HPU2 Journal of Sciences:
Natural Sciences and Technology

journal homepage: https://sj.hpu2.edu.vn

Article type: Research article

Antifungal activity of purple amomum (*Amomum longiligulare* T.L. Wu) plant extracts against *Fusarium oxysporum* Schlecht. and *Helminthosporium sativum* P. K. & B.

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Abstract

Purple amomum (*Amomum longiligulare* T.L. Wu), which belongs to the monophyletic Zingiberaceae family, is a valuable medicinal plant in Vietnam. The studies on the biological activities of the extracts or essential oils of this species are very limited. So far, there has been no evaluation of the antifungal activity of purple amomum extracts. *Fusarium oxysporum* Schlecht. and *Helminthosporium sativum* P. K. & B. fungi which are the causal agent of a wide variety of cereal and many economically important crop species diseases, causing significant losses to agricultural production. The use of plant extracts to control these fungi is an alternative to synthetic fungicides. The attempt of this study was to investigate the antifungal activity of ethanol extracts from different organs of purple amomum against both fungi species. The antifungal effect of purple amomum extracts (including ethanol extract of leaf, seed, rhizome and root, and pseudo-stem) concentrations of 0.05 and 0.10 mg/mL were investigated. Our present study showed that the extracts of purple amomum had different effects on the two fungi species. Extracts of 0.10 mg/mL seed and 0.10 mg/mL leaf in most of the treatments showed the best antifungal effect on both studied fungal species. In all the treatments using seed extract, the lowest indicator of the specific growth rate (μ) of the fungi was observed. On the other hand, the antifungal effect of purple amomum extracts on *H. sativum* fungus appeared to be better than on *F. oxysporum* fungus. The results of this study open up the prospect of developing new fungicides from purple amomum extracts to treat diseases caused by *F. oxysporum* and *H. sativum* fungi in crops.

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https://doi.org/10.56764/hpu2.jos.2022.1.2.52-62

Received date: 23-12-2022 ; Revised date: 26-12-2022 ; Accepted date: 28-12-2022

Keywords: Purple amomum, phytopathogenic fungi, *Fusarium oxysporum*, *Helminthosporium sativum*, percent inhibition, colony growth

1. Introduction

Fusarium Link is a genus of fungi in the family Nectriaceae Tul. & Tul. C., widely distributed in soil and associated with plants. The taxonomy of this genus is complex and inconsistent in taxonomic systems. Many species of this genus (*Fusarium oxysporum* being one of these) are pathogens of crops, costing agricultural production around the world millions of dollars each year [1]. *F. oxysporum* (Schlecht. emend. Snyder & Hansen) is a species complex that comprises soil-borne species, varieties and forms fungi, found in cultivated or fallow soils in a variety of climates [2-6]. Although most fungal strains of the *F. oxysporum* complex are harmless or even beneficial plant endophytes, many strains are pathogens of many agricultural crops. They can cause wilting or rot of roots and tops on many economically valuable crops, such as bananas, cotton, soybeans, melons, tomatoes, gerberas, and orchids, etc. [7].

Similar to *F. oxysporum*, *Helminthosporium sativum* P. K. & B. (Syn. *Cochliobolus sativus* Drechsler ex Dastur) which is the causal agent of a wide variety of cereal diseases. It attacks all parts of the plants from roots to seeds, at all stages of plant growth. Depending on the site of infection, it causes diseases such as spot blotch, seedling blight, foot rot, crown rot and root rot on cereals [8].

In fact, to treat diseases caused by *F. oxysporum* and *H. sativum* infections on agricultural crops, agrochemicals are often used. Agrochemicals have made an important contribution to global agricultural production, and without them, the yields of crops will be reduced by more than 30% [3]. However, the uncontrolled use of these agrochemicals often causes undesirable consequences such as ecological imbalance of the soil, reduced resistance of crops to pests and diseases, and the arising of new strains of fungi that are difficult to control [9]. Therefore, it is necessary to create new disease management systems that are less dependent on chemicals and safer for the environment [10].

Recent reports of antifungal and phytotoxic activities in many plant species have opened up a potential solution to this problem of creating biopesticides derived from extracts of plant species [11]. These plant extracts can be rapidly degraded in soil thereby reducing their environmental impact, and they can have an effective role in sustainable agriculture [12].

Purple amomum (*Amomum longiligulare* T.L. Wu), which belongs to the monophyletic Zingiberaceae family, is a valuable medicinal plant in Vietnam [13, 14]. The purple amomum contains essential oils with many valuable chemical compounds, such as α-humulene, pinene, caryophyllene, limonene, camphor, borneol, and saponins [15–17]. In Vietnamese traditional medicine, dried purple amomum is used to treat flatulence in pregnant women, diarrhea, indigestion, vomiting, abdominal pain, toothache, and numbness, etc [13, 18]. Besides, its fruits are not only widely used clinically but also often used as a type of condiment. However, the studies on the biological activities of the extracts or essential oils of this species are very limited. Several compounds isolated from the pseudo-stem and fruit possessed an immune enhancement ability [19-22]. Essential oils extracted from the leaf, rhizome, and fruit of purple amomum were shown to exhibit antibacterial properties against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* with the minimum inhibitory concentration (MIC) values ranging from 200 to 400 μg/mL [23]. There has been no evaluation of the antifungal activity of purple amomum extracts.

The overall goal of the present study was to investigate the antifungal activity of ethanol extracts of different organs of purple amomum against *F. oxysporum* and *H. sativum*. Thereby, the potential of purple amomum in the development of biopesticides to treat diseases caused by *F. oxysporum* and *H. sativum* in crop plants was determined.

2. Materials and methods

2.1. Plant and fungus material

Mature purple amomum plants were collected from the forest on the rocky mountain (Lat 22° 46' 08.9" N and Long 104° 59' 18.4" E) at the Lang Cung village, Dao Duc commune, Vi Xuyen district, Ha Giang province, Vietnam, during August 2020. After being collected, plant materials were carried to the Botanical laboratory, Hanoi Pedagogical University 2. Their leaf, pseudo-stem, rhizome, root, and seed were rinsed with tap water 3-4 times, then again with distilled water twice. In the next step, they were dried at 40°C using a Memmert UF55 universal oven and ground into powder in liquid nitrogen. These powders were preserved by vacuum packing, transported to the biotechnology laboratory of the Moscow Agricultural Academy, and stored at room temperature (25±2°C) [24, 25]. A voucher specimen (n. QA001) was deposited at the herbarium of Hanoi Pedagogical University 2, Vietnam. Botanical identification was achieved by Dr. Minh-Tam Ha.

F. oxysporum (strain M-10-1 isolated from wheat plants, 2009, Moscow region) and *H. sativum* (strain M-20-1 isolated from wheat plants, 2009, Moscow region) fungi were provided by the biotechnology laboratory of the Moscow Agricultural Academy.

2.2. Antifungal activity assays

In preparation for antifungal assay, extracts of each plant material powder were prepared according to the following steps: in the first step, 2.0 grams of plant material powder were homogenized with 20.0 mL of 96% cold ethanol for 5 min with a pestle and mortar. Homogenized material was extracted at room temperature for 1 hour and then filtered with Whatman no.1 filter paper twice to obtain an extract solution. In the second step, the extract solution was poured into a pre-weighed empty test tube and left open for a week to evaporate the alcohol. The stock extract was left at the bottom of the test tube after it was evaporated. Such a test tube was weighed, and by subtracting the weight of the empty test tube from this value, the amount of extracted substance was determined [24, 25]. In the third step, the stock extract in the tube was dissolved in 0.03% (v/v) aqueous solution of dimethyl sulfoxide (DMSO) and added in different concentrations (0.05 and 0.10 mg/mL) to the Murashige and Skoog (MS) medium [26]. MS medium containing 0.03% DMSO and without plant extracts was used as a control. In the last step, these media were poured into separate petri dishes. After solidification, the mycelium plugs (2 mm in diameter) of *F. oxysporum* and *H. sativum* were placed at the center of the separate dishes. After incubation for various periods of time (7 days for *F. oxysporum* and 10 days for *H. sativum*) at 25±2°C and a 16 h-photoperiod under illumination with white fluorescent lamps (brand “OSRAM AG”, production - Germany), with light intensity 3000 lux., the diameter colony diameter (mm) was measured. The antifungal activity of the purple amomum extracts was calculated in terms of percent inhibition (I) of colony growth according to the following equation [27]:

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100$$

where, C and T represent the diameter of the fungal colony on control medium and extract-added media, respectively.

On the other hand, the specific daily growth rate (μ) of fungus was calculated according to the following equation [28]:

$$\mu = (\ln D2 - \ln D1)/(t2 - t1)$$

where, D1 and D2 represent the diameter of the fungal colony on the control medium or extract-added media at a moment of time, t1 and t2, respectively.

2.3. Statistical processing of experimental results

Five replicates were maintained in a randomized design for each treatment. Mean values of all data were calculated using Microsoft Excel 2013 (Microsoft Corporation, USA). Analysis of variance (ANOVA) was performed using Statistica version 10.0 and means were compared using Fisher's Least Significant Difference (LSD) test at a significance level of $p \leqslant 0.05$.

3. Results

3.1. Antifungal effect of purple amomum extracts on colony growth of F. oxysporum at seven days after incubation

The antifungal effect of ethanol extracts of leaf, seed, pseudo-stem, and rhizome and root from purple amomum on the colony growth of *F. oxysporum* was determined (Table 1, Figure 1, 2). Ethanol extracts of purple amomum caused a significant ($p \leq 0.05$) decrease or inhibition in the colony growth of *F. oxysporum*. The general trend in all treatments showed that the antifungal effect of purple amomum extracts on *F. oxysporum* increased where concentration of these extracts was increased in the medium (Table 1). The effect was clear at seven days after incubation.

Table 1. Antifungal effect of purple amomum extracts on colony growth of *F. oxysporum* at seven days after incubation at 25°C

Extract type	Concentration (mg/mL)	Days of incubation					
		2	3	4	5	6	7
Rhizome and Root	0.05	15.2 ± 0.44 [1]	28.2 ± 0.60	38.5 ± 0.87	46.1 ± 1.69	55.1 ± 1.35	66.2 ± 1.29 b (8.3)
	0.10	14.8 ± 1.07	26.9 ± 1.02	36.8 ± 1.12	44.1 ± 0.24	52.9 ± 1.20	62.9 ± 1.36 bc (12.9)
Pseudo-stem	0.05	15.7 ± 0.19	28.6 ± 0.41	39.2 ± 0.29	47.0 ± 0.43	56.2 ± 0.64	67.5 ± 0.98 ab (6.5)
	0.10	14.9 ± 0.68	26.0 ± 1.03	35.9 ± 1.17	42.4 ± 1.22	50.2 ± 1.43	59.8 ± 2.60 cd (17.2)
Seed	0.05	13.7 ± 0.19	24.6 ± 0.67	33.6 ± 0.43	39.8 ± 0.47	47.2 ± 0.59	56.2 ± 2.27 def (22.2)
	0.10	13.2 ± 0.64	23.1 ± 0.27	31.2 ± 0.29	37.0 ± 0.40	44.0 ± 2.76	51.6 ± 1.60 f (28.5)
Leaf	0.05	14.1 ± 0.95	24.8 ± 0.54	34.1 ± 0.83	40.6 ± 1.07	48.3 ± 2.09	57.3 ± 2.47 de (20.6)
	0.10	13.4 ± 0.48	23.5 ± 0.34	32.1 ± 0.46	37.9 ± 0.33	45.0 ± 1.08	53.0 ± 1.20 ef (26.6)
Control		15.9 ± 1.17	29.8 ± 0.89	41.5 ± 0.52	50.1 ± 0.82	60.5 ± 0.19	72.2 ± 0.68 a (0.0)
$LSD_{0.05}$							4.85

[1] *Means (colony diameter, mm) ± standard error (SE); means followed by the same letter are not significantly different at p ≤ 0.05 according to the Fisher's Least Significant Difference (LSD) test. Values in parentheses are percent of mycelial growth inhibition over the control.*

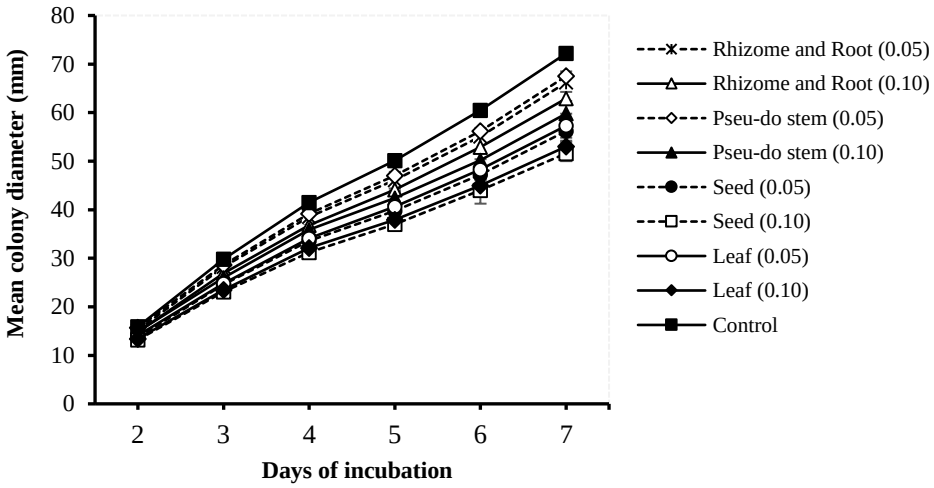


Figure 1. Fungal colony growth of *F. oxysporum* on extract-added media compared with control after seven days of incubation at 25°C.

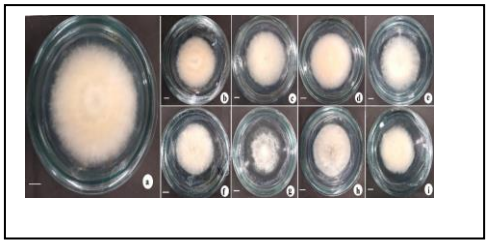


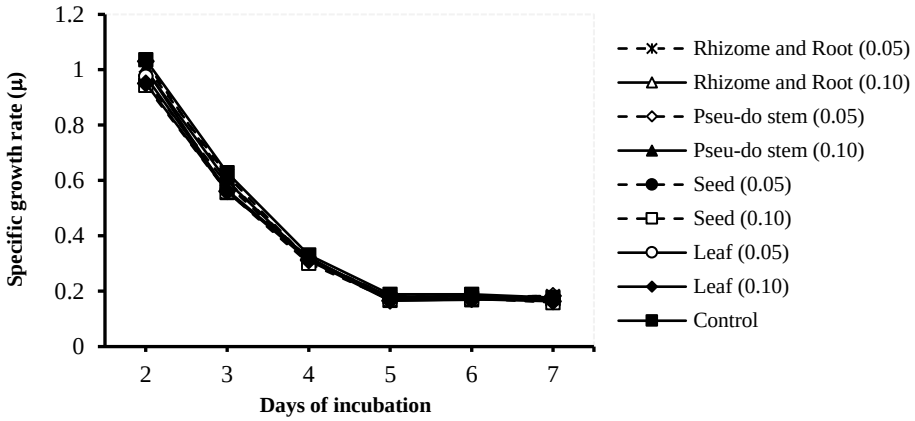
Figure 2. *F. oxysporum* colony after seven days of incubation at 25°C on media supplemented with different extracts of purple amomum: a) Control; b) Rhizome and Root (0.05 mg/mL); c) Rhizome and Root (0.10 mg/mL); d) Pseudo-stem (0.05 mg/mL); e) Pseudo-stem (0.10 mg/mL); f) Seed (0.05 mg/mL); g) Seed (0.10 mg/mL); h) Leaf (0.05 mg/mL); i) Leaf (0.10 mg/mL). Scale bars = 1 cm.

On the other hand, results also indicated that the studied extracts had different antifungal effects on the colony growth of *F. oxysporum*. In descending order, the antifungal activities of the studied extracts were seed, leaf, rhizome and root, and pseudo-stem, respectively. Significant inhibitory effect on the colony growth of *F. oxysporum* was obtained using 0.10 mg/mL seed extract. In this treatment, a 28.5% reduction in the colony growth of *F. oxysporum* was observed compared to the control at seven days after incubation. In addition, the lowest indicator of the daily growth rate (μ) of the *F. oxysporum* fungus was observed in this treatment (Table 2, Figure 3).

Table 2. Effect of extracts obtained from various organs of purple amomum on the specific daily growth rate (μ) of the *F. oxysporum* at seven days after incubation at 25°C

Extract type	Concentration (mg/mL)	Days of incubation					
		2	3	4	5	6	7
Rhizome and Root	0.05	1.01	0.62	0.31	0.18	0.18	0.18
	0.10	1.00	0.60	0.31	0.18	0.18	0.17
Pseudo-stem	0.05	1.03	0.60	0.32	0.18	0.18	0.18
	0.10	1.00	0.56	0.32	0.17	0.17	0.17

Seed	0.05	0.96	0.59	0.31	0.17	0.17	0.17
	0.10	0.94	0.56	0.30	0.17	0.17	0.16
Leaf	0.05	0.98	0.56	0.32	0.17	0.17	0.17
	0.10	0.95	0.56	0.31	0.17	0.17	0.16
Control		1.04	0.63	0.33	0.19	0.19	0.18



Control		1.04	0.63	0.33	0.19	0.19	0.18

Figure 3. Specific daily growth rate (μ) of *F. oxysporum* cultivated *in vitro* on MS medium with the addition of various purple amomum extracts at seven days after incubation at 25°C.

3.2. Antifungal effect of purple amomum extracts on colony growth of H. sativum at ten days after incubation

The antifungal effect of ethanol extracts of leaf, seed, pseudo-stem, and rhizome and root from purple amomum on the colony growth of *H. sativum* was determined (Table 3, Figure 4, 5).

Table 3. Antifungal effect of purple amomum extracts on colony growth of H. sativum at ten days after incubation at 25°C

Extract type	Concentration (mg/mL)	Days of incubation				
		2	4	6	8	10
Rhizome and Root	0.05	11.7 ± 0.58 [1]	21.5 ± 0.70	26.9 ± 0.99	32.2 ± 0.95	37.6 ± 0.67 b (14.4)
	0.10	11.3 ± 0.49	20.5 ± 1.17	25.8 ± 1.32	30.6 ± 1.17	35.9 ± 0.91 bc (18.2)
Pseudo-stem	0.05	11.4 ± 0.54	20.7 ± 0.89	25.1 ± 1.04	29.8 ± 1.06	34.8 ± 0.45 cd (20.7)
	0.10	10.8 ± 0.19	19.4 ± 1.06	23.6 ± 1.25	28.3 ± 1.18	32.9 ± 1.05 de (25.1)
Seed	0.05	10.9 ± 0.37	19.6 ± 0.41	24.1 ± 0.77	28.1 ± 0.79	32.6 ± 0.59 de (25.7)
	0.10	10.3 ± 0.60	18.1 ± 0.98	21.8 ± 0.78	24.9 ± 0.70	28.2 ± 1.09 f (35.8)
Leaf	0.05	11.3 ± 0.66	20.1 ± 1.47	25.2 ± 1.15	28.9 ± 1.11	33.5 ± 0.74 cd (23.7)
	0.10	10.7 ± 0.51	19.1 ± 1.24	23.5 ± 1.21	26.8 ± 1.16	30.2 ± 1.34 ef (31.2)
Control		13.1 ± 0.96	24.1 ± 1.89	30.7 ± 1.61	37.0 ± 1.74	43.9 ± 0.92 a (0.0)
$LSD_{0.05}$						2.52

[1] *Means (colony diameter, mm) ± standard error (SE); means followed by the same letter are not significantly different at $p \leq 0.05$ according to the Fisher's Least Significant Difference (LSD) test. Values in parentheses are percent of mycelial growth inhibition over the control.*

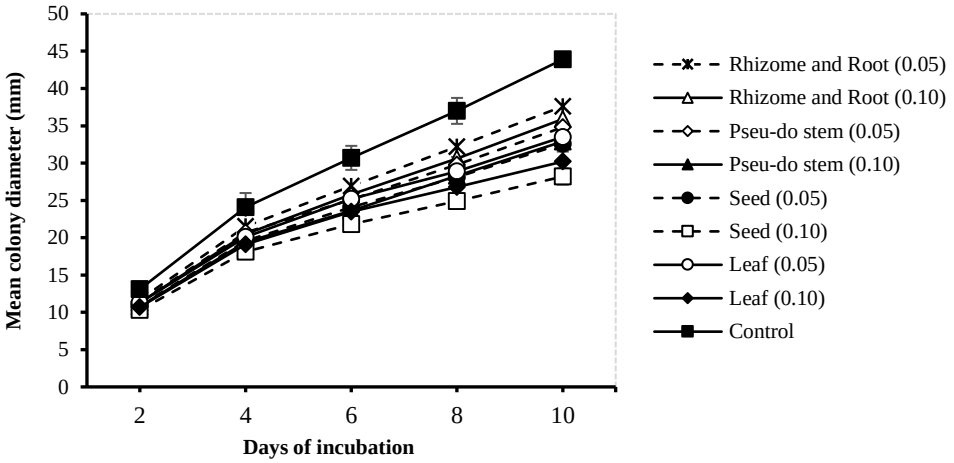


Figure 4. Fungal colony growth of *H. sativum* on extract-added media compared with control after ten days of incubation at 25°C.

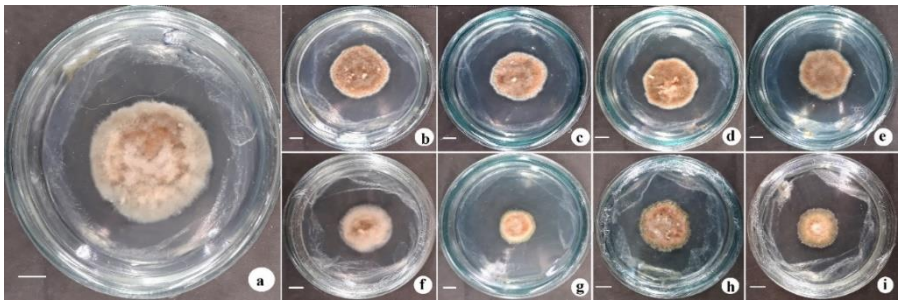


Figure 5. *H. sativum* colony after ten days of incubation at 25°C on media supplemented with different extracts of purple amomum: a) Control; b) Rhizome and Root (0.05 mg/mL); c) Rhizome and Root (0.10 mg/mL); d) Pseudo-stem (0.05 mg/mL); e) Pseudo-stem (0.10 mg/mL); f) Seed (0.05 mg/mL); g) Seed (0.10 mg/mL); h) Leaf (0.05 mg/mL); i) Leaf (0.10 mg/mL). Scale bars = 1 cm.

There was a significant ($p \leq 0.05$) decrease or inhibition in the colony growth of *H. sativum*, similar to what happened to *F. oxysporum*. The general trend in all treatments showed that the antifungal effect of purple amomum extracts on *H. sativum* increased where concentration of these extracts was increased in the medium (Table 3). The effect was clear at ten days after incubation. In descending order, the antifungal activities of the studied extracts were seed, leaf, pseudo-stem, and rhizome and root, respectively. Treatments using seed extract at a concentration of 0.10 mg/mL showed the highest inhibitory effect on fungal colony growth compared with the control at ten days after incubation (reaching 35.8%). In addition, the lowest indicator of the daily growth rate (μ) of the *H. sativum* fungus was observed in treatments using seed and leaf extracts (Table 4, Figure 6).

Table 4. Effect of extracts obtained from various organs of purple amomum on the specific daily growth rate (μ) of the *H. sativum* at ten days after incubation at 25°C.

Extract type	Concentration (mg/mL)	Days of incubation				
		2	4	6	8	10
Rhizome and Root	0.05	0.88	0.30	0.11	0.09	0.08
	0.10	0.87	0.30	0.11	0.09	0.08
Pseudo-stem	0.05	0.87	0.30	0.10	0.09	0.08
	0.10	0.84	0.29	0.10	0.09	0.08
Seed	0.05	0.85	0.29	0.10	0.08	0.07
	0.10	0.82	0.28	0.09	0.07	0.06
Leaf	0.05	0.87	0.29	0.11	0.07	0.07
	0.10	0.84	0.29	0.10	0.07	0.06
Control		0.94	0.30	0.12	0.09	0.09

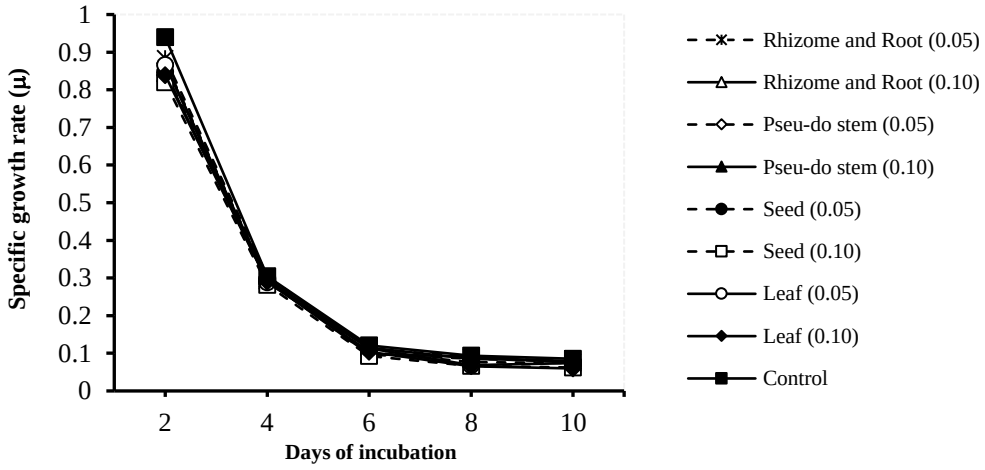


Figure 6. Specific daily growth rate (μ) of *H. sativum* cultivated *in vitro* on MS medium with the addition of various purple amomum extracts at ten days after incubation at 25°C.

4. Discussion

Extracts or essential oils of many plant species of the *Amomum* genus (Ginger family) have been reported to be resistant to many species of fungi, for example, the essential oils extracted from leaf and rhizome of *A. glabrum* were able to resist against *Candida albicans* with MIC values of 10.23 μg/mL [26]; the essential oils extracted from rhizome and stem of *A. rubidum* exhibited significant inhibitory activity on *Aspergillus niger* and *C. albicans* with MIC values of 50 μg/mL [27]; the fruit oils of three different cultivars of *A. subulatum* such as varlangy, seremna and sawney inhibited the growth of

fungal pathogens *C. albicans* (MIC values of 2.5 mg/mL, 1.25 mg/mL, 1.25 mg/mL, respectively) [28]; the fruit oils of *A. subulatum* exhibited significant inhibitory activity on 3 fungal strains (*C. albicans*, *A. niger*, and *Saccharomyces cerevisiae*) [29]; the essential oils isolated from the seeds and rinds of *A. subulatum* showed significant activity against *A. niger* (MIC values of 313 μg/mL and 19.5 μg/mL) [30]; the leaf oils of *A. subulatum* were found effective against two strains of *Aspergillus*

Author contributions

Conceptualization: E.-A. Kalasnikova; methodology: E.-A. Kalasnikova and R.-N. Kirakosyan; validation: E.-A. Kalasnikova, R.-N. Kirakosyan, and T.-H. Nguyen; formal analysis: V.-Q. Khuat and M.-T. Ha; investigation: V.-Q. Khuat and M.-T. Ha; writing original draft: R.-N. Kirakosyan and V.-Q. Khuat; review and editing: E.-A. Kalasnikova, T.-H. Nguyen, and M.-T. Ha. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

We are thankful to the Department of Biotechnology, Russian State Agrarian University—Moscow Timiryazev Agricultural Academy and Department of Biology and Agriculture Engineering, Hanoi Pedagogical University 2.

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