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RESEARCH PAPER

The bioherbicidal potential of isolated fungi cultivated in microalgal biomass

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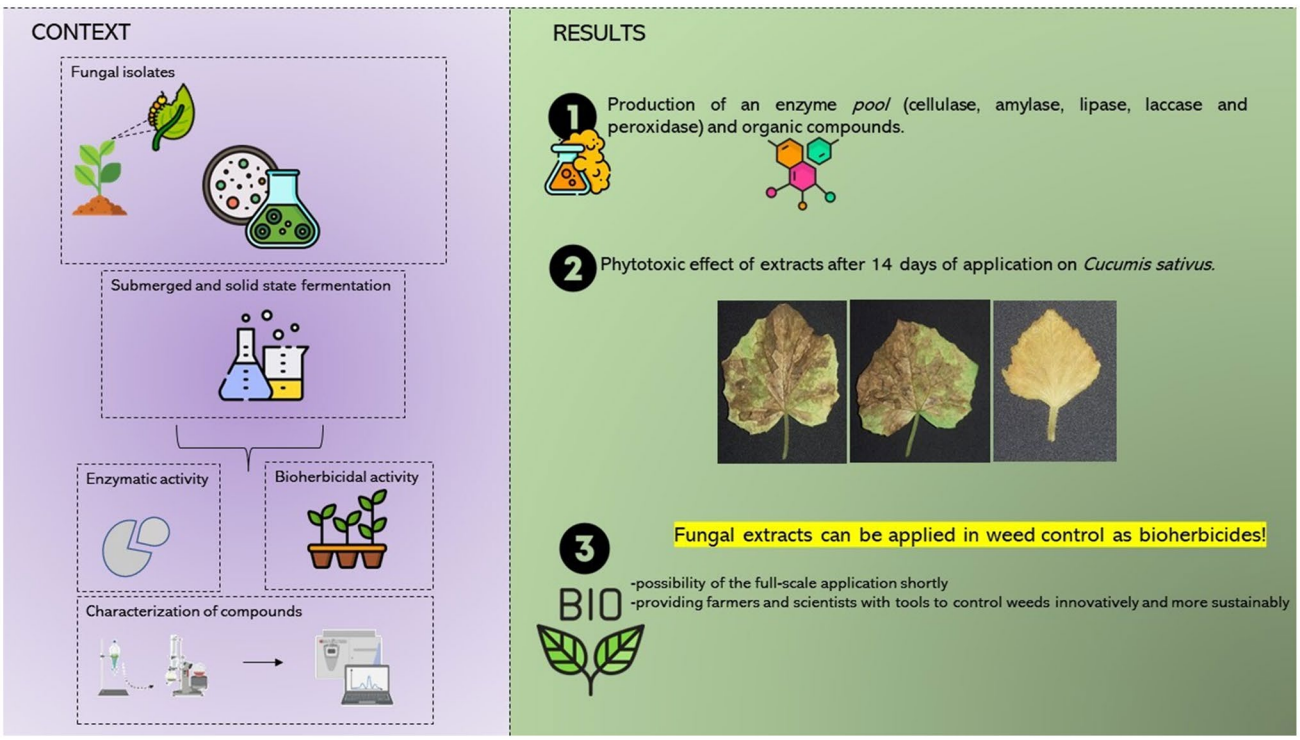
Abstract

This study evaluated the bioherbicidal potential of wild fungi grown on microalgal biomass from the digestate treatment of biogas production. Four fungal isolates were used and the extracts were evaluated for the activity of different enzymes and characterized by gas chromatography coupled with mass spectrometry. The bioherbicidal activity was assessed by application on *Cucumis sativus,* and the leaf damage was visually estimated. The microorganisms showed potential as agents producing an enzyme pool. The obtained fungal extracts presented different organic compounds, most acids, and when applied to *Cucumis sativus,* showed high levels of leaf damage (80–100 ± 3.00%, deviation relative to the observed average damage). Therefore, the microbial strains are potential biological control agents of weeds, which, together with the microalgae biomass, offer the appropriate conditions to obtain an enzyme pool of biotechnological relevance and with favorable characteristics to be explored as bioherbicides, addressing aspects within the environmental sustainability.

Graphical abstract



Extended author information available on the last page of the article

Keywords Biological control · Biotechnological processes · Enzymes · Food safety · Microbial strains

Introduction

In the agricultural sector, there are activities aimed at providing food to meet the needs of a constantly developing population. Over the years, agricultural productivity has suffered successive decreases due to different factors, among which weeds are responsible for significant losses and can affect food security [1]

Herbicides have been widely used as a strategy to ensure crop safety and also to prevent weeds from increasing rapidly in these environments. This control strategy has been used since the late 1960s. Still, its continued use leads to plant resistance and negatively affects the environment and human and animal health, resulting in reduced yields and high costs to sustain healthy food production [2, 3].

Ending hunger, achieving food security, improving nutrition, and promoting sustainable agriculture are targets for achieving the Sustainable Development Goals by 2030; an exciting strategy to ensure the availability of healthy and more sustainable food is the use of bioherbicides as a way to reduce the use of synthetic herbicides [4].

Using natural products as a form of weed management offers some advantages over synthetic herbicides, such as specificity of action, rapid degradation, and low risk of environmental contamination. Bioherbicides are products based on biologically active organisms or secondary metabolites and enzymes produced by them. Although several natural products have been tested for bioherbicidal activity, few can be obtained commercially due to the need for rigorous validation to assess their efficacy and reliability in weed control [5–7].

The essence of bioherbicide formulation lies in defining the biologically active compound, which will make the necessary interactions with the target plant. The environment harbors many microorganisms that can be applied in various biotechnological areas. In this sense, bioprospecting microbial strains are fundamental to discovering new relevant species in this scenario. Fungi of the genera *Fusarium* and *Trichoderma* are already widely used as biological control agents; they act by forming metabolites (phytohormones, 2-(hydroxymethyl) phenol, organic acids, alcohols, plant growth regulators, among others) and enzymes (lipases, peroxidases, cellulases, amylases, among others) that may act degrading the cell wall of plants [8–12].

The production of a bioherbicide is still expensive, which does not make it competitive with other chemicals available in the market. To overcome this bottleneck, an alternative would be using a cheaper fermentation medium, adding value to the final product. To this end, microalgae resulting from the treatment of swine manure present a possible application as a readily available substrate rich in carbohydrates and proteins, which would make it possible to use them as a raw material to produce bioherbicides. This would be a sustainable solution to add value to microalgae and reduce the final cost of bioherbicide [13, 14].

Thus, this study evaluated the potential of wild fungi cultivated on microalgae biomass from the digestate treatment of biogas production, aiming to obtain fungal bioherbicides.

Materials and methods

Microalgae biomass: raw material for bioherbicide production

The microalgae used as fermentative substrate belong to the genus *Chlorella* spp. and come from the phytoremediation treatment of wastewater from biogas production (digestate), implemented at EMBRAPA Swine and Poultry (Concordia, SC, Brazil). The biochemical of microalgae (dry weight basis) is composed of $58.90 \pm 1.30\%$ protein (proportional to the nitrogen content), $25.20 \pm 0.90\%$ carbohydrates, $3.00 \pm 0.50\%$ lipids, $12.8 \pm 0.60\%$ minerals [15, 16] and has a moisture content of $88.99 \pm 0.50\%$.

Microbial strains

Four fungal isolates were used to produce extracts with bioherbicidal potential. The first of them, from the species *Trichoderma koningiopsis* (identification code in GenBank MK860714), was isolated from the weed *Digitaria ciliaris* and showed promising results for enzyme production and weed control in other studies [8, 11, 17].

Three other microbial strains were isolated from the gut of military caterpillars (*Spodoptera frugiperda*). For this, caterpillars were subjected to the dissection process. Subsequently, the intestines were placed in Erlenmeyer flasks containing synthetic minimal YNB medium (6.7 g/L yeast nitrogenous base—SIGMA-Aldrich) plus 10 g/L xylose and 0.2 g/L chloramphenicol. The flasks were kept at 30 °C under 145 rpm until turbidity resulting from microbial growth could be perceived [18, 19]. Next, depletions were made in Petri dishes with the same culture medium described above to obtain isolated colonies containing 20 g/L of agar. Among the isolated strains, two cultures showed potential and were molecularly identified (Neoprospecta, Florianópolis-Brazil) as *Fusarium* sp. and two fungi with associated growth, *Fusarium denticulatum* and *Mucor circinelloides*. The DNA sample (extracted from mycelium grown in a culture medium) was subjected to a polymerase chain reaction (PCR) to amplify the rDNA internal transcribed spacer

(ITS) region. The primers for the ITS region were: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCC TCCGCTTATTATTGATATGC-3'). The fragments were sequenced by chain termination analysis with the Big Dye 3.1 reagent (Applied Biosystems) in an automated capillary sequencer 3500 XL (Applied Biosystems). After, the sequences obtained for the isolated fungi were compared with the GenBank database using the BLAST (Basic Local Alignment Search Tool) [20].

The strains were repotted and kept in Petri plates containing Potato Dextrose Agar (PDA) culture medium (Merck, Germany) for 7 days at 28 ºC for fungal growth.

Submerged and solid-state fermentation

The fermentations to obtain extracts with bioherbicidal potential were performed in a submerged and solid state.

The submerged fermentation (SF) process was performed in 300 mL Erlenmeyer with a proper volume of 100 mL. The medium was composed of 10 g of wet microalgal biomass, aiming to meet the supplementation of a synthetic fermentative medium for bioherbicide production [8] and 90 mL of distilled water. The inoculated Erlenmeyers were kept on an orbital shaker (New Brunswick TM, Germany) at 120 rpm for 72 h and 28 °C.

Solid-state fermentation (SSF) was performed in polypropylene beakers (250 mL) containing a medium composed of 10 g of wet microalgal biomass without supplementation. The fermentation occurred in a B.O.D type incubator (Solab, Brazil) for 72 h at a fixed temperature of 28 ºC and relative humidity of 70%. After fermentation, 90 mL of distilled water was added to the fermented samples to perform the extraction. This solution was kept under 200 rpm agitation for 60 min at 28ºC.

The flasks were autoclaved at 120 ºC for 20 min at 101.325 kPa, to ensure that the rupture of microalgal biomass cells occurred, which led to the release of intracellular lipids, in addition to complete sterilization of the culture medium. After sterilization, the media were inoculated with a suspension of 10^6 spores/mL of each microorganism: *Trichoderma koningiopsis*, *Fusarium* sp., *Fusarium denticulatum*, and *Mucor circinelloides*. After fermentation, the extracts were filtered by manual pressing in synthetic fabric, the solid retained was sterilized and discarded, and the liquid permeate was centrifuged (NT 815-NovaTecnica, Brazil) at 2000 rpm, 4 ºC for 10 min. The supernatant from the centrifugation was used for the subsequent steps.

Enzymatic activity

The following enzymes were selected to evaluate the presence of an enzymatic pool in the extracts: amylase, cellulase, laccase, lipase, and peroxidase. The enzyme assays were performed in triplicate and always contained a reaction control without the enzyme extracts.

The quantification of the enzyme's amylase [21, 22] and cellulose [23] occurred by quantifying the release of total reducing sugars, measured by the DNS (3,5 dinitrosalicylic acid) method [24]. One unit of enzyme activity (U) was defined as the amount of enzyme capable of releasing 1 µmol of glucose per minute under the reaction conditions.

To determine laccase activity, 2,2′-azino-di-3-ethylbenzotialozin-6-sulfonic acid (ABTS) was used as the substrate for the enzyme reaction [25]. One unit of laccase activity (U) was defined as the amount of enzyme capable of forming 1 µmol of ABTS + per minute under the reaction conditions.

Lipase quantification was performed by preparing an emulsion [26]. One unit of lipase activity (U) was defined as the enzyme able to hydrolyze 1 µmol of substrate per minute under the reaction conditions.

Peroxidases were quantified by one unit of peroxidase activity (U), defined as the amount of enzyme capable of causing an absorbance unit increase of 0.001 per minute in the reaction conditions [27, 28].

Bioherbicide activity

The evaluation of the bioherbicidal activity of the extracts produced by the fungi through submerged and solid fermentations and the combinations of extracts was performed through the application in *Cucumis sativus*; the choice for this species is due to its potential as a model plant because it is used in tests with chemical herbicides [29].

For the *Cucumis sativus* trials, each treatment was composed of 36 plants and 12 controls (no treatment), all with 4 repetitions. The extracts were applied in a greenhouse with controlled temperature and humidity conditions (26 ºC and 70%). The seeds were grown for 10 days, and when they reached three leaves, approximately 5 mL of the extracts were manually applied to the plant's leaf surface.

For the application tests, the control samples were: control A consisted of the culture medium (microalgae + distilled water) used in the submerged fermentations without the addition of microorganisms; control B consisted of the culture medium (microalgae) used in the solid fermentations, without the addition of microorganisms and after the extraction process; control C consisted of distilled water.

The extracts' bioherbicidal potential was evaluated 7 and 14 days after application. Leaf damage was visually estimated as a percentage reduction in growth compared to controls. The percentage of leaf damage was established according to the scale recommended by the Brazilian Weed Science Society (SBCPD) [30].

Characterization of compounds present in the extracts

The compounds are characterized in the fermented extracts through the analysis of the products generated from the liquid–liquid extraction of the fermented extracts via submerged and solid processes of the fungi. In addition, the fermentation medium of each fermentation was evaluated and considered a control.

For this, ethyl acetate (Et_2O) was used as an organic solvent, mixed with the extracts in a separation funnel at a ratio of 1:1 (v/v). The samples were followed to a rotary evaporator (Rotary Evaporator—Q344B, QUIMIS, Brazil) until the reduction of the sample volume was observed due to the evaporation of the organic solvent. The conditions of the evaporation process were: bath at 30 ºC and agitation of 120 rpm (under vacuum). The aqueous phase was discarded, and the organic phase was filtered and dried with ammonium sulfate.

The reaction products were identified by gas chromatography coupled to mass spectrometry (GC/MS—QP2010, Shimadzu, Japan) using an NST 05 ms—3,025,025 column (30 m × 0.25 mm × 0.25 µm). The column temperature followed the schedule: 80 °C/3 min, 6 °C/min up to 218 °C, 2 °C/min up to 240 °C, and 7 °C/min up to 300 °C. The carrier gas was helium (He). The injector and detector temperature were set to 280 °C and 1.0 µL of the solution (Derivatization:1:1:1 (extract: ethyl acetate: BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide) + TMCS (Trimethylchlorosilane) (1%) at 60 °C for 1 h) was injected into the GC/MS system with a split ratio of 1:10. The apparatus operated at a flow rate of 19.1 mL/min in 70 V electronic impact mode and with the column pressure at 100 kPa. The compounds were identified by comparing the mass spectra with those from the Wiley library and by comparing the GC retention time of standard compounds. The products were partially quantified by the percentage of the area of each peak relative to the total peak area [31].

Statistical analysis

The data were statistically treated by analysis of variance followed by Tukey's test, considering a significance level of 95% ($p < 0.05$). Statistica 8.0 software (Statsoft Inc., Tulsa, OK, USA) was used for this.

Results and discussion

Evaluation of enzyme production

Table 1 shows the results of the enzymatic quantification of the extracts from the submerged and solid fermentations.

The microorganisms showed potential as agents for producing an enzyme pool via submerged and solid fermentation. The microalgae biomass used as substrate provided a suitable culture medium for the microorganisms to produce some enzymes of interest in the biological control of weeds.

As for the results regarding submerged fermentation, the extract fermented by the fungi *Fusarium denticulatum* and *Mucor circinelloides* showed the highest values of lipase activity, 1.80 ± 0.34 (U/mL), probably related to the fact that the fungus *Mucor circinelloides* is known to accumulate high lipid content in its metabolism and, consequently, to be a potential producer of microbial lipases [32]. Peroxidase activities were the highest in the extracts obtained in submerged fermentation. Previous studies using the fungus *Trichoderma koningiopsis* and species of the *Fusarium* already showed that these fungi are good producers of peroxidases [8, 11]. Using the associated

Table 1 Results of the enzymatic production of amylase, cellulase, laccase, lipase, and peroxidase present in the extracts obtained by submerged and solid-state fermentation with the fungi *Trichoderma koningiopsis*, *Fusarium denticulatum* in consortium with *Mucor circinelloides* and *Fusarium* sp

Microorganism	Enzymatic activity SF (U/mL)				
	Amylase	Cellulase	Laccase	Lipase	Peroxidase
T. koningiopsis	10.29 ± 0.01^{a}	1.11 ± 0.01^{a}	nd	0.80 ± 0.09^{a}	36.03 ± 0.01^{a}
F. denticulatum M. circinelloides	3.43 ± 0.01^{b}	0.43 ± 0.01^{b}	nd	1.80 ± 0.34^{b}	37.15 ± 0.01^{b}
Fusarium sp.	2.80 ± 0.01^{c}	0.33 ± 0.01^{c}	nd	nd	18.63 ± 0.01^{c}
	Enzymatic activity SSF (U/mL)				
T. koningiopsis	0.83 ± 0.06^{a}	0.83 ± 0.01^{a}	nd	nd	9.25 ± 0.01^{a}
F. denticulatum M. circinelloides	1.78 ± 0.05^{b}	1.78 ± 0.05^{b}	nd	nd	12.53 ± 0.01^{b}
Fusarium sp	2.54 ± 0.28^{c}	2.54 ± 0.02^{c}	nd	nd	16.88 ± 0.01^{c}

[a,b,c]Different lowercase letters in the columns indicate statistical differences by Tukey's test at a 95% confidence level ($p < 0.05$) among the fermenting microorganisms, each type of fermentation was evaluated separately. Equal letters do not differ significantly

nd: not detected

fungi *Fusarium denticulatum* and *Mucor circinelloides*, an enzyme activity of 37.15 ± 0.01 (U/mL) was reached, the maximum obtained among the enzyme extracts produced by submerged fermentation. This fact may be related to the fungi's joint action, establishing synergism and potentiating enzymatic production [33].

Among the extracts obtained by solid fermentation, the extract fermented by the microorganism *Fusarium* sp. reached the highest enzyme concentrations of amylase, cellulase, and peroxidase, demonstrating that this fungus is an exciting producer of enzymes in the fermentative conditions that were made available. The metabolism of species of the genus *Fusarium* is related to the nutritional source used; it is versatile, adapting quickly to different fermentative conditions to which it is submitted [34]. Hydrolytic enzymes such as amylase and cellulase can be detected when their metabolism is associated with nitrogen. On the other hand, exposure to mild stress can produce oxidative enzymes such as peroxidases [35].

The enzyme lipase was not produced via solid-state fermentation, which may be related to the composition of the culture medium since when you have fermentative media with the presence of some oil, the fungus *Fusarium* sp. becomes a potential producer of microbial lipases [36]. On the other hand, when *Fusarium denticulatum* associated with *Mucor circinelloides* were subjected to submerged fermentation, they produced lipase, probably because, during the production of this enzyme, in environments with high lipid content and low amount of water (solid-state fermentation), the formation of phospholipid-based reverse micelles that capture water occurs, and may result in enzyme inactivation. In this sense, the higher the water content available in the substrate, the greater the possibility of lipase production; perhaps, for this reason, the submerged medium (water availability and low agitation) was the most suitable for these fungi to achieve microbial lipase production [37, 38].

The fungus *Trichoderma koningiopsis* obtained the lowest values of enzyme activity when compared to the other fungi. Although *T. koningiopsis* presents itself in a versatile way, to its performance observed in other studies, possibly this fungus adapts better in submerged fermentations [8, 11, 17].

The laccase enzyme was not quantified in the extracts obtained by submerged and solid fermentation. Generally, fungal laccases are difficult to obtain because they require substrates that contain chemical inducers such as phenolic compounds, aromatics, or copper ions; this makes laccase production only detected after a more complex and optimized fermentation [39]. In addition, the tested fungi may have produced distinct forms (isoforms) of this enzyme, which could be detected after an enzyme purification process [40].

The low production of cellulase obtained in the different fermentative processes may be linked to the composition of the microalgae biomass since the carbohydrate fraction may be formed by polysaccharides such as starch and cellulose. The (possibly low) content of these polysaccharides may limit enzyme production since the presence of these compounds in the biomass used as a substrate is not specific [41].

Analysis of bioherbicidal activity

Recent studies consider microalgae as potential biological control agents because they produce biologically active compounds, such as secondary metabolites [13]. Table 2 shows the values in the percentage of leaf damage observed 7 and 14 days after the applications, according to the scale recommended by the SBCPD. These values may be related to a higher presence of free water in the extract obtained by submerged fermentation (control A), which would facilitate the entry of metabolites in the microalgae on the leaf surface, causing minor damage. Control A, which refers to the culture medium of fermentation in a submerged, showed low foliar damage of 4.00 ± 0.06% after 14 days of application, which may be linked to a low bioherbicidal potential in the microalgal biomass. No foliar damage was observed for control B (solid-state fermentation medium) Table (3).

Using the fungi *Trichoderma koningiopsis* and Fusarium sp., complete control of the crops was obtained, with only a few living plants, according to the scale recommended by the SBCPD. The extracts produced by submerged fermentation showed a higher damage percentage than those produced by solid fermentation extract made by *T. koningiopsis* that showed the production of the peroxidase enzyme in significant concentrations; this enzyme is one of the responsibilities for eliminating reactive oxygen species (ROS), which are indicators of stress in the plant. When applied to the plant, this extract rich in peroxidases increases the concentration of superoxide radicals and hydrogen peroxide, damaging DNA and cell membranes. The genus *Fusarium* exhibits controlling characteristics in invasive plants, acting mainly on ethylene biosynthesis, stimulating ripening and yellowing leaves [6, 11]. These effects can be visualized in Fig. 1, in which the extract of *Trichoderma koningiopsis* acted to inhibit plant growth, causing necrosis on the leaf surface, and the extract produced by the fungus *Fusarium* sp. caused early leaf ripening (Table 4).

Fusarium sp. in which the presence of the enzymes cellulase, peroxidase, and amylase was identified, the last two may have acted in the degradation of leaf structures together with cellulase responsible for serving as a gateway for the fungus into the leaf structures, working in the degradation of cellulose present in the cell wall of plants [10, 11]. The symptoms caused by the extracts produced by solid fermentation could already be identified 7 days after application

Table 2 Results of the application in *Cucumis sativus* of the extracts obtained by submerged, solid-state fermentation of the fungi *Trichoderma koningiopsis*, *Fusarium denticulatum* in consortium with *Mucor circinelloides,* and *Fusarium* sp

Microorganism	SF		SSF	
	7 days (%)	14 days (%)	7 days (%)	14 days (%)
Control A and B	2.00 ± 0.05^{a}	4.00 ± 0.06^{a}	nd	nd
Control C	nd	nd	nd	nd
Trichoderma koningiopsis	8.00 ± 2.00^{b}	98.00 ± 0.01^{b}	40.00 ± 3.00^{a}	70.00 ± 3.00^{a}
Fusarium denticulatum Mucor circinelloides	8.00 ± 1.90^{b}	94.00 ± 6.80^{b}	20.00 ± 2.50^{b}	95.00 ± 1.00^{b}
Fusarium sp.	4.00 ± 0.50^{c}	100.00 ± 0.01^{b}	20.00 ± 2.50^{b}	98.00 ± 3.00^{b}
	7 days (%)		14 days (%)	
Crude microalgae	nd		20.00 ± 1.00^{a}	

[a,b,c]Different lowercase letters in the columns indicate statistical difference by Tukey's test, with a 95% confidence level ($p < 0.05$). Equal letters do not differ significantly

Control A = culture medium (microalgae + distilled water) used in submerged fermentations, without the addition of microorganisms and after the extraction process;

Control B = culture medium (microalgae) used in solid fermentations, without the addition of microorganisms and after the extraction process;

Control C = control group, which received no treatment;

Crude microalgae = microalgae *in nature*, unfermented

nd: not detected

on *Cucumis sativus*, different from those produced by submerged fermentation. These symptoms (Fig. 1) may be associated with specific characteristics of the enzymes in these extracts, such as those produced by the fungus *Fusarium denticulatum* in consortium with *Mucor circinelloides* (Table 5).

The application of the crude microalgae demonstrates that even if the microalgae present a mild level of phytotoxicity, the phytotoxic potential can be amplified when it is used as a substrate for fungal bioherbicide production. This can be observed in the bioherbicide activities of the extracts applied alone from the submerged and solid fermentations. Thus, it can be stated that the microalgae alone do not have the same potential for biological control as when applied in consortium with the fungi and metabolites produced by them.

Characterization of compounds present in the fermented extracts

About 40 compounds (Tables 3, 4, 5, 6, 7, 8, 9, 10) were identified in the submerged fermentation and 26 in the solid fermentation control. In contrast, for the fungal extracts, the values were reduced between 21 and 17 identified compounds (see supplementary material). This fact may be related to fermentation, in which the fungi can synthesize and/or assimilate some compounds [42, 43]. Among the compounds identified in the submerged fermentation control, 48.6% referred to hexadecanoic (22.9%), octadecanoic (14.93%), and propanoic (10.76%) acids. These same acids were identified in different percentages in the fermented extracts. The hexadecanoic acid compound is more observed in the extracts obtained from the submerged fermentations (41.65% in the ethanolic extract derived from *Trichoderma koningiopsis* and 35.36% in the extract derived from *Fusarium* sp.). In the derivatized extract of *Fusarium denticulatum* in consortium with *Mucor cicinelloides* of submerged fermentation, ethylene glycol was the primary compound with 36.98%. In the solid-state fermentation control, the main compounds identified were: ethylene glycol (26.96%), hexadecanoic acid (20.22%), octadecanoic acid (12.18%), and propanoic acid (4.71%). These compounds were also observed mainly in the extracts resulting from solid-state fermentation. Hexadecanoic acid is the most observed compound presenting up to 40.46% in the extract derived from *Trichoderma koningiopsis*. In addition to these compounds, linolenic acid was detected in all extracts of the solid fermentations, presenting up to 16.54% in the extract derived from *Fusarium* sp.

The fraction of fatty acids present in the extracts, such as pentanoic acid, hexadecanoic acid, octanoic acid, and linolenic acid, among others, when applied to *Cucumis sativus* plants, may have acted on the permeability of metabolic exchanges in the cell wall, resulting in membrane destruction and even altering photosynthesis processes [44]. Fatty acids can present phytotoxic effects on weeds and, for this reason, are considered compounds with allelopathic properties, defined as the condition in which plants have a competitive advantage, and through secondary metabolites produced

Table 3 Compounds identified in the derivatized ethanolic extract of the submerged fermentation control in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular formula	Mol Wt
1	3.22	2,4,4-Trimethyl-1 pentanol	80	0.24	C8H18O	130
2	3.37	2-Penten-1-ol	92	0.51	C8H18OSi	158
3	3.49	Butanoic acid, 2-methyl	93	1.36	C8H18O2Si	174
4	3.64	Butanoic acid, 3-methyl	96	1.66	C8H18O2Si	174
5	4.01	Ethylamine	77	0.51	C8H23NSi2	189
6	4.17	Heptane, 2,2,4-trimethyl	83	0.21	C10H22	142
7	4.30	Ethylene glycol	90	4.16	C8H22O2Si2	206
8	4.43	Pentanoic acid	94	0.34	C8H18O2Si	174
9	5.73	Propane-1,2-diol	93	2.27	C9H24O2Si2	220
10	5.85	cis-2-Hexen-1-ol	79	0.87	C9H20OSi	172
11	5.94	Butane, 2,3-dihydroxy	97	2.74	C10H26O2Si	234
12	6.22	1,3 Propanediol	95	1.14	C9H24O2Si2	220
13	6.33	Propanoic acid, 2hydroxy	96	10.76	C9H22O3Si2	234
14	6.50	Hexanoic acid	96	1.16	C9H20O2Si	188
15	10.30	2-phenylethyl	94	3.77	C11H18OSi	194
16	10.76	Pentanoic acid, 4-methyl2-hydroxy	78	0.29	C12H28O3Si	276
17	10.91	Diethylene glycol	88	0.26	C10H26O3Si	250
18	11.27	Octanoic acid	90	0.44	C11H24O2Si	216
19	11.38	3-Acetoxybutyric acid	78	1.28	C9H18O4Si	218
20	11.66	Dodecane, 4,6-dimethyl	92	0.26	C14H30	198
21	11.86	Glycerol	95	3.17	C12H32O3Si	308
22	12.74	Nonanoic acid	86	2.43	C12H26O2Si	230
23	13.18	Pyrimidine, 2,4dihydroxy	94	3.73	C10H20N2O	256
24	14.69	Pyrimidine, 5-methyl2,4-dihydroxy	93	2.03	C11H22N2O	270
25	16.66	Tetradecane	93	0.93	C14H30	198
26	17.62	Hexadecane	91	0.45	C16H34	226
27	17.76	1,3-Benzoxazol-2-amine	78	1.13	C13H22N2O	278
28	17.82	Dodecanoic acid, 2,3bis(acetyloxy)propyl ester	73	0.32	C19H34O6	358
29	18.34	4-Hydroxyphenylethanol	87	0.47	C14H26O2Si	282
30	20.11	3-hydroxy stearic acid	79	1.36	C24H52O3Si	444
31	21.06	Heptadecane	92	0.69	C17H36	240
32	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	88	0.84	C16H22O4	278
33	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	85	0.91	C17H24O3	276
34	25.00	Eicosane	92	0.51	C20H42	282
35	26.68	Alpha.-Linolenic acid	88	3.79	C21H38O2Si	350
36	27.10	Hexadecanoic acid	94	22.91	C19H40O2Si	328
37	30.70	17-Octadecynoic acid	72	1.64	C21H40O2Si	352
38	31.12	Octadecanoic acid	86	14.93	C21H44O2Si	356
39	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	90	1.57	C25H54O4Si	474
40	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	91	1.96	C27H58O4Si	502

by microorganisms or plants influence in a way that stimulates or regulates the development of other plants or organisms [45, 46].

On the other hand, organic and volatile compounds may be responsible for some phytotoxic effects in plants. One has only a few indications of possible phytotoxic compounds, such as eicosane [47] and 2-phenylmethyl [48].

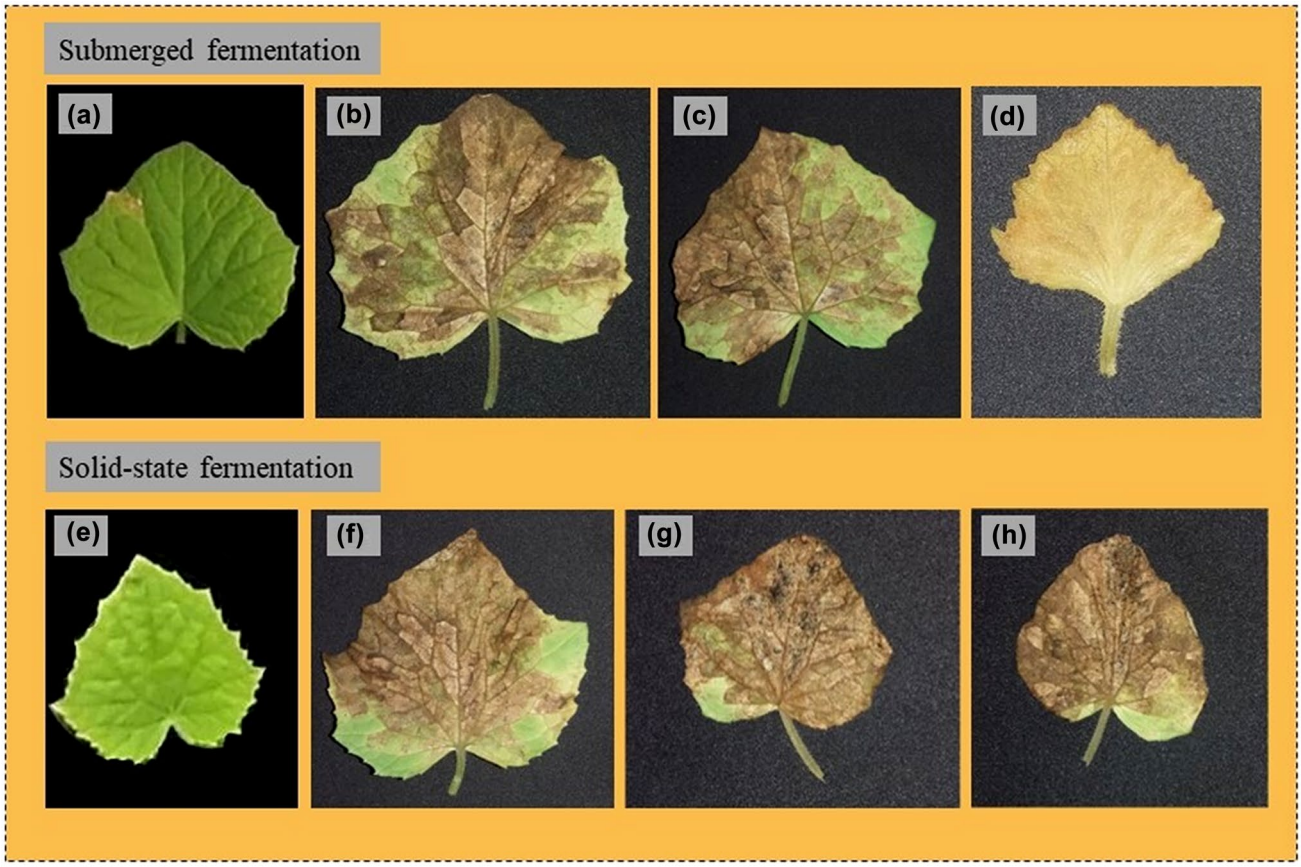


Fig. 1 Phytotoxic effect of extracts obtained by submerged fermentation: control A (**a**) and of the fungi *Trichoderma koningiopsis* (**b**), *Fusarium denticulatum* in consortium with *Mucor circinelloides* (**c**), and *Fusarium* sp (**d**). Phytotoxic effect of extracts obtained by solid fermentation: control B (**e**) and of the fungi *Trichoderma koningiopsis* (**f**), *Fusarium denticulatum* in consortium with *Mucor circinelloides* (**g**) and *Fusarium* sp. (**h**) (after 14 days of application on *Cucumis sativus*)

Table 4 Compounds identified in the derivatized ethanolic extract of *Trichoderma koningiopsis* from submerged fermentation in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular formula	Mol Wt
1	4.17	Heptane, 2,2,4-trimethyl	86	0.81	C10H22	142
2	6.33	Propanoic acid, 2hydroxy	96	5.91	C9H22O3Si2	234
3	16.66	Tetradecane	93	1.64	C14H30	198
4	21.06	Heptadecane	94	1.29	C17H36	240
5	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	90	3.13	C16H22O4	278
6	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	89	2.20	C17H24O3	276
7	25.00	Eicosane	93	1.11	C20H42	282
8	27.10	Hexadecanoic acid	88	41.65	C19H40O2Si	328
9	29.67	3,7,11,15-tetramethyl-2hexadecen-1-ol	87	1.14	C23H48OSi	368
10	30.70	17-Octadecynoic acid	72	1.54	C21H40O2Si	352
11	31.12	Octadecanoic acid	92	31.66	C21H44O2Si	356
12	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	92	2.70	C25H54O4Si	474
13	42.72	2-Monopalmitin	80	1.99	C25H54O4Si2	474
14	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	92	3.23	C27H58O4Si	502

Table 5 Compounds identified in the derivatized ethanolic extract of *Fusarium* sp. from submerged fermentation in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular formula	Mol Wt
1	4.01	Ethylamine	81	1.60	C8H23NSi2	189
2	4.17	Heptane, 2,2,4-trimethyl	82	0.35	C10H22	142
3	4.30	Ethylene glycol	90	10.15	C8H22O2Si2	206
4	6.22	1,3 Propanediol	89	0.42	C9H24O2Si2	220
5	6.33	Propanoic acid, 2hydroxy	95	6.16	C9H22O3Si2	234
6	11.38	3-Acetoxybutyric acid	77	0.66	C9H18O4Si	218
7	11.66	Dodecane, 4,6-dimethyl	92	0.47	C14H30	198
8	11.86	Glycerol	95	3.30	C12H32O3Si	308
9	16.66	Tetradecane	93	1.50	C14H30	198
10	17.62	Hexadecane	92	0.65	C16H34	226
11	17.76	1,3-Benzoxazol-2-amine	77	1.69	C13H22N2O	278
12	21.06	Heptadecane	93	1.32	C17H36	240
13	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	91	2.19	C16H22O4	278
14	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	79	1.04	C17H24O3	276
15	25.00	Eicosane	91	0.94	C20H42	282
16	27.10	Hexadecanoic acid	95	35.36	C19H40O2Si	328
17	30.70	17-Octadecynoic acid	72	2.35	C21H40O2Si	352
18	31.12	Octadecanoic acid	93	22.60	C21H44O2Si	356
19	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	91	2.79	C25H54O4Si	474
20	42.72	2-Monopalmitin	83	1.18	C25H54O4Si2	474
21	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	91	3.27	C27H58O4Si	502

Table 6 Compounds identified in the derivatized ethanolic extract of *Fusarium denticulatum* in consortium with *Mucor cicinelloides* from submerged fermentation in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular formula	Mol Wt
1	4.30	Ethylene glycol	86	36.98	C8H22O2Si2	206
2	6.33	Propanoic acid, 2hydroxy	96	4.49	C9H22O3Si2	234
3	11.38	3-Acetoxybutyric acid	76	1.13	C9H18O4Si	218
4	11.66	Dodecane, 4,6-dimethyl	93	0.49	C14H30	198
5	11.86	Glycerol	94	2.13	C12H32O3Si	308
6	16.66	Tetradecane	91	1.46	C14H30	198
7	17.62	Hexadecane	90	0.61	C16H34	226
8	17.76	1,3-Benzoxazol-2-amine	77	1.72	C13H22N2O	278
9	21.06	Heptadecane	94	1.27	C17H36	240
10	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	90	1.72	C16H22O4	278
11	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	79	0.86	C17H24O3	276
12	25.00	Eicosane	89	0.96	C20H42	282
13	27.10	Hexadecanoic acid	95	24.60	C19H40O2Si	328
14	30.70	17-Octadecynoic acid	72	2.67	C21H40O2Si	352
15	31.12	Octadecanoic acid	93	12.74	C21H44O2Si	356
16	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	91	2.61	C25H54O4Si	474
17	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	92	3.56	C27H58O4Si	502

Table 7 Compounds identified in the derivatized ethanolic extract of the solid fermentation control in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular formula	Mol Wt
1	3.49	Butanoic acid, 2-methyl	92	0.85	C8H18O2Si	174
2	3.64	Butanoic acid, 3-methyl	94	0.92	C8H18O2Si	174
3	4.30	Ethylene glycol	89	26.96	C8H22O2Si2	206
4	5.85	cis-2-Hexen-1-ol	80	2.05	C9H20OSi	172
5	5.94	Butane, 2,3-dihydroxy	93	0.52	C10H26O2Si	234
6	6.22	1,3 Propanediol	92	0.82	C9H24O2Si2	220
7	6.33	Propanoic acid, 2hydroxy	95	4.71	C9H22O3Si2	234
8	6.50	Hexanoic acid	93	0.55	C9H20O2Si	188
9	7.38	2-ethyl-1-decanol	82	0.62	C15H34OSi	258
10	10.30	2-phenylethyl	94	1.42	C11H18OSi	194
11	11.38	3-Acetoxybutyric acid	77	1.45	C9H18O4Si	218
12	11.86	Glycerol	94	2.54	C12H32O3Si	308
13	12.74	Nonanoic acid	85	1.77	C12H26O2Si	230
14	13.18	Pyrimidine, 2,4dihydroxy	94	3.64	C10H20N2O	256
15	14.69	Pyrimidine, 5-methyl2,4-dihydroxy	91	1.54	C11H22N2O	270
16	16.66	Tetradecane	92	1.37	C14H30	198
17	17.76	1,3-Benzoxazol-2-amine	77	1.35	C13H22N2O	278
18	21.06	Heptadecane	94	1.17	C17H36	240
19	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	91	1.57	C16H22O4	278
20	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	82	1.07	C17H24O3	276
21	25.00	Eicosane	91	0.80	C20H42	282
22	27.10	Hexadecanoic acid	95	20.22	C19H40O2Si	328
23	30.70	17-Octadecynoic acid	72	3.47	C21H40O2Si	352
24	31.12	Octadecanoic acid	91	12.18	C21H44O2Si	356
25	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	90	3.36	C25H54O4Si	474
26	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	91	3.08	C27H58O4Si	502

The action of each of the identified compounds is not sure since the bioherbicidal activity is possibly the result of the interactions between all the phytotoxins present in each extract [49, 50] since new fungal molecules may present different mechanisms of action that may even be unknown until now [51].

Among the identified compounds, linolenic acid is a compound that, if applied at lower concentrations, can stimulate plant growth. This is a positive point for a selective bioherbicide if its formulation can reduce the growth of invasive plants and promote the development of crops of agronomic interest [14, 52, 53]. In this scenario, we would encompass a healthier form of production if compared to conventional techniques, using a product that is both a biostimulant and a bioregulator for plants.

Linolenic acid is one of the main precursors in the biosynthetic pathway that leads to plant jasmonates. The jasmonate group of plant hormones acts mainly in plant defense processes and is essential concerning the development and growth of plants, serving in several methods such as the induction of ethylene biosynthesis; retard or inhibit plant growth by blocking glucose incorporation; stimulating stomatal closure; induce or inhibit seed germination due to altered sensitivity; under stress conditions, it can increase plant tolerance to pathogens and pests and attract natural enemies because they are volatile compounds. For the synthesis of jasmonates, the presence of linolenic acid is essential. For this reason, plants with concentrations of this substance may have greater efficiency in the signaling system, increasing tolerance to stress [54, 55].

Linolenic acid and other fatty acids can be incorporated into structural elements in the lipid bilayers of the cell membrane and cuticular wax, helping plants to reduce water loss and pathogen invasion. Studies have shown that linolenic acid concentrations may indicate increased lipid biosynthesis in plants. Furthermore, the study demonstrated that all treated plants exhibited higher total concentrations of fatty acids, meaning the biostimulating effects of microalgae extracts on the lipid profile of the plants [54, 55].

Table 8 Compounds identified in the derivatized ethanolic extract of *Trichoderma koningiopsis* from solid fermentation in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular formula	Mol Wt
1	4.10	Heptane, 2,2,4-trimethyl	88	0.43	C10H22	142
2	4.30	Ethylene glycol	90	11.24	C8H22O2Si2	206
3	6.22	1,3 Propanediol	92	0.53	C9H24O2Si2	220
4	6.33	Propanoic acid, 2hydroxy	95	4.71	C9H22O3Si2	234
5	11.38	3-Acetoxybutyric acid	77	1.57	C9H18O4Si	218
6	11.86	Glycerol	94	1.90	C12H32O3Si	308
7	13.18	Pyrimidine, 2,4dihydroxy	93	1.09	C10H20N2O	256
8	16.66	Tetradecane	93	1.25	C14H30	198
9	17.62	Hexadecane	89	0.49	C16H34	226
10	17.76	1,3-Benzoxazol-2-amine	77	1.69	C13H22N2O	278
11	21.06	Heptadecane	93	1.00	C17H36	240
12	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	91	1.27	C16H22O4	278
13	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	85	1.45	C17H24O3	276
14	25.00	Eicosane	91	0.69	C20H42	282
15	26.63	Alpha-linolenic acid	89	14.81	C21H38O2Si	350
16	27.10	Hexadecanoic acid	93	40.46	C19H40O2Si	328
17	30.58	9,12-octadecadienoic acid (Z,Z)	85	6.08	C21H40O2Si	352
18	30.70	17-Octadecynoic acid	72	2.08	C21H40O2Si	352
19	31.12	Octadecanoic acid	91	2.58	C21H44O2Si	356
20	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	90	2.27	C25H54O4Si	474
21	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	92	2.41	C27H58O4Si	502

Table 9 Compounds identified in the derivatized ethanolic extract of *Fusarium* sp. from solid fermentation in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular formula	Mol Wt
1	4.30	Ethylene glycol	90	09.16	C8H22O2Si2	206
2	6.33	Propanoic acid, 2hydroxy	95	2.27	C9H22O3Si2	234
3	11.38	3-Acetoxybutyric acid	77	0.93	C9H18O4Si	218
4	11.86	Glycerol	94	1.84	C12H32O3Si	308
5	13.18	Pyrimidine, 2,4dihydroxy	94	1.74	C10H20N2O	256
6	14.69	Pyrimidine, 5-methyl2,4-dihydroxy	93	1.24	C11H22N2O	270
7	16.66	Tetradecane	94	1.02	C14H30	198
8	17.76	1,3-Benzoxazol-2-amine	77	1.28	C13H22N2O	278
9	21.06	Heptadecane	93	0.75	C17H36	240
10	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	90	1.38	C16H22O4	278
11	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	88	1.47	C17H24O3	276
12	25.00	Eicosane	91	0.67	C20H42	282
13	26.63	Alpha-linolenic acid	90	16.54	C21H38O2Si	350
14	27.10	Hexadecanoic acid	92	29.38	C19H40O2Si	328
15	30.58	9,12-octadecadienoic acid (Z,Z)	92	12.90	C21H40O2Si	352
16	30.70	17-Octadecynoic acid	72	3.57	C21H40O2Si	352
17	31.12	Octadecanoic acid	91	8.28	C21H44O2Si	356
18	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	91	2.58	C25H54O4Si	474
19	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	92	3.00	C27H58O4Si	502

Table 10 Compounds identified in the derivatized ethanolic extract of *Fusarium denticulatum* in consortium with *Mucor circinelloides* from solid fermentation in GC–MS

N°	RT (Min.)	Compound name	Quality (%)	% of total	Molecular Formula	Mol Wt
1	4.30	Ethylene glycol	90	13.03	C8H22O2Si2	206
2	6.22	1,3 Propanediol	95	1.32	C9H24O2Si2	220
3	6.33	Propanoic acid, 2hydroxy	96	5.56	C9H22O3Si2	234
4	10.91	Diethylene glycol	92	0.61	C10H26O3Si2	250
5	11.38	3-Acetoxybutyric acid	77	1.41	C9H18O4Si	218
6	11.66	Undecane,3,8-dimethyl	92	0.41	C13H28	184
7	11.86	Glycerol	93	2.03	C12H32O3Si	308
8	13.18	Pyrimidine, 2,4dihydroxy	94	2.59	C10H20N2O	256
9	14.69	Pyrimidine, 5-methyl2,4-dihydroxy	93	1.88	C11H22N2O	270
10	16.66	Tetradecane	92	1.58	C14H30	198
11	17.76	1,3-Benzoxazol-2-amine	77	2.10	C13H22N2O	278
12	21.06	Heptadecane	93	1.32	C17H36	240
13	23.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	91	2.53	C16H22O4	278
14	24.86	7,9-Di-tert-butyl-1oxaspiro(4,5)deca-6,9diene-2,8-dione	80	1.10	C17H24O3	276
15	25.00	Eicosane	91	0.86	C20H42	282
16	26.63	Alpha-linolenic acid	89	8.25	C21H38O2Si	350
17	27.10	Hexadecanoic acid	93	29.24	C19H40O2Si	328
18	30.70	17-Octadecynoic acid	72	3.92	C21H40O2Si	352
19	31.12	Octadecanoic acid	91	10.78	C21H44O2Si	356
20	39.92	Hexadecanoic acid, 2,3dihydroxy propyl ester	91	4.25	C25H54O4Si	474
21	43.26	Octadecanoic acid, 2,3dihydroxy propyl ester	93	5.23	C27H58O4Si	502

Conclusion

Microalgae biomass can be considered an alternative culture medium to synthetic ones for fermentative purposes, rich in nutrients and with added value, taking into account its obtaining and recycling process.

The fungi used in this study highlight the relevance of research in bioprospecting new microbial strains with biotechnological potential. The microorganisms demonstrated pertinent characteristics, such as good enzyme producers and ease in adapting to different fermentative conditions and an alternative substrate. The enzyme production from the fungal strains and the microalgae biomass offered suitable conditions to obtain an enzyme cocktail for bioherbicidal purposes.

Through the preliminary tests performed on *Cucumis sativus*, it can be inferred that the microbial strains are potential biological control agents. Studies still need to be outlined regarding the impact of the permanence of these bioherbicides in the soil and the survival period of residues from the application of these extracts in the environment, besides the adaptation of these applications to the field.

With the characterization of the extracts, it was possible to obtain important information about the fermented extracts, identify some compounds, and associate their probable phytotoxicity effects to understand better the mechanisms of action involved in the final bioherbicidal activity.

Finally, some challenges were overcome in turning successful wild strains into viable bioprocesses. Results suggest that fungal extracts can be applied in weed control as bioherbicides, presenting the possibility of the full-scale application shortly, providing farmers and scientists with tools to control weeds innovatively and more sustainably.

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Author contributions AFC, GF, and HT conceived and designed the study. AFC analyzed the data. AFC and CB drafted the manuscript. CD, AU, TS, CB, NK, WM, and LL helped in the experimental part and carefully revised the manuscript. SLAJ, MAT, and OB helped isolate strains from the intestines of caterpillars. SLAJ, AJM, MAT, OB,

SRP, GF, HT critically reviewed and supervised the development of the paper. All authors reviewed and approved the final manuscript.

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Data availability The datasets generated for this study are available on request to the corresponding author.

Declarations

Conflict of interest There are no competing interests.

Ethical approval and consent to participate Not applicable.

Consent for publication All authors agreed with this publication.

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