2):11-18.
Kumari D, Achal V (2008). Effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* (Oyster mushroom). Life Science Journal 5(3):73-76.
Kumla J, Suwannarach N, Sujarit K, Penkhrue W, Kakumyan P, Jatuwong K, Vadthanarat S, Lumyong S (2020). Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. Molecules *2*5(12):2811.
Liang CH, Lee YL, Kuo HC, Wu TP, Jian, SY, Huang WL (2009). Antioxidant properties of novel solid-state fermented culinary-medicinal mushroom and fungi products. International Journal of Medicinal Mushrooms 11(3):259-268.
Muswati C, Simango K,Tapfumaneyi L, Mutetwa M, Ngezimana W (2021). The effects of different substrate combinations on growth and yield of oyster mushroom (*Pleurotus ostreatus*). International Journal of Agronomy 32(4):23-25.
Nadir H, Ali A, Muhammed GAR (2016). Determination of yield and Quality of Oyster Mushroom (*Pleurotus florida*) using Different Substrates in Halabja, Kurdistan Reign-Iraq. Journal of Plant Production 7(7):787-790.
Naraian R, Sahu RK, Kumar S, Garg SK, Singh CS, Kanaujia RS (2009). Influence of different nitrogen rich supplements during cultivation of Pleurotus florida on corn cob substrate. The Environmentalist 29(1):1-7.
National Farmers Information Service (NAFIS) 2014. Mushroom production in kenya. Copeel Agrisience.
Musanze R (2013). Relative performance of oyster mushroom (Pleurotus florida) on agro-industrial and agricultural substrate. International Journal of Agronomy and Plant Production 4(1):109-116.
Nongthombam J, Kumar A, Ladli B, Madhushekhar M, Patidar S (2021). A review on study of growth and cultivation of oyster mushroom. Plant Cell Biotechnology and Molecular Biology 22(5&6):55-65.
Obodai M, Cleland–Okine J, Vowotor KA (2003). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. Journal of Industrial Microbiology and Biotechnology 30(3):146-149.
Odendo M, Kirigua V, Kimenju JW, Wasilwa L, Musieba F, Orina M (2009). Analysis of Mushroom Value Chain in Kenya. East African Agricultural and Forestry Journal 76(3&4):
Oei P (2005). Mushroom Cultivation*:* Appropriate Technology for Mushroom Growers. The Netherlands Backhuys Publishing: Leiden. No.Ed.3 pp.xii + 429 pp.
Ojwang DO (2014). Molecular characterization of the wild edible mushrooms of the *Pleurotus* species in Kenya (Doctoral dissertation).
Onyango BO, Palapala VA, Arama PF, Wagai SO, Gichimu BM (2011). Morphological characterization of Kenyan native wood ear mushroom [*Auricularia auricula* and the effect of supplemented millet and sorghum grains in spawn production. Agriculture And Biology Journal of North America 2(3):407-414.
Oseni TO, Dlamini SO, Earnshaw DMT, Masarirambi M (2012). Effect of substrate pre-treatment methods on oyster mushroom (Pleurotus ostreatus) production. International Journal of Agriculture and Biology 14(2):32-36.
Pardo-Giménez A, Picornell Buendia MR, de Juan Valero JA, Pardo-González JE, Cunha Zied D (2010). Cultivation of *Pleurotus ostreatus* using supplemented spent oyster mushroom substrate. In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 933:267-272.
Pathmashini L, Arulnandhy V, Wijeratnam RW (2008). Cultivation of oyster mushroom (*Pleurotus ostreatus*) on sawdust. Ceylon Journal of Science (Biological Science) 37(2):177-182.
Porselvi A, Vijayakumar R (2019). Evaluation of paddy straw varieties on the cultivation and nutritional value of two oyster mushroom species. International Journal of Research in Advent Technology *7*(5):556-563.
Rajasekar M, Arumugam T, Kumar SR (2013). Influence of weather and growing environment on vegetable growth and yield. Journal of Horticulture and Forestry 5(10):160-167.
Rambey R, Sitepu IDB, Siregar EBM (2019). Productivity of oyster mushrooms (Pleurotus ostreatus) on media corncobs mixed with sawdust. In IOP Conference Series: Earth and Environmental Science 260(1):012076.
Randive S (2012). Cultivation and Study of growth of oyster mushroom on different agricultural waste substrate and its nutrient Analysis. Advances in Applied Science Research 3(4):1938-1949.
Ritota M, Manzi P (2019). *Pleurotus* spp. cultivation on different agri-food by-products: Example of biotechnological application. Sustainability (Switzerland*)* 11(18):5049.
Sánchez C (2010). Cultivation of Pleurotus ostreatus and other edible mushrooms. Applied Microbiology and Biotechnology 85(5):1321-1337.
SAS Institute Inc. (2017). SAS® 9.4 System Options: Reference, Fifth Edition. Cary, NC: SAS Institute Inc. AS® pp27513-2414.
Shah ZA, Ashraf M, Ch MI (2004). Comparative study on cultivation and yield performance of oyster mushroom *(Pleurotus ostreatus*) on different substrates (Wheat Straw, Leaves, Saw Dust). Pakistan Journal of Nutrition 3(3):158-160.
Sharma S, Yadav RKP, Pokhrel CP (2013). Growth and Yield of Oyster

mushroom (Pleurotus ostreatus) on different substrates. Journal on New Biological Reports 2(1):3-8.
Singh R, Yadav DB, Ravisankar N, Yadav A, Singh H (2020). Crop residue management in rice–wheat cropping system for resource conservation and environmental protection in north-western India. Environment, Development and Sustainability 22(5):3871-3896.
Stanley OH, Awi-Waadu G D (2010). Effect of substrates of spawn production on mycelial growth of oyster mushroom species. Agriculture and Biology Journal of North America 1(5):817-820.
Tavarwisa DM, Govera C, Mutetwa M, Ngezimana W (2021). Evaluating the suitability of baobab fruit shells as substrate for growing oyster mushroom (*Pleurotus ostreatus*). International Journal of Agronomy 45:24-32.
Tekeste N, Dessie K, Taddesse K, Ebrahim A (2020). Evaluation of different substrates for yield and yield attributes of oyster mushroom (*Pleurotus ostreatus*) in crop-livestock farming system of Northern Ethiopia. The Open Agriculture Journal 14(31):30-35.
Tesfay T, Godifey T, Mesfin R, Kalayu G (2020). Evaluation of waste paper for cultivation of oyster mushroom (Pleurotus ostreatus) with some added supplementary materials. AMB Express 10(1):1-8.
Thakur MP (2020). Advances in mushroom production: key to food, nutritional and employment security: A review. Indian Phytopathology 73(3):377-395.
Vetayasuporn S (2006). Oyster Mushroom Cultivation on Different Cellulosic Substrates. Research Journal of Agricultural and Biological Science 2(6):548-551.
Waiganjo MW, Ngeli P, Gateri MW, Muriuki AW (2008). Cultivation and commercialization of edible mushrooms in Kenya: A review of prospects and challenges for smallholder production. In International Symposium on Underutilized Plants for Food Security, Nutrition, Income and Sustainable Development 806:473-480.
Yang D, Liang J, Wang Y, Sun F, Tao H, Xu Q, Wan X (2016). Tea waste: an effective and economic substrate for oyster mushroom cultivation. Journal of the Science of Food and Agriculture 96(2):680-684.
Zakil FA, Xuan LH, Zaman N, Alan NI, Salahutheen NAA, Sueb MS, Isha R (2022). Growth performance and mineral analysis of Pleurotus ostreatus from various agricultural wastes mixed with rubber tree sawdust in Malaysia. Bioresource Technology Reports 17:100873.

Vol. 16(6), pp. 138-147, July 2022
DOI: 10.5897/AJPS2022.2245
Article Number: EE0227569451
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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African Journal of Plant Science

Full Length Research Paper

Antibacterial activity of some substances against citrus bacterial canker caused by *Xanthomonas citri* pv*. citri* (Hasse) in Burkina Faso

Kevin Ben Fabrice ZERBO[1,2], Issa WONNI[1*], Florence YAMEOGO[1,2] and Irénée SOMDA[2]

[1]Centre National de la Recherche Scientifique et Technologique/Institut de l'Environnement et de Recherches Agricoles (INERA)/Laboratoire Mixte International/Observatoire des Agents Pathogènes, Biosécurité et Biodiversité (LMI PathoBios), 01 BP 910 Bobo Dioulasso 01, Burkina Faso.
[2]Université Nazi BONI/Institut de Développement Rural/Clinique des Plantes; 01 BP1091, Bobo Dioulasso, Burkina Faso.

Received 24 March, 2022; Accepted 8 June, 2022

Citrus bacterial canker (CBC) threatens citrus fruit production in Burkina Faso due to the lack of effective control methods. In this study, the antimicrobial effect of copper hydroxide and sulphate, the biopesticide from *Bacillus amyloliquefaciens*, the essential oil of *Ocimum gratissimum* and aqueous extracts of *Eucalyptus camadulensis, Azadirachta indica* and *Cymbopogon citratus* were tested *in vitro* and *in vivo* against *Xanthomonas cirri* pv*. citri* (Hasse) (*Xcc*890) strain from Burkina Faso. The dilution method in solid medium and macro-method dilution in liquid medium were, respectively used in order to determine the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC). Hydroxide and sulphate copper tested at 25 mg/ml induced 13 mm in diameter of inhibition zone, followed by *B. amyloliquefaciens* at 25 µl/ml (11.6±3.9 mm). *O. gratissimum* induced 10.5 ± 0.11 mm of inhibition zone at 5 µl/ml. Aqueous extract of *E. camadulensis* at 100 mg/ml induced 11.4 ± 1 mm of inhibition diameter. Based on MBC/MIC ratio, copper hydroxide and sulphate, *O. gratissimum* and *E. camadulensis* have a bactericidal effect *in vitro. In vivo* test, copper products at 25 mg/ml and *O. gratissimum* at 10 µl/ml were the most effective and significantly reduced the CBC incidence. These substances can be evaluated under natural conditions in order to determine their effectiveness against CBC.

Key words: *Citrus* species, citrus bacterial canker, *Xanthomonas citri* pv*. citri,* antibacterial activity, substances.

INTRODUCTION

Citrus bacterial canker (CBC) caused by *Xanthomonas citri* pv. *citri* (*Xcc*) represents the most devastating bacterial disease on citrus production through the world (Salifou and Bounou, 2020). The disease causes defoliation, fruit cankers, a premature fall of fruit and a general withering of trees (Gottwald et al., 2002; CABI,

*Corresponding author. E-mail: wonniissa@gmail.com. Tel: +22670156832.

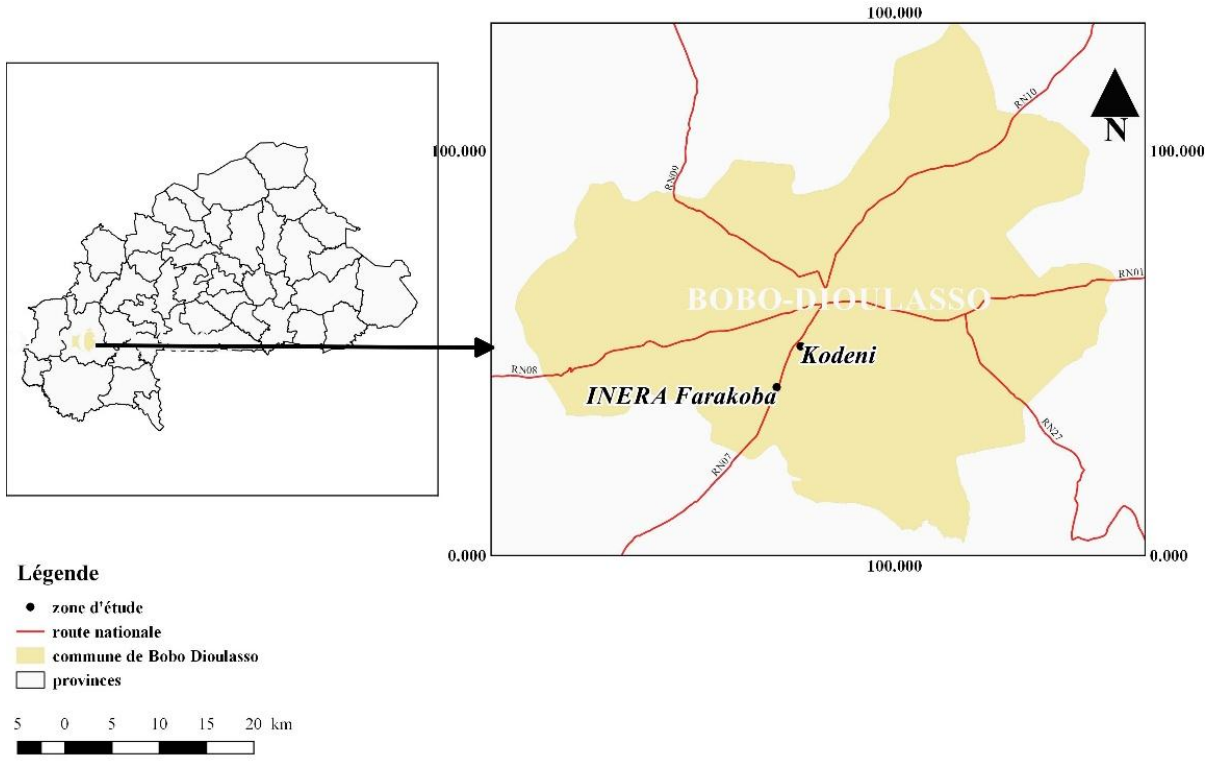

Figure 1. Location of *Xcc* 890 strain isolation site.
Source: Authors

2019). There are no highly efficient strategies for controlling CBC disease. In Burkina Faso, CBC was reported on all commercial citrus cultivars in the provinces of Comoé, Houet and Kénédougou (Juhasz et al., 2013). In endemic areas, the quarantine and eradication are the major means of control against *Xcc* (OEPP/CABI, 1990). However, the use of windbreaks, destruction of infected branches and spraying of copper-based products during periods of susceptibility, can reduce the severity of the disease (Civerolo, 1984; Behlau, 2021).

Indeed, copper-based products, despite their effectiveness (Stall et al., 1980; Idrissou-Touré et al., 2017), have an adverse effect on human and environmental health (Villeneuve, 2008; Damien et al., 2017). In addition, their repetitive and uncontrolled use causes resistance phenomenon in pathogens.

In order to prevent major damage caused by CBC, alternative less harmful tools have been extensively studied in recent years (Oliveira et al., 2021; Raveau, 2020). For example, several studies have shown that many plants from the West African flora have an enormous biocidal potential (toxic, repellent, anti-appetizing) against a broad spectrum of pests. They can be used in the form of aqueous extracts or essential oils (Saha et al., 2013; Zombré et al., 2015). In Burkina Faso, despite the high prevalence of CBC and the resulting crop losses, no means of control, including the use of copper-based products, have been deployed. Wonni et al. (2016) showed that aqueous extract and essential oil of *Eucalyptus camaldulensis* have an antibacterial activity *in vitro* test against both pathovars of *Xanthomonas oryzae*. The results of Rabbee et al. (2021) showed that ethyl acetate extract from *Bacillus velezensis*, have antibacterial activity against *Xcc* wildtype strains, in contrast to *Bacillus amyloliquefaciens* strains which had no antibacterial activity.

In order to promote an effective control of *Xcc*, the present study aimed to evaluate *in vitro* and *in vivo,* the antibacterial activity of some chemical, biological substances, plant aqueous extracts, and essential oils.

MATERIALS AND METHODS

Bacterial strain and inoculum's preparation

Strain *Xcc* 890 was isolated in 2020 from samples of tangelo species (*Citrus reticulata* × *Citrus paradisi*) from the citrus orchard of the locality of Kodeni which is located in the commune of Bobo-Dioulasso (Figure 1), on the Bobo-Banfora axis (National Road No. 7). The geographic coordinates of the site are 11°7'40.5372" North Latitude, 4°19'6.34332" West Longitude. The inoculum was prepared from a 24 h bacterial culture. It was suspended in 5 ml of 0.85% physiological NaCl solution and adjusted to 10^8 bacterium/ml using a spectrophotometer at A_{600nm}. The different activities were

Table 1. Spectrum of substances concentration used in this study.

Substance	Concentration					
	C1	C2	C3	C4	C5	C6
Copper hydroxide (mg/ml)	25	12.5	6.25	3.12	1.56	0.78
Copper sulfate (mg/ml)	25	12.5	6.25	3.12	1.56	0.78
Essentiel oil of *Ocimum gratissimum (*µl/ml)	5	2.5	1.25	0.62	0.31	0.15
Aqueous extracts of *Azadirachta indica* (µl/ml)	100	50	25	12.5	6.25	3.12
Aqueous extracts of *Eucalyptus camadulensis* (µl/ml)	100	50	25	12.5	6.25	3.12
Aqueous extracts of *Cymbopogon citratus* (µl/ml)	100	50	25	12.5	6.25	3.12
Bacillus amyloliquefaciens (Serenade) (µl/ml)	25	12.5	6.25	3.12	1.5	0.78
Thymol gamma terpène (Neco 50EC) (µl/ml)	50	25	12.5	6.25	3.12	1.56
Thymol eugenol (Proraly 50EC) (µl/ml)	50	25	12.5	6.25	3.12	1.56

Source: Authors

carried out in the bacteriology laboratory of INERA/FARAKOBA.

Substances tested

Five types of substances were tested. They were (i) Chemicals; sulphate and hydroxide copper, (ii) plant aqueous extracts; of *Azadirachta indica* (A.) JUSS, *Cymbopogon citratus* (D.C.) STAFF, and *E. camadulensis* DEHNH, (iii) essential oils; *Ocimum gratissimum* LINNE, (iv) biological substance derived from *B. amyloliquefaciens* supplied by BAYER company and (v) formulations based on essential oils that were Neco 50EC and Proraly 50EC from Côte d'Ivoire.

Preparation of aqueous plant extracts

The aqueous extracts of leaves of *A. indica* (A.) JUSS., *C. citratus* (D.C.) STAFF and *E. camadulensis* DEHNH, were prepared according to the method described by Guédé-Guina et al. (1996).

The leaves were collected at the Farako-Ba station with coordinates Longitude 4°20' West, Latitude 11°6' North and an altitude of 405 m. The leaves were firstly dried in the shade for two weeks, and then powdered using a mortar. The powders were macerated in the proportion of 1 g of powder/1 ml of distilled water and the suspension was kept for 24 h. The mixture was then, centrifuged at 4000 rpm for 10 min and the supernatant was sterilized in order to remove contaminants. The final substrate was stored in the refrigerator for its use.

Concentration range

Six concentrations per substance were used in this study. They were prepared by the method of double dilution in according to a geometric progression of ½ reason (Toty et al., 2013) from a maximal concentration (C1) to a minimal concentration (C6) (Table 1).

Efficiency test *in vitro*

It was performed using the standard disc or antibiogram method (Abo-Elyousr and Asran, 2009). Bacterial suspension of 40 µl at 10^8 CFU/ml was spread uniformly over the entire surface of the LPGA solid medium (LPGA for 1 L: Yeast 7 g, Peptone 7 g, Glucose 7 g, Agar 18 g) contained in Petri dishes. After drying, sterile blotting paper discs of 6 mm diameters were placed and inoculated with 10 µl of each substance. A negative control was inoculated with sterile distilled water. Petri dishes were firstly incubated for 1 h at room temperature, and then at 30°C in inverted position into incubator. After 72 h incubation, the diameters of inhibition (ID) zones were measured.

The efficacy of each substance was determined based on the criteria of Ponce et al. (2003) as follows: (i) ineffective: ID <8 mm, (ii) effective: 9 mm < ID <14 mm; (iii) very effective: 15 mm <ID <19 mm, and (iv) highly effective: ID > 20 mm.

Determination of antibacterial parameters

It was carried out using the technique of microdilution in nutrient liquid. This technique consisted of inoculate in a standardized inoculum, and decrease concentrations of the tested substances. For this, a series of seven test tubes, each containing 3 ml of LPGA nutrient liquid, were inoculated with 50 µl of bacteria inoculum at 10^8 colonies/ml, and then 50 µl of each substance according to the concentrations defined in Table 1 except for the control tube. The tubes were placed at 28°C under shaking for 24 h, and then at 28°C for 72 h in a bacteriological incubator. Three parameters were assessed in the following.

Bacterial growth (T_{survey})

It was determined to measure the optical density (DO) of each tube using spectrophotometer before and after incubation and to calculate with the formula used by Moroh et al. (2008).

T_{survey} (x) = (T_i -T_f) / (T_{it} –T_{ft}),

where T_i: initial DO; T_f: final DO; T_{it}: initial DO of control without substance; T_{ft}: final DO of control without substance.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

They were determined to assess the bactericidal or bacteriostatic effect (Berche et al., 1991) of each substance by calculating the MBC/MIC ratio as follows:

(1) bactericidal if MBC/MIC ≤ 2;
(2) bacteriostatic if MBC/MIC > 2

Table 2. Inhibition's diameter (mm) of the bacterial growth induced by the tested substances.

Concentration	Inhibition diameter (mm)								
	Copper hydroxide	**Copper sulphate**	**Serenade**	***Ocimum. gratissimum***	**Neco 50EC**	**Proraly 50EC**	***Azadiractha indica***	***Cymbopogon citratus***	***Eucalyptus camadulensis***
C1	13.3±1.6^{a}	13±01.3^{a}	11.6±3.9^{a}	10.5±0.11^{a}	9.4±1.3^{a}	9.6±0.9^{a}	10.1±1.3^{a}	10.9±1.2^{a}	11.4±1^{a}
C2	12±4.5^{a}	10.7±1.2^{b}	10.4±2.5^{ab}	8.9±0.07^{b}	6±0.0^{b}	8.7±0.9^{b}	7.6±1.1^{b}	8±0.7^{b}	8.9±0.9^{b}
C3	9.1±2.9^{b}	9.2±1.0^{c}	8.7±1.1^{c}	8±0.11^{c}	6±0.0^{b}	7.7±0.7^{c}	7.3±0.9^{b}	7.5±0.0^{C}	7.9±1.1^{b}
C4	7.2±1.1^{c}	8.6±0.7^{c}	7.1±0.9^{d}	6±0.00^{d}	6±0.0^{b}	6±0.0^{d}	6±0.0^{e}	6±0.0^{d}	6.4±1.0^{c}
C5	6.4±0.7^{C}	7.6±0.9^{d}	6±0.0^{e}	6±0.00^{d}	6±0.0^{b}	6±0.0^{d}	6±0.0^{e}	6±0.0^{d}	6±0.0^{d}
C6	6.1±0.3^{c}	7±0.8^{d}	6±0.0^{e}	6±0.00^{d}	6±0.0^{b}	6±0.0^{d}	6±0.0^{e}	6±0.0^{d}	6±0.0^{d}
P-value	0.001	0.002	0.001	0.003	0.005	0.005	0.003	0.003	0.001

a-e: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source : Authors

***In vivo* test**

Young plants of the tangelo species with leaves infected by CBC, from nurseries in the city of Orodara, were used under greenhouse conditions, to test the effective concentrations of the substances which were found to be effective *in vitro*. Therefore, copper hydroxide and sulfate (25 and 12.5 mg/ml), HE of *O. gratissimum* (5 and 10 µl/ml), the aqueous extract of *E. camadulensis* (50 and 100 mg/ml), and *B. amyloliquefaciens* formulation (30 and 60 µl/ml) were tested every 15 days.

Before foliar treatment with the substances, we counted (i) the number of symptomatic leaves per plant, (ii) the proportion of canker on each symptomatic leaf per plant, and (iii) the number of symptomatic leaves of the plant. These data were used to calculate the foliar incidence and severity of CBC according to the following formulae defined respectively by Idroussou-Touré et al. (2017) and Mayee and Datar (1986).

Disease incidence (%) = (NSL/NTL) × 100

where NSL: the number of symptomatic leaves; NTL: the number of total leaves.

The severity was evaluated using the scale of Lakshmi et al. (2011).
Severity = Addition of numerical notation × 100 / Number of examined unit × Maximal note.

Statistical analysis

The analysis of variance and Tukey's test were carried out with the Minitab18 software in order to compare the effectiveness of the substances used at different doses *in vitro* and *in vivo*, but also to compare the variations between MIC and BMC.

RESULTS

Sensitivity of the *Xcc* strain to the tested substances

All the tested substances showed an inhibitory effect at the different concentrations used, with a very remarkable effect of copper hydroxide at 12.5 mg/ml, inducing 13.3 ± 1.6 mm of inhibition diameter (Table 2). In general, the inhibition diameter was proportional to the concentration of each tested substance. In fact, the highest inhibition diameters were obtained with the maximum concentrations (C1).

Efficiency of the different tested concentrations

Tables 3 to 6 show the efficiency of the antibacterial activity of each substance in terms of the tested concentrations. The products based on copper were efficient from the concentration of 3.12 mg/ml for copper hydroxide and 6.25 mg/ml for copper sulphate (Table 3). The antibacterial activities of Proraly 50EC and Neco 50EC were efficient, respectively from 25 and 50 µl/ml concentrations (Table 4). Below these doses, they became inefficient. Furthermore, the antibacterial activity of the formulation based on *B. amyloliquefaciens* was efficient to the concentration of 6.25 µl/ml, whereas the essential oil of *O. gratissimum* was efficient from 1.5 µl/ml (Table 5). The efficiency of plant aqueous extracts was very variable according to the vegetal species. So, the aqueous extracts of *C. citratus* and *E. camadulensis* were efficient to the

Table 3. Efficiency of copper hydroxide and copper sulphate against *Xcc*.

Concentration (mg/ml)	Substance	Inhibition diameter (mm)	Efficiency
25	Copper hydroxide	13.3±1.6^{a}	Efficient
	Copper sulphate	13±1.3^{a}	Efficient
12.5	Copper hydroxide	12±4.5^{ab}	Efficient
	Copper sulphate	10.7±1.2^{bc}	Efficient
6.25	Copper hydroxide	9.1±2.9^{cd}	Efficient
	Copper sulphate	9.2±1.0^{cd}	Efficient
3.12	Copper hydroxide	8.6±0.7^{cde}	Efficient
	Copper sulphate	7.2±1.1^{def}	Inefficient
1.56	Copper hydroxide	6.6±0.8^{ef}	Inefficient
	Copper sulphate	7.6±0.9^{def}	Inefficient
0.78	Copper hydroxide	6.1±0.3^{f}	Inefficient
	Copper sulphate	7.6±0.9^{def}	Inefficient

a-f: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source : Authors

Table 4. Efficiency of Neco 50EC and Proraly 50EC against *Xcc*.

Concentration (µl/ml)	Substance	Inhibition diameter (mm)	Efficiency
50	Proraly 50EC	9.6±0.9^{a}	Efficient
	Neco 50EC	9.4±1.3^{ab}	Efficient
25	Proraly 50EC	8.7±0.9^{b}	Efficient
	Neco 50EC	6±0.0^{d}	Inefficient
12.5	Proraly 50EC	7.7±0.7^{c}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient
6.25	Proraly 50EC	6±0.0^{d}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient
3.12	Proraly 50EC	6±0.0^{d}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient
1.56	Proraly 50EC	6±0.0^{d}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient

a-d: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source: Authors

concentration of 50 mg/ml, whereas that of *A. indica* was efficient from 100 mg/ml (Table 6).

Survival rate of *Xcc*

The survival rates of *Xcc* at the different tested concentrations were inversely proportional to the increase of the concentration of each substance (Table 7). Indeed, the lowest rate of survival (-9.2±3.26%) was registered with *O. gratissimum* at 5 µl/ml. However, the highest rate of survival (124.32±31.54%) was registered with the aqueous extract of *A. indica* at 6.25 mg/ml.

Characteristics of the tested substances

The different values of Minimal Inhibitory Concentrations

Table 5. Efficiency of *Bacillus amyloliquefaciens* formulation and essential oil of *Ocimum gratissimum* against *Xcc*.

Substance	Concentration (µl/ml)	Diameter (mm)	Efficiency
Bacillus amyloliquefaciens	25	11.6±3.9[a]	Efficient
	12.5	10.4±2.5[ab]	Efficient
	6.25	8.7±1.1[c]	Efficient
	3.12	7.1±0.9[d]	Inefficient
	1.56	6±0.0[e]	Inefficient
	0.78	6±0.0[e]	Inefficient
Ocimum gratissimum	5	10.5±0.11[a]	Efficient
	2.5	8.9±0.07[b]	Efficient
	1.5	8±0.11[c]	Efficient
	0.62	0.6±0.00[d]	Inefficient
	0.31	0.6±0.00[d]	Inefficient
	0.15	0.6±0.00[d]	Inefficient

a-e: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source: Authors

Table 6. Efficiency of plant aqueous extracts against *Xcc*.

Concentration (mg/ml)	Plant aqueous extract	Inhibition diameter (mm)	Efficiency
100	*Eucalyptus camadulensis*	11.4±1.5[a]	Efficient
	Cymbopogon citratus	10.9±0.7[ab]	Efficient
	Azadirachta indica	10.1±0.7[bc]	Efficient
50	*Eucalyptus camadulensis*	8.9±0.9[b]	Efficient
	Cymbopogon citratus	8±0.9[b]	Efficient
	Azadirachta indica	7.6±1.1[de]	Inefficient
25	*Eucalyptus camadulensis*	7.9±1.3[de]	Inefficient
	Cymbopogon citratus	7.6±1.1[e]	Inefficient
	Azadirachta indica	7.3±0.9[e]	Inefficient
12.5	*Eucalyptus camadulensis*	6.4±0.8[f]	Inefficient
	Cymbopogon citratus	6±0.0[f]	Inefficient
	Azadirachta indica	6±0.0[f]	Inefficient
6.25	*Eucalyptus camadulensis*	6±0.0[f]	Inefficient
	Cymbopogon citratus	6±0.0[f]	Inefficient
	Azadirachta indica	6±0.0[f]	Inefficient
3.12	*Eucalyptus camadulensis*	6±0.0[f]	Inefficient
	Cymbopogon citratus	6±0.0[f]	Inefficient
	Azadirachta indica	6±0.0[f]	Inefficient

a-f: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p \leq 0.05$).
Source: Authors

(MIC) and Minimal Bactericidal Concentrations (MBC) are shown in Table 8. Referring to survival rates, MBC/MIC ratios ≤ 2 were obtained with the copper sulphate, essential oil of *O. gratissimum* and aqueous extract of *E. camadulensis* confirming their antibacterial properties. However, we were unable to determine the MIC and MBC of *B. amyloliquefaciens*, *A. indica*, *C. citratus* and Proraly 50 EC due to their opacity.

Table 7. Survival rate (%) of *Xcc* colonies at different concentrations of substances tested.

Concentration	Copper hydroxide	Copper sulphate	*Bacillus amyloliquefaciens*	*Ocimum gratissimum*	Proraly 50EC	Neco 50EC	Azadirachta *indica*	*Cymbopogon citratus*	*Eucalyptus camadulenssis*
Survival rate%									
C1	-4.9±6.2^{a}	-5.7±3.8^{a}	9.7±1.71^{a}	-9.2±3.26^{a}	5±1.3^{a}	7.24±1.2^{a}	7.7±1.1^{a}	4.38±1.9^{a}	-2.8±1.8^{a}
C2	-0.6±6.7^{a}	6.3±4.64^{b}	30.5±6.43^{b}	1.3±2.99^{b}	19.3±3.2^{b}	26.01±4.4^{b}	43.8±11.2^{b}	38±11.2^{b}	5±1.76^{b}
C3	16.4±7.4^{b}	27.5±7.7^{c}	54.6±5.3^{c}	15.7±4.7^{c}	30.9±6.2^{c}	44.32±5.4^{c}	70.6±17.6^{c}	62.3±17.6^{C}	25.3±5.3^{c}
C4	37.2±13.9^{c}	49.7±10.8^{d}	75.1±4.12^{d}	30.9±4.5^{d}	67.2±10.5^{d}	76.7±9.4^{d}	90.5±23.1^{d}	89.1±24.3^{d}	40.3±7.2^{d}
C5	48.7±15.7^{cd}	64.3±16.4^{e}	96.4±5.6^{e}	37.9±4.4^{e}	90.9±3.4^{e}	100.6±8.9^{e}	109.1±29.2^{e}	99.3±26.3^{e}	64.8±9.5^{e}
C6	57.6±18.8^{d}	74.5±14.8^{e}	110±7.7^{f}	45.4±5.76^{f}	102.5±8.5^{f}	119.1±7.45^{f}	124.3±31.5^{f}	111.6±29.1^{f}	80.3±12.5^{f}
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

a-f: Different letters indicate statistically relevant differences among survival rates within each treatment using the Tukey test ($p < 0.05$).
Source : Authors

Table 8. Antibacterial parameters of the different substances.

Substance	Antibacterial parameter			
	MIC	MBC	MBC/MIC	Activity
Copper hydroxide (mg/ml)	12.5	12.5	1	Bactericidal
Copper sulphate (mg/ml)	12.5	25	2	Bactericidal
Ocimum gratissimum (µl/ml)	2.5	5	2	Bactericidal
Eucalyptus camadulensis (mg/ml)	50	100	2	Bactericidal

MIC: Minimum inhibitory concentration; MBC: minimum bactericidal concentration.
Source : Authors

Effect of treatments on the incidence and severity of citrus bacterial canker

The results showed that the averages of incidence and severity of CBC significantly differed among the treated substances. We noted a significant decrease of the incidence and severity on the treated plants compared to untreated controls, which incidence progressed from 41.32 to 56.12% after 120 days of observation (Figures 2 and 3). The C2 concentrations tested were the most effective. Analysis showed that the strongest disease reduction rates were obtained with copper hydroxide and sulfate at 12.5 mg/ml. However, with *O. gratissimum* at 10 µl/ml, we recorded a reduction in disease without significant difference with chemical products.

DISCUSSION

Through this study, we evaluated the antibacterial activity of the diverse substances *in vitro* and *in vivo* on the growth of *X. citri* pv. *citri*.

The two chemical products based on copper at 25 mg/ml, were the most active with the high inhibition diameters. Furthermore, the aqueous extract of *A. indica*, *C. citratus* and *E. camadulensis* and essential oils of *O. gratissimum* were each active inducing an inhibition diameter superior to 10 mm at the maximal tested concentrations. The lowest inhibition diameters were obtained with Neco 50EC and Proraly 50EC.

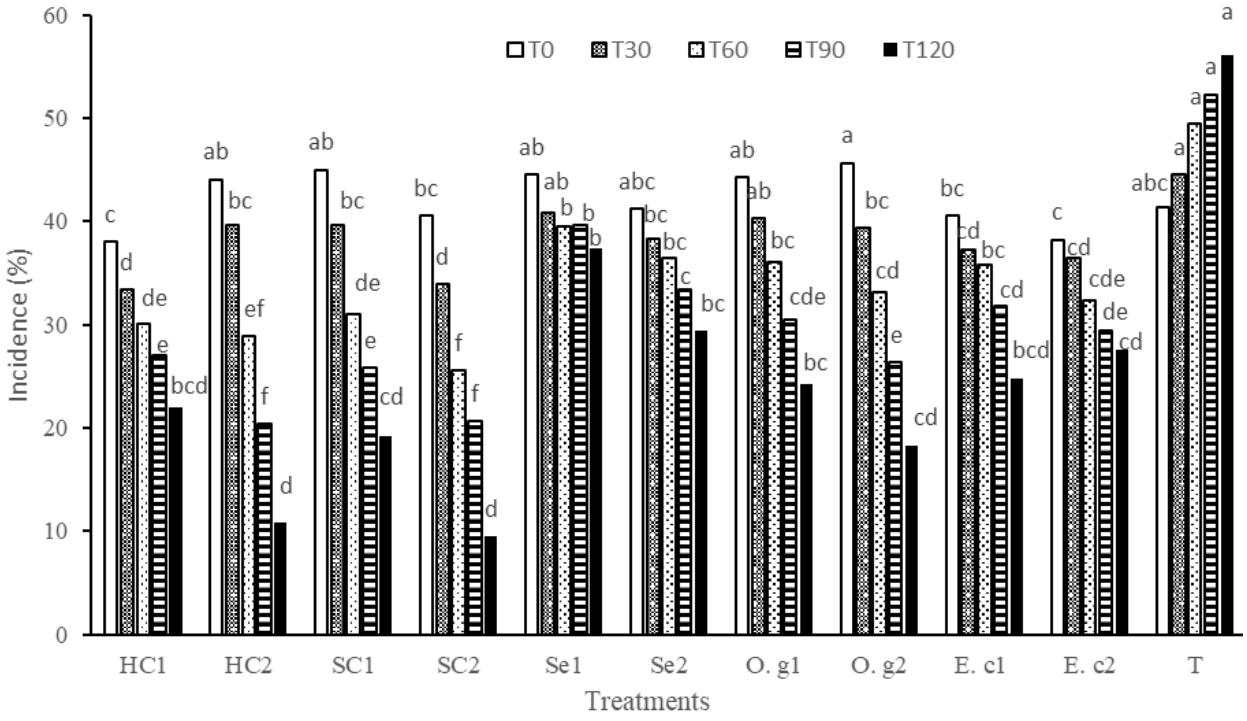

Figure 2. Disease incidence after treatment with different substances. **HC1 and HC2**: copper hydroxide at 25 and 12.5 mg/ml; **SC1 and SC2**: copper sulfate at 25 and 12.5 mg/ml; **Se1 and Se2**: *Bacillus amyloliquefaciens* formulation at 30 and 60 µl/ml; **O.g1 and O.g2**: He of *O. gratissimum* at 5 and 10 µl/ml; **E.c1 and E.c2**: Aqueous extract of *E. camadulensis* at 50 and 100 mg/ml; **T**: Control.
Source : Authors

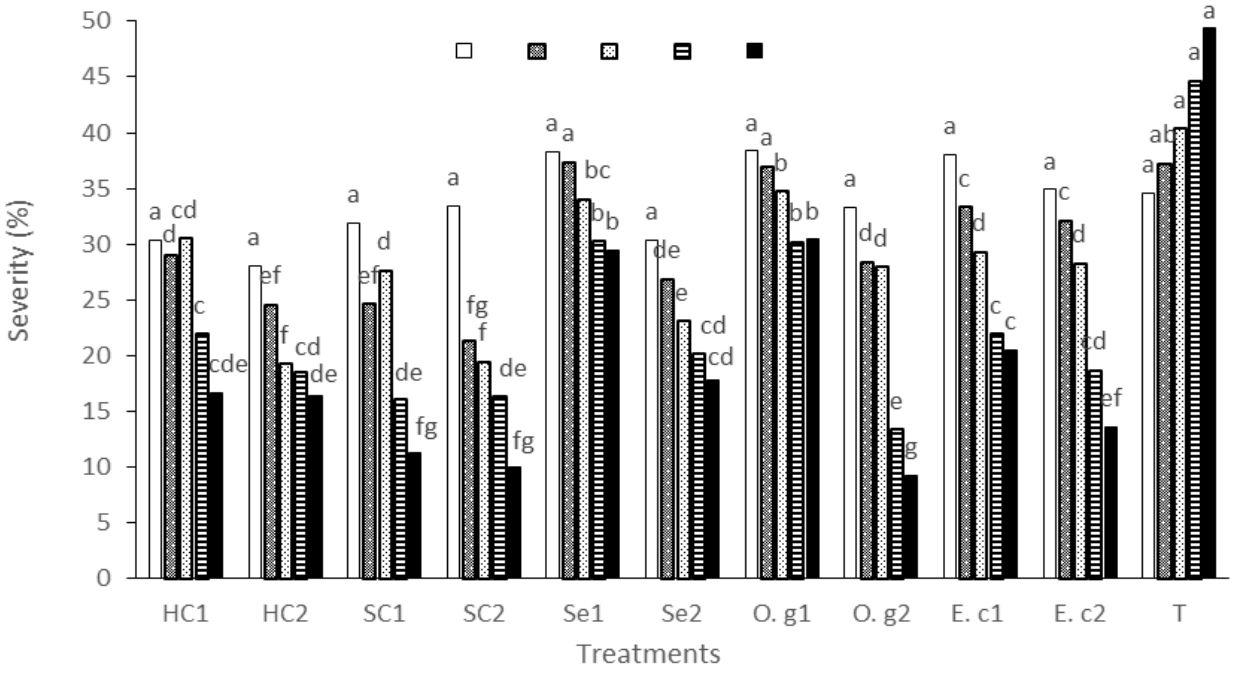

Figure 3. Disease severity after treatment with different substances. HC1 and HC2 : copper hydroxide at 25l and 12.5mg/ml; SC1 and SC2: copper sulfate at 25 and 12.5 mg/ml; Se1 and Se2: *Bacillus amyloliquefaciens* formulation at 30 and 60 µl/ml; O.g1 and O.g2: He of *O. gratissimum* at 5 and 10 µl/ml; E.c1 and E.c2: Aqueous extract of *E. camadulensis* at 50 and 100mg/ml; T: Control.
Source: Authors

According to MBC/MIC ratios of copper hydroxide (12.5 mg/ml), copper sulphate (25 mg/ml), *O. gratissimum* and *E. camadulensis* (50 mg/ml), the results showed that these substances have antibacterial properties.

For *in vivo* tests, the results showed that chemical products based on copper and the essential oil of *O. gratissimum* were the most effective, when they were used in foliar treatment each two week intervals.

In fact, the antibacterial action of copper hydroxide and copper sulphate *in vitro* and *in vivo* has been demonstrated by several studies (Girard, 2004; Idrissou-Touré et al., 2020; Behlau et al., 2021). However, some authors claimed that the use of copper can induce resistance to pathogens (Giraud et al., 2007; Villeneuve, 2008; Damien et al., 2017), create environmental pollution and copper accumulation in soils (Alva et al., 1995).

To attenuate the harmful effect of copper, some authors recommended its use in combination with mancozeb (Roberts et al., 2008; Fayette et al., 2012) or other biological components such as *B. subtilis* (Provost et al., 2012).

Studies have shown that the essential oil of *O. gratissimum* contains components such as thymol over 43%, gamma-terpinene 18.77% and para-cymene 6.77% (Soro et al., 2011), which would give it a bactericidal activity. Thus, thymol was one of the components of essential oils that are the most active against pathogens (Ajjouri et al., 2008).

The results confirmed those finding of Kpodekon et al. (2013) that demonstrated that the MICs and CMBs of *O. gratissimum* would be respectively between 6.10^{-3} and 144.10^{-3} mg/ml. In addition, Zombré et al. (2015) have shown that *O. gratissimum* was effective against bacterial blight of cashew and mango caused by *X. citri* pv. *mangiferaindica*. Saha et al. (2013) have also reported the antibacterial activity of *O. gratissimum* against Gram negative bacteria.

The effectiveness of aqueous plant extracts of *A. indica*, *C. citratus* and *E. camadulensis* has been proven in numerous studies, especially against fungi of the *Fusarium* genus (Dao, 2013; Tiendrebeogo, 2011).

As for the results obtained in the study with the biopesticide, the component of *B. amyloliquefaciens* would be the active molecule. Chen et al. (2009) reported that *B. amyloliquefaciens* produces various antibiotics which confer antibacterial activity. Roberts *et al*. (2008) and Ibrahim *et al.* (2016) have shown its efficacy on numerous species of *Xanthomonas*, *Pseudomonas* and *Erwinia*. Ye et al. (2016) reported that *in vivo* treatment with the combination of copper products and *B. subtilis* formulations reduced the incidence of CBC by only 19%, whereas untreated plants had 43%.

Conclusion

All the substances tested showed antibacterial properties against *Xcc* and their efficacy changes with concentration. The copper products were the most effective followed by the essential oil of *O. gratissimum*, the aqueous extracts of *E. camadulensis* and the substance formulation of *B. amyloliquefaciens* respectively. These substances were induced reductions of the incidence and severity of CBC, when applied at certain concentrations at two weeks intervals. Therefore, they can be tested in the field to determine their effectiveness.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abo-Elyousr KAM, Asran MR (2009). Antibacterial activity of certain plant extracts against bacterial wilt of tomato. Archives of Phytopathology and Plant Protection 42(6):573-578.

Ajjouri ME, Satrani B, Ghanmi M, Aafi A, Farah A, Rahouti M, Amarti F, Aberchane M (2008). Activité antifongique des huiles essentielles de Thymus bleicherianus Pomel et *Thymus capitatus* (L.) Hoffm. Link contre les champignons de pourriture du bois d'œuvre. Base 12(4):345-351.

Alva AK, Graham JH, Anderson CA (1995). Soil pH and copper effects on young 'Hamlin' orange trees. Soil Science Society America Journal 59(2):481-487.

Behlau F, Lanza FE, da Silva Scapin M, Scandelai LHM, Silva Junior GJ (2021). Spray volume and rate based on the tree row volume for a sustainable use of copper in the control of citrus canker. Plant Disease 105(1):183-192.

Berche P, Gaillard JL, Simonet M (1991). Les bactéries des infections humaines", Éditeur : Flammarion. Médecine & Sciences. 660 p.

CABI (2019). Invasive species compendium *Xanthomonas citri* (citrus canker). Disponible sur. Avalable at: https://www.cabi.org/isc/datasheet56921

Chen XH, Koumoutsi A, Scholz R, Schneider K, Vater J, Sussmuth R, Piel J, Borriss R (2009). Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. Journal Biotechnology 140(1-2):27-37.

Civerolo EL (1984). Bacterial canker disease of citrus (*Xanthomonas campestris*). Journal of the Rio Grande Valley Horticultural Society 37:127-146.

Damien R, Tribot N, Boyer C, Terville M, Boyer K, Javegny S, Roux-Cuvelier M, Pruvost O, Moreau A, Chabirand A, Vernière C (2017). First report of copper resistant *Xanthomonas citri* pv. *citri* pathotype A causing Asiatic citrus canker in Réunion France. Plant Disease 101(3):503.

Dao K (2013). Étude de la variabilité de *Fusarium verticilloides* (Sacc.) Nirenberg isolé des semences paysannes de maïs au Burkina Faso et recherche de méthodes de lutte alternatives basées sur les extraits de plantes *in vitro*. Mémoire du Diplôme d'Etude Approfondie, Institut du Développement Rural, Université Polytechnique de Bobo-Dioulasso, Burkina Faso 54p.

Fayette J, Roberts PD, Pernezny KL, Jones JB (2012). The role of cymoxanil and famoxadone in the management of bacterial spot on tomato and pepper and bacterial leaf spot on lettuce. Crop Protection 31:107-112.

Girard H (2004). Le cuivre : origines. Usages et différentes formes. Conférence Journées techniques Nationales Viticulture Biologique Avignon. Editions ITAB. pp 73-89.

Giraud M, Prunet JP, Chevallier A, Ramain S (2007). La bactériose du noyer. Evolution des populations de *Xanthomonas* en verger. Infos Ctifl 230:38-42.

Gottwald TR, Graham JH, Schubert TS (2002). Citrus canker: the pathogen and its impact. Plant Health Progress 3(1):15.

Guédé-Guina F, Vangah-Manda M, Harounad D, Bahi C (1996). Potencies of MISCA a plant source concentrate against fungi. Journal of Ethnopharmacology 14(2):45-53.

Idrissou-Touré M, Sirikou R, Bello S, Oussou F, Dossoumou MEEA (2017). Efficacité de l'hydroxyde de cuivre contre la nervation noire du chou (*Brassica oleracea* L.) causée par la bactérie *Xanthomonas campestris* pv. *campestris.* Agronomie Africaine 29(2):149-157.

Ibrahim YE, Saleh AA, El_Komy MH, Al_Saleh MA (2016). *Bacillus subtilis* QST 713, copper hydroxide, and their tank mixes for control of bacterial citrus canker in Saudi Arabia. Journal of Citrus Pathology 3(1):1-6.

Juhasz CC, Leduc A, Vernière C, Pruvost O, Wonni I, Ouedraogo L (2013). First report of *Xanthomonas citri* pv. *citri* Causing Asiatic Citrus Canker in Burkina Faso. Plant Disease 97(12):1653.

Kpodekon MT, Boko CK, Mainil JG, Farougou S, Sessou P, Yehouenou B, Gbenou, J, Duprez JN, Bardiau M (2013). Composition chimique et test d'efficacité *in vitro* des huiles essentielles extraites de feuilles fraîches du basilic commun (*Ocimum basilicum*) et du basilic tropical (*Ocimum gratissimum*) sur *Salmonella enterica* sérotype Oakland et *Salmonella enterica sérotype* Legon. Journal de la Société Ouest-Africaine de Chimie 35:41-48.

Mayee CD, Datar VV (1986). Phytopathometry. Department of Plant Pathology, Marathwada Agricultural University, Parbhani. Technical Bulletin 3:110.

Moroh JLA, Bahi C, Dje K, Loukou YG, Guede-Guina F (2008). Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de *Morinda morindoides* (Baker) milne-redheat (rubiaceae) sur la croissance *in-vitro* des souches d'*Escherichia coli*. Bulletin de la Société Royale des Sciences de Liège 77:44-61.

OEPP/CABI (1990). Fiche informative sur les organismes de quarantaine. *Xanthomonas axoponodis* pv. *Citri*. Préparé par le CABI et l'OEPP pour l'UE sous Contrat 90/39900.

Oliveira Filho JGD, da Cruz Silva G, de Aguiar AC, Cipriano L, de Azeredo HMC, Bogusz Junior S, Ferreira MD (2021). Chemical composition and antifungal activity of essential oils and their combinations against *Botrytis cinerea* in strawberries. Journal of Food Measurement and Characterization 15(2):1815-1825.

Ponce AG, Fritz R, del Valle C, Roura SI (2003). Antibacterial activity of essential oils on the native microflora of organic Swiss chard. Society of Food Science and Technology 36(7):679-684.

Provost C, Guerra N, Caron L (2012). Evaluation de nouveaux agents bactéricides contre deux maladies bactériennes des tomates de champ. Programme de Soutien à l'Innovation Horticole. Québec, Canada 3 p.

Rabbee MF, Ahmed CR, Roy A, Chowdhury R, Kubra K, Banik A, Chowdhury MMH, Baek KH (2021). Promesses thérapeutiques des plantes médicinales au Bangladesh et de leurs composés bioactifs contre les ulcères et les maladies inflammatoires. Plantes 10(7):1348.

Raveau R, Fontaine J, Lounès-Hadj Sahraoui A (2020). Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds: A Review. Foods 9(3):365.

Roberts PD, Momol MT, Ritchie L, Olson SM, Jones JB, Balogh B (2008). Evaluation of spray programs containing famoxadone plus cymoxanil. acibenzolar-S-methyl and *Bacillus amyloliquefaciens* compared to copper sprays for management of bacterial spot-on tomato. Crop Protection 27:1519-1526.

Saha S, Dhar TD, Sengupta C, Ghosh P (2013). Biological activities of essential oils and methanol extracts of five *Ocimum* Species against pathogenic bacteria. Czeh Journal of Food Sciences 31(2):194-202.

Salifou A, Bounou BI (2020). Attaque du chancre bactérien des agrumes sur des pieds de tangelos de pamplemoussier et de citronnier dans la commune de Youri (département de Say), *VegNote.* Vol 1: N°1, Clinique des plantes. Bioengineering and Agri-business consulting. Disponible sur. Available at: https://www.csan-niger.com/chancre-bactérien-agrumes-niger-xanthomonas-axonopodis-pv-citri-php

Soro S, Abo K, Kone D, Coffi K, Kouadio JY, Ake S (2011). Comparaison de l'efficacité antifongique de l'huile essentielle d'*Ocimum gratissimum* L. et du fongicide de synthèse mancozebe contre le mycopathogene tellurique, *Fusarium oxysporum* f. Sp. *Radicis-lycopersici* en cultures de tomate (*Lycopersicon esculentum* Mill.) Sous abri en côte d'ivoire. Agronomie Africaine 23(1):43-52.

Stall RE, Miller JW, Marco GM, Echenique BIC (1980). Population dynamics of *Xanthomonas citri* causing cancer of citrus in Argentina. Proceedings of the Florida State, Horticultural Society 93:10-14.

Tiendrebeogo A (2011). Étude de l'efficacité des extraits aqueux de plantes locales (*Edipta alba* L. *Cymbopogon citratus* (D. C.) Stapf. *Agavae sisalana* Perr. et *Lippia multiflora* (Moldenke) contre les principaux champignons seminicoles du riz. Mémoire d'Ingénieur du Développement Rural. Institut du Développement Rural. Université Polytechnique de Bobo Dioulasso. Burkina Faso 67 p.

Toty AA, Guessennd N, Bahi C, Kra AM, Otokore DA, Dosso M (2013). Evaluation *in vitro* de l'activité antibactérienne de l'extrait aqueux de l'écorce de tronc de *Harungana madagascariensis* sur la croissance de souches multi-résistantes. Bulletin de la Société Royale des Sciences de Liège 82:12-21.

Villeneuve C (2008). Lutte aux bactéries et résistance au cuivre dans le poivron et la tomate. Conférence des journées horticoles de Saint Remy. Document Agri Réseau 7 p.

Wonni I, Ouedraogo SL, Ouedraogo I, Sanogo L (2016). Antibacterial activity of extracts of three aromatiplants from Burkina Faso against rice pathogen, *Xanthomanas oryzae*. African Journal of Microbiology Research 10(20):681-686.

Zombré C, Sankara BC, Ouédraogo SL, Wonni I, Pruvost O, Boyer C, Vernière C, Adandonon A, Vasyssière JF, Ahohuendo BC (2015). First report of *Xanthomonas citrus pv.mangiferaeindicae* causing Mango bacterial canker on *Mangifera indica* L. in Benin. Plant Disease 99(12):1854.

Vol. 16(6), pp. 148-156, July 2022
DOI: 10.5897/AJPS2017.1617
Article Number: 4E2661469455
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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Traditional knowledge and economic importance of *Ferula assa-foetida* in the rural areas of southeastern Iran

Farhood Golmohammadi

Department of Agricultural Extension and Education, Islamic Azad University, Birjand Branch, Birjand, Iran.

Received 26 October, 2017; Accepted 17 December, 2017

Traditional knowledge is considered as knowledge, innovations, practices of indigenous and local communities embodying traditional life-styles; the wisdom developed over many generations, of holistic traditional scientific utilization of lands, natural resources and environment. Traditional knowledge is valid and necessary, and due to its relevance, it currently has wide application for human benefit. Many people in Mediterranean region who consult spiritual healers, homeopaths and herbalists are utilizing traditional therapies. These are the first choice for problems such as liver diseases, inflammation, skin diseases, infertility, impotence, diabetes, obesity, epilepsy, psychosomatic troubles and many other diseases. *Ferula assa-foetida* L. (Apiaceae) is one of the most important among the thirty species of *Ferula* distributed in Iran. One part used is an oleo-gum resin, called asa-foetida or Anghoze in Persian, which is obtained by incision from the roots. *Ferula* is one of the most important endangered medicinal plants, which is rare in nature due to poor seed germination. In this article, qualitative and participatory study on main characteristics, harvesting and economic importance of this valuable medicinal plant in South-Khorasan province, East of Iran, as a major producer and exporter, is introduced.

Key words: Ferula, traditional knowledge, medical plant, rural regions, Iran.

INTRODUCTION

Medicinal plants are an important element of the medical system. These resources are usually regarded as part of cultural traditional knowledge (Golmohammadi, 2013). The genus, Ferula belongs to the Umbelliferae family and consists of 140 species which are widespread from the Mediterranean region to central Asia. *Ferula assa-foetida* L. (Apiaceae) is one of the most important species of this genus which is native to Iran and Afghanistan, and commonly known as *asa foetida*. It is a herbaceous, monoecious and perennial plant that grows up to 2 m in height, and is in two types, bitter and sweet (Iranshahy and Iranshahi, 2011). *F. assa-foetida* L. (Apiaceae) is a medicinal plant indigenous to Iran and Afghanistan. This plant is one of the most important among the thirty

E-mail: farhood.gol@gmail.com. Tel: (0098) 913 -326 -1040.

Table 1. Names of *Ferula assa-foetida* in different languages

Persian	English	French	German	Hindi	Arabic	Botanical name	Family
Anghose	Stinking assa	Stinkender assand	Teufels treck stinkender assand	Hing, Hingra	Zallouh	Ferula assa – foetida	Apiaceae

Golmohammadi (2013).

Figure 1. Medicinal plant collectors that are usually poor villagers and plant collection is their part time activity besides farming and herds keeping IN Sorond village of Tabas City, 300 km distance to Birjand, centre of South Khorasan province. 1a. Pool for storaging mountain water for utilization by villagers and their herds, 1b. Herds of the villagers beside the shrubs of *F. assa foetida* in mountains and pasturelands (May 23, 2016).
Source: Golmohammadi (2016).

species of *Ferula* distributed in Iran. One part used is an oleo-gum resin, called *asa-foetida* or anghouzeh in Persian, obtained by incision from the roots. It has been reported in Iranian folk medicine to be antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, anthelmintic, aphrodisiac and antiseptic.

Asafoetida's English and scientific name is derived from the Persian word resin (*asa*) and Latin *foetida*, which refers to its strong sulfurous odour. Its pungent odour has resulted in it being called by many unpleasant names; in French it is known (among other names) as *merde du diable* (devil's faeces); in English, it is known as devil's dung, and equivalent names can be found in most Germanic languages (e.g. German *Teufelsdreck*, Swedish *dyvelstr¿ck*, Dutch *duivelsdrek*, Afrikaans *duiwelsdrek*), also in Finnish, it is called *pirunpaska* or *pirunpihka*. In Turkish, it is known as *şeytantersi* (devil's sweat), *şeytan boku* (devil's crap) or *şeytanotu* (the devil's herb) (Hassani et al., 2009) (Table 1).

Medicinal plant collectors are usually poor villagers. Plant collection is their part time activity besides farming and livestock keeping (Hamayun et al., 2003). This situation has also been seen in South Khorasan province, south east of Iran (Figure 1).

MATERIALS AND METHODS

The study area is South Khorasan province, east of Iran. The present study was conducted from 2010 to 2017 in rural regions of South Khorasan province, east of Iran (Figure 2). South Khorasan province consists of 11 counties namely: Birjand, Ferdows, Tabas, Qaen, Nehbandan, Darmian, Sarbisheh, Boshruyeh, Sarayan, Zirkouh and Khusf.

Two main regions in mountains and pastures where *Ferula assa-foetida* L. are grown are, Tabas and Darmian counties, thus majority of field research has been done in these locations. For this article, recent and most important articles in the domain of the research wereas utilized. Main instruments for gathering information in this article were qualitative and participatory research methods and place-based approaches, using in-depth semistructured interviews and participatory observation, discussions, experiments, documents, pictures and nonformal interview with villagers, related officers, specialists and professors in the domain of this research from 2010 to 2017.

Various stages for harvesting Ferula gum, plus producing and sowing its seeds

F. assa foetida grows up to 2 m high, with a circular mass of 30 to 40 cm leaves. Stem leaves have a wide sheathing petioles. Flowering stems are 2.5 to 3 m high and 10 cm thick and hollow, with a number of schizogenesis ducts in the cortex containing the resinous gum. Flowers are pale greenish yellow in color, produced in large compound umbels. Fruits are oval, flat, thin, reddish brown and have a milky juice. Roots are thick, massive and pulpy. They yield a resin similar to that of the stems. All parts of the plant have a distinctive fetid smell (Table 2).

Resin-like gum is obtained from the dried sap, extracted from the stem and roots and is used as a spice. The resin is grayish-white when fresh but dark amber in color when dry. The *asa foetida* resin is difficult to grate and is traditionally crushed between stones or with a hammer. Today, the most commonly available form is

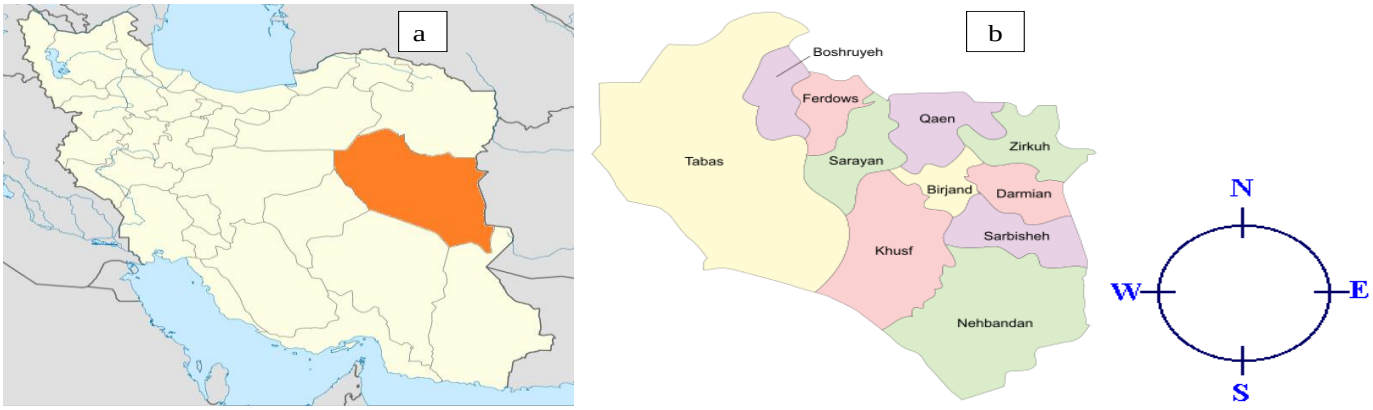

Figure 2. Maps of South Khorasan province in eastern part of Iran. a. Map of the main study area besides borders of Afghanistan; Coordinates: 32.8653°N 59.2164°E; b. Map of the cities of South Khorasan province (at a map scale of 1:1000000).
Source: Information and Statistical Department (2016).

Table 2. Some of the meteorology, geology and botanical information of growth regions of *Ferula assa foetida* in South Khorasan province.

Average amount of rainfall in province (2005-2016)	81/03 mm
Maximum temperature (in June and July months)	46.59°C
Minimum temperature (in December and January months)	-2.11°C
Time for maturation of *Ferula assa foetida* shrubs and production of gum	5 years
Time of growth of *Ferula assa foetida* shrubs	From end of winter till end of June
Time of dormancy of immature *Ferula assa foetida* shrubs	From beginning of July till end of winter
Main type of lands where *Ferula* can grow	Sandy and lime
Main locations where *Ferula* can grow	Mountains and pastures
Main type of reproduction by Ferula	Only by seed
Average rainfall for growth of *Ferula* shrubs	90 – 150 mm
Slope of growth regions of *Ferula* shrubs	30-60%
Main origin regions of *Ferula* shrubs	Iran and Afghanistan
Acreage of potential pasture areas for production of medicinal fresh gum of *Ferula assa foetida*	100000 ha
Number of rural households that their income are dependent on *Ferula*	2000

Information and Statistical Department (2016).

Figure 3. Shrub of *F. assa foetida* in mountains and pasturelands of South Khorasan province.
Source: Golmohammadi (2016).

compounded *asa foetida*, a fine powder containing 30% *asa foetida* resin, together with rice flour and gum Arabic. *F. assa foetida* exudations are obtained by tapping the root stock of the plant.

The root of this plants have been used for perceived anthelmintic, antimicrobial, antispasmodic, aromatic, laxative, antispasmodic, diuretic and antiseptic actions in folk medicine. *F. assa-foetida* L. (Apiaceae) is one of the most important endangered medicinal plants, which is rare in nature due to poor seed germination. Although, the flora of Persia is thus fairly well known, there are still very few works on its overall vegetation (Information and Statistical Department, 2016; Golmohammadi, 2013). In this regard, various stages for harvesting and obtaining Ferula gum are as follows:

i. Selecting shrubs of *F. assa Foetida* which are ready for use as medicinal gum and marking them by rural people from April and November (Figure 3).
ii. Fencing these selected shrubs with stones (Figure 4).
iii. Harvesting gum; this is the final stage of producing dried gum, and supply to the market is done in June, July and August. In this stage, in every 4 to 5 days of these months, native medicinal plant collectors with their traditional tools (Figures 4 and 5) create a thin cutting on the stem and after this period, they gather resin-like gum discharges from the stem, which is again replicated with 12 to 16 rounds, each in 4 to 5 days on the stems of Ferula in these months (Figures 7 and 8). In gathering of the gum, before thin cutting of Ferula shrubs stems by native medicinal plant collectors with the above 12 to 16 rounds, they are collected and dried to obtain dried stem cutting of *F. assa* (Keshteh- in endemic Persian language), which will be taken to the market and processed by foreign medicinal factories (Figure 7 and Table 3). Each skillful native medicinal plant collector can in one day cut stems of 1000 Ferula shrubs and gather 4 to 10 g of resin-like gum from each stem cutting in 4 to 5 days. This means that a skillful native medicinal plant collector can obtain 2 to 3 kg gum from Ferula shrubs in dry and rainy seasons in the months of harvest (Figure 4).
iv. Producing seeds from shrubs of *F. assa foetida* are set by the end of summer.
v. Sowing seeds of *F. assa foetida* by rural people in winter and beginning a new germination of plant by the end of winter, originates a new spring (Figure 6) (Information and Statistical Department, 2016) (Table 3).

Essential oil of Ferula

Essential oils (volatile oils) are aromatic oily liquids obtained from plant materials such as flowers, herbs, buds, fruits, twigs, bark, seeds, wood, roots, resin, gum and latex. Essential oil components are chemically derived from terpenes and their oxygenated derivatives, which are aromatic and aliphatic acids, esters and phenolic compounds.

The percentage of components of the essential oils varies among species and plant parts, depending on the species, climate and altitude, time of collection and growth stage. The composition of essential oils might be different qualitatively and quantitatively.
F. assa-foetida is a herbaceous perennial plant with an unpleasant odour and is often considered to be the main source of oleo-gum-resin (OGR, a milky exudation from certain plants that coagulates on exposure to air), which has a characteristic sulfurous odour and bitter taste (Kavoosi and Rowshan, 2013). Oleo-gum resin is obtained as, secretions of the upper parts of the roots in plant by incision. It is a dark brown to black resin-like gum obtained from the juice of the rhizome. After drying, it becomes dark brown in color with resin-like mass. Different grades of resins, dried granules, chunks or powders are sold. It is marketed in three forms: tears, mass and pastes. Chemical composition and antibacterial activity of essential oils of commonly consumed herbs, such as *Citrus aurantium, Citrus limon, Lavandula angustifolia, Matricaria chamomilla, Mentha piperita, Mentha spica, Ocimum basilicum, Origanum vulgare, Thymus vulgaris, Salvia officinalis* and *Zataria multiflora* and their main components have been evaluated in many countries. The main constituent of OGR is essential oil which contains ferulic acid, sesquiterpene, sulfur-containing compounds, monoterpenes and other volatile terpenoids. Although, advances in chemical and pharmacological evaluation of *F. assa-foetida* have occurred in recent past, several useful features of this plant remains unknown (Kavoosi and Rowshan, 2013). Accordingly, essential oils obtained from *F. assa-foetida* OGRs in different collections had different chemical composition, antioxidant, reactive oxygen species (ROS), reactive nitrogen species (RNS), H_2O_2 and TBARS scavenging activities. The essential oil of OGR1 had high levels of acyclic sulfur-containing compounds [(E)-1-propenyl sec-butyl disulfide and (Z)-1-propenyl secbutyl disulfide] and bicyclic sesquiterpenes (10-epi-c-eudesmol) which showed the highest radical scavenging and the lowest antibacterial and antifungal activities.

Essential oil of OGR2 had high levels of acyclic sulfur-containing compounds [(Z)-1- propenyl sec-butyl disulfide and (E)-1-propenyl sec-butyl disulfide] and bicyclic monoterpenes (b-pinene and a-pinene) which showed moderate radical scavenging, antibacterial and antifungal activities. Essential oil of OGR3 had high levels of bicyclic monoterpenes (b-pinene and a-pinene) and heterocyclic disulfide (1, 2-dithiolane) which showed lowest radical scavenging and highest antibacterial and antifungal activities. For this reason, the essential oil obtained from the earlier stages of *F. assa-foetida* growth could be used as safe and effective natural antioxidants in food industry, to improve the oxidative stability of fatty foods during storage, while the essential oil obtained from the later stages of *F. assa-foetida* growth could be used in health industry, as a safe and effective source of antimicrobial agents. However, this is the first report on the effect of growth stage on the essential oil profile of *F. assa-foetida*. More professional studies are required to examine phenolic and flavonoid biosynthetic pathways and expression profiles of the related enzymes. With these expertise studies, tentative applications of essential oils can be discussed (Kavoosi and Rowshan, 2013).

Ferula and traditional medicine

In traditional medicine, this plant is used for the treatment of different diseases, such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion and influenza (Kavoosi and Rowshan, 2013).

The old traditional phytomedicine asafoetida, an oleo-gum-resin obtained from the roots of different *F. assa-foetida*, is used in different countries for various purposes. This oleo-gum-resin has been known to possess antifungal, anti-diabetic, anti-inflammatory, anti-mutagenic and antiviral activities. A wide range of chemical compounds including sugars, sesquiterpene coumarins and polysulfides have been isolated from this plant. Recent studies have shown new promising antiviral sesquiterpene coumarins from this old phytomedicine. *Asa foetida* has been used as a spice and a folk phytomedicine for centuries and has a characteristic sulfurous odor and a bitter taste. It is used as a flavoring spice in a variety of foods, particularly in India. In addition, Nepali people regularly consume it in their daily diets, and it is believed that asafoetida has aphrodisiac, sedative and diuretic properties.

Another biological activity of *F. assa-foetida*, which has been confirmed by a number of new studies, is cancer chemoprevention. Antihelminthic property (or anthelmintic) is another emphatically reported traditional use of *asa foetida* in different countries. In Iran, China and Nepal, it is traditionally used for the treatment of intestinal parasites infestation (Iranshahy and Iranshahi, 2011) and

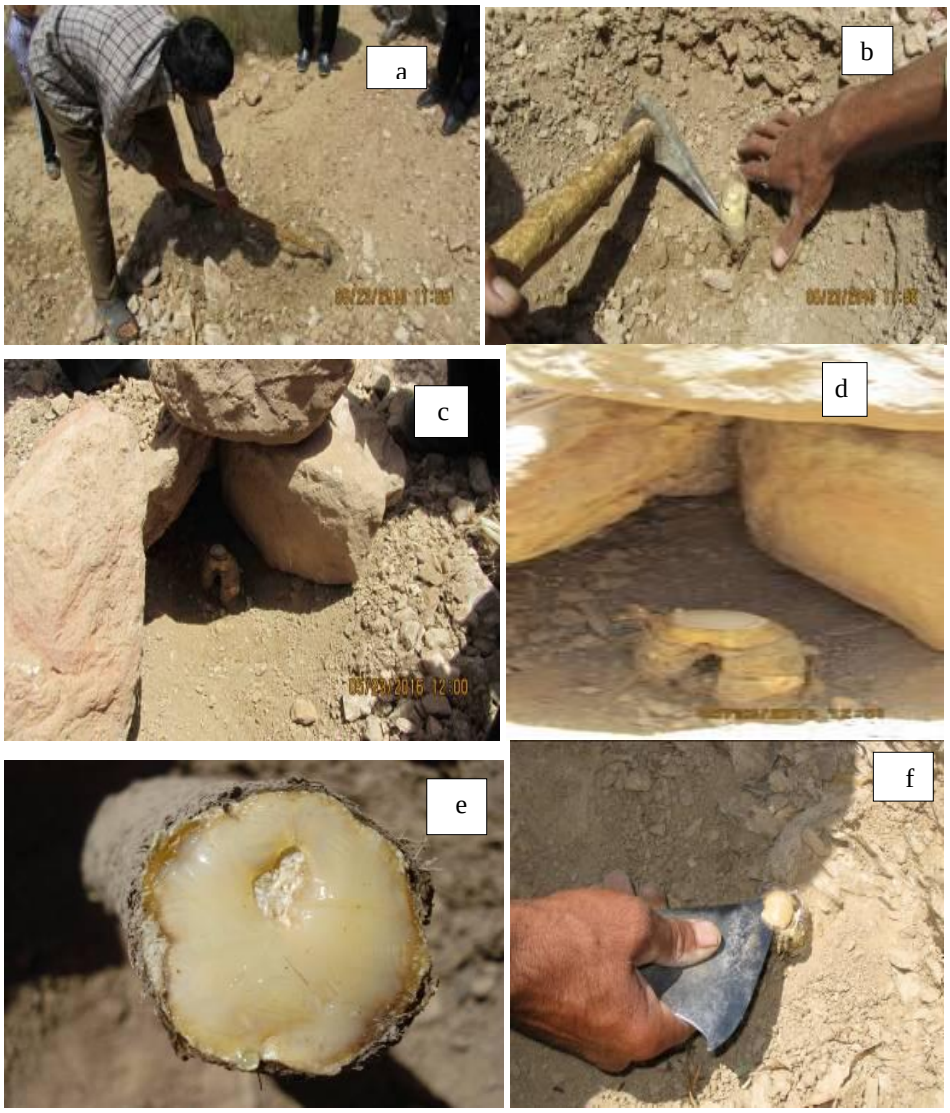

Figure 4. Villagers performing various stages of preparing shrubs of *Ferula assa* and stem cutting harvesting for medicinal gum (Shireh). Sorond village of Tabas City, 300 km distance from Birjand centre of South Khorasan province. These stages are in the order:a. Establishing a hole around stem of the shrub; b. Preparing stem of shrub for next steps;c. Providing a stone sunshade for protecting stem from high temperature and drying; d. Cutting head of the stem for oozing and exudation of juice of *F. assa*; e. Juice of *Ferula assa*; f. Gathering of the juice by iron shaver (May 23, 2013, 2016).
Source: Golmohammadi (2016).

(Gundamaraju, 2013). According to the Chinese, European, Iranian and Indian traditional medicines, oleo gum resin of *F. assa-foetida* (*asa foetida*) has therapeutic effects on different kinds of diseases. Some of these effects are related to the diseases of nervous system such as hysteresis and convulsion (Moghadam et al., 2014).

DISCUSSION

The demand for medicinal plants has increased globally due to the resurgence of interest in and acceptance of herbal medicine. Most of the demand is being met through collection of large quantities of medicinal plants and plant parts from wild populations. The methods of extraction employed are almost invariably crude and unsystematic. As a consequence, the rates of exploitation may exceed those of local natural regeneration.

Water is a major natural resource which is a limiting factor in the development of agriculture and natural resources, especially in a dry region such as Iran. Therefore, it is necessary to adopt water management technologies for utilizing the available water resources. Water is the most precious commodity in the arid region

Figure 5. Traditional tools of villagers for preparing shrubs of *Ferula assa* and cutting of stems for harvesting medicinal gum, in Sorond village of Tabas City, 300 km from Birjand, Centre of South Khorasan province. These tools are: a. A leather sack for storing and gathering juices; b. an adze for establishing holes around stem of shrubs; c. Iron shavers etc. for gathering juice of *Ferula assa* from stem cuttings (May 23, 2016). Source: Golmohammadi (2016).

of Iran due to prevalence of unfavorable hydro meteorological condition (Golmohammadi, 2012). We are living in a knowledge driven world where knowledge is the ultimate power (Kumari et al., 2014).

In this regard, indigenous traditional and local knowledge on medicinal plants are important elements of herbal and medicinal system. These resources are usually regarded as part of the cultural traditional knowledge. Despite all kinds of technological advances, the geographic variation is one element that is far from human control because of the different climatic conditions and edaphic factors that exist in each region.

Essential oil quality and quantity in general are extremely dependent on the weather conditions; also, several authors considered that the physico chemical characteristics are determinant factors in secondary metabolites composition especially for quality of volatiles (Moghaddam and Farhadi, 2015).

Also, the natural habitats are quickly being depleted. There is thus an urgent need to develop and implement conservation strategies to exploit medicinal plant species. The medicinal plant is propagated through seeds. However, its natural populations are very limited in native habitats, which may be due to poor seed germination. Low seed germination in Apiaceae is also known (Moghadam et al., 2014; Golmohammadi, 2012).

F. assa-foetida L. (Apiaceae) is one of the most important and valuable medicinal plants in pastures of Iran and especially, South-Khorasan province with the majority of its products (about 99%) being exported to foreign countries (especially for utilizing by medicinal factories in developed countries). Because of the above

Figure 6. Various stages of production of seeds from shrubs of *Ferula assa foetida*. These stages are: a. Recognizing shrubs of *Ferula assa foetida* that are ready for seeds production; b. Gathering seeds of *Ferula assa*; c. Preparing appropriate foothills and mountains for sowing *Ferula assa* seeds at the end of winter by local rural people; d. Germinating shrubs of *Ferula assa foetida* in winter (May, 2012, 2013). Source: Golmohammadi (2016).

Figure 7. Final production of dried thin stems cutting of *Ferula assa-foetida*, Keshteh- in endemic Persian language, for market supply and processing by medicinal factories in Sorond village of Tabas City, 300 km from Birjand centre of South Khorasan province (May 23, 2016). Source: Golmohammadi (2016).

reasons, main goals of managers of Natural Resources and Watershed Administration of South Khorasan province are sustainable exploitation plus maximum economic efficiency of this plant. *Ferula* is one of the most important endangered medicinal plants, which is rare in nature due to poor seed germination (Information and Statistical Department, 2016). The production of this valuable plant in South-Khorasan province in about 100000 ha, is 60 (in rainy years) and 15 to 20 tons (in dried years) annually and almost all of this production is exported because of, lack of processing industries in this province. This plant generates income for many rural and nomadic households, which in the conditions of their subsistence agriculture, have high dependence on this production.

South Khorasan province in the east of Iran has good ethnobotanical potential for medicinal plants. With the presence of the above mentioned cases, the author stated the following recommendations for sustainable management and exploitation with increased economic efficiency of this plant:

i. Utilizing *F. assa-foetida* in the present traditional form namely, cutting its stem from above its root for producing

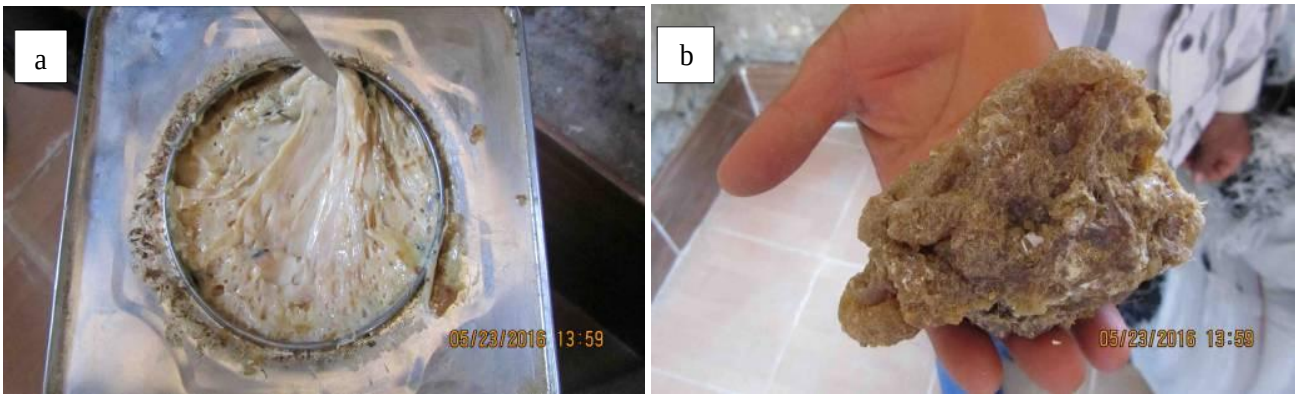

Figure 8. Essential medicinal oil (oleo gum resin, OGR) of *Ferula assa-foetida*. a. Shireh (in endemic Persian language) in jelly form (major), and b. solid form (final). These are obtained from pastures of Sorond village of Tabas City, 300 km from Birjand centre of South Khorasan province (May 23, 2016).
Source: Golmohammadi, (2016).

Table 3. Some of the economic information of *Ferula assa foetida* in South Khorasan province.

Amount of medicinal fresh gum produced by one shrub of *Ferula assa foetida*	20-40 g
Value of annually exported dried gum of *Ferula assa foetida*	4000000 $ USD
Total amount of dried gum of *Ferula assa foetida* in rainy years	60 tons
Amount of dried gum of *Ferula assa foetida* in dried years	15-20 tons
Main cities that produce medicinal fresh gum of *Ferula assa foetida* in their pasture areas	Tabas, Qaen, Ferdows and Boshrooyeh
Value of one kg dried gum of *Ferula assa foetida* in global markets	130-17 $ USD
Value of one kg dried gum of *Ferula assa foetida* bought by middlemen from native medicinal plant collectors (in 2016)	60-70 $ USD
Value of one kg. dried stem of *Ferula assa foetida* bought by middlemen from native medicinal plant collectors (in 2016)	25-30 $ USD
Selecting shrubs of *Ferula assa foetida* that are ready for obtaining their gum and marking on them by rural people	April and November
Harvesting gum for market supply	June, July and August
Sowing seeds of *Ferula assa foetida* by rural people	Winter
New germinating shrubs of *Ferula assa foetida* plants	End of winter and beginning of spring
Main pests of *Ferula assa foetida* shrubs	Desert mice, one type of worm and grasshopper
Main targeted global markets for exporting dried gum of *Ferula assa foetida*	India, Europe Union, and Arab countries around the Persian Gulf
Percent of dried gum that are exported to global markets each year	Approximately 100%*
Main method of consumption of dried gum of *Ferula assa foetida*	Medicinal factories
Number of medicinal factories in South Khorasan province	Zero
Years for production of seeds by shrub of *Ferula assa foetida*	4 - 5 years
Number of times each shrub of *Ferula assa foetida* can produce seeds in its life time	Once **

*Consumption of dried gum among locally people is very limited and mainly in medicinal plants shops,****** Each shrub of *Ferula assa foetida* after producing seeds will die because of the use of all its fresh gum (Information and Statistical Department, 2016).

gum is not a sustainable way for exploitation because after one season, production of gum by mature plant in the next year will result in the death of the plant. In this regard, in the research centers of Natural Resources and Watershed Organization of Iran, researchers have found methods for sustainable utilization of *Ferula* such as concave and staircase methods. These new, scientific and sustainable methods must be extended among farmers (especially by agriculture and natural resources extension workers), replacing the present prevailing hazardous traditional method.

Reduction in the number of small (especially goats and

sheep herds) and big livestocks (cow's herds in second order) is the major weakness in pastures of South Khorasan province. Also, prevention from grazing by these livestock herds is mainly during germination and exploitation seasons of *Ferula.* In this regard, Natural Resources and Watershed Organization of Iran must allow livestock grazing in these pastures only after the last exploitation stage of *Ferula.*

ii. Development of strategic planning for appropriate future exploitation-utilization of *Ferula* and other medicinal plants.

iii. Controlling exploitation stages and monitoring the quantity and quality of *Ferula* products.

iv. Conserving and restoring to life the germinating locals and pastures of *Ferula*.

v. Absorbing participation of local people in implementing *Ferula* plans and projects of Natural Resources and Watershed Organization by strengthening the local organizations of medicinal plant collectors.

vi. Establishing factories in the field of medicinal plants especially, *Ferula* in the South Khorasan province. This can create jobs for unemployed local youth, preventing them from raw and cheap selling and exporting of *Ferula* gum and increasing its final value. This is also an important step for accessing sustainable development and poverty alleviation goals, and criteria in this deprived, dried and remote region of Iran.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

Golmohammadi F (2016). Certification for scientific collaboration and scientific field participation in Medical plant of Ferula assa Foetida-with research team from: General Director, Mangyshlak Experimantal, Botanical Garden, Candidate of Biology Science, Akzhunis Imanbayeva, Aktau, Kazakhstan. Belonged to Ministries of Education and science of the Republic of Kazakhstan.

Golmohammadi F (2012). Sustainable agriculture and rural development in Iran, Some modern issues in sustainable agriculture and rural development in Iran. LAP Lambert Academic Publishing Germany.

Golmohammadi F (2013). Medical plant of Ferula assa-foetida and its cultivating, main characteristics and economical importance in South khorasan province - east of Iran. Technical Journal of Engineering and Applied Sciences 3(18):2334-2346.

Gundamaraju R (2013). Evaluation of anti-helmintic activity of Ferula foetida “Hing- A natural Indian spice” aqueous extract. Asian Pacific Journal of Tropical Disease 3(3):189-191.

Information and Statistical Department (2016). Annually Reports of Natural Resources and Watershed Organization of South Khorasan province, Iran.

Iranshahy M, Iranshahi M (2011). Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin)-A review. Journal of Ethnopharmacology 134(1):1-10.

Kavoosi G, Rowshan V (2013). Chemical composition, antioxidant and antimicrobial activities of essential oil obtained from Ferula assa-foetida oleo-gum-resin: Effect of collection time. Food Chemistry 138:2180-2187.

Kumari NC, Choudhary SB, Jha SK, Singh SRK (2014). Radio: An educational media to transfer agricultural information among farmers. Indian Research Journal of Extension Education 14(2):130-132.

Moghadam FH, Dehghan M, Zarepur E, Dehlavi R, Ghasemini F, Ehsani S, Barzegar K (2014). Oleo gum resin of *Ferula assa-foetida L*. ameliorates peripheral neuropathy in mice. Journal of Ethnopharmacology 154(1):183-189.

Moghaddam M, Farhadi N (2015). Influence of environmental and genetic factors on resin yield, essential oil content and chemical composition of *Ferula assa-foetida* L. populations. Journal of Applied Research on Medicinal and Aromatic Plants 2(3):69-76.

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