



# Article In Vitro Effect of Purple Amomum (*Amomum longiligulare* T.L. Wu) Extracts on Seed Germination and Seedling Growth of Different Crop Species

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Abstract: The ginger family member purple amomum (Amomum longiligulare T.L. Wu) is an important medicinal plant in Vietnam. Although there have been studies on the chemical composition of essential oils and extracts of purple amomum, as well as their antibacterial, antifungal, activating macrophages, and immune enhancement effects, there is still a lack of evaluation of the phytotoxicity of this plant. In this study, the total content of phenolic (TPC) and flavonoid (TFC) in extracts of leaf, seed, pseudo-stem, rhizome, and root from purple amomum and the phytotoxic effect of these extracts against five test plant species, including four dicotyledonous: camelina (Camelina sativa Crantz), quinoa (Chenopodium quinoa Willd.), cabbage (Brassica oleracea var. capitata L.), tomato (Solanum lycopersicum L. cv. Dubrava), and one monocotyledonous: onion (Allium cepa L. cv. Stuttgarter risen), were investigated. Results showed that the seed and leaf extracts had higher total phenolic and flavonoid contents than the other two extracts (highest TPC value:  $4.30 \pm 0.03$  mg GAE/mg dry weight of seed powder; highest TFC value:  $1.32 \pm 0.07$  mg QE/mg dry weight of leaf powder). Furthermore, it was observed that the extracts of purple amomum inhibited seed germination and the growth of seedlings of all test plant species with different inhibition values. The general trend in all treatments showed that, when increasing the concentration of extracts from 0.10–0.20 mg/mL, the ability to inhibit seed germination, hypocotyl length, radicle length, fresh weight, and dry weight increased. Seed extract at a concentration of 0.20 mg/mL in most treatments showed the highest percentage inhibition of seed germination and growth of seedlings of the tested species. Onion was the most sensitive to purple amomum extracts among the five species tested. Based on these results, we conclude that extracts of different parts of the purple amomum exhibited phytotoxicity for the tested species. Further evaluation of the phytotoxic potential of the extracts on weed species and under field conditions is also recommended for the purpose of developing bio-herbicides for future weed management that are less toxic to the environment and human health.

**Keywords:** purple amomum; ethanol extract; bioassay plant; phytotoxic activity; total phenolic content; total flavonoid content

# 1. Introduction

Most countries around the world rely on synthetic herbicides to control weeds. Their abuse has harmed the environment and human health, so finding alternative weed control



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). technology to traditional methods is an urgent task [1,2]. Phytotoxic plants can help solve the problems created by synthetic herbicides because they possess growth retarding substances [3,4]. A number of plant species have been reported to be phytotoxic to some of the weeds and crops studied [4–7]. Due to the possibility that medical plants may contain more bioactive compounds than other plants, researchers have recently been more and more interested in phytotoxic medicinal plants [8–11].

Zingiberaceae, or the ginger family, has about 50 genera and a total of about 1600 recognized species of aromatic perennial herbs, many of which are significant medicinal plants [12]. Several studies on the phytotoxic effects of extracts and essential oils of Zingiberaceous species have been reported. The aqueous extract of ginger's rhizome, stem, and leaf (Zingiber officinale Rosc.) has been shown to decrease soybean and chives seed germination and seedling growth [13]. According to Pukclai et al. (2013) [14], five test plant species (cress, lettuce, alfalfa, timothy, and crabgrass) were inhibited by krawan (Amomum krervanh Pierre ex Gagnep.) aqueous methanol extracts. According to Satyal et al. (2012) [15], exposure to essential oils from the seeds and rinds of large cardamom (Amonum subulatum Roxb.) hindered the seed germination of two test plant species (lettuce and perennial ryegrass). White turmeric (Curcuma zedoaria Rosc.) essential oil has also been shown to have phytotoxic effects on the seed germination and seedling growth of two bio-indicator species, lettuce achenes and tomato [16]. Ibanez et al. (2019) [17] reported the phytotoxic activity of essential oils extracted from the rhizomes of turmeric (Curcuma longa L.) and ginger (*Zingiber officinale* Rosc.) against five weeds and three food crops, and discovered that turmeric essential oil could be an effective bioherbicide against weeds without being harmful to crops. Most recently, Mahanta et al. (2022) [18] carried out an essential oil screening from the rhizomes of six species of Curcuma, including C. caesia Roxb., C. longa L., C. zedoaria Roscoe, C. amada Roxb., C. angustifolia Roxb., and C. aromatic Salisb. They found that black turmeric (*C. caesia* Roxb.) oil had the most effective growth inhibitor in a wheatgrass bioassay. On the other hand, the phytotoxic actions of groups of compounds extracted from different parts of some ginger species have been reported. Fujita et al. (1994) [19] reported that  $\alpha$ -pyrones from n-hexane and benzene extracts of shell ginger (*Alpinia specioca* K. Schum.) leaves showed inhibitory activity against lettuce seedlings. In a different study, Mongkol et al. (2015) [20] observed that the development of lettuce and Italian ryegrass was significantly inhibited by the 1'-acetoxyl group (1'-acetoxychavicol acetate) obtained from galangal (*Alpinia galanga* Willd.).

The ginger family member purple amomum (*Amomum longiligulare* T.L. Wu) is an important medicinal plant in Vietnam [21,22]. In several phytochemical studies of purple amomum species, the chemical compositions of the volatile oil and non-volatile fractions were determined [21,23–29]. Accordingly, the volatile oil of the purple amomum plant was determined to contain abundant amounts of oxidizing monoterpenes (e.g.,  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, camphene, D-limonene, and camphor, etc.) and sesquiterpenes (e.g., caryophyllene and humulene) [21,26–29]. Other groups of compounds isolated and identified from purple amomum extracts, in addition to essential oils, include phenolic acids, flavonoids, diarylheptanoids, saponins-steroids, tannins, etc. [23–25]. It is thought that a few of these chemicals—including p-hydroxybenzoic, vanillic, p-coumaric, and ferulic acids—are the ones most frequently connected to phytotoxic action [30–32]. In the wild, populations of purple amomum grow and thrive, sometimes invading other nearby plant species. To date, studies on the phytotoxic action of purple amomum species are still lacking.

In the present study, seedling development bioassays were performed to determine the phytotoxic potential of different parts of the purple amomum (including the leaf, pseudo-stem, rhizome and root, and seed) on seed germination and seedling growth of the five bio-indicator species that have short germination times and are sensitive to phytotoxic extracts.

# 2. Materials and Methods

# 2.1. Plant Material

In August 2020, mature purple amonum plants were harvested from the forest on the rocky mountain at Lang Cung village, Vi Xuyen district, Ha Giang province, Vietnam (Lat 22°46′08.9″ N and Long 104°59′18.4″ E). After being collected, plant materials were carried to the botanical laboratory at Hanoi Pedagogical University 2. Their leaf, pseudostem, rhizome, root, and seed were rinsed with tap water three to four 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 vacuum-packed, delivered to the biotechnology laboratory of the Russian State Agrarian University, and stored at room temperature ( $25 \pm 2$  °C). A voucher specimen (n. QA001) was deposited at the herbarium of Hanoi Pedagogical University 2, Vietnam. Botanical identification was achieved by Dr. Ha, M.T.

Four dicotyledonous groups: camelina (*Camelina sativa* Crantz), quinoa (*Chenopodium quinoa* Willd.), cabbage (*Brassica oleracea* var. *capitata* L.), tomato (*Solanum lycopersicum* L. cv. Dubrava), and one monocotyledonous group: onion (*Allium cepa* L. cv. Stuttgarter risen), were selected as test plant species.

## 2.2. Total Content of Phenolic and Flavonoid

#### 2.2.1. Preparation of Extract Solution

Using a pestle and mortar, 10.0 mL of 96% cold ethanol was homogenized with 1 g of powdered plant material for 5 min. The homogenized material was extracted at room temperature for 72 h and then filtered twice with Whatman No. 1 filter paper to obtain the extract solution [33]. (Figure 1).



**Figure 1.** Preparation of extract solution: (**a**) plant material powders are preserved by vacuum packing; (**b**) one gram of each plant material powder; (**c**) the plant material powder is homogenized with ethanol with a pestle and mortar; (**d**) homogenized material is kept at room temperature for 72 h; (**e**) filter with Whatman No.1 filter paper; (**f**) types of extract solutions obtained. Note: (1) leaf powder; (2) seed powder; (3) pseudo-stem powder; (4) rhizome and root powder.

# 2.2.2. Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent method was used to estimate the total phenolic content in the extract solutions of purple amomum [33–37]. A mixture of 200  $\mu$ L of extract solution and 400  $\mu$ L diluted Folin-Ciocalteu reagent (10%) was thoroughly mixed. Next, 1600  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> solution (700 mM) was added and incubated for 2 hrs at room temperature. The OD optical density was read at 765 nm by the UV-Vis spectrophotometer. The standard gallic acid curve was obtained under the same conditions as above using a range of concentrations (including 25, 50, 75, 100, 125, 150, 175, 200, and 250  $\mu$ g/mL). Gallic acid

equivalents ( $\mu$ g gallic acid equivalent GAE/mg dry matter (DM)) were used to measure the total phenolic content.

#### 2.2.3. Determination of Total Flavonoid Content (TFC)

The aluminum chloride method was used to estimate the total flavonoid content in the extract solutions of purple amomum [33,38]. A mixture of 1500  $\mu$ L of extract solution with 75  $\mu$ L of aluminum chloride solution (10%), 75  $\mu$ L of potassium acetate (1 M), and 2100  $\mu$ L distilled water was thoroughly mixed. The mixture was kept at room temperature for 30 min. The optical density reading was taken at 415 nm with a UV-Vis spectrophotometer. The standard quercetin curve was obtained under the same conditions as above using a range of concentrations (including 25, 50, 75, 100, 125, 150, 175, 200, and 250  $\mu$ g/mL). The quercetin equivalent ( $\mu$ g quercetin equivalent QE/mg dry matter (DM)) was used to measure the total flavonoid content.

#### 2.3. Bioassay to Assess Seed Germination and Seedling Growth of the Five Bio-Indicator Species

Bioassays on seed germination and seedling growth of the five bio-indicator species were carried out to ascertain the phytotoxic potential of different parts of the purple amomum, including the leaf, pseudo-stem, rhizome and root, and seed [8,34,35,39–45].

In preparation for each bioassay, extracts of each plant material powder were prepared according to the following steps: In the first step, a certain amount of plant material powder was homogenized in a pestle and mortar for five minutes with 96% cold ethanol in a ratio of one-tenth. Homogenized material was extracted at room temperature for 72 h, followed by filtering twice with Whatman No. 1 filter paper to obtain an extract solution. The second step involved adding the extract solution to an empty, pre-weighed test tube and leaving it open for a week to let the alcohol evaporate. After evaporating, the stock extract was left at the bottom of the test tube (Figure 2a). The test tube was weighed, and the amount of extracted substance was calculated by deducting the weight of the empty test tube from this value. In the last step, the stock extract in the tube was dissolved in a 0.03% (v/v) aqueous solution of dimethyl sulfoxide (DMSO) to obtain extract concentrations of 0.10, 0.15, and 0.20 mg/mL for bioassay [8,33,40]. A bioassay was performed on sterilized 6-well cell culture plates (Figure 2b). Seeds of the tested plants were surface sterilized in a 1:10 (v/v) dilution of sodium hypochlorite (NaOCl) for 5 min and rinsed several times with distilled water. Thirty-six seeds of each tested plant were placed on two layers of paper in separate plates (six seeds per well) (Figure 2c). A total of 1 mL of the extracts at different concentrations (0.10, 0.15, and 0.20 mg/mL) were applied to each plate. A control culture plate was treated with 1 mL of a 0.03% (v/v) aqueous solution of DMSO [33]. Three replicates were maintained in a randomized design for each bioassay experiment.



**Figure 2.** Preparation of germination bioassay: (**a**) a test tube contains the stock extract at the bottom; (**b**) a sterilized 6-well cell culture plate; (**c**) seeds of the five bio-indicator species are sown in separate plates (six seeds per well).

All culture plates were put in a bright room with a temperature of  $25 \pm 2$  °C and a 16-h photoperiod, and the lighting was provided by white fluorescent lamps with a light

intensity of 3000 lux that were made in Germany under the "OSRAM AG" brand. Germination was counted every two days until the 12th day. The radicle emergence of the seeds was recorded as germinated [46]. Three germination parameters, including germination percentage (GP), mean germination time (MGT), and germination rate index (GRI), were determined according to the formulas mentioned in previous studies [8,34,35,39–48]. On the 12th day, radicle length, hypocotyl length, and the fresh weights of the tested seedlings were also measured. Subsequently, the dry weights of the seedlings were determined by drying them in an oven at 60 °C for 24 h. Equation (1), given by Kordali et al. (2009) [49], was used to calculate the inhibitory or stimulatory percentage (I, %):

$$(I, \%) = \left(\frac{\text{control} - \text{extracts}}{\text{control}}\right) \times 100 \tag{1}$$

#### 2.4. Statistical Processing of Experimental Results

Microsoft Excel 2013 (Microsoft Corporation, Redmond, Washington, DC, USA) was used to calculate the means of all the data. In Statistica Version 10.0 software (StatSoft, Inc., Tulsa, OK, USA), analysis of variance (ANOVA) was carried out, and means were compared using Duncan's multiple range test at a significance level of  $p \le 0.05$ .

#### 3. Results

#### 3.1. Total Content of Phenolic and Flavonoid

Gallic acid was used as a standard to determine the total phenolic content of the extract. An experiment was conducted to establish a standard curve between the concentration of gallic acid and the increase in optical density value with the Folin-Ciocalteu reagent. The results showed that there was a positive relationship between the content of gallic acid and the increase in the optical density measurement value produced when reacting with the Folin-Ciocalteu reagent: Y = 0.0141X - 0.0078,  $R^2 = 0.9975$ , and *p*-value < 0.001 (Figure S1). When reacting with the Folin-Ciocalteu reagent; this correlation function was used to convert the increase in optical density value produced by the extracts. The results showed that seed extract had the highest total phenolic content at [( $4.30 \pm 0.03$ ) µg GAE/mg DM], followed by leaf, pseudo-stem, and rhizome and root extracts, which were ( $2.43 \pm 0.02$ ) µg GAE/mg DM, ( $1.80 \pm 0.01$ ) µg GAE/mg DM, and ( $1.63 \pm 0.01$ ) µg GAE/mg DM, respectively (Figure 3).



#### Extract solution type

**Figure 3.** Total content of phenolic and flavonoid of purple amonum. Means (total content phenolic or flavonoid) with a different letter (s) within the bars differ significantly at a 0.05 probability level using Duncan's multiple range test.

Quercetin was used as a standard to determine the total flavonoid content of the extract. An experiment was also conducted to establish a standard curve between the concentration of quercetin and the increase in optical density value with aluminum chloride. The results showed that there was a positive relationship between the content of quercetin and the increase in the optical density measurement value produced when reacting with the aluminum chloride in the presence of acetate solution: Y = 0.0246X - 0.0169,  $R^2 = 0.9974$ , and the *p*-value < 0.001 (Figure S2). When reacting with the aluminum chloride, this correlation function was used to convert the increase in optical density value produced by the extracts. The results indicated that the leaf extract had the highest flavonoid content, which was [( $1.32 \pm 0.07$ ) µg QE/mg DM], followed by the seed, pseudo-stem, and rhizome and root extracts, which were ( $1.05 \pm 0.01$ ) µg QE/mg DM, ( $0.79 \pm 0.01$ ) µg QE/mg DM, and ( $0.60 \pm 0.02$ ) µg QE/mg DM, respectively (Figure 3).

#### 3.2. Effect of Purple Amomum Plant Extracts on Seed Germination of Five Bio-Indicator Species

The data obtained in this study showed that purple amomum extracts at the studied concentrations had significant ( $p \le 0.05$ ) effects on all calculated germination indices. Purple amomum extracts caused a significant ( $p \le 0.05$ ) decrease or inhibition in the germination percentage (GP) of camelina, quinoa, cabbage, tomato, and onion (Table 1). Among these test species, onion was the most affected by inhibition. Purple amomum extracts did not show significant inhibition on the seed germination of camelina seeds except for the seed extract at a concentration of 0.20 mg/L. For the remaining three species, the inhibitory effect of the extracts was moderate. The general trend in all treatments showed that, when increasing the concentration of extracts from 0.10–0.20 mg/mL, the ability to inhibit germination increased. Seed extract at a concentration of 0.20 mg/mL in all treatments showed the highest percentage inhibition of seed germination of the tested species (camelina, 2.8%; quinoa, 9.3%; cabbage, 19.8%; tomato, 23.5%; onion, 50.3%).

Extract Type	Concentration $mg mL^{-1}$	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	100 a* ( <b>0.0</b> )	$93.5\pm0.9~\text{bc*}~\textbf{(6.5)}$	$75.0 \pm 1.6 \text{ cde}**$ (9.9)	72.2 ± 4.2 ab** ( <b>3.8</b> )	25.0 ± 1.6 bcde** ( <b>29.0</b> )
Leaf	0.15	$99.1\pm0.9$ ab (0.9)	$92.6\pm0.9$ c (7.4)	72.2 $\pm$ 1.6 def (13.2)	$65.7\pm4.6$ abc (12.5)	$20.4\pm1.8$ defg (42.3)
	0.20	$98.1\pm0.9$ ab (1.9)	$90.7\pm0.9$ c ( <b>9.3</b> )	$70.4\pm2.5$ efg (15.4)	$61.1\pm2.8$ bc (18.5)	$18.5\pm2.4$ fg (47.7)
Seed	0.10	100 a ( <b>0.0</b> )	$93.5\pm0.9$ bc (6.5)	$74.1\pm0.9$ cdef ( <b>11.0</b> )	$69.4\pm4.2$ abc (7.5)	$22.2\pm1.6~\text{defg}~\textbf{(37.0)}$
	0.15	$99.1\pm0.9$ ab (0.9)	$93.5\pm0.9$ bc (6.5)	$69.4\pm1.6$ fg (16.5)	$67.6\pm4.0$ abc (10.0)	$19.4\pm1.6$ efg (44.9)
	0.20	$97.2\pm1.6~b~\textbf{(2.8)}$	$90.7\pm0.9$ c ( <b>9.3</b> )	$66.7\pm3.2~g~\textbf{(19.8)}$	$57.4\pm3.3$ c (23.5)	$17.6\pm1.8$ g (50.3)
	0.10	100 a (0.0)	99.1 $\pm$ 0.9 a (0.9)	$81.5\pm0.9$ ab (2.2)	$72.2 \pm 4.2 \text{ ab} (3.8)$	$28.7 \pm 1.9$ bc (18.5)
Pseudo-stem	0.15	100 a ( <b>0.0</b> )	97.2 $\pm$ 1.6 a ( <b>2.8</b> )	$78.7\pm0.9$ abc (5.4)	$71.3\pm4.6~\text{ab}~(\textbf{5.0})$	$25.9\pm1.9$ bcd (26.4)
	0.20	100 a ( <b>0.0</b> )	97.2 $\pm$ 1.6 a ( <b>2.8</b> )	$75.9 \pm 0.9 \text{ cd} (8.8)$	$69.4\pm4.2$ abc (7.4)	$24.1\pm2.5~\text{cdef}~\textbf{(31.8)}$
	0.10	100 a ( <b>0.0</b> )	$99.1\pm0.9$ a (0.9)	$82.4\pm0.9$ ab (1.1)	$74.1\pm3.3$ ab (1.2)	$30.6 \pm 1.6 \text{ ab} (13.2)$
Rhizome and root	0.15	100 a ( <b>0.0</b> )	99.1 $\pm$ 0.9 a ( <b>0.9</b> )	$78.7\pm0.9$ abc (5.5)	$73.2\pm4.0$ ab ( <b>2.6</b> )	$25.0 \pm 1.6$ bcde ( <b>29.0</b> )
	0.20	100 a ( <b>0.0</b> )	96.3 $\pm$ 1.9 ab (3.7)	77.8 $\pm$ 0.0 bc (6.6)	71.3 $\pm$ 3.3 ab (5.0)	$24.1 \pm 2.5 \text{ cdef } (31.8)$
Control		100 a ( <b>0.0</b> )	100 a ( <b>0.0</b> )	83.3 ± 1.6 a ( <b>0.0</b> )	$75.0\pm3.2$ a (0.0)	35.2 ± 0.9 a ( <b>0.0</b> )

**Table 1.** Effect of purple amomum plant extracts on seed germination percentage (GP, %) of five bio-indicator species.

(\*, \*\*) Means (%, mean  $\pm$  SE) followed by the same letter within a column are not significantly different at  $p \le 0.05$  according to the Duncan's multiple range test. (\*) Values of germination percentage were square root transformed prior to statistical analysis. (\*\*) Values of germination percentage were arcsin  $\sqrt{X}$  transformed prior to statistical analysis. Values in parentheses are inhibition percentages over the control.

7 of 15

On the other hand, the data obtained in this study also showed that increasing the concentration of extracts increased the MGT value and decreased the GRI value (Tables 2 and 3). The higher the GRI value compared to the control, the lower the inhibition, and vice versa. However, the meaning is reversed for the MGT value. According to these two indices, a significant inhibitory effect was observed in all of the treatments conducted. In all five species, the highest value of MGT and the lowest value of GRI were observed using the seed extract at a concentration of 0.20 mg/mL. In contrast, the lowest values of MGT and the highest values of GRI were observed when using the pseudo-stem or rhizome and root extract at a concentration of 0.10 mg/mL.

**Table 2.** The effect of purple amonum plant extracts on mean germination time (MGT, days) of five bio-indicator species.

Extract Type	Concentration mg mL $^{-1}$	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	$2.80\pm0.08~cde*$	$3.80\pm0.07~cd$	$3.48\pm0.05~bcd$	$5.10\pm0.04~bcd$	$5.72\pm0.17~\mathrm{cde}$
Leaf	0.15	$2.92\pm0.06~bcd$	$3.96\pm0.09~bc$	$3.56\pm0.03~\text{abc}$	$5.18\pm0.01~\text{ab}$	$6.17\pm0.08~\mathrm{ab}$
	0.20	$3.00\pm0.02~ab$	$4.08\pm0.12~ab$	$3.68\pm0.08~\text{a}$	$5.27\pm0.03~\mathrm{a}$	$6.27\pm0.17~\mathrm{a}$
Seed	0.10	$2.78\pm0.03~de$	$3.92\pm0.05bc$	$3.50\pm0.07~bcd$	$5.04\pm0.02~cde$	$5.74\pm0.14~\rm cd$
	0.15	$2.95\pm0.03~bc$	$4.12\pm0.03~\text{ab}$	$3.57\pm0.06~\text{abc}$	$5.12\pm0.02~bc$	$6.08\pm0.08~abc$
	0.20	$3.10\pm0.05~a$	$4.24\pm0.07~\mathrm{a}$	$3.61\pm0.04~\text{ab}$	$5.29\pm0.05~\mathrm{a}$	$6.42\pm0.08~\mathrm{a}$
	0.10	$2.56\pm0.03~\text{fg}$	$3.23\pm0.08~\text{f}$	$3.36\pm0.04~de$	$4.95\pm0.04~\text{ef}$	$5.47\pm0.09~def$
Pseudo-stem	0.15	$2.74\pm0.07~\mathrm{e}$	$3.58\pm0.10~de$	$3.43\pm0.05~bcde$	$5.02\pm0.03~\mathrm{cde}$	$5.63\pm0.09~def$
	0.20	$2.80\pm0.07~\mathrm{cde}$	$3.66\pm0.12~de$	$3.49\pm0.05~bcd$	$5.07\pm0.04~bcde$	$5.83\pm0.09~bcd$
	0.10	$2.48\pm0.05~g$	$3.27\pm0.06~\text{f}$	$3.28\pm0.05~\text{ef}$	$4.88\pm0.03~\text{f}$	$5.33\pm0.07~\mathrm{ef}$
Rhizome and root	0.15	$2.57\pm0.05~\text{fg}$	$3.46\pm0.02~ef$	$3.34\pm0.06~def$	$4.96\pm0.06~\text{ef}$	$5.48\pm0.16~def$
	0.20	$2.67\pm0.06~\text{ef}$	$3.52\pm0.01~\mathrm{e}$	$3.40\pm0.06~\text{cde}$	$4.99\pm0.07~def$	$5.68\pm0.17~\mathrm{de}$
Control		$2.04\pm0.02~h$	$2.24\pm0.04~g$	$3.18\pm0.07~\text{f}$	$4.76\pm0.03~g$	$5.26\pm0.06~\text{f}$

(\*) Means (days, mean  $\pm$  SE) followed by the same letter within a column are not significantly different at  $p \le 0.05$  according to Duncan's multiple range test.

**Table 3.** The effect of purple amomum plant extracts on the germination rate index (GRI) of five bio-indicator species.

Extract Type	Concentrration mg mL $^{-1}$	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	$15.36\pm0.20~def*$	$11.55\pm0.12~\mathrm{e}$	$10.03\pm0.06~cd$	$5.56\pm0.32~abc$	$1.82\pm0.20~\text{cd}$
Leaf	0.15	$14.93\pm0.26~efg$	$11.10\pm0.03~efg$	$9.64\pm0.02~def$	$5.03\pm0.39~bcd$	$1.32\pm0.12~efg$
	0.20	$14.49\pm0.22~\mathrm{gh}$	$10.69\pm0.13~\mathrm{gh}$	$9.24\pm0.23$ ef	$4.58\pm0.25~cd$	$1.18\pm0.15~\text{fg}$
Seed	0.10	$15.32\pm0.10~\text{def}$	$11.31\pm0.03$ ef	$9.83\pm0.21~de$	$5.39\pm0.34~abcd$	$1.61\pm0.14~def$
	0.15	$14.81\pm0.13~\text{fgh}$	$10.89\pm0.04~\mathrm{fgh}$	$9.34\pm0.08$ ef	$5.20\pm0.30~abcd$	$1.28\pm0.10~\text{fg}$
	0.20	$14.26\pm0.30~h$	$10.34\pm0.23~h$	$8.98\pm0.16~\text{f}$	$4.37\pm0.27~d$	$1.11\pm0.11~{\rm g}$
	0.10	$16.15\pm0.10~bc$	$13.71\pm0.13~\text{b}$	$10.89\pm0.32~ab$	$5.73\pm0.32~ab$	$2.26\pm0.11bc$
Pseudo-stem	0.15	$15.68\pm0.14~cd$	$12.69\pm0.35~\mathrm{d}$	$10.53\pm0.14bc$	$5.54\pm0.38~\mathrm{abc}$	$1.80\pm0.13~\mathrm{cd}$
	0.20	$15.49\pm0.11~\mathrm{de}$	$12.54\pm0.44~d$	$10.17\pm0.11~\rm cd$	$5.36\pm0.38~\mathrm{abcd}$	$1.64\pm0.16~def$
	0.10	$16.49\pm0.17\mathrm{b}$	$13.82\pm0.06~\text{b}$	$11.03\pm0.34~ab$	$5.93\pm0.27~\mathrm{ab}$	$2.43\pm0.11~ab$
Rhizome and root	0.15	$16.18\pm0.17\mathrm{bc}$	$13.35\pm0.07~bc$	$10.54\pm0.24bc$	$5.74\pm0.33~ab$	$1.87\pm0.18~\mathrm{cd}$
	0.20	$15.88\pm0.19~cd$	$12.81\pm0.29~cd$	$10.33\pm0.17bcd$	$5.61\pm0.29~\mathrm{abc}$	$1.75\pm0.21~\mathrm{de}$
Control		$17.83\pm0.08~\mathrm{a}$	$17.08\pm0.10~\mathrm{a}$	$11.35\pm0.39~\text{a}$	$6.20\pm0.18~\mathrm{a}$	$2.83\pm0.11~\mathrm{a}$

(\*) Means (mean  $\pm$  SE) followed by the same letter within a column are not significantly different at  $p \le 0.05$  according to Duncan's multiple range test.

### 3.3. Effect of Purple Amomum Plant Extracts on Seedling Growth of Five Bio-Indicator Species

Radicle length, hypocotyl length, fresh weight, and dry weight per seedling of five test species were used to evaluate the phytotoxic activity of purple amonum extracts. Similar to germination, the results showed that purple amonum extracts had a significant effect ( $p \le 0.05$ ) on the seedling growth of camelina, quinoa, cabbage, tomato, and onion (Tables 4 and 5; Figures S3–S7).

**Table 4.** Effect of purple amomum plant extracts on hypocotyl length (mm) of five bio-indicator species.

Extract Type	Concentration mg mL <sup><math>-1</math></sup>	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	$8.4\pm0.13$ c* (28.2)	$10.4\pm0.42~\text{bcd}$ (29.8)	$7.5\pm0.06$ bc (4.6)	$9.4\pm0.09$ b (26.2)	$\textbf{22.3}\pm0.36~\text{b}~\textbf{(33.2)}$
Leaf	0.15	$7.2\pm0.11$ ef ( <b>38.1</b> )	$9.7\pm0.17$ cdef (34.5)	$7.0\pm0.07$ ef (11.2)	$8.9\pm0.08$ bc (29.9)	$15.9 \pm 1.07$ e ( <b>52.4</b> )
	0.20	$7.0\pm0.06$ f (39.6)	$8.5\pm0.09$ ef (42.5)	$6.6\pm0.05$ g (15.9)	$8.2\pm0.09$ c (35.7)	$11.5\pm1.05$ f (65.7)
	0.10	$8.6\pm0.12$ c (25.9)	$9.3\pm0.39$ def (37.6)	$7.4\pm0.13$ cd (6.1)	$8.9\pm0.04$ bc (29.7)	$\textbf{22.6}\pm0.03~b~\textbf{(32.3)}$
Seed	0.15	$8.4\pm0.16$ c (27.6)	$8.4\pm0.19$ ef (43.6)	$6.7\pm0.05$ fg (14.9)	$8.8\pm0.10$ bc (30.5)	$16.8\pm0.64$ de (49.8)
	0.20	$7.6\pm0.08$ de (34.8)	$8.2\pm0.33~\text{f}~\text{(45.1)}$	$6.6\pm0.19$ g (16.5)	$8.0\pm0.07$ c (37.0)	$10.8\pm0.77~\mathrm{f}$ (67.7)
Pseudostem	0.10	$9.4\pm0.16$ b (19.1)	$11.7\pm0.67$ b (20.9)	$7.8\pm0.05$ a (0.5)	$9.4\pm0.26$ b (26.2)	$31.0\pm0.91$ a (7.2)
	0.15	$9.4\pm0.08$ b (19.3)	$9.7\pm0.45$ cdef (34.7)	$7.4\pm0.08$ cd (6.5)	$9.0\pm0.07$ bc (29.0)	$20.1\pm0.74$ bc (39.8)
	0.20	$8.0\pm0.18$ cd ( <b>31.1</b> )	$9.4\pm0.50$ def (36.5)	$7.1\pm0.08$ de (10.0)	$8.9\pm0.11$ bc ( <b>29.6</b> )	$18.5\pm0.35$ cde (44.7)
	0.10	$9.3\pm0.10\text{ b}\text{ (20.1)}$	$11.1\pm0.69$ bc (25.0)	$7.8\pm0.14$ a (0.9)	$9.7\pm0.20$ b (23.3)	$\textbf{22.6} \pm \textbf{0.84} \text{ b} \textbf{ (32.4)}$
Rhizome and root	0.15	$8.5\pm0.14$ c (27.0)	$10.0\pm0.31$ cde ( <b>32.8</b> )	$7.2\pm0.03$ de ( <b>9.2</b> )	$9.3 \pm 0.12$ b ( <b>26.5</b> )	$19.0\pm1.01~\text{cd}~\textbf{(43.1)}$
	0.20	$8.1\pm0.09$ c (30.1)	$9.2\pm0.78$ def ( <b>38.1</b> )	$7.0\pm0.11$ ef (11.7)	$9.1\pm0.10$ bc (28.2)	17.0 $\pm$ 0.82 de (49.1)
Control		$11.6\pm0.46$ a (0.0)	$14.9\pm1.03$ a (0.0)	$7.9\pm0.22$ a (0.0)	$12.7\pm1.15$ a (0.0)	$33.4 \pm 1.31$ a ( <b>0.0</b> )

(\*) Means (mm, mean  $\pm$  SE) followed by the same letter within a column are not significantly different at  $p \le 0.05$  according to Duncan's multiple range test. Values in parentheses are inhibition percentages over the control.

**Table 5.** The effect of purple amomum plant extracts on radicle length (mm) of five bio-indicator species.

Extract Type	Concentration mg mL $^{-1}$	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	$41.4\pm1.93$ c* (34.4)	$14.6\pm0.73$ de* (44.9)	$36.8\pm0.96$ cd* (26.7)	$30.0\pm0.65~ab*~\textbf{(4.8)}$	$12.6\pm0.75$ c* (39.6)
Leaf	0.15	$32.2\pm1.23~\text{def}~(\textbf{49.0})$	$13.5 \pm 0.66$ e ( <b>48.9</b> )	$35.8 \pm 1.50$ d (28.7)	$24.3\pm0.53$ de ( <b>22.9</b> )	$12.3\pm1.15$ c (41.3)
	0.20	$30.6\pm0.74$ ef (51.5)	$13.0\pm0.52$ e ( <b>50.9</b> )	$30.8 \pm 1.21$ e ( <b>38.6</b> )	22.9 $\pm$ 1.11 ef (27.2)	$7.4\pm0.55$ d (64.6)
	0.10	$44.1\pm1.67~\mathrm{c}~\textbf{(30.1)}$	$16.4\pm0.37~\mathrm{cd}~(38.1)$	$36.0 \pm 1.29$ d ( <b>28.4</b> )	$27.5\pm0.51$ bc (12.6)	$7.3\pm0.51$ d (65.4)
Seed	0.15	$32.4\pm0.41$ de (48.7)	$14.8\pm0.98$ de (44.2)	$32.0 \pm 2.15$ e ( <b>36.3</b> )	$22.8\pm1.06$ ef (27.6)	$6.5\pm0.79$ d (69.0)
	0.20	$28.5\pm0.61~\text{f}~\textbf{(54.9)}$	$12.9\pm0.55~\mathrm{e}~(\textbf{51.1})$	$30.5\pm2.00$ e ( <b>39.4</b> )	$21.4\pm0.82~\text{f}~\textbf{(32.1)}$	$3.4\pm0.60$ e (83.8)
Pseudo-stem	0.10	$51.9 \pm 1.82$ b (17.7)	$18.5\pm0.77$ bc (30.1)	$42.6\pm0.78\:b\:(\textbf{15.1})$	$30.9\pm1.10$ a (1.9)	$18.9\pm0.37$ ab (9.6)
	0.15	$41.8\pm0.92\ \text{c}\ \textbf{(33.7)}$	$17.4\pm1.29$ c (34.3)	$40.1\pm0.86$ bc (20.2)	$30.5\pm0.85$ a (3.3)	$18.2\pm0.17b~\textbf{(13.1)}$
	0.20	$33.5 \pm 0.58$ de ( <b>46.9</b> )	$14.4\pm0.46$ de (45.6)	$36.7\pm0.66$ cd (27.0)	$27.8\pm1.21~\text{bc}~(\textbf{11.8})$	$6.6\pm0.21~\textrm{d}~\textbf{(68.4)}$
Rhizome and	0.10	$50.8\pm1.16$ b (19.4)	$20.3\pm0.74~b~\textbf{(23.4)}$	$46.9\pm1.11$ a ( <b>6.6</b> )	$31.1\pm0.17$ a ( <b>1.1</b> )	$11.7\pm0.93$ c (44.0)
root	0.15	$41.5\pm1.94$ c (34.3)	17.7 $\pm$ 0.87 c (33.1)	$37.1\pm0.56~\mathrm{cd}$ (26.2)	$26.7\pm0.68$ cd (15.2)	$10.5\pm0.34$ c (50.0)
	0.20	$35.1\pm0.94~d~\textbf{(44.3)}$	14.1 $\pm$ 0.72 de (46.8)	$36.3\pm0.37$ cd (27.7)	$26.5\pm0.56$ cd (15.8)	$6.2\pm0.64$ d (70.5)
Control		$63.1\pm0.46$ a ( <b>0.0</b> )	$26.5\pm0.79$ a ( <b>0.0</b> )	$50.3\pm0.78$ a (0.0)	$31.5\pm0.81$ a (0.0)	$20.9\pm1.32$ a (0.0)

(\*) Means (mm, mean  $\pm$  SE) followed by the same letter within a column are not significantly different at  $p \le 0.05$  according to Duncan's multiple range test. Values in parentheses are inhibition percentages over the control.

The results showed that purple amomum extract showed inhibitory activity on shoot and root growth of all five tested species, and the degree of inhibition increased with increasing extract concentration (Tables 4 and 5). In most of the treatments, the leaf and seed extracts showed significantly higher inhibitory effects on hypocotyl length and radicle length than the other two extracts at the same concentration (Figures S3–S7). Seed extracts at a concentration of 0.20 mg/mL in most treatments showed the highest percentage of inhibition for hypocotyl length and radicle length (hypocotyl: camelina, 34.8%; quinoa, 45.1%; cabbage, 16.5%; tomato, 37.0%; onion, 67.7%; radicle: camelina, 54.9%; quinoa, 51.1%; cabbage, 39.4%; tomato, 32.1%; onion, 83.8%).

On the other hand, purple amomum extracts resulted in a decrease in the fresh weight and dry weight of the seedlings of the five tested species (Tables 6 and 7). In all plants tested, the control treatment showed the maximum fresh weight and dry weight. The general trend showed that increasing the concentration of the extracts reduced the fresh weight and dry weight of the seedlings. Seed extracts at a concentration of 0.20 mg/mL reduced the fresh weight and dry weight of the seedlings the most in most of the treatments.

**Table 6.** The effect of purple amomum plant extracts on the fresh weight (mg) of five bio-indicator species.

Extract Type	Concentration mg mL $^{-1}$	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	9.48 ± 0.14 bc* (17.4)	8.38 ± 0.04 de ( <b>25.0</b> )	29.39 ± 0.42 bc (24.5)	17.98 ± 1.21 ab ( <b>3.0</b> )	11.94 ± 0.66 bc ( <b>34.6</b> )
Leaf	0.15	9.28 ± 0.11 bcd ( <b>19.2</b> )	8.28 ± 0.11 e ( <b>25.9</b> )	27.62 ± 1.34 bcde ( <b>29.1</b> )	$15.34 \pm 0.56 \text{ cd}$ (17.2)	9.82 ± 0.26 d ( <b>46.2</b> )
	0.20	8.76 ± 0.13 cde (23.7)	8.08 ± 0.07 ef (27.7)	23.43 ± 1.66 f ( <b>39.8</b> )	13.77 ± 0.64 de ( <b>25.7</b> )	6.52 ± 0.46 e ( <b>64.3</b> )
	0.10	9.47 ± 0.09 bc (17.5)	8.39 ± 0.07 de ( <b>24.9</b> )	29.27 ± 0.48 bc (24.8)	16.64 ± 0.38 abc ( <b>10.2</b> )	10.89 ± 0.25 cd ( <b>40.4</b> )
Seed	0.15	8.35 ± 0.17 de (27.3)	7.72 ± 0.10 fg ( <b>30.9</b> )	24.47 ± 1.30 ef ( <b>37.1</b> )	$14.61 \pm 0.43$ cde (21.2)	9.77 ± 0.14 d ( <b>46.5</b> )
	0.20	7.98 ± 0.03 e ( <b>30.5</b> )	$\begin{array}{c} 7.54 \pm 0.18 \text{ g} \\ \textbf{(32.5)} \end{array}$	$\begin{array}{c} 20.05 \pm 0.06 \text{ g} \\ \textbf{(48.5)} \end{array}$	12.77 ± 0.43 e ( <b>31.1</b> )	6.49 ± 0.54 e ( <b>64.5</b> )
	0.10	10.97 ± 0.44 a (4.4)	9.38 ± 0.09 b ( <b>16.0</b> )	$30.34 \pm 0.37$ b (22.1)	18.53 ± 0.59 a ( <b>0.0</b> )	18.27 ± 0.88 a ( <b>0.0</b> )
Pseudo-stem	0.15	9.89 ± 0.07 b ( <b>13.9</b> )	8.72 ± 0.03 cd (22.0)	28.86 ± 1.17 bcd (25.9)	16.29 ± 0.67 abc ( <b>12.1</b> )	12.65 ± 0.93 b ( <b>30.7</b> )
	0.20	9.51 ± 0.18 bc (17.2)	8.19 ± 0.10 e ( <b>26.7</b> )	26.47 ± 1.25 cdef ( <b>32.0</b> )	15.75 ± 0.26 bcd ( <b>15.0</b> )	9.93 ± 0.20 d ( <b>45.6</b> )
	0.10	10.85 ± 0.76 a (5.5)	9.25 ± 0.17 b (17.2)	$29.54 \pm 0.70 \mathrm{bc}$ (24.1)	18.53 ± 1.22 a ( <b>0.0</b> )	13.47 ± 0.53 b ( <b>26.3</b> )
Rhizome and root	0.15	$\begin{array}{c} 9.86 \pm 0.15 \text{ b} \\ \textbf{(14.1)} \end{array}$	8.79 ± 0.26 c ( <b>21.3</b> )	27.48 ± 0.29 bcde ( <b>29.4</b> )	$18.05\pm0.76~\mathrm{ab}$ (2.6)	$10.13 \pm 0.48 \text{ d}$ (44.5)
	0.20	9.39 ± 0.31 bc (18.2)	8.37 ± 0.05 de (25.1)	25.76 ± 1.79 def (33.8)	16.79 ± 0.90 abc (9.4)	9.22 ± 0.10 d (49.5)
Control		11.48 ± 0.58 a ( <b>0.0</b> )	11.17 ± 0.15 a ( <b>0.0</b> )	38.93 ± 0.81 a ( <b>0.0</b> )	18.53 ± 0.52 a ( <b>0.0</b> )	18.27 ± 0.67 a (0.0)

(\*) Means (mg, mean  $\pm$  SE) followed by the same letter within a column are not significantly different at  $p \le 0.05$  according to Duncan's multiple range test. Values in parentheses are inhibition percentages over the control.

**Table 7.** The effect of purple amomum plant extracts on the dry weight (mg) of five bio-indicator species.

Extract Type	Concentration mg m $L^{-1}$	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	1.33 ± 0.04 b* ( <b>24.2</b> )	1.63 ± 0.07 a ( <b>3.0</b> )	$4.02 \pm 0.08 \text{ cd}$ (19.5)	1.76 ± 0.03 ab (5.7)	$1.64 \pm 0.12$ abc (16.1)
Leaf	0.15	1.31 ± 0.08 b ( <b>25.0</b> )	1.60 ± 0.01 a ( <b>4.8</b> )	3.91 ± 0.07 cd ( <b>21.8</b> )	1.64 ± 0.07 ab ( <b>12.2</b> )	1.28 ± 0.09 cde ( <b>34.5</b> )
	0.20	$1.27 \pm 0.02 \text{ b}$ (27.4)	1.45 ± 0.06 a ( <b>14.1</b> )	3.38 ± 0.17 f ( <b>32.2</b> )	1.59 ± 0.10 ab ( <b>14.8</b> )	1.16 ± 0.20 de ( <b>40.7</b> )

Extract Type	Concentration mg mL <sup><math>-1</math></sup>	Camelina	Quinoa	Cabbage	Tomato	Onion
	0.10	$1.32 \pm 0.03 \text{ b}$ (24.4)	1.62 ± 0.11 a ( <b>3.6</b> )	3.89 ± 0.13 cd ( <b>22.0</b> )	1.75 ± 0.06 ab ( <b>5.9</b> )	1.56 ± 0.12 abcd ( <b>19.8</b> )
Seed	0.15	$1.28 \pm 0.08 \text{ b}$ (26.9)	1.57 ± 0.10 a ( <b>6.9</b> )	$3.44 \pm 0.04 \text{ f}$ (31.2)	$1.61 \pm 0.06 \text{ ab}$ (13.6)	1.22 ± 0.02 cde ( <b>37.4</b> )
	0.20	$1.22 \pm 0.06 \text{ b}$ (30.3)	$1.44 \pm 0.08$ a (14.3)	3.37 ± 0.10 f ( <b>32.5</b> )	1.55 ± 0.06 b (17.0)	1.12 ± 0.15 е ( <b>42.6</b> )
Pseudo-stem	0.10	1.37 ± 0.04 b ( <b>21.7</b> )	1.68 ± 0.11 a ( <b>0.4</b> )	$4.37 \pm 0.10 \text{ b}$ (12.5)	$1.82 \pm 0.04$ ab (2.3)	1.95 ± 0.23 a ( <b>0.2</b> )
	0.15	$1.34 \pm 0.06 \text{ b}$ (23.2)	$1.64 \pm 0.05$ a (2.6)	3.96 ± 0.09 cd ( <b>20.8</b> )	$1.71 \pm 0.17$ ab (8.1)	1.52 ± 0.12 bcde ( <b>22.2</b> )
	0.20	$1.31 \pm 0.06 \text{ b}$ (25.3)	1.62 ± 0.12 a ( <b>3.6</b> )	3.76 ± 0.08 de ( <b>24.7</b> )	$1.63 \pm 0.02$ ab (12.5)	1.30 ± 0.06 cde ( <b>33.3</b> )
	0.10	$1.36 \pm 0.03 \text{ b}$ (22.5)	1.68 ± 0.12 a ( <b>0.4</b> )	$4.18 \pm 0.08  ext{ bc}$ (16.2)	1.86 ± 0.05 a ( <b>0.4</b> )	1.87 ± 0.13 ab ( <b>4.1</b> )
Rhizome and root	0.15	$1.33 \pm 0.07 \text{ b}$ (24.2)	1.66 ± 0.08 a ( <b>1.4</b> )	$3.94 \pm 0.17 \text{ cd}$ (21.1)	1.78 ± 0.15 ab (4.7)	1.48 ± 0.09 bcde ( <b>24.3</b> )
	0.20	$1.29 \pm 0.05 \text{ b}$ (26.3)	$1.65 \pm 0.04$ a (2.0)	$3.51 \pm 0.05 \text{ ef}$ (29.8)	$1.68 \pm 0.07 \text{ ab}$ (10.0)	1.32 ± 0.05 cde ( <b>32.3</b> )
Control		1.75 ± 0.06 a ( <b>0.0</b> )	1.68 ± 0.08 a ( <b>0.0</b> )	4.99 ± 0.09 a ( <b>0.0</b> )	1.86 ± 0.07 a ( <b>0.0</b> )	1.95 ± 0.06 a ( <b>0.0</b> )

Table 7. Cont.

(\*) Means (mg, mean  $\pm$  SE) followed by the same letter within a column are not significantly different at  $p \le 0.05$  according to Duncan's multiple range test. Values in parentheses are inhibition percentages over the control.

#### 4. Discussion

In this study, the total phenolic and flavonoid contents of purple amonum extracts were determined. Although the results showed that the seed and leaf extracts had higher total phenolic and flavonoid contents than the other two extracts, these concentrations were quite low (Figure 3). Similar results were also recorded in ethanol extracts of ginger (Zingiber officinale Rosc.) and cardamom (Elettaria cardamomum L.) (TPC: 10.8 mg GAE/g dry weight and 2.2 mg GAE/g weight, respectively; TFC: 6.5 mg catechin/g weight and 1.43 mg catechin/g weight, respectively) [50]. Nurcholis et al. (2021) [51] determined that the *Amomum compactum* Sol. ex Maton extracts, other species of the genus *Amomum*, also had low TFC values (ethanol extract: 0.19 mg QE/g dry weight; ethyl acetate extract: 2.26 mg QE/g dry weight). A study by Amir et al. (2011) [52] showed that the methanol rhizome extract of ginger had low TPC and TFC values ( $1.45 \pm 0.01$  mg GA/g dry weight and  $0.84 \pm 0.03$  mg QE/g dry weight, respectively). On the other hand, some studies that determined TPC and TFC present in methanol extracts of some ginger species recorded moderate to high values. The methanol rhizome extract of Amonum nilgiricum VP. Thomas & M. Sabu species had a TPC value of 6.96 mg GAE/g dry weight [53], while the leaf extract was  $95.4 \pm 1.19$  mg GAE/g dry weight [54]. A study by Sinitha et al. (2017) [55] showed that the methanol extract rhizome of *Amomum masticatorium* Thwaites had a TPC value of 89.54  $\pm$  4.67 mg GAE/g dry weight and a TFC of 58.28  $\pm$  3.32 mg QE/g dry weight. Through some of the above reports, it can be seen that different species and parts of the ginger family plant have different TPC and TFC values.

Regarding the main body of this study, the results indicated that purple amonum extracts showed phytotoxic effects on camelina, quinoa, cabbage, tomato, and onion. Similar to the reports of many previous authors [8,35,40,45], we found that the phytotoxic effect of purple amonum extract depends on three main factors, including: (1) extract type; (2) extract concentration; and (3) bio-indicator plant.

First, the analysed results showed that the magnitude of the phytotoxicity effects at the same concentration levels in the test plants was dependent on the source of the purple amonum extract (seed, leaf, pseudo-stem, and rhizome and root). In most of the treatments, the seed and leaf extracts had stronger inhibitory properties than the other two extracts (Tables 1–7). The higher TPC and TFC values in seed and leaf are probably responsible for the difference (Figure 3). Phytochemical studies of purple amomum have shown that there are differences in the chemical composition and content of substances in the essential oils and extracts from different parts of the plant. As reported by Do (2001) [23], Liu et al. (2013) [24], and Hao et al. (2014) [25], the seed extract of purple amomum contains a variety of phenolic acids, flavonoids, and diarylheptanoids, with a high potential for phytotoxic actions. These compounds can control the growth and development of recipient plants by reducing their photosynthetic and respiratory capacities [56]. Several studies have also revealed that phenolic acids can severely interfere with metabolism during germination, seedling development, and later growth stages in recipient plants [57–59]. Several authors have reported the high phytotoxicity of leaf extracts on tested plants. Khanh et al. (2005) [60] reported that the herbicide impact on rice weed control of leaf extracts of five medicinal plants and leguminous plants was better than that of root and stem extracts. The percentage inhibition of Aloe ferox Mill. leaf extract against turnip (Brassica rapa L.), beetroot (Beta vulgaris L.), and carrot (Daucus carota L.) was higher than that of the root extract, according to Arowosegbe and Afolayan (2012) [61]. Similarly, Turk and Tawaha (2003) [62] also reported that the leaf extract of black mustard (Brassica nigra L.) had the greatest inhibitory effect, while the stem extract had the least inhibitory effect (the test species is lentil: Polygala tatarinowii Regel). In contrast, according to Han et al. (2008) [13], the ginger rhizome extract was the least inhibitory, while the ginger stem extract demonstrated the maximum inhibition at all dosages.

Secondly, the different concentrations of purple amomum extracts had various inhibitory impacts on the germination and growth of five tested species. According to our study, the lowest concentration of the extracts (0.10 mg/mL) showed a significant inhibitory effect on the initial growth of seedlings of the studied species (via the following evaluation criteria: hypocotyl length, radicle length, and fresh weight). On the other hand, the extracts at this concentration reduced the germination rate and increased the germination time of the seeds of three species, including cabbage, tomato, and onion. For camelina and quinoa, the seed germination inhibitory effect of the extracts at a concentration of 0.10 mg/mL was not observed. At the highest concentrations of the extracts (0.20 mg/mL), most of them showed a clear inhibition of seed germination and early seedling growth in all five species studied (Tables 1–7). On the other hand, the concentrations required for 50% growth inhibition (IC50) in onion species were 0.152-0.195 mg/mL (data not shown). In the remaining four tested species, no IC50 value could be determined. The larger amount of inhibitor in the extract used at high concentrations may be one of the reasons for this difference. This finding is consistent with the results of Randhawa et al. (2002) [63], Batlang et al. (2007) [64], and Ashrafi et al. (2009) [65], who found that the degree of inhibition increased with increasing extract concentration. As reported by Han et al. (2008) [13], aqueous extracts from the rhizome, stem and leaf of ginger at studied concentrations, including 10, 20, 40, and 80 g dry tissue/L, all showed inhibitory effects on seed germination and early seeding growth of soybean and chive. According to this report, at the highest extract concentration (80 g dry tissue/L), the inhibitory effect on seed germination and early seeding growth of the two studied species was maximal. According to Pukclai et al. (2013) [14], methanol extract at a concentration of 30 mg dry weight equivalent extract/mL had an inhibitory effect on the early growth of seedlings of the five species tested (cress, lettuce, alfalfa, timothy, and crabgrass). At a concentration of 100 mg dry weight equivalent extract/mL, the growth and development of seedlings of the test species were almost completely inhibited.

Thirdly, our study showed that the purple amonum extracts inhibited onion growth more strongly than the other four test species. The effects of the extracts on seed germination and seedling development, however, varied. According to our findings, the extracts had the greatest inhibitory effect on onion at the seed germination stage, followed by tomato, cabbage, quinoa, and camelina, in that order. But at the seedling growth stage, quinoa and camelina were more affected than tomato and cabbage, although onion remained the most affected species (Tables 1, 4 and 5). The different sensitivity of different plant species to different compounds may be the reason for the results. According to Kobayashi (2004) [66], the difference in the physiological and biochemical characteristics of each species is the cause of the difference. Differences in seed structure and seed germination physiology may also be responsible [67]. Haq et al. (2010) [68] suggested that allelochemicals in plant extracts may act in different ways (antagonistic or synergistic) on different plant species. Mahdavikia et al. (2015) [35] found that the peppermint plant water extract (WE) exhibited different inhibitory effects against the two horticultural crops (tomato and radish) and three weed species (field bindweed, purslane, and jungle rice). According to Han et al. (2008) [13], the aqueous extract of ginger had a greater effect on chive than on soybean, which suggests species specificity. Pukclai et al. (2013) [14] reported that the aqueous methanol extract of siam cardamom on dicotyledonous plants (cress, lettuce, and alfalfa) were more sensitive to the extract than monocotyledonous plants (timothy and crabgrass). Islam et al. (2013) [69] also indicated that lettuce seeds were more affected by the extracts of lychee (*Litchi chinensis* Sonn.) than they were by barnyard grass.

In addition, radicle growth appeared to be more sensitive to extracts than hypocotyl growth. Many previous reports also showed similar results on other plants [8,13,35]. This may be because radicles are in close contact with the allelochemicals in the extract solution [70].

#### 5. Conclusions

Although purple amomum has been extensively researched for medicinal purposes, there are only a few reports that address these. This is the first report to evaluate the phytotoxicity of purple amomum. The results of this study showed that the five species tested (camelina, quinoa, cabbage, tomato, and onion) were all sensitive to compounds present in the purple amomum extracts. Seed extract at a concentration of 0.20 mg/mL in most treatments showed the highest percentage inhibition of seed germination and growth of seedlings of the tested species. On the other hand, at the seed germination stage, the extracts had the highest inhibitory effect on onion, tomato, cabbage, quinoa, and camelina, in that order. But at the seedling growth stage, quinoa and camelina were more affected than tomato and cabbage, although onion remained the most affected species. However, these are preliminary studies, and further evaluation of the phytotoxic potential of purple amomum extracts on weed species is needed to determine the appropriate concentration that is sufficient to inhibit the germination and growth of weeds while doing little harm to plants. On the other hand, the further evaluation of the phytotoxic potential of the extracts under field conditions is needed. These results suggest that purple amomum could be utilized in the development of bio-herbicides for future weed management that are less toxic to the environment and human health.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9050554/s1.

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