

Mycochemical contents of higher fungi and their *in vitro* effect on plant growth

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Abstract

This research work is part of a project to develop the natural substances of higher basidiomycetes from Algeria's forest. This study aimed to evaluate the bioactive compounds of higher fungi, by quantifying phenols and flavonoids using spectrophotometry. The assay, showed that the highest quantity of flavonoids was recorded in the extract of *Trametes quercina* (10.27 ± 0.015 mg QE/g), followed by *Suillus mediterraneensis* (7.82 ± 0.011 mg QE/g). For phenols, amount of 442.23 and 432.88 mg GAE/g were respectively found in *Lactarius zonarioides* and *S. mediterraneensis*. In addition, TLC chromatographic screening was carried out for the first time in order to select higher basidiomycetes containing secondary metabolites. The results reported that all the basidiomycete extracts are rich in phenols, tannins and flavonoids with a similarity of secondary metabolites between the different extracts. Following these chemical investigations, eight extracts showing a phyto-stimulating effect were selected for analysis by high-performance liquid chromatography (HPLC). The presence of flavones and flavonols was detected: Flavones were found in *Agaricus bisporus*, *Boletus* sp., *Pleurotus dryinus*, *S. mediterraneensis*, *Coprinus atramentarius*, *T. quercina* and *Xerocomus* sp. On the other hand, flavonols were found in *C. atramentarius*, *Pleurotus ostreatus* and *P. dryinus*. Additionally, tannic acid was found in *S. mediterraneensis*, chromatographic profiles show that basidiomycete extracts produce a wide range of secondary metabolites, with many compounds common to all extracts. The study of plant growth enhancement by extracts of basidiomycetes on eggplant, in hydroponic conditions, is a novelty. The chemical study confirmed that these extracts do indeed contain a diversity of bioactive products that contribute to increasing plant yield, thus offering strong agricultural potential.

Keywords: higher fungi; high performance liquid chromatography; plant growth enhancer; secondary metabolites; thin layer chromatography

Abbreviations: ADW: Aerial Dry Weight; AFW: Aerial Fresh Weight; AL: Aerial Length; ANOVA: Analysis of variance; EXT: Extract; FR Frontal Ratio; GAE: Gallic Acid Equivalent; HPLC: High Performance Liquid Chromatography; NL: Number of Leaves; PGPR: Plant Growth-Promoting Rhizobacteria; QE: Quercetin Equivalent; RDW: Root Dry Weight; RFW: Root Fresh Weight; RL: Root Length; RT: Retention Time; TLC: Thin Layer Chromatography; UV-Vis: Ultra-violet-visible

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Introduction

Higher basidiomycetes have been targeted as promising therapeutic agents, as it has been postulated that fungal diversity (up to 3 to 5 million species) exceeds that of land plants by an order of magnitude (Dai, 2010; Blackwell, 2011; De silva *et al.*, 2013). Higher basidiomycetes develop a diversity of prolific products of secondary metabolites (phenolic acids, flavonoids, tannins, sesquiterpenoids, triterpenoids) with antimicrobial activities (Sum *et al.*, 2019). These products differ in their biogenetic origin and structure remarkably from metabolites of ascomycetes or other prolific producers such as actinomycetes or myxobacteria (Schüffler, 2018).

Basidiomycetes, as bioactive fungi, provide an exciting opportunity to obtain new natural products with high application potential (Sandargo *et al.*, 2019). Recently, many new active compounds discovered in these higher basidiomycetes have demonstrated their ability to produce numerous other unknown natural products, most of which remain unexploited for their applications (Tian *et al.*, 2020). Scientists are led to explore different natural sources capable of acting without toxicity. Fungi such as: *Agaricus bisporus*, *Amanita virosa*, *Pleurotus* spp have been shown to be sources of a wide variety of metabolites with effective biological activity (Zhang *et al.*, 2016; Bhardwaj *et al.*, 2020). According to Loiseleur (2017), fungal bioactive metabolites contribute to plant growth thus having a strong agricultural potential. These compounds are called biostimulants, which are a group of substances of natural origin that help increase plant yield and nutrient uptake (Xu and Geelen, 2018).

Biostimulants, which comprise various organic materials and microorganisms, are gaining popularity as a valuable agricultural aid. Unlike chemical fertilizers, these novel compounds can enhance plant stress resistance, nutrient use efficiency, and rhizosphere solubility. By employing them alongside fertilizers and phytosanitary products, plant immune systems can be strengthened, making them economically feasible for supporting increased yields while reducing ecological impact (Nephali *et al.*, 2020; Chanthini *et al.*, 2022; Tahir *et al.*, 2022; Del Buono *et al.*, 2023). Recently, biostimulants based on living microorganisms have been increasingly in demand, supporting a revival of organic agriculture. Studies have shown that less than 25% of the commercial products currently available on the global market are plant-growth-promoting microbial biostimulants, which can be composed of PGPR from the genera *Bacillus* and *Pseudomonas*, as well as *Azotobacter*, *Azospirillum*, and *Rhizobium* species capable of nitrogen fixation (Bulgari *et al.*, 2019; Hamid *et al.*, 2021). On the other hand, biostimulants based on extracts from macro and microalgae have long been regarded as valuable materials that provide benefits for soil health, plant growth, and development (Carillo *et al.*, 2020; Ali *et al.* 2021; Engel *et al.*, 2023; Kergosien *et al.*, 2023; Mostafa *et al.*, 2023; Prisa and Spagnuolo, 2023).

There is a paucity of scientific data on the effect of basidiomycete extracts on seed and plant growth. This research section was initiated to address this gap, as biostimulants contribute to sustainable agriculture by improving soil health.

In this context, the study aimed to evaluate the chemical determination of basidiomycete extracts, primarily through spectrophotometric methods. Specifically, we determined phenols using the Folin-Ciocalteu reagent and flavonoids using aluminum trichloride. We then expanded the study by employing chromatographic analyses, including TLC (Thin Layer Chromatography) and HPLC (High-Performance Liquid Chromatography), for the identification of bioactive compounds. Ultimately, we tested the effect of the extracts on the growth of eggplants cultivated in a hydroponic system.

Materials and Methods

Collection and extraction of higher basidiomycetes

Samples of higher fungi are collected in different sites and forests in northwestern Algeria during the rainy season (AIT Hamadouche *et al.*, 2022).

For the detection of bioactive molecules contained in the higher basidiomycete's extracts, a chemical extraction was carried out. 10 g of each species of mushroom (16 samples) were weighed and continuously extracted using ethyl acetate in a Soxhlet apparatus for 2 hours.

The extraction solvent was evaporated to dryness at 70 °C using an evaporator Heidolph (Dib Bellahouel and Fortas, 2011; AIT Hamadouche *et al.*, 2021).

Determination of bioactive compounds

Total phenolic content

The phenolic content in the ethyle acetate extracts were estimated using the Folin–Ciocalteu reagent, according to the method of Li *et al.*, (2007). The phenolic content was expressed using the regression equation of the calibration range $R^2 = 0.938$, established with gallic acid (0-150 $\mu\text{g}/\text{mL}$) and is expressed as micrograms of gallic acid equivalent per milligram of extract ($\mu\text{g GAE}/\text{mg extract}$) at 725 nm using UV spectrophotometer.

All measurements are performed in triplicate and averages calculated.

Total flavonoids content

Flavonoids were characterized using methanolic extracts. We therefore macerated 8 samples of basidiomycetes: *Lactarius zonarioides*, *Amanita proxima*, *Amanita virosa*, *Agaricus bisporus*, *Suillus mediterraneensis*, *Coprinus atramentarius*, *Pleurotus pulmonarius*, and *Trametes quercina*, under agitation using a Gerhabdt Laboshake shaker to obtain extracts with a concentration of 0.2 mg/mL.

Flavonoids were quantified by the aluminum trichloride (AlCl_3) method adopted by Djeridane *et al.* (2006) using quercetin as a standard. The flavonoids content was done according to a linear calibration curve $R^2 = 0.989$, generated by quercetin standards at different concentrations (0-20 $\mu\text{g}/\text{mL}$) under the same conditions as the sample. Each experiment was conducted in triplicate. Absorbance was taken at 415 nm by UV spectrophotometer.

Qualitative screening by thin-layer chromatography

Phytochemical screening was performed using TLC (Abedini, 2013). 2 μl of each ethylacetate extract were analysed on a chromatoplate (silica gel 60 F254, rigid aluminium support, 20×20, Fluka-Silica Merck), testing different solvent systems as developers with few modifications: Petroleum Ether/Ethyl Acetate (5/5) (8/2) (V/V) (Jazy *et al.*, 2018); Hexane / diethyl ether / acetic acid (7/2/1) (V/V) (Berger *et al.*, 2004); Hexane / Ethyl acetate / diethyl ether (7/2/1) (V/V) (Melouka 2018) and Hexane / Ethyl Acetate (5/1) (V/V) (Brou *et al.*, 2010). The TLC plates were observed under UV lamp light at 254 and 365 nm (Özer *et al.*, 2004). After spot revelation, chromatographic plate separation profiles were compared between isolates and frontal ratios were calculated for each compound. The FR value was calculated according to the following formula:

$$\text{FR} = \frac{X}{Y} = \frac{\text{Distance travelled by the constituent}}{\text{Distance covered by the front of the eluent}}$$

We primarily searched for compounds with antifungal properties. Depending on the type of secondary metabolites to be identified, several specific reagents were used: the reagent of ethanolic AlCl_3 , NH_3 and Godin for flavonoids, FeCl_3 at 2% (v/v) for tannins and phenolic compounds (N'guessan *et al.*, 2011).

High performance liquid chromatography

The HPLC is the most useful analytical technique to characterize polyphenolic compounds (Gomez-Caravaca *et al.*, 2006). This analysis was carried out at the scientific research center CRAPC of Bousmail "Tipaza, Algeria".

The obtained extracts were analyzed for amount of: flavones (2,3,4,5,7 penta-hydroxy flavone; 3hydroxy flavone; 5. hydroxy flavone; acacetin; apigenin, tangeritin, flavone and diosmin), flavanols (catechin), flavonols (galangin, kaemferide, myricetin, rutin and quercetin) and flavanones (hesperetin).

We have analyzed the extracts with phytostimulant properties. In this context, we have selected 8 extracts of higher basidiomycetes: EXT 4 (*Agaricus bisporus*), EXT 5 (*Suillus mediterraneensis*), EXT6 (*Coprinus atramentarius*), EXT 10 (*Boletus* sp.), EXT 11 (*Xerocomus* sp.), EXT13 (*Pleurotus dryinus*), EXT 15 (*Pleurotus ostreatus*) and EXT 16 (*Trametes quercina*). Among them, three extracts (EXT 11, 15 and 16) which gave a significant antifungal power on three phytopathogenic strains (AIT Hamadouche *et al.*, 2021).

The HPLC was performed using a YL9100 HPLC apparatus with YL9110 quaternary pump with a 20 μ L injection loop, a column type AGILENT XDB ECLIPSE (4.6 \times 250 mm, 5 μ m) protected with a pre-column.

A quantity of ethyl acetate extract of higher basidiomycetes was filtered through syringe membranes (0.45 μ m) to remove all impurities. 20 μ L of each extract was injected onto a C18 reverse phase (RP-C18) column (4.6 \times 250 mm, 5 μ m). The mobile phase used was MeOH/ H₂O 1% acetic acid. An elution gradient mode applied was used (A: B ratio): t=0 min (95%:5%); t=55 min (5%:95%); t=60 min (95%:5%). The flow rate was 1 mL/min. The temperature was fixed at 30 °C and the detection was performed by a UV-Vis detector at a wavelength equal to 254 and 325 nm.

In order to confirm the presence of substances (flavonoids, phenolic acids, tannins, vitamins) in each of the 8 extracts tested, the analysis was performed with standard solutions of these compounds as standards. The presence of the tested metabolites in the sample showed an increase in peak height for the appropriate retention time (RT).

Enhancing the growth of eggplant plants in hydroponics using basidiomycete extracts

Preparing eggplant seeds

The eggplant seeds (*Solanum melongena*) utilized in this study were obtained from commercial source, imported from Spain. The seeds used were disinfected following the protocol recommended by Yala *et al.* (2020), with a few modifications.

Disinfected with sodium hypochlorite for 15 min, then aseptically processed under axenic conditions by abundant rinsing with sterile distilled water until clarified, and finally, then dried on sterile filter paper.

The seeds were then placed on 1% agar water in Petri dishes using sterile forceps. Two series of seed dishes are made: one with 1.5 mL of basidiomycete extracts added (30 μ g/mL), and another without extracts (control). These dishes were then incubated at 25 °C for 15 days.

Effect of crude basidiomycete extracts on eggplant seed germination

We prepared a concentration of 30 μ g/mL of basidiomycete extract in sterile distilled water, which we filtered through 0.22 μ m millipore membrane to eliminate possible contamination. After filtration, we injected the various extracts (16 extracts) into the compartments of the Petri dishes and aseptically deposited our seeds using sterile forceps under a hood. In addition, seeds used as a control were deposited without the addition of extracts under the same aseptic conditions. These dishes were then incubated at 25 °C for 15 days.

Effect of basidiomycete crude extracts on eggplant plant growth

After 15 days of germination, we transferred the pre-germinated seeds (control and extracted) *in vitro* (hydroponically), into Gibson tubes containing a 1% agar nutrient solution (Fahraeus, 1957). Plants were placed in a culture chamber at a temperature of 24 ± 1 °C and a photoperiod of 16 h (Zayova *et al.*, 2017).

Remember that there are two series of seeds: one with added 30 µg/mL basidiomycete extracts and one without extracts (Control).

Estimation of seed germination parameters

The study of the biostimulant effect of extracts on eggplant seed germination was carried out by correlating the various growth parameters with the extracts. The germination parameters are presented in Table 1 where germinated seeds are counted every day for 15 days.

Table 1. Parameters for seed germination

No.	Germination parameters	Mathematical formulas	References
1	Cumulative Seed Germination Rate	$(L/S) \times 100$	Côme, 1970
2	Mean Germination Time	$\frac{N1T1 + N2T2 + N3T3 + \dots + N8T8}{N1 + N2 + N3 + \dots + N8}$	Czabator, 1962
3	Vigor Index (Vgl)	$[a/1 + b/2 + \dots + z/n] \times 100/S$	Jain and Saha, 1971
4	Kotowski coefficient	$\left(\frac{(N1 + N2 + \dots + Nx)}{(N1T1 + N2T2 + \dots + NxTx)} \right) \times 100$	Kotowski, 1926
5	Germination index (GI)	cumulative number of germinated seeds / number of days since test began	Scott <i>et al.</i> , 1984
6	Germination rate index (GRI)	$ITG = G1 / 1 + G2 / 2 + \dots + Gn/n$	Al-Karaki, 1998
7	Final Germination Percentage (FG %)	(Final number of germinations / nombre de graines testées) $\times 100$	Osborne <i>et al.</i> , 1993
8	Daily Average Germination	Final germination percentage/ 15 days	Osborne <i>et al.</i> , 1993

1. **L**: cumulative numbers of germinated seeds; **S**: total number of seeds;

2. **N**: number of newly germinated seeds at time T_i . (1st, 2nd, up to 15th day)

3. **a**: number of seeds germinated on day 1; **b**: number of seeds germinated on day 2; **Z**: number of seeds germinated on the 15th and last day; **n**: total number of days; **S**: total number of germinated seeds.

4. **N**: number of seeds germinated each day (1st, 2nd, up to 15th day); **T**: duration in days corresponding to N.

6. **G1**: germination percentage on day 1; G2 on day 2, etc...

Estimation of eggplant plant growth parameters

After two months of *in vitro* culture, eggplant plants are carefully removed from the agar tubes in order to measure: height, fresh and dry weight of above-ground and root parts as well as root length (Kpinkoun *et al.*, 2019; Yala *et al.*, 2020). Fresh weight was measured directly using a precision balance, while dry weight was determined after depositing the plant material at 70 °C for 48 h.

Statistical analysis

Results were expressed as mean values and standard deviations (means \pm SD). The data were analyzed using "Statistica, version 12" software and subjected to one-way ANOVA, with multiple comparisons done using Duncan's test at $\alpha=0.05$.

Results

Determination of total phenolic compounds

All the extracts studied contained noticeable phenolic content with significant variations ($P = 0,000000$).

The results below (Figure 1) show that the amount of phenolic compounds present in the extracts varies between 78.47 and 442.23 mg GAE/g extract. The extracts of *L. zonarioides* and *S. mediterraneensis* of 442.23 and 432.88 mg GAE/g extract respectively, are significant and gave the highest contents of total phenols. Followed by *P. ostreatus*, *Boletus* sp. and *A. bisporus* which are significant and have contents are respectively: 319.65; 252.12 and 243.09 mg GAE/g extract respectively.

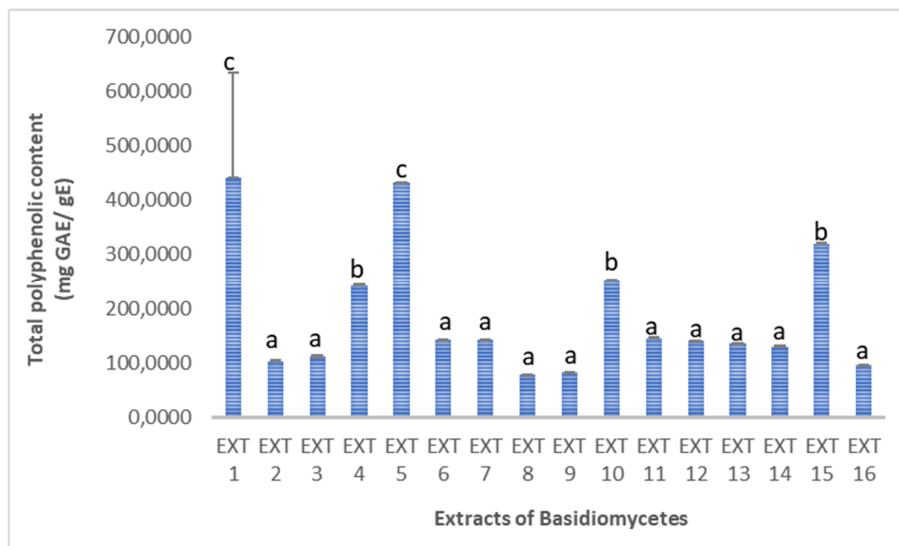


Figure 1. Determination of the total phenolic compounds of 16 extracts of higher basidiomycete. Bars with different letters indicate significantly different activities ($P \leq 0.05$)

EXT1: *Lactarius zonarioides*, EXT2: *Amanita proxima*, EXT 3: *Amanita virosa*, EXT 4: *Agaricus bisporus*, EXT 5: *Suillus mediterraneensis*, EXT6: *Coprinus atramentarius*, EXT7: *Pleurotus pulmonarius*, EXT8: *Lepiota* sp., EXT9: *Cortinarius orellanus*, EXT 10: *Boletus* sp., EXT 11: *Xerocomus* sp., EXT 12: *Pleurotus eryngii*, EXT13: *Pleurotus dryinus*; EXT14: *Armillaria mellea*, EXT 15: *Pleurotus ostreatus*, EXT 16: *Trametes quercina*

Determination of total flavonoids

The statistical analysis using ANOVA variance and the Duncan test allowed us to observe a significant difference of flavonoid compounds contained in the extracts, which is highly significant ($P=0.00$), except for *P. pulmonarius* and *C. atramentarius*. The amount of flavonoid compounds varied between 2.45 and 10.27 mg QE/mg extract. The highest total content was observed in *T. quercina* (10.27 ± 0.015 mg QE/g), followed by *S. mediterraneensis* (7.82 ± 0.011 mg QE/g) which were highly significant (Figure 2). The lowest content was recorded in *A. proxima* (3.50 ± 0.030 mg QE/mg) $> P. pulmonarius$ (2.82 ± 0.055 mg QE/mg) $> C. atramentarius$ (2.79 ± 0.055 mg QE/mg) $> A. bisporus$ (2.58 ± 0.020 mg QE/mg) $> A. virosa$ (2.52 ± 0.025 mg QE/mg) $> L. zonarioides$ (2.45 ± 0.036 mg QE/mg).

If we compare the dosage of these extracts with those of the phenolic compounds, we notice that *S. mediterraneensis* was very rich in phenols as well as in flavonoids, while *T. quercina* was among the extracts with the lowest phenol content but the highest flavonoid content.

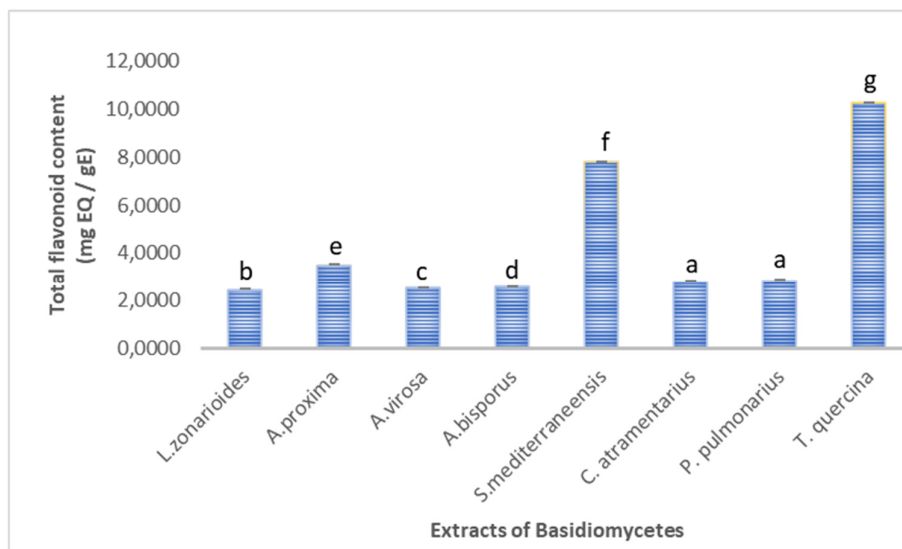


Figure 2. Quantification of total flavonoid compounds of higher fungi extracts
Bars with different letters (a, b, c, d, e, f, g) indicate significantly different activities ($P \leq 0.05$)

Chemical screening by thin layer chromatography

Mycochemical screening was carried out on ethylacetate extracts of basidiomycetes. We highlighted the presence of flavonoids, tannins and phenolic compounds using specific developers for each metabolite. This analysis showed that our basidiomycete extracts are revealed under UV/254 nm but not visible under UV/365 nm. On the other hand, after using the developers, we were visualizing UV/254 and 365 nm.

Detection of phenolic compounds and tannins by TLC

The results of this screening on TLC allowed us to visually observe that our extracts are very rich in phenolic compounds and tannins with a dominance of tannic compounds (Figure 3). All extracts present at least one tannin compound, except for Ext 7 (*P. pulmonarius*) and EXT 16 (*T. quercina*). On the other hand, Ext 9 (*C. orellanus*) and EXT 13 (*P. dryinus*) are the richest in tannins with a maximum of 4 compounds, while Exts 3 (*A. virosa*), 5 (*S. mediterraneensis*), 10 (*Boletus* sp.) et 15 (*P. ostreatus*) contain 3 compounds.

During this screening, we noted that both amanites, *S. mediterraneensis*, *C. atramentarius*, *P. eryngii* and *P. ostreatus* show the same compounds ($R_f=0.95$), either phenolic or tannic, despite the genetic variation but these higher basidiomycetes have similarities in their metabolomes. There is a lot of similarity of compounds between the different basidiomycete extracts, as in the case of *Xerocomus* sp., *P. dryinus* and *A. mellea* ($R_f=0.98$) noted as phenolic compounds. Additionally, we observed that the extracts of the two amanites, *S. mediterraneensis*, *A. bisporus*, *C. atramentarius* and *C. lepiota* sp., which gave the same tannic compound ($R_f=0.18$). The amanites (*A. virosa* and *promxima*) gave the same tannic compound ($R_f=0.95$ and 0.18) and others different one. We also observed that oyster mushrooms that gave different compounds, while others showed similarities, such as *P. dryinus* and *P. eryngii* (tannic compound of $R_f=0.23$); *P. dryinus* and *P. ostreatus* (tannic compound of $R_f=0.55$).

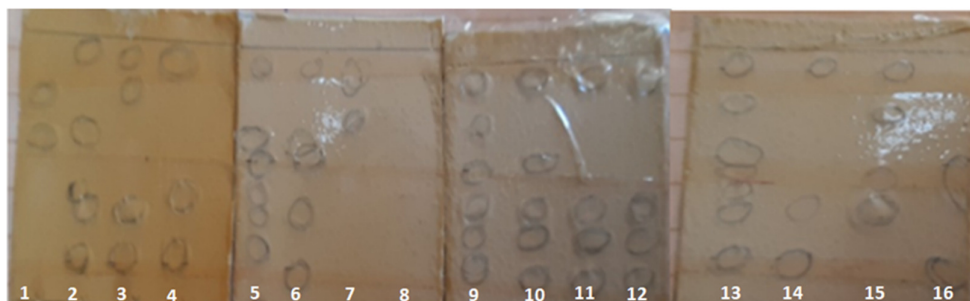


Figure 3. Detection chromatogram of tannins and phenolic compounds revealed by FeCl_3 2% reagent in the visible; Developing: n-C₆H₁₄/AcOEt, 5:1 (v/v)

1: *Lactarius zonarioides* 2: *Amanita proxima* 3: *Amanita virosa* 4: *Agaricus bisporus* 5: *Suillus mediterraneensis* 6: *Coprinus atramentarius* 7: *Pleurotus pulmonarius* 8: *Lepiota* sp. 9: *Cortinarius orellanus* 10: *Boletus* sp. 11: *Xerocomus* sp. 12: *Pleurotus eryngii* 13: *Pleurotus dryinus* 14: *Armillaria mellea*, 15: *Pleurotus ostreatus*; 16: *Trametes quercina*

Detection of flavonoids

For the characterization of flavonoids, several specific developers were used. Our tested extracts gave yellow, light yellow, and orange-yellow colours in the visible range when in contact with AlCl_3 , but showed no visibility under UV/366 nm. However, they fluoresce under UV/254 nm, giving various colours (blue, green, purple and mauve) (Figure 4).

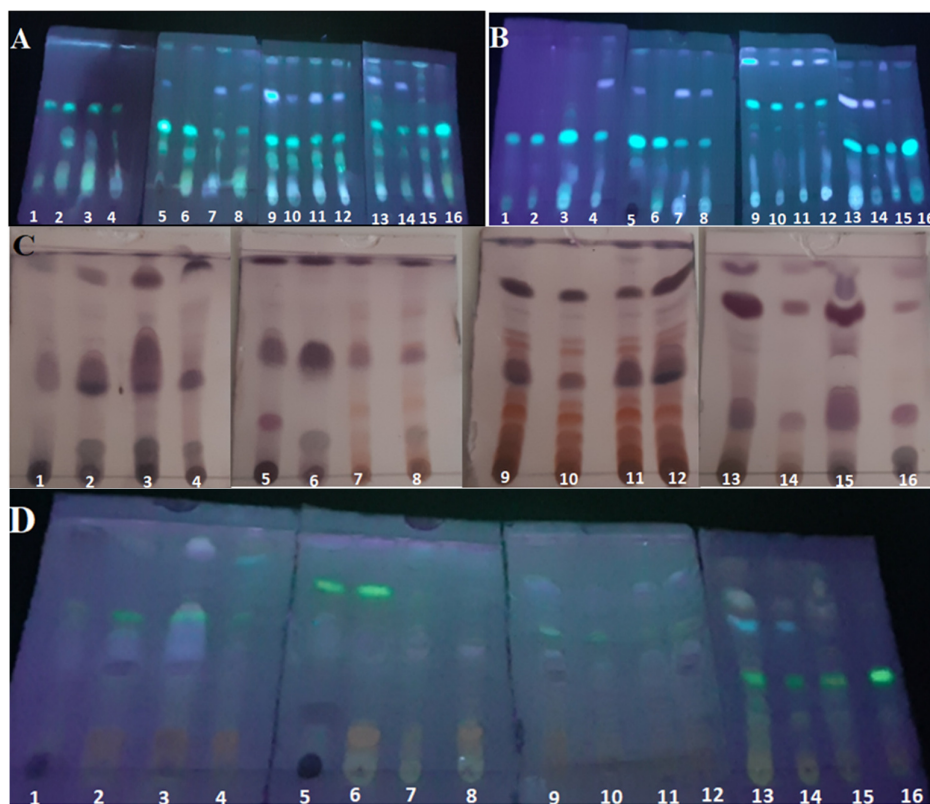


Figure 4. Detection chromatogram of flavonoids revealed by AlCl_3 (A), NH_3 (B) reagent under UV/254 nm and Godin's reagent (C) in the visible, (D) under UV/254 nm. Developing: n-C₆H₁₄/AcOEt, 5:1 (v/v)

1: *Lactarius zonarioides* 2: *Amanita proxima* 3: *Amanita virosa* 4: *Agaricus bisporus* 5: *Suillus mediterraneensis* 6: *Coprinus atramentarius* 7: *Pleurotus pulmonarius* 8: *Lepiota* sp. 9: *Cortinarius orellanus* 10: *Boletus* sp. 11: *Xerocomus* sp. 12: *Pleurotus eryngii* 13: *Pleurotus dryinus* 14: *Armillaria mellea* 15: *Pleurotus ostreatus* 16: *Trametes quercina*

Analysis of higher basidiomycetes extracts by high performance chromatography

Research into the qualitative analysis of bioactive compounds in mushrooms has become increasingly important. Among the standards sought (Table 2), we have the 2,3,4,5,7 penta-hydroxy flavone present in *A. bisporus*, *Boletus* sp. and *P. dryinus*; the 3-hydroxy flavone present in *S. mediterraneensis*, *C. atramentarius*, *Boletus* sp. and *T. quercina*; the flavone tangeritin is present in *Xerocomus* sp. and for diosmin which is a flavone or bioflavonoid has been found in *T. quercina*. For flavonols (rutin and galangin, myricetin, quercetin), they were found respectively in *C. atramentarius*, *P. ostreatus* and *P. dryinus*. From these results, we note that our higher basidiomycetes extracts contain only flavonols and flavones. In addition to these compounds, we have tannic acid in *S. mediterraneensis* and α -tocopherol which is a vitamin E in *Boletus* sp.

From the chromatograms of the different macrofungi extracts, in addition to the peaks previously identified, we estimated the presence of a significant number of unidentified peaks (Table 3), which differ from one extract to another. According to the data in the Table 2, the number of peaks recorded during this analysis varied between 31 to 78 peaks under UV light at 254 nm for the 8 extracts, while their number was lower (38 to 62) under UV light at 325 nm. The extracts (EXTs 4, 10, 11, 15 and 16) absorb better at 254 nm, while EXTs 5, 6, 13 show a higher absorbance at 325 nm. Each chromatogram of each extract tells us about their richness in metabolites, which can be primary or secondary as mentioned in the literature.

Table 2. Compounds present in higher fungi extracts in HPLC analysis

Standards	TR (min)	λ max (nm)	Ext 4	Ext 5	Ext 6	Ext 10	Ext 11	Ext 13	Ext 15	Ext 16
2.3.4.5.7 PHF	36.2	254	+	-	-	+	-	+	-	
3 Hydroxy Flavon	52.2	254	-	+	+	+	-	-	-	+
5. Hydroxy Flavon	54.2	-	-	-	-	-	-	-	-	-
Stearic acid	55.8	-	-	-	-	-	-	-	-	-
Gallic acid	10.3	-	-	-	-	-	-	-	-	-
Vannillic acid	24.7	-	-	-	-	-	-	-	-	-
Acacétin	49.2	-	-	-	-	-	-	-	-	-
α -tocophérol	11.5	254	-	-	-	+	-	-	-	-
Apegin	25.2	-	-	-	-	-	-	-	-	-
Apigenin	43.7	-	-	-	-	-	-	-	-	-
Cafein	22.1	-	-	-	-	-	-	-	-	-
Catechin	17.9	-	-	-	-	-	-	-	-	-
Diosmin	49.8	254	-	-	-	-	-	-	-	+
Flavon	25.2	-	-	-	-	-	-	-	-	-
Galangin	47.3	325	-	-	+	-	-	-	-	-
Hesperitin	30	-	-	-	-	-	-	-	-	-
Kaemferid	49.30	-	-	-	-	-	-	-	-	-
Myricetin	33.9	325	-	-	-	-	-	-	+	-
Quercetin	35.7	325	-	-	-	-	-	+	-	-
Rutin	18	325	-	-	+	-	-	-	-	-
Tangeritin	51.7	325	-	-	-	-	+	-	-	-
Tannic acid	6.6	254	-	+	-	-	-	-	-	-

(+) presence, (-) absence, P.H.F: Penta-Hydroxy Flavon, EXT 4: *Agaricus bisporus*; EXT 5: *Suillus mediterraneensis*; EXT 6: *Coprinus atramentarius*; EXT 10: *Boletus* sp.; EXT 11: *Xerocomus* sp.; EXT 13: *Pleurotus dryinus*; EXT 15: *Pleurotus ostreatus*; EXT 16: *Trametes quercina*.

Table 3. Number of peaks in the chromatographic profiles

Extracts of basidiomycetes	A 254 nm	A 325 nm
EXT 4 (<i>Agaricus bisporus</i>)	71	38
EXT 5 (<i>Suillus mediterraneensis</i>)	31	46
EXT6 (<i>Coprinus atramentarius</i>)	55	58
EXT 10 (<i>Boletus</i> sp.)	65	47
EXT 11 (<i>Xerocomus</i> sp.)	78	46
EXT13 (<i>Pleurotus dryinus</i>)	51	62
EXT 15 (<i>Pleurotus ostreatus</i>)	49	40
EXT 16 (<i>Trametes quercina</i>)	77	58

Estimation of eggplant seed germination parameters by PCA

Principal component analysis (PCA), was used to graphically represent the growth factors of seed supplemented with higher fungi extracts compared with the control (without extract) (Figure 5).

The two axes describe 98.32% of the total variation. The first axis (factor 1) expresses the highest rate of variation, of the order of 69.78%, and is negatively correlated with the vigor index (VgI), the germination rate index and the Kotowski coefficient. The germination parameters mentioned were improved by the addition of extracts EXT1 (*L. zonarioides*), EXT 3 (*A. virosa*), EXT 4 (*A. bisporus*), EXT6 (*C. atramentarius*) in contrast to the control.

The second axis (factor 2) accounts for 28.54% of the information and is associated with the germination index, cumulative germination rate, percentage of final germination, average daily germination. This was improved by both extracts EXT 9 (*C. orellanus*) and EXT 15 (*P. ostreatus*). This same axis is positively correlated with the mean germination time, which is positively influenced by EXT 11 (*Xerocomus* sp.), EXT 12 (*P. eryngii*) and EXT 14 (*A. mellea*). As for extracts EXT 5 (*S. mediterraneensis*), EXT7 (*P. pulmonarius*), EXT8 (*Lepiota* sp.) and EXT 10 (*Boletus* sp.), they negatively influenced the germination of eggplant seeds.

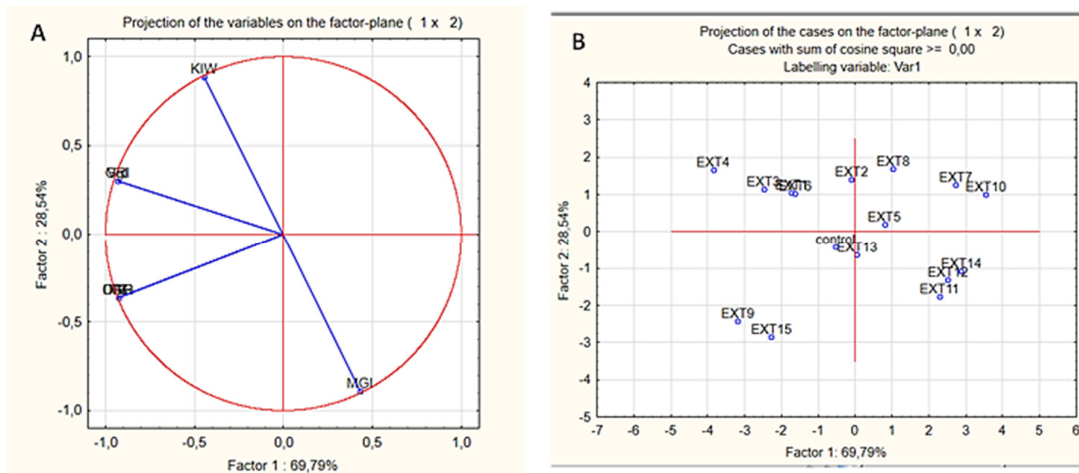


Figure 5. A) Contribution of germination parameters analyzed on the two second axes of the axes; B) Distribution of higher fungi extracts on the two second PCA

EXT 1: *Lactarius zonarioides*; EXT 2: *Amanita proxima*; EXT 3: *Amanita virosa*; EXT 4: *Agaricus bisporus*; EXT 5: *Suillus mediterraneensis*; EXT 6: *Coprinus atramentarius*; EXT 7: *Pleurotus pulmonarius*; EXT 8: *Lepiota* sp.; EXT 9: *Cortinarius orellanus*; EXT 10: *Boletus* sp.; EXT 11: *Xerocomus* sp.; EXT 12: *Pleurotus eryngii*; EXT 13: *Pleurotus dryinus*; EXT 14: *Armillaria mellea*; EXT 15: *Pleurotus ostreatus*; MGT: Mean Germination Time; VgI: Vigor Index; KTW: Kotowski coefficient; GI: Germination index; GRI: Germination rate index; FG: Final Germination Percentage; DAG: Daily Average Germination

Estimation of eggplant seedlings growth parameters using PCA

A principal component analysis (PCA) was performed to assess the correlations between the effect of higher fungi extracts on eggplant growth parameters, which are as follows: aerial length (AL), root length (RL), number of leaves (NL), root and aerial fresh weight (RFW, AFW), root and aerial dry weight (RDW, ADW).

PCA was used to graphically represent the growth factors of eggplant plants supplemented with higher fungi extracts compared with the control (Figure 6).

The two axes describe 68.97% of the total variation. The first axis (factor 1) expresses the highest rate of variation, of the order of 49.46%, it is negatively correlated with the AL and the AFW. These growth parameters were improved by the addition of extracts EXT 2 (*A. proxima*), EXT 6 (*C. atramentarius*), EXT 8 (*Lepiota* sp.), EXT 9 (*C. orellanus*), EXT 12 (*P. eryngii*) and EXT 14 (*A. mellea*).

The second axis (factor 2) accounts for 19.51% of the information, and is associated with RL, NL, FRW, DRW. This was improved by both extracts EXT 3 (*A. virosa*) and EXT 13 (*P. dryinus*). This same axis is positively correlated with the mean germination time, and is negatively correlated with aerial dry weight, which is positively influenced by EXT 1 (*L. zonarioides*), EXT 4 (*A. bisporus*), EXT 5 (*S. mediterraneensis*), EXT 10 (*Boletus* sp.), and EXT 15 (*P. ostreatus*). *P. pulmonarius* (extract 7) had a negative effect on eggplant growth.

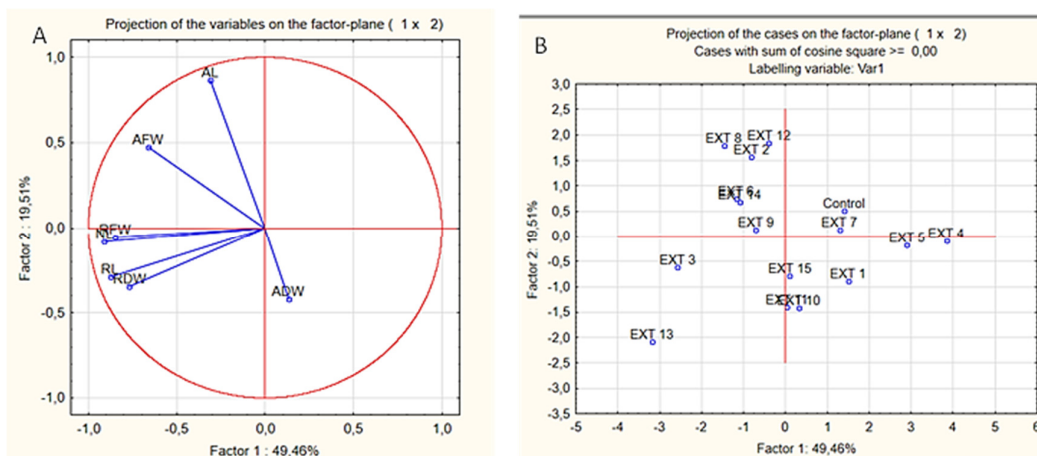


Figure 6. A) Contribution of plant growth parameters analyzed on the two second axes of the PCA; B) Distribution of higher fungi extracts on the two second axes of the PCA

EXT 1: *Lactarius zonarioides*; EXT 2: *Amanita proxima*; EXT 3: *Amanita virosa*; EXT 4: *Agaricus bisporus*; EXT 5: *Suillus mediterraneensis*; EXT 6: *Coprinus atramentarius*; EXT 7: *Pleurotus pulmonarius*; EXT 8: *Lepiota* sp.; EXT 9: *Cortinarius orellanus*; EXT 10: *Boletus* sp.; EXT 11: *Xerocomus* sp.; EXT 12: *Pleurotus eryngii*; EXT 13: *Pleurotus dryinus*; EXT 14: *Armillaria mellea*; EXT 15: *Pleurotus ostreatus*; ADW: Aerial Dry Weight; AFW: Aerial Fresh Weight; AL: Aerial Length; NL: Number of Leaves RDW: Root Dry Weight; RFW: Root Fresh Weight; RL: Root Length

Effect of basidiomycete extracts on aerial fresh weight

Statistical analysis using the ANOVA test showed a highly significant difference ($P = 0.0012$) in fresh weight of aerial part (FWP) of eggplants. The addition of extracts: EXT 1, 13, 9, 10, 6, 2, 3, 8, 12 and 14 considerably improved AFW and proved significant difference according to Duncan test when, compared to with the control (T) (Figure 7).

Extract 8 (*Lepiota* sp.) gave the best result with an average of 0.031 ± 0.012 g, followed by the two amanites (EXT 2 and 3) and EXT 6 (*A. proxima*, *A. virosa* and *C. atramentarius*) which stimulated plant growth in the same way, giving an average of 0.030 ± 0.009 g, followed by extracts (EXT 12, 10, 14, 9 and 1) (*P.*

eryngii, *Boletus* sp., *A. mellea*, *C. orellanus* and *L. zonarioides*) with an average ranging from 0.020 ± 0.005 g to 0.025 ± 0.005 g compared with the control (0.02 ± 0.005 g).

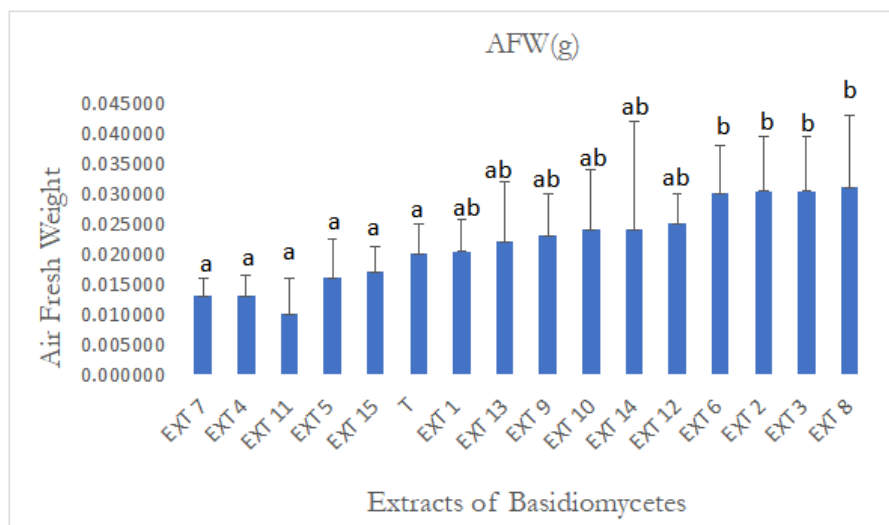


Figure 7. Higher fungi extract effects on aerial fresh weight. Bars with different letters indicate significant differences ($p \leq 0.05$)

EXT 1: *Lactarius zonarioides*; EXT 2: *Amanita proxima*; EXT 3: *Amanita virosa*; EXT 4: *Agaricus bisporus*; EXT 5: *Suillus mediterraneensis*; EXT 6: *Coprinus atramentarius*; EXT 7: *Pleurotus pulmonarius*; EXT 8: *Lepiota* sp.; EXT 9: *Cortinarius orellanus*; EXT 10: *Boletus* sp.; EXT 11: *Xerocomus* sp.; EXT 12: *Pleurotus eryngii*; EXT 13: *Pleurotus dryinus*; EXT 14: *Armillaria mellea*; EXT 15: *Pleurotus ostreatus*; T: control

Effect of basidiomycete extracts on aerial dry weight

The ANOVA variance test showed a highly significant difference ($P=0.013$) in aerial dry weight. Extract 10 (*Boletus* sp.) gave the best result, with an average of 0.004 ± 0.007 g, and was the only significant extract to improve aerial dry weight compared to the other extracts and the control tested (0.0004 ± 0.0004 g) (Figure 8).

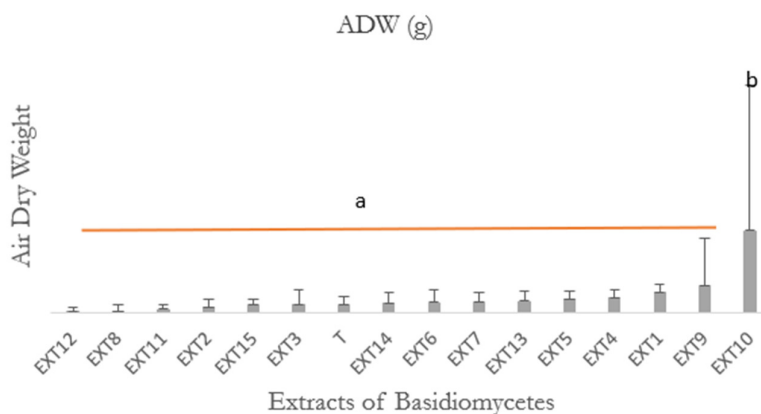


Figure 8. Higher fungi extract effects on aerial dry weight of eggplant seedlings

Bars with different letters indicate significant differences ($p \leq 0.05$)

EXT 1: *Lactarius zonarioides*; EXT 2: *Amanita proxima*; EXT 3: *Amanita virosa*; EXT 4: *Agaricus bisporus*; EXT 5: *Suillus mediterraneensis*; EXT 6: *Coprinus atramentarius*; EXT 7: *Pleurotus pulmonarius*; EXT 8: *Lepiota* sp.; EXT 9: *Cortinarius orellanus*; EXT 10: *Boletus* sp.; EXT 11: *Xerocomus* sp.; EXT 12: *Pleurotus eryngii*; EXT 13: *Pleurotus dryinus*; EXT 14: *Armillaria mellea*; EXT 15: *Pleurotus ostreatus*; T: control

Effect of basidiomycete extracts on root fresh weight

Root fresh weight was highly significant ($P=0.002$) according to extract addition. Duncan's test showed that only the addition of extract 1 (*L. zonarioides*) and 4 (species) were non-significant and therefore did not stimulate root fresh weight. All other tested fungal extracts stimulated fresh root biomass (Figure 9) of eggplant seedlings compared to control.

The addition of extract 3 (*A. virosa*) considerably improved this growth parameter and gave the best result with an average of 0.0175 ± 0.0051 g

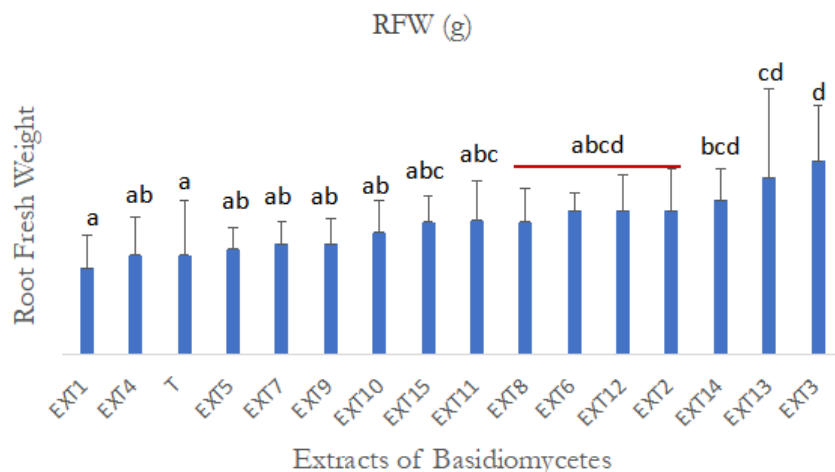


Figure 9. Effect of higher fungi extracts on fresh root weight of eggplant seedlings

Bars with different letters indicate significant differences ($p \leq 0.05$)

EXT1: *Lactarius zonarioides*; EXT 2: *Amanita proxima*; EXT 3: *Amanita virosa*; EXT 4: *Agaricus bisporus*; EXT 5: *Suillus mediterraneensis*; EXT 6: *Coprinus atramentarius*; EXT 7: *Pleurotus pulmonarius*; EXT 8: *Lepiota* sp.; EXT 9: *Cortinarius orellanus*; EXT 10: *Boletus* sp.; EXT 11: *Xerocomus* sp.; EXT 12: *Pleurotus eryngii*; EXT 13: *Pleurotus dryinus*; EXT 14: *Armillaria mellea*; EXT 15: *Pleurotus ostreatus*; T: control

Effect of basidiomycete extracts on root dry weight

Statistical analysis using the ANOVA test of root dry weight averages results demonstrated that variation of this parameter was non-significant ($P=0.058$) according to extract types.

Effect of basidiomycete extracts on root length

Root length variations was found to be highly significant ($P=0.000041$) according to extract types. Duncan's test showed that extract 13 (*P. dryinus*) was the most significant and gave the best result with an average of 5.53 ± 1.83 cm, followed by EXT 3 (*A. virosa*) (4.15 ± 1.69 cm) then extract 14 (*A. mellea*) 4.083 ± 0.74 cm compared with the control (3.33 ± 0.59 cm) and the other extracts (Figure 10).

The RL of eggplant seedlings in the presence of the two basidiomycete extracts (Ext 4 and 5) is low compared with the rest of the extracts, with an average of 1.28 ± 0.56 cm and 2.16 ± 0.078 cm respectively.

The rest of the extracts were all biostimulatory compared to the control (3.33 ± 0.59 cm), with the exception of EXT 12 (*P. eryngii*) which had an average of 3.03 ± 0.51 cm, less than that recorded in the control.

Therefore, this extract doesn't stimulate root length as much in eggplant.

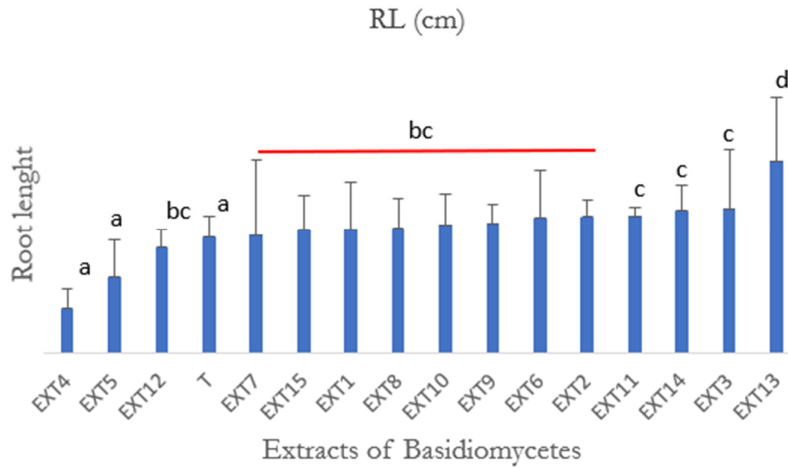


Figure 10. Effect of higher fungi extracts on root length of eggplant seedlings. Bars with different letters indicate significant differences ($p \leq 0.05$)

EXT1: *Lactarius zonarioides*, EXT2: *Amanita proxima*, EXT 3: *Amanita virosa*, EXT 4: *Agaricus bisporus*, EXT 5: *Suillus mediterraneensis*, EXT6: *Coprinus atramentarius*, EXT7: *Pleurotus pulmonarius*, EXT8: *Lepiota* sp., EXT9: *Cortinarius orellanus*, EXT 10: *Boletus* sp., EXT11: *Xerocomus* sp., EXT 12: *Pleurotus eryngii*, EXT13: *Pleurotus dryinus*; EXT 14: *Armillaria mellea*, EXT 15: *Pleurotus ostreatus*; T: control

Effect of basidiomycete extracts on aerial length

According to the ANOVA test, aerial length was highly significant ($P=0.011$).

Duncan's test revealed that extract 8 (*Lepiota* sp.) was the most significant and gave the best result with an average of 6.23 ± 0.65 cm, followed by extract 12 (*P. eryngii*) 6.15 ± 0.71 cm. On the other hand, extracts 2 and 9 respectively (*A. proxima* and *C. orellanus*) recorded the same length with an average of 5.83 cm, indicating that these two extracts stimulate the AL of eggplant seedlings in the same way (Figure 11).

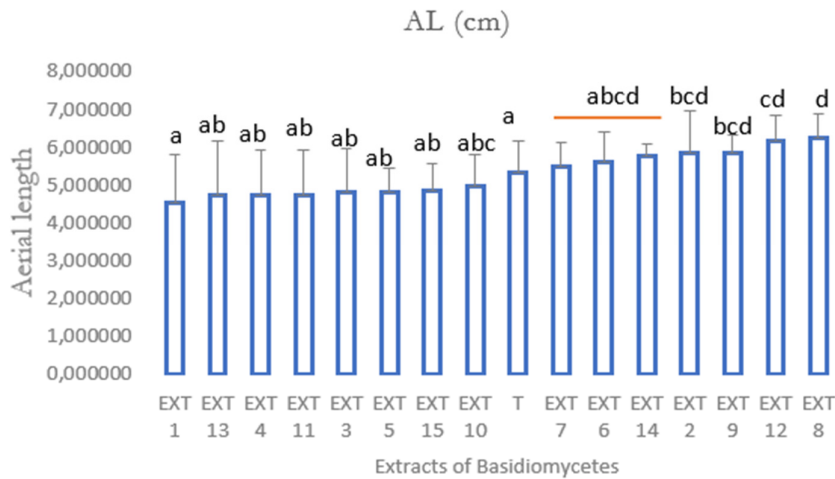


Figure 11. Effect of higher fungi extracts on the aerial length of eggplant seedlings. Bars with different letters indicate significant difference ($p \leq 0.05$)

EXT1: *Lactarius zonarioides*, EXT2: *Amanita proxima*, EXT 3: *Amanita virosa*, EXT 4: *Agaricus bisporus*, EXT 5: *Suillus mediterraneensis*, EXT6: *Coprinus atramentarius*, EXT7: *Pleurotus pulmonarius*, EXT8: *Lepiota* sp., EXT9: *Cortinarius orellanus*, EXT 10: *Boletus* sp., EXT 11: *Xerocomus* sp., EXT 12: *Pleurotus eryngii*, EXT13: *Pleurotus dryinus*; EXT14: *Armillaria mellea*, EXT 15: *Pleurotus ostreatus*; T: control

Effect of basidiomycete extracts on leaf number

The last parameter studied was the number of leaves (LN) of eggplants, which proved highly significant (P=0.00) difference according to extracts type. All fungal extracts stimulated LN in eggplant seedlings, except EXT 4 and 5 from *A. bisporus* and *S. mediterraneensis* respectively, which gave no leaves (Figure 12). Extracts 3 and 13 of *A. virosa* and *P. dryinus* gave the best leaf stimulation with an average of 2.5, followed by extracts 8 and 11 (*Lepiota* sp. and *Xerocomus* sp.) respectively, with averages of 2.17 and 2.33.

This study confirmed that basidiomycete extracts are indeed biostimulants for eggplant growth (Table 4). Each higher fungi extract stimulates a part of the eggplant seedlings, resulting a better yield. *Lepiota* sp., the two amanites (*A. proxima* and *A. virosa*), *C. atramentarius*, *C. orellanus*, boletus (*Boletus* sp. and *Xerocomus* sp.) and the two oyster mushroom species (*P. dryinus* and *P. eryngii*) were particularly effective. Extracts of *A. bisporus* and *S. mediterraneensis* only stimulate root fresh mass, aerial and root length, but to a lesser extent than observed for the control.

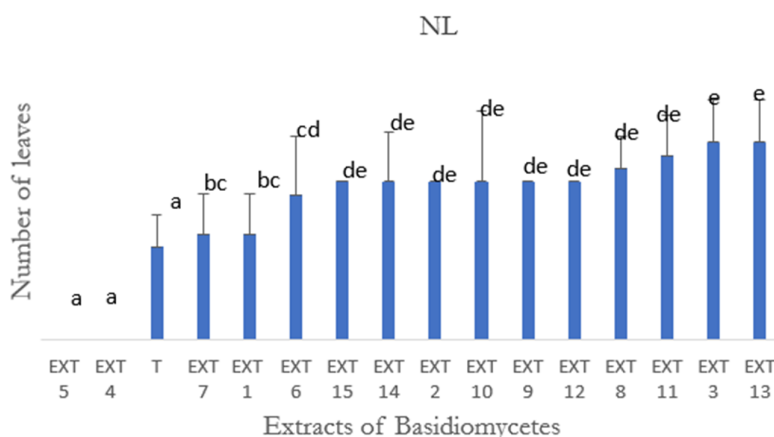


Figure 12. Effect of higher basidiomycetes extracts on the number of leaves on eggplant seedlings. Bars with different letters indicate significant differences ($p \leq 0.05$)

EXT1:*Lactarius zonarioides*, EXT2:*Amanita proxima*, EXT 3: *Amanita virosa*, EXT 4: *Agaricus bisporus*, EXT 5: *Suillus mediterraneensis*, EXT6: *Coprinus atramentarius*, EXT7: *Pleurotus pulmonarius*, EXT8: *Lepiota* sp., EXT9: *Cortinarius orellanus*, EXT 10:*Boletus* sp.,EXT11: *Xerocomus* sp., EXT 12: *Pleurotus eryngii*, EXT13: *Pleurotus dryinus* ; EXT14 :*Armillaria mellea*, EXT 15:*Pleurotus ostreatus* ; T: control

Table 4. The impact of higher basidiomycetes extracts used as biostimulants on the growth parameters of eggplant plants

Eggplant growth parameters	Extracts of higher basidiomycetes														
	EXT 1	EXT 2	EXT 3	EXT 4	EXT 5	EXT 6	EXT 7	EXT 8	EXT 9	EXT 10	EXT 11	EXT 12	EXT 13	EXT 14	EXT 15
AFW	+	+	+	-	-	+	-	+	+	+	-	+	+	+	-
ADW	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
RFW	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RL	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AL	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NL	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+

(+): Stimulating effect, (-): without stimulating effect, EXT1: *Lactarius zonarioides*, EXT2:*Amanita proxima*, EXT 3:*Amanita virosa*, EXT 4: *Agaricus bisporus*, EXT 5: *Suillus mediterraneensis*, EXT6:*Coprinus atramentarius*, EXT7: *Pleurotus pulmonarius*, EXT8:*Lepiota* sp.,EXT9: *Cortinarius orellanus*, EXT 10:*Boletus* sp., EXT 11: *Xerocomus* sp.,EXT 12:*Pleurotus eryngii*, EXT13:*Pleurotus dryinus*; EXT14 :*Armillaria mellea*, EXT 15:*Pleurotus ostreatus*; ADW: Aerial Dry Weight; AFW: Aerial Fresh Weight; AL: Aerial Length; NL: Number of Leaves; RFW: Root Fresh Weight; RL Root Length

Discussion

Mushroom extracts and their phenolic acids, have been largely for antimicrobial (antibacterial and antifungal) and antiviral activity (Heleno *et al.*, 2013; Attarat and Phermthai, 2015). The main phenolic compounds present in fungi are mostly phenolic acids and flavonoids (Ferreira *et al.*, 2009; Heleno *et al.*, 2015; Soković *et al.*, 2017). However, tannins can also be found (Pavithra *et al.*, 2016). It is well known that the quantities and types of phenolic compounds varies among families and varieties (Sini *et al.*, 2010). This difference can also be explained by variation of the environmental conditions, climatic and sampling period as well as by the genetic factors and experimental conditions.

The total phenol and flavonoid content of 10 species of edible mushrooms, including cultivated (*Pleurotus ostreatus*, *Agaricus bisporus*) and wild (*P. ostreatus*, *Boletus edulis* and *Agaricus campestris*), were determined to be very rich in these compounds. This contributes to the understanding of mushrooms as sources of biologically active compounds (Buruleanu *et al.*, 2018).

In a study of, N'Guessan *et al.* (2011) detected the presence of these secondary metabolites, which in the visible light tannins give grey and brown spots; phenolic compounds in red, blue and green. These compounds form well-colored with several specific reagents (AlCl₃, Neu) in the visible or under UV/ 366 nm (Georgirskii *et al.*, 1990).

After treatment with ethanolic AlCl₃, they turn yellow in the visible light and exhibit various colors such as blue-green, fluorescent blue, green, brown, yellow and yellow-green under UV/366 nm (Brou *et al.*, 2010; N'Guessan *et al.*, 2011). Xanthenes take on an orange colour (Ladigina *et al.*, 1983); yellow spots correspond to flavonols and/or aurones. Methylated flavones are blue or purple (Dawson *et al.*, 1991). Markham (1982) and Mohammadi (2006) have shown that anthocyanidin-3-glycosides give orange, red and purple spots; flavonols are detected as yellow and green spots; flavones, methylated flavones, isoflavones, flavanones and chalcones appear as blue spots as in the case of our extracts: *A. mellea* and *P. ostreatus*, *C. orellanus*, *P. pulmonarius* (Spot of Rf=0,98); *P. dryinus*, *A. mellea*, *P. ostreatus* (Spot of Rf=0,89). Flavanols and aurones are characterised by the appearance of green spots and this is the case of all our extracts (Figure 4); flavones and chalcones present a purple and orange coloration and this is the case of *P. dryinus* (Spot of Rf=0, 82); *A. mellea* and *P. ostreatus* (Spot of Rf=0, 80); *S. mediterraneensis* (Spot of Rf=0, 71); *C. orellanus*, *Boletus* sp, *Xerocomus* sp., *P. eryngii* (Spot of Rf=0, 69) of purple spots (Figure 4).

By comparing the results of the frontal ratios (Rf) of this analysis with those of Brou *et al.* (2010) tested on plants, we obtained a compound (Spot of Rf=0, 58) of a green fluorescence for *L. zonarioides*, *A. proxima*, *A. virosa* and *A. bisporus*, it could be a phenolic acid or catechol. On the other hand, a compound (Spot of Rf=0, 36) of a green fluorescence in the two amanites (*A. proxima* and *A. virosa*) and finally the compound (Spot of Rf=0,8) of the extracts *P. ostreatus* and *A. mellea*, of a purple colour under UV/254 nm which is a flavonoid compound. The rest of the compounds could not be identified.

In 2004, Chaib highlighted flavonoids under UV/366 nm by the appearance of yellow, orange coloration after revelation with Godin's reagent. For each specific colour spot with a given Rf, we obtained by using the AlCl₃ reagent a presence of various flavonoid structural types in the extracts studied.

Comparing the Rfs of the compounds revealed by the AlCl₃ reagent and those of Godin's reagent (Figures 5), we found 3 compounds from 3 extracts that agree with the flavonoid compounds revealed by AlCl₃.

We refer to: *P. dryinus* (yellow spot of Rf=0.29); *T. quercina* (yellow spot of Rf=0.22) and *C. orellanus* (orange-brown spot of Rf=0.22). Our higher basidiomycetes are rich in flavonoids except for *L. zonarioides*, *A. proxima*, *A. virosa*, and *A. bisporus* which are poor (1 to 2 compounds). According to the results obtained during this analysis, and what the AlCl₃ reagent was able to reveal, and by comparing with the literature, we assume the presence of the following flavonoid compounds: flavanols and aurones, flavones and chalcones, flavones, methylated flavones, isoflavones, flavanones and chalcones.

From the results of the comparison of chromatographic profiles, we found that higher basidiomycetes extracts produce a wide range of secondary metabolites, and many compounds are common between the different extracts with identical R_f values, despite the genetic variations between the fungi. However, their metabolomes seem to have some similarities. The screening done by TLC reveals that the fungal isolates that produced a lot of biomass were not those that produced the most secondary metabolites. These mycochemical investigations revealed the presence of phenolic, flavonoid and tannic compounds in the extracts studied.

The presence of these compounds explained the antifungal property of our higher basidiomycetes' extracts (Ait Hamadouche *et al.*, 2021).

One of the main analytical techniques used to obtain the chemical profile of the mushroom is liquid chromatography (HPLC) (De Souza Campos Junior *et al.*, 2019).

Regarding *P. ostreatus*, the presence of a flavonol compound has been reported; however, other research has reported the richness of this mushroom in phenolic acids (Reis *et al.*, 2012; Muszyńska *et al.*, 2013). We detected the presence of only one flavonoid compound in *A. bisporus*, which may be due to the availability of the range of standards tested. Furthermore, many researchers have revealed the richness of this extract in phenolic acids (Reis *et al.*, 2012).

For *C. atramentarius*, we recorded the presence of flavonoids but Heleno *et al.* (2015), reported that this extract contained highly phenolic acids such as p-hydroxybenzoic acid, p-coumaric acid.

Ayvaz *et al.* (2019) reported that myricetin and quercetin are not included in the choice of standards because they were not detectable when flavonoids were assayed in one of the samples. It is an accepted fact since only plants have the biosynthetic capacity to produce flavonoids, but animals and fungi do not. With the exception of the filamentous fungus *Aspergillus candidus* and the two higher basidiomycetes *Phallus impudicus* and *Fistulina hepatica*, whose were confirmed as containing flavonoids (Barros *et al.*, 2009). In contrast, the results of our HPLC analysis revealed that the higher basidiomycetes extracts contained at least one flavonoid molecule.

The present chemical analysis of the eight extracts is consistent with previous studies in the literature (Alam *et al.*, 2011; Özyürek *et al.*, 2014). Eight flavonoid compounds (catechin, rutin, myricetin, quercetin, apigenin...) are detected from several mushroom species (Kaewnarin *et al.*, 2016; Yahia *et al.*, 2017; De Souza Campos Junior *et al.*, 2019).

The results obtained in this study show similarities and differences with the literature research related to cultivation techniques, ripening process, processing and storage conditions, extraction methods, as well as stress conditions such as UV radiation, infection by pathogens and parasites, air pollution and exposure to extreme temperatures (Dinçer *et al.*, 2013). It can be suggested that differences in the qualitative and quantitative composition of phenolic compounds may be caused by genetic differences between species, habitats, growth conditions and maturation of fungi (Çayan *et al.*, 2020).

The chemical results confirm that the macrobasidiomycete are rich in bioactive molecules, which act in various ways as biostimulants, on a biological level, by improving nutrient absorption (Mishra *et al.*, 2015; Singh *et al.*, 2017; Varma *et al.*, 2017). These molecules also act on flowering, fruit set, crop productivity, and are also capable of improving tolerance against a wide range of stress factors (Colla and Roupael, 2015).

Conclusions

The spectrophotometric assay really showed that the tested macrofungi extracts, in this study are rich in bioactive compounds, particularly, *Suillus mediterraneensis*, one, which was demonstrated as the richest one in both phenolics and flavonoids contents, while *Trametes quercina* extract, contains the lowest phenolics but the highest flavonoids quantities. We also conclude that extracts screened by Thin Layer Chromatography are

hyperproductive in metabolites; and HPLC profiles revealed their abundance in phenolic compounds, flavonoids, tannic acid and vitamin E. All the results, and those presented in the chemical analysis, confirmed that indeed these higher fungi extracts develop a diversity of prolific secondary metabolites products that contribute to plant growth, thus offering strong agricultural potential. These compounds, known as biostimulants, are of natural origin, and help increase plant yields and nutrient uptake. Their use could improve crop productivity and tolerance against a wide range of stress factors. Finally, fungal extracts when applied to hydroponics culture, could offer new promising technological strategy, for various crops. These natural bioactive molecules demonstrate the many biological activities that these fungi present, highlighting their importance in the environment, agriculture and human health.

Authors' Contributions

YA and SD performed the sampling, methodology, data collection, supervision and article writing; OM supervised and edited the article. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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