

Article

# Evaluation of Potential Volatile Allelopathic Plants from Bangladesh, with *Sapindus mukorossi* as a Candidate Species

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**Abstract:** This study focuses on identifying volatile emissions from plants with potential plant growth inhibitory activity. The aim of this study was to evaluate plant species from the Asian country Bangladesh for new, potential volatile allelopathic species. A total of 103 plant samples from 40 different families were assessed with the dish pack (DP) method. About 25% of the evaluated plant samples influenced (inhibited or stimulated) the growth of lettuce, due to the presence of potentially volatile allelochemicals. The pericarp of *Sapindus mukorossi* Gaertn. caused the lowest radicle elongation (3% of control) of the lettuce. This was followed by the leaves of *Cassia nodosa* Roxb. (34.4%) and the root of *Kaempferia galangal* L. (43.4%), in that order. Therefore, the pericarp of *S. mukorossi* is reported from this study as a new potential volatile allelopathic species. On the contrary, the leaves of *Gynostemma pentaphyllum* Thunb. had a stimulatory effect on the hypocotyl elongation of lettuce seedlings (156% of control). The single petri dish (SPD), a new method, was also adapted to justify the potentiality of the growth control of particular allelopathic species. This study revealed that the new potentially volatile allelopathic plant species could be further explored in sustainable weed management.

**Keywords:** *Sapindus mukorossi* Gaertn.; dish pack method; single petri dish method; volatile allelopathy; weed management; sustainability

## 1. Introduction

The "2030 Agenda for Sustainable Development" adopted by the United Nations' Sustainable Development Goal emphasized "zero hunger" planning all over the world through sustainable agriculture [1]. Sustainability in agriculture intensifies as an essential implementation of the evaluation and execution of crop cultivation for reliable conservation of agricultural systems. In many developed countries, sustainable farming practices have become a popular concept. However, the concept of sustainable agriculture remains a challenging notion in many developing countries. Bangladesh, a substantially populated, developing Asian country, has intensified agrarian production due to the remarkable food demand coupled with several natural calamities. A survey report from Intergovernmental Panel on Climate Change (IPCC) estimated that by 2050, natural disasters like



cyclones, extreme temperature increase, flooding, and drought could reduce rice and wheat production by 8% and 32%, respectively, when compared to yield in the 1990s [2].

In recent years, improved and health-conscious crop protection technologies, coupled with high food demand with limited arable land, have become an indispensable concern in Bangladesh. Protection of field crops from weeds, diseases, and other crop pests are part of the critical factors for sustainable crop production. Weed management strategy plays a vital role and directly influences food security and productivity worldwide. Studies in 2006 and 2015 reported that about 34% of yield loss occurred due to the interference of weeds compared to other pests [3,4]. Rice is the major food in Bangladesh and mostly cultivated all over the country [5]. Mamun [6] reported that weeds reduced the yield of rice productivity in the range of 68%–100% for Aus rice, 16%–48% in Aman rice, and 22%–36% in Boro rice. Weeds compete directly with the crops for space, nutrition, light, and moisture. Hence, the physiological activity and growth of crop plants are significantly affected by weed interference [4,7].

In managing weed interference, the extensive use of synthetic herbicides have led to the emergence of herbicide resistant/tolerant weeds [8]. Currently, there are about 256 weed species that have developed resistance to different herbicide sites of action [9]. Consequently, there is the need to diversify the herbicide application practices or identify new herbicide sites of action. The natural process of releasing bioactive secondary metabolites from organisms (plants, microbes, insects, fungus, algae), and the subsequent interaction with another organisms, causing beneficial or harmful effects, is referred to as allelopathy [10–13]. The utilization of allelopathic species and allelochemicals for sustainable weed management in agriculture has been explored in various studies [10,14–16]. Allelochemicals are released into the environment through different routes, such as leaching, volatilization, root exudation, and the decomposition of plant residues [12,17]. Using natural products in terms of allelopathy is a primary way of conserving the habitat and managing weeds in agriculture. On the other hand, there are increasing possibilities of developing bio-herbicides from plants that can be used for weed control, in order to minimize the heavy reliance on synthetic herbicides [18].

Consequentially, current research has focused on the prospects of volatile allelopathic species as natural pesticide candidates to promote sustainability in agriculture [19]. In this study, we screened plants for allelopathic species with the ability to release inhibitory bioactive compounds through volatilization. Based on volatile allelochemicals, the commercial herbicide cinmethylin, derived from the natural product 1,4-cineole, was developed [20]. Cinmethylin causes the inhibition of mitosis in meristematic tissue of shoots and roots that selectively controls annual grasses in many crops [21,22]. Also, cinmethylin targets the protein extracts of *Lemna paucicostata* Hegelm. that bind and validate FAT (fatty acid thioesterase) inhibition as a new site of action, different from other lipid biosynthesis inhibitors [23].

Besides, researchers have been exploiting the processes of isolating new bioactive compounds from higher plants, especially from medicinal plants [24]. However, several novel bioactive substances that can lead to the identification of new herbicide sites of action that remain untapped in medicinal plants [25]. Potential allelopathic tree species may promote sustainability in agroecology, including weed control [26,27]. This study focuses on identifying new volatile allelochemical containing species that have the potential to suppress weeds in agricultural farming. Identified allelopathic species can be utilized in sustainable weed management, to minimize any adverse effects of synthetic herbicides [28]. Identification of the new volatile allelopathic species can contribute to the prospect of finding novel volatile allelochemicals, which may promote the development of new natural herbicides for weed management in agriculture.

## 2. Materials and Methods

#### 2.1. Plant Samples and Preparation

Fresh plant samples (different parts of plants) were collected from different areas of the sub-tropical country Bangladesh, in order to evaluate their potential volatile allelopathy. The evaluation of volatile

allelopathy of the plant samples included different plant parts, such as leaves, flowers, barks, peels, fruits, roots, and seeds (Figure 1).



Leaf Seed

Figure 1. Different plant parts used for the evaluation of potential volatile allelopathic species.

The samples were collected from Dhaka National Botanic Garden, Dhaka (25-29 January 2018), Sher-e-Bangla Agriculture University, Dhaka (16–17 January and February 1, 2018), Bangladesh Agriculture University, Mymensingh (21–22 January 2018), Chittagong University (3–6 February 2018), Jahangirnagar University (Savar, Dhaka, on 19 and 30 January 2018), and different local markets (Dhaka on 9–12 February 2018). Each sample was kept in separate paper bags and oven-dried for 10 h at 60°C. The dried samples were preserved separately in air-tight, zip-lock polyethylene bags in a refrigerator (4°C) until further use. The authority of the collected plant species were confirmed from International Plant Names Index (IPNI) https://www.ipni.org/. The dried plant samples were sent to the Laboratory of International Agrobiological Resources and Allelopathy at the Tokyo University of Agriculture and Technology, Japan, for further study.

## 2.2. Dish Pack (DP) Method

The dish pack (DP) method [29] is a reliable and established bioassay for detecting the presence of volatile allelochemicals. This method was adopted [29–33] for the evaluation of a large number of plant samples, to identify volatile allelopathic plants. For this study, the DP method was adopted to evaluate the volatile allelopathic potential of 103 plant samples (March-June 2018). The multi-well plastic dish contains six wells, and the dimension of each well was  $36 \text{ mm} \times 18 \text{ mm}$ . One well in the corner of the six-well dish was designated as the source well, with 200 mg dried plant sample. The distances from the centre of the source well to the centre of the other five test wells were 41 mm, 58 mm, 82 mm, and 92 mm (Figure 2). Filter papers (size = 33 mm; Toyo Rosha Kaisha, Ltd., Tokyo, Japan) were placed in the test wells, except for the source well, and soaked with 0.75 mL distilled water. Plant samples were not added to the source well in the control DP. Five uniformly sized seeds of Lactuca sativa var. legacy, (Takii Seed Co., Ltd., Kyoto, Japan) were placed in the test wells. The dish was tightly sealed using cellophane tape to avoid desiccation or loss of volatile compounds. Aluminium foil was used to cover the dish to prevent contact with light, and it was placed in an incubator (NTS Model MI-25S) at 25°C for three days. The lengths of the radicle and hypocotyl were measured after three days of incubation and data recorded for further analysis. The 41 mm distance from the source well was considered for the optimum inhibitory evaluation. Each experiment was replicated three times. The inhibition of radicle and hypocotyl growth was calculated by comparing to the control. The

relationship between the inhibition of *L. sativa* seedling growth and the distance of the source well was determined by the degree of inhibition or control level of growth.



**Figure 2.** The layout of the dish pack (DP) method. The six-well plastic dish was used to test the volatile allelopathy of plants. A total of 200 mg of dry plant samples were used in the corner well (source well), and five lettuce seeds were used for rest of the wells, to design the volatile allelochemicals assessment.

The DP method was adopted again for the selected candidate allelopathic species (*S. mukorossi*) with different amounts (5 mg, 25 mg, 50 mg, 100 mg, and 200 mg), in order to evaluate the inhibition and regression analysis. Individual replication was set to obtain data at the 24th, 48th, and 72nd hours separately. Data were collected every 24 h during the incubation period, in order to understand the status of growth inhibition, relationship with the variation of crude concentration, distance, and duration of time, as well as to understand the effective concentration.

## 2.3. Single Petri Dish (SPD) Method

The single petri dish (SPD) method was used after the evaluation by DP method and the selection of candidate allelopathic plant species, to understand the inhibition potentiality, accuracy, and justification. A single glass petri dish with a lid was used in this method. The inner diameter of the petri dish was 145 mm, the height 20 mm, and the volume 240 mL (Figure 3).



**Figure 3.** The layout of the single petri dish (SPD) method: the control level of growth of the *L. sativa* seedling by volatile allelochemicals in the glass petri dish desk. Different coloured dots represent the various distances (20 mm, 40 mm, and 60 mm) and directions of the tested *L. sativa* seeds.

A dry, 145 mm diameter filter paper was placed on the plate and soaked with 1.5 mL distilled water. A small, hard, unscented plastic disk of 10 mm diameter containing the test sample (pericarp of *Sapindus mukorossi*) for evaluation was placed at the centre of the petri dish. Three uniformly sized *L. sativa* seeds were placed at each point of different distances (20 mm, 40 mm, and 60 mm) at four different directions from the centre of the dish. Thus, the number of seeds used for each distance was 12. The amount of the sample used in the single petri dish method was 100 mg (half of the amount used in the aluminum foil to properly prevent luminous interaction and incubated (NTS Model MI-25S) at 25 °C for three days. In this method, the inhibition of *L. sativa* seedlings was determined by replications at each point. Each experiment was replicated three times. The growth of seedlings was checked, as well as the data on radicle and hypocotyl lengths.

## 2.4. Comparison of Volatile Allelopathy of S. mukorossi by Dish Pack and Single Petri Dish Methods

Unlike the DP method, the SPD method is more pragmatic. In the field or greenhouse trial, the test sample can spread volatile allelochemicals towards all directions. Besides, the petri dish used for the SPD method is readily available and cost-efficient. The method is easier to handle, accurate, and convenient for initial understanding and identification of the volatile allelopathic species. This study is the first and fundamental introduction to the SPD method for evaluating the potential volatile allopathy of plant species (Table 1).

Dish Description	Dish Pack (DP) Method (Multiple Six-Wells Plastic Dish)	Single Petri Dish (SPD) Method (Single Glass Petri Dish)		
Initial sample used	200 mg	100 mg		
Sample placement	On a corner	At the centre		
Diffusion of volatile compound	Might have equal diffusion	Might have equal diffusion to all directions		
Volume	120 mL	240 mL		
Size of dish	$[36 \text{ mm} (\phi) \times 18 \text{ mm} (h)] \times 6 \text{ wells}$	145 mm ( $\phi$ ) × 20 mm (h)		
Establishment	Already established [29].	Developed in this study (resembles all sides of the DP method).		
Interaction with light	Wrapped with aluminum foil. No interaction with light.	Wrapped with aluminum foil. No interaction with light.		
Avoid contact with test plant	The test sample placed at the corner well of the six-well multiple dishes. Therefore, no contact with the test plant.	10 mm small dish used at the centre of the 145 mm glass petri dish to place the sample. Therefore, no contact with the test plant.		
Test seed	Test seed Five seeds in each well.			
Data collection	Three replications each.	Three replications each.		

## Table 1. Brief description of the DP method and the SPD method.

The comparative experiments of DP and SPD methods were done by simultaneous experiment with the same sample amounts (25, 50, and 100 mg). The distances for the SPD method were 20, 40, and 60 mm, and these same distances were measured in the DP method from the source well. Small petri dishes with 10 mm diameter were used for placing the crude sample and test seeds. Except for the distance, the rest of the protocol was followed as before (Section 2.4). Data from both DP and SPD methods were taken after a 72-h incubation period and analysed.

#### 2.5. Analytical Study

The results of radicle and hypocotyl growth conformed to normal distribution. The statistical analysis was performed by evaluating the mean (M), standard deviation (SD), and the standard deviations value (SDV), using a frequency distribution curve for Microsoft Excel Ekuseru-Toukei 2012 by a special program (Social Survey Research Information Co. Ltd.). The concept of the SDV has been used by previous reports [28–32] to evaluate the criteria of allelopathic activity among the samples. The criterion of the standard deviation value (SDV) was used to estimate the range of significant effects of the species. The criteria index was set as \* = M – 0.5 (SD), \*\* = M – 1 (SD), \*\* \* = M – 1.5 (SD), \*\* \*\* = M – 2.5 (SD), and \*\* \*\* \*\* = M – 3 (SD) of the radicle and hypocotyl elongation rate. The total number of criteria, defined as a total score, would provide an index for ranking all the samples.

The potential volatile allelopathy of plant species was evaluated by the DP method, comparing the variations of the elongations of radicles and hypocotyls of the *L. sativa*. The estimation of the control level of growth was performed by using the following equations (1) and (2) [17,30] below:

$$E \text{ or } Gr \% = \frac{(Av. L. \text{ of } Tr / H) \times 100}{Av. L \text{ of } Cr / H}$$
(1)

where E is the elongation, Gr is the growth rate, *Av* is the average, *L* is the length, *Tr* is the treatment radicle, *Cr* is the control radicle, and *H* is the hypocotyl; and

I or 
$$Clg \% = 100 - E \text{ or } Gr \%$$
 (2)

where I is the inhibition, Clg is the control level of growth, *E* is elongation, and *Gr* is the growth rate.

The DP and SPD method comparison test and ranking data were calculated by Microsoft Excel 2016 and Tukey's HSD (Honestly Significant Difference) test in RStudio software version 1.1.423.0.

## 3. Results and Discussion

#### 3.1. Volatile Allelopathic Effect of Evaluated Plant Species

The radicle and hypocotyl elongation of *L. sativa* seedlings were evaluated by the DP method, with 200 mg dried plant parts from 103 different plant species. The leaves were the dominant plant parts used in this study. Usually, the most abundant source of chemicals appears in leaves [34], but other plant parts are also noticeable for the production and release of allelochemicals. Different plant parts from plants also showed different levels of allelopathic effect on lettuce seedling growth. The results from this study indicated that the fruits showed the most substantial growth inhibitory activity on the test plant compared with other plant parts. The results showed that the elongation of the radicle and hypocotyl ranged from 3% to 100% and 44.6% to 156%, respectively. The radicle elongation was affected by the volatile emissions more than the hypocotyl elongation (Figure 4, Table S1). The results of this screening of plant species by dish pack (DP) method was evaluated by the standard deviation value (SDV), in order to indicate the various criteria or scoring of the radicle and hypocotyl growth. The degree of scoring was estimated from the relationship between *L. sativa* seedling growth inhibition or elongation and its distance from the source well. The 41 mm distance was considered for the standard of estimation, compared with the corresponding test plant. The highest controlled growth was shown in five families, including Sapindaceae, Fabaceae, Zingiberaceae, Acanthaceae, and Piperaceae.



**Figure 4.** Effect of volatile allelopathic potentialities of 103 plant specimens on test plant (*L. sativa*) (a) radicle elongation and (b) hypocotyl elongation.

From the results of this study, the dried pericarp of *Sapindus mukorossi* showed the highest reduction of lettuce radicle growth elongation (Table 2, Table S1). Thus, the results indicated that *Sapindus mukorossi* reduced lettuce radicle elongation to 3% of the control, followed by the leaves of *Cassia nodosa* (34.4%) and roots of *Kaempferia galanga* (43.4%), in that order. Mardani et al. [35] reported that the volatile allelopathic plant *Crocus sativus* (saffron) showed the most significant inhibitory activity on radicle growth of lettuce, followed by *Dracocephalum kotschyi, Solanum nigrum,* and *Artemisia aucheri*. Appiah et al. [33] observed that the released volatile allelochemical from *Photinia glabra* affected the radicle elongation of *L. sativa* seedlings by more than 90%, followed by *Liquidambar styraciflua* and *Cinnamomum camphora* among the 69 plant species evaluated.

				Elongation Status			
Family	Site Code	Botanical Name	Plant Part	41 mm Wells			
			-	<b>R%</b>	Score	H%	Score
Sapindaceae	LM	Sapindus mukorossi	Fruit	3.00	** ** **	59.5	**
Fabaceae	DNBG	Cassia nodosa	Leaf	34.4	** ** **	76.7	
Zingiberaceae	LM	Kaempferia galanga	Root	43.4	** **	46.0	** *
Acanthaceae	CU	Justicia adhatoda	Leaf	52.1	** *	74.6	
Piperaceae	DNBG	Piper longum	Fruit	53.5	** *	61.6	*
Apiaceae	SAU	Cuminum cyminum	Seed	56.1	**	90.5	
Apocynaceae	DNBG	Tabernaemontana dichotoma	Flower	58.6	**	44.6	** **
Myristicaceae	DNBG	Myristica fragrance	Leaf	59.6	**	82.2	
Asteraceae	SAU	Tagetes erecta	Flower	59.7	**	44.9	** **
Commelinacea	ae SAU	Commelina benghalensis	Leaf	59.8	**	77.5	
Euphorbiacea	e BAU	Euphorbia neriifolia	Leaf	60.3	**	86.4	
Lecythidaceae	SAU	Couroupita guianensis	Fruit	61.4	**	69.6	
Anacardiaceae	e LM	Mangifera indica	Seed	62.0	**	51.4	** *
Sapindaceae	JU	Lepisanthes rubiginosa	Leaf	62.8	*	91.3	
Combretaceae	DNBG	Terminalia chebula	Leaf	63.1	*	45.8	** *
Asteraceae	SAU	Helianthus annuus	Leaf	63.2	*	56.5	**
Lamiaceae	CU	Gmelina arborea	Bark	63.7	*	81.0	
Malvaceae	DNBG	Sida cordifolia	Leaf	64.4	*	50.8	** *
Apiaceae	JU	Cordia dichotoma	Leaf	65.9	*	45.6	** *
Rubiaceae	DNBG	Mitragyna parvifolia	Leaf	65.9	*	80.8	

**Table 2.** The radicle and hypocotyl elongation (%) of *L. sativa* seedlings using the DP method (top 20 samples).

\* Score indicates the strength of elongation of the tested plant samples on the radicle of the *L. sativa* (control plant) by the standard deviation variance (SDV), where:  $* = M - 0.5 \times SD$ ,  $** = M - 1 \times SD$ ,  $** = M - 1.5 \times SD$ ,  $** = M - 2 \times SD$ ,  $** ** = M - 3 \times SD$ ; these refer to radicle elongation shorter than the mean (M) value. Each additional \* indicates a stronger level of growth control. M: mean of radicle elongation, SD: standard deviation of the length of the tested *L. sativa* radicle, *R*%: radicle elongation percentage, *H*%: hypocotyl elongation percentage. \* Site codes indicate the areas of sample collection. DNBG: Dhaka National Botanic Garden, Dhaka; SAU: Sher-e-Bangla Agriculture University, Dhaka; BAU: Bangladesh Agriculture University, Mymensingh; CU: Chittagong University, Chittagong; JU: Jahangirnagar University, Savar, Dhaka; LM: local market, Dhaka.

agricultural cultivation.

The results showed that the lettuce hypocotyl elongation was also significantly inhibited by the flowers of Tabernaemontana dichotoma (44.6%), followed by the flowers of Tagetes erecta (44.9%) and leaves of Cordia dichotoma (45.6%). In contrast, some of the plants released volatile substances that stimulated the growth of the test plant. The results of this study showed that leaves of Gynostemma pentaphyllum (Cucurbitaceae) caused a significant plant growth stimulatory effect on lettuce. The leaves caused 156% hypocotyl elongation of lettuce seedlings (Table S1). This traditional medicinal plant is well distributed from Bangladesh to Southeast Asia to Japan and Korea, as well as to New Guinea [36]. The leaves of G. pentaphyllum could be another potential candidate for plant growth stimulation in

The DP method assessment of 103 plant species showed that the pericarp of S. mukorossi (Sapindaceae) showed the highest (97%) inhibition on radicle elongation of test plants by releasing potential volatile allelochemicals. From this study, the fruits of *Sapindus mukorossi* (Sapindaceae), which have many herbal medicine values and are distributed in Asian countries like Bangladesh, India, and Japan, show potential allelopathic activity [37–39]. The primary phytochemicals of S. mukorossi galls and pericarps are saponins, triterpenoids, flavonoids, and fatty acids [40-42]. Saponins isolated from S. mukorossi fruit have antibacterial and anticancer activities [43,44], and sesquiterpene glycosides showed potent antimicrobial activity against dermatophytes [37]. Moreover, crude saponins from the seed of S. mukorossi had insecticidal and molluscicidal activities [45], as well as potential herbicidal properties [18,44]. This plant sample is relatively essential for human welfare due to its medicinal values (Figure 5). Nevertheless, there is no report on the biological activity of the volatile emissions from the S. mukorossi, and this fact partly led to this study.



41 mm distance

Figure 5. Growth level evaluated by DP method, considering 200 mg by dry pericarp of S. mukorossi and 41 mm distance growth status, compared with the control treatment on L. sativa seedlings' radicle and hypocotyl.

It was found that the ethanol extract of the leaves of S. mukorossi exhibited growth inhibitory activity against Avena fatua L. and Amaranthus retroflexus L. in a petri plate test and pot culture assay [46]. However, there is no previous report concerning the volatile phytochemicals of the pericarp of S. mukorossi as an allelochemical. This is the first report indicating the pericarp of S. mukorossi as a potential volatile allelopathic species, and S. mukorossi might be a new candidate for natural weed management in the agricultural system.

#### 3.2. Growth Inhibitory Activity of S. mukorossi by DP Method

The variation of the time and the amount of dried pericarp of *S. mukorossi* was used to evaluate the inhibition of *L. sativa* seedlings elongation. The highest application rate was considered as 200 mg, followed by 100 mg, 50 mg, 25 mg, and 5 mg. By estimation, the EC<sub>50</sub> (Effective Concentration that induces half-maximal inhibitory activity) of the pericarp of *S. mukorossi*, due to the volatile emission, was 22.6 mg/dish, with 41 mm distance and 72 h time consideration (Figure 6). Octyl acetate, the identified volatile allelochemical from *Heracleum sosnowskyi* fruit, slightly suppressed the radicle and hypocotyl elongation of *L. sativa* seedlings at EC<sub>50</sub> values of 64 and 57 ng/cm<sup>3</sup>, respectively [47].



**Figure 6.** The effective concentration that induces half-maximal inhibitory activity ( $EC_{50}$ ) of the radicle growth inhibition and the crude amount of dry plant samples of *S. mukorossi* on test plant *L. sativa*, with 41 mm distance and 72 h time consideration.

The results showed that the lower distance with increasing sample amount exhibited the highest level of controlled radicle growth associated with extended time duration. The dried pericarp of *S. mukorossi* at a distance of 41 mm and 72 h time period of incubation showed 97.5%, 97.2%, 75%, 59%, and 6.2% growth inhibition with 200 mg, 100 mg, 50 mg, 25 mg, and 5 mg, respectively. The results of this experiment revealed that the degree of control or inhibition by 200 mg (97.5%) and 100 mg (97.2%) of *S. mukorossi* dried pericarp has nearly similar inhibitory activity compared to the control treatment after 72 h (Figure 7a). However, at a 58 mm distance and after 72 h, 5 mg (2.98%), 25 mg (17.2%), 50 mg (15.9%), 100 mg (62.9%), and 200 mg (66.8%) showed inhibition as well (Figure 7b).

At a distance of 82 mm and 72 h time duration, the radicle growth controlled by 5 mg, 25 mg, 50 mg, 100 mg, and 200 mg were -8.63%, 5.26%, 11.1%, 32.9%, and 32.1%, respectively (Figure 7c). The results indicate that the released strong volatile allelochemicals had a significant effect, even at an increased distance from the source of the donor. In contrast, a distance of 92 mm and 72 h time consideration exhibited controlled growth of the radicle by 5 mg (0.156%), 25 mg (6.08%), 50 mg (9.38%), 100 mg (9.90%), 200 mg (8.46%) (Figure 7d). The percentage of *L. sativa* seedling growth inhibition expressed the interaction of volatile allelochemicals, which decreased with distance. Based on this concept, test plants close to the source of volatile emissions showed a high suppression effect. The result showed that the highest inhibitory activity was detected at 41 mm distance and 72 h after incubation of the *L. sativa* seedling.



**Figure 7.** Control level of radicle growth percentage: (a) 41 mm distance from source well, (b) 58 mm distance from source well, (c) 82 mm distance from source well, and (d) 92 mm distance from source well, considering the amount of variation of *S. mukorossi* dry samples (200 mg, 100 mg, 50 mg, 25 mg, 5 mg) on the growth control variable by the DP method of *L. sativa* seedlings. The data compared by Tukey's HSD test and values are the averages of three replications of  $(\pm)$  standard error.

## 3.3. Inhibitory Effect of S. mukorossi in the Single Petri Dish (SPD) Method Evaluation

The radicle growth status of the test plant by the DP method, using 100 mg and 200 mg pericarp of *S. mukorossi*, showed nearly the same results. Therefore, 100 mg of the plant sample was considered the maximum amount of sample and 72 h the period for the SPD method evolutionary study. The single petri dish method justified the inhibitory effect of *S. mukorossi* on the radicle of *L. sativa* seedlings, by understanding the efficacy of the volatile allelochemicals, considering the number of seedlings with a smaller amount of plant sample (Figure 8).



**Figure 8.** Growth level evaluated by the SPD, method considering 100 mg dry pericarp of *S. mukorossi* with a distance of 20 mm, compared with a control treatment on *L. sativa* seedlings' radicle and hypocotyl.

Based on a 72 h time consideration, the distance of seedlings from the target place and the number of seedlings showed significant changes from each range. Firstly, a 20 mm distance for 25 mg, 50 mg, 100 mg showed 30.2%, 65.7%, and 97.2% inhibition of radicle elongation, respectively. In addition, a 40 mm distance with the amounts of 25 mg, 50 mg, and 100 mg showed -11.3%, 15.7%, and 55.9% controlled status of seedling radicles, respectively, while a 60 mm distance with the amounts of 25 mg, 50 mg, and 100 mg showed 4.66%, 15.0%, and 21.9%, respectively (Figure 9a). The inhibitory effect on plant growth had a positive correlation with seedling distance and the amount of plant materials. The results showed that the controlled effect of the volatile allelochemicals was reduced when the number of seeds and distance from the source increased. Appiah et al. [48] reported that the inhibitory effect of carnosic acid as an allelochemical decreased when the number of seeds of each test plant was increased. Similarly, the inhibitory effects of l-3-(3,4-Dihydroxyphenyl) alanine (L-DOPA) diminished when the number of seeds of the test plants increased [49]. From the surface plot, it was clear that for 25 mg, 50 mg, and 100 mg plant samples at a 20 mm distance showed 13.0%, 34.8%, and 61.9% of hypocotyl growth controlled. At a 40 mm distance, 25 mg, 50 mg, and 100 mg amount showed -0.52%, 2.7%, and 24.3% controlled condition, respectively, and at a 60 mm distance 25 mg, 50 mg, and 100 mg amounts it showed 6.72%, 2.0%, and 24.5%, respectively (Figure 9b).



**Figure 9.** Single petri dish method evaluation. (**a**) Radicle inhibition (**b**) Hypocotyl inhibition. Distance consideration (20 mm, 40 mm, 60 mm), amount variation of *S. mukorossi* dry samples (100 mg, 50 mg, 25 mg), and correlation investigated control level of the growth variable of the (**a**) radicle and (**b**) hypocotyl of *L. sativa* seedlings. The data compared by Tukey's HSD test and values are the average of three replications of (±) standard error.

The negative value represents the stimulatory effect when compared to the control. The result showed a relative hormesis effect with the biphasic dose-response, with a low-dose stimulatory or

beneficiary effect and a high-dose inhibitory effect [50,51]. This experiment also revealed that radicle growth was more affected than that of the hypocotyl. Radicles of the seedlings were more susceptible to allelochemicals due to early emergence and exposure to the allelochemicals [36,52].

#### 3.4. Evaluation of Volatile Allelopathy of S. mukorossi by Dish Pack Method and Single Petri Dish Methods

The present study showed the relative effect of the candidate species (*S. mukorossi*) on *L. sativa* seedlings by the DP and SPD methods. For this evaluation, both methods were conducted again, and nine replications were considered for analytical study by Tukey's test. The two methods were different in technical orientation, but almost identical in the inhibitory activity. The growth inhibitory effect was found to be similar on test plants for both DP at 50 mg and SPD at 100 mg per dish. Germination percentages depended on the dose or the amount of the plant materials, which influences the number of constituents released in both the DP and SPD methods. The phytotoxic effects on germination of test plants was also dose-dependent [53]. In contrast, the effect of potential volatile allelochemical diffusion from *S. mukorossi* by the DP method showed a difference from the SPD method. Nonetheless, the comparison between DP and SPD showed variability in inhibition status among the distances in addition to the variation of the sample amounts.

At the same amount, (100 mg, 50 mg, 25 mg), the SPD method exhibited a lower inhibitory effect than the DP method on the lettuce (Figure 10a). It also represented a remarkable difference regarding the two different methods. On the other hand, the variation of the volume of the dish also varied the results. The volume of the DP was 120 mL and that of SPD was 240 mL, which indicates that the volume of the SPD was twice that of the DP. However, the result of the activation of allelochemicals from both methods also indicates that the inhibition of the radicle growth of the test plant by *S. mukorossi* for 100 mg SPD method was equivalent to DP 50 mg method. The method comparison test revealed that there is possibly no significant difference between these two results. It also showed a controlled level slope with the variance of the distance (20 mm, 40 mm, and 60 mm) for both methods (Figure 10b).



**Figure 10.** Comparison of the radicles of the *S. mukorossi* test plants between the DP method and the SPD method. (**a**) Control level of growth (%) with different amounts of samples with different distances. (**b**) Comparison of the control level of growth (%) status in DP with 50 mg of crude dry plant sample and SPD with 100 mg of the sample. The data was compared by Tukey's HSD test (the same alphabet indicates there is no significant difference between two treatments). Values are the average of nine replications (±) standard error.

In this study, the implemented SPD method is a new method to justify the inhibition status by volatile allelochemicals. This is the first report to introduce the SPD method for evaluating the volatile allelopathy of plant species. Nevertheless, this method can be used for further study of volatile allelopathy for searching for new species, and to prove their efficacy in the agricultural field. Moreover, the volatile allelochemical dispersal process by the SPD method represents the field condition metaphorically, with regard to the cylindrical diffusion model [54]. The results state that the inhibition by both methods was similar. The SPD method is an authentic method that could justify the findings of the DP method.

## 4. Conclusions

The discovery of natural herbicides by the evaluation of the volatile allelopathic species can be a precedent for sustainable weed management in agricultural systems. Finding unknown volatile allelopathic plants from Bangladesh might be a benchmark for new natural and eco-friendly weed management candidates. However, further evaluation is necessary to identify the allelochemicals responsible for the plant growth inhibitory effects of the candidate species. This is the first report that the pericarp of *S. mukorossi* as a potential volatile allelopathic candidate for weed management in agroecosystems. The new SPD screening method might be an affordable and convenient way to test plant samples and identify new volatile allelopathic species for developed and developing countries. New volatile allelopathic species may provide efficacy for growth control activities on weeds, further pursuing sustainable weed management practices in agriculture.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4395/10/1/49/s1, Table S1: The radicle and hypocotyl elongation percentage of *L. sativa* seedlings in the DP method containing 200 mg of dry plant materials (103 plant samples).

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