

**PHYTOTOXICITY OF DIFFERENT AVAILABLE SPICES IN BANGLADESH**

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**DHAKA -1207**

**December, 2020**

**PHYTOTOXICITY OF DIFFERENT AVAILABLE SPICES IN BANGLADESH**

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A Thesis  
*Submitted to the Department of Agricultural Chemistry*  
*Sher-e-Bangla Agricultural University, Dhaka*  
*In partial fulfillment of the requirements*  
*For the degree of*

**MASTER OF SCIENCE (MS)**  
**IN**  
**AGRICULTURAL CHEMISTRY**  
**SEMESTER: JULY- DECEMBER, 2020**

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**CERTIFICATE**

This is to certify that the thesis entitled “**PHYTOTOXICITY OF DIFFERENT AVAILABLE SPICES IN BANGLADESH**” submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.) in AGRICULTURAL CHEMISTRY**, embodies the result of a piece of faithful research work carried out by **REZOANA FERDOUSI**, Registration No. **18-09238** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged.

**December, 2020**

**Dhaka, Bangladesh**

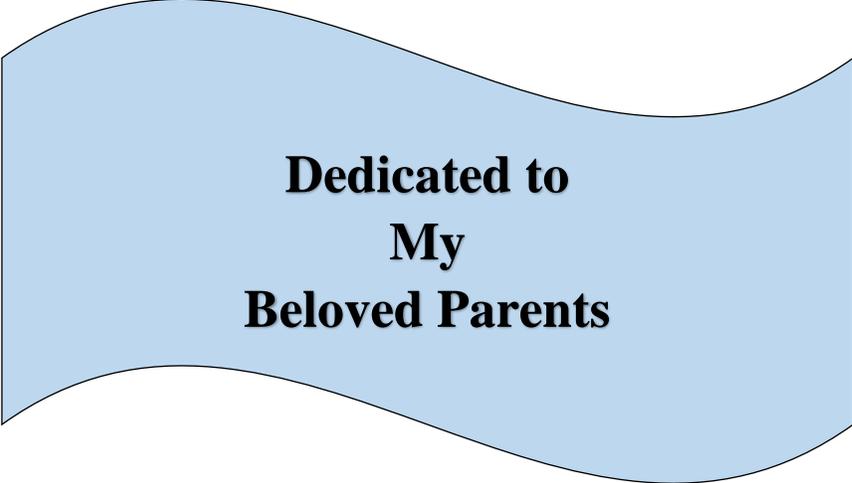
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**Dedicated to  
My  
Beloved Parents**

## ACKNOWLEDGEMENT

All praises to the “**Almighty Allah**” Who enable the author to complete a piece of research work and prepare this thesis for the degree of Master of Science (M.S.) in Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207.

The author feels much pleasure to express her gratefulness, sincere appreciation and heartfelt liability to her venerable research supervisor **Dr. Md. Sirajul Islam Khan**, Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 for his scholastic guidance, support, uninterrupted encouragement, valuable suggestions and constructive criticism throughout the study period.

The author also expresses her gratitude and thankfulness to co-supervisor **Dr. Rokeya Begum**, Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, for her constant inspiration, valuable suggestions, cordial help, heartiest co-operation and supports throughout the study period.

The author deeply desires to express her cordial thanks to chairman of the department, **Dr. Md. Tazul Islam Chowdhury**, Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University. His cordial help, heartiest supports and worthwhile suggestions are remarkable throughout the study period.

The author would like to express her grateful thanks to all teachers of the Department of Agricultural Chemistry for their constructive suggestions and advice during the study period.

The author deeply acknowledges the profound dedication to her beloved parents: **Shahjahan Mia** and **Laila Iasmin**, and **Husband** for their moral support, steadfast encouragement and continuous prayer in all phases of academic pursuit from the beginning to the completion of study successfully

Finally, the author is deeply indebted to her friends and well-wishers for their kind help, constant inspiration, co-operation and moral support which can never be forgotten.

**December, 2020**

**Dhaka, Bangladesh**

**The Author**

## PHYTOTOXICITY OF DIFFERENT AVAILABLE SPICES IN BANGLADESH

### ABSTRACT

The phytotoxic effect of different spices plant extract was investigated on the germination and seedling growth of Okra (*Abelmoschus esculentus*) and barnyard grass (*Echinochloa crus-galli* L.) with experiment carried out in Laboratory, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, during the period from October 2019 to September 2020. The effect of spices plant extracts of Clove, Black paper, Bay leaf, Cinnamon, Cardamom at the concentration of E0 (control; no extract), E1 (0.01 mg dry wt. eq. extract/mL), E2 (0.03 mg dry wt. eq. extract/mL), E3 (0.1 mg dry wt. eq. extract/mL) and E4 (0.3 mg dry wt. eq. extract/mL) were studied on germination, root and shoot length of okra and barnyard grass. The experiment was done under Completely Randomized Design (CRD) with three replications. Results showed that all the test plant species were inhibited under extracts of all spices plant. Results indicated that all concentrations of plant extract had phytotoxic effect on okra and barnyard grass. The concentration of 0.3 mg dry wt. eq. extract/mL completely inhibited the germination, root and shoot growth both of okra and barnyard grass among all the concentration. At the same concentration Clove extract showed the highest phytotoxic effect on seed germination of okra (31%) and barnyard grass (30%). Again, in terms of root length of okra, Bay leaf extract at the 0.3 mg dry wt. eq. extract/mL concentration showed highest phytotoxic effect and gave lowest root length (0.71 mm). Similarly, Cinnamon extract at the 0.3 mg dry wt. eq. extract/mL concentration on barnyard grass had the highest phytotoxic effect for shoot and root length (0.75 and 1.3 mm, respectively). The above results suggested that tested spices plant may have phytotoxins. Therefore, it is possible to use these extracts as a component for production of bio-herbicides due to their phytotoxic effects on weeds and crops and considered as a natural way for sustainable weed management.

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## ABBREVIATIONS

|                 |   |
|-----------------|---|
| BBS             | Bangladesh Bureau of Statistics                     |
| BCSRI           | Bangladesh Council of Scientific Research Institute |
| cm              | Centimeter  |
| CV %            | Percent Coefficient of Variation                    |
| DMRT            | Duncan's Multiple Range Test                        |
| <i>et al.</i> , | And others  |
| e.g.            | exempli gratia (L), for example                     |
| etc.            | Etcetera  |
| FAO             | Food and Agricultural Organization                  |
| g               | Gram (s)  |
| i.e.            | id est (L), that is                                 |
| Kg              | Kilogram (s)  |
| LSD             | Least Significant Difference                        |
| m <sup>2</sup>  | Meter squares                                       |
| ml              | MiliLitre   |
| M.S.            | Master of Science                                   |
| No.             | Number  |
| SAU             | Sher-e-Bangla Agricultural University               |
| var.            | Variety   |
| °C              | Degree Celceous                                     |
| %               | Percentage  |
| GM              | Geometric mean                                      |
| mg              | Miligram  |
| L               | Litre   |
| µg              | Microgram   |
| USA             | United States of America                            |
| WHO             | World Health Organization                           |

# CHAPTER-1

## INTRODUCTION

Phytotoxicity is defined as detrimental effects on various physiological processes (e.g. seed germination, seedling growth, and water uptake) of plants caused by specific substances or growing conditions (Raven *et al.*, 2016). Phytotoxicity in plants usually occurs in those that are overly sensitive to chemicals. Phytotoxicity may be caused by a wide variety of compounds, including trace metals, salinity, pesticides and phytochemicals. It is the degree of toxic effect of these chemical compounds on plant growth which creates a condition in a given substance in the environment is harmful to plants. Phytotoxins with negative allelopathic effects are an important part of plant defense against herbivore. Phytochemicals have shown far-reaching effect on the growth and development on plants (Arshad and Frankenberger, 1998).

Spices are very important food crop as food as well as medicine. Spices are commonly used for cooking and seasoning of foods. They are so important in ancient times and still today almost all people are habituated to use spices in curries and other food. They are known in different flavors and aroma.

Spices have been in use for centuries both for culinary and medicinal purposes. Spices not only enhance the flavor, aroma, and color of food and beverages, but they can also protect from acute and chronic diseases. Now, Americans are considering the use of spices and herbs for medicinal and therapeutic/remedy use, especially for various chronic conditions. There is now ample evidence that spices possess antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, and glucose- and cholesterol-lowering activities as well as properties that affect cognition and mood. Research over the past decade has reported on the diverse range of health properties that they possess via their bioactive constituents, including sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins, especially flavonoids and polyphenols. Spices such as clove and cinnamon are excellent sources of antioxidants with their high content of phenolic compounds (De soysa *et al.*, 2016). It is evident that frequent consumption of spicy foods was also linked to a

lower risk of death from cancer and ischemic heart and respiratory system diseases. However, the actual role of spices in the maintenance of health, specifically with regards to protecting against the development of chronic, noncommunicable diseases, is currently unclear. Cardamom contains phytochemicals that have anti-inflammatory and antibacterial properties.

Phytotoxicity is a biological phenomenon by which an organism produces one or more biochemicals that influence the germination, growth, survival, and reproduction of other organisms. These biochemicals are known as phytotoxins and can have beneficial or detrimental effects on the target organisms and the community. Phytotoxins are a subset of secondary metabolites, which are not required for metabolism (i.e. growth, development and reproduction) of the phytotoxic organism. Phytotoxins with negative phytotoxic effects are an important part of plant defense against herbivore (Ali *et al.*, 2010)

Phytotoxicity has been widely discussed and reviewed in recent years (e.g. Barner, 2000; Evenari, 2005; Grodzinsky, 1998). Many plants have been shown to contain toxins and these have been specifically identified in numerous instances. Similarly, interference by one plant with the growth of another has in many cases been shown to be chemical in nature. Significant quantities of plant toxins in the environment have also been demonstrated. But in very few studies has a single instance of apparent Phytotoxicity been successfully investigated from all these points of view.

Phytotoxicity must be considered as an ecological factor of wide significance, capable of influencing succession, dominance, vegetation dynamics, species diversity, community structure and productivity. Floyd and Rice (1967) and Wilson and Rice (1968) demonstrated the importance of phenolic compounds in altering the rate and nature of old field succession. Muller (2001) discussed the role of terpenes from *Salvia leucophylla* and other aromatic shrubs in the production of stands of single species dominance. Such stands alter immunity, productivity and diversity. The production and release of toxic chemicals by plants and their subsequent effective action in the environment constitute a process of obvious ecological significance.

The interaction of plants through chemical signals (phytotoxicity) has many possible agricultural applications. Decline in crop yields in cropping and agro-forestry system in

recent years has been attributed to allelopathic effects. phytotoxicity associated problems have been observed both in monocultures and multiples cropping system, continuous monoculture causes the accumulation of phytotoxins and harmful microbes in soil, which give rise to phytotoxicity and soil thickness (Bhatt and Todaria, 1990).

Root Crop rotation is practiced to eliminate the effect of monoculture, but the succeeding crop may be influenced by the phytotoxins released by the preceding crop. A large number of weeds and trees possess allelopathic properties which have growth inhibiting effect on crops. Phytotoxicity also plays an important role in suppressing the growth of weed plants. Struggle for space and nutrients for propagation, continuity and universality is the most powerful law of nature. In this trend, some plants have allelopathic potential by releasing allelochemicals to their surrounding that have either deleterious or beneficial effects on other plants. These compounds inhibited plant growth by affecting many physiological processes among them, the effect on ion uptake and hydraulic conductivity (i.e. water uptake) are particularly important since the root is the first organ to come into contact with the allelochemicals in the rhizosphere. The degree of inhibition depends on their concentration. Some plant genotypes are likely to escape the allelopathic chemical(s) by being "hypersensitive". In this regards the tip may actually be strongly affected by phytochemical(s) and have its growth rate nearly stopped. Chemicals with phytotoxic activity are present in many plants and in various organs, including leaves and fruits and have potential as either herbicides or templates for new herbicide classes (Cheng and cheng, 2015). Many allelopathic compounds produced by plants are regulated by environmental factors, such as water potential of the environment, temperature, light intensity, soil moisture, nutrients, soil microorganisms and perhaps others. The compounds are released to the environment by means of volatilization, leaching, decomposition of residues and root exudation. They are; firstly, the terpenoids cineole, are released to the environment by volatilization, which is noticeable under drought conditions; secondly, the water born phenolic and alkaloids are washed out by rainfall through leaching; thirdly, phytotoxic aglycones, such sphenolics and others are produced during the decomposition of plant residues in soil; fourthly, many secondary metabolites such as scopoletin, may be released to the surrounding soil through root exudation. Several researches of phytotoxic effect of spices have emphasis on the effect of leaf of the plant while other phytopathic

sources of spices were investigated, including root and affected soils, so as to detect the origin of any allelopathic effect and to analyze the effects of these sources on native species (Gomiero *et al.*, 2011).

It is a novel work, so that no significant work has been done on this subject that's why I have decided to research this topic. Considering the above facts, the present study has been under taken to fulfill the following objectives:

- I. To assess the phytotoxicity of five spices plant extract on germination and seedling growth against Okra and Barnyard grass
- II. To evaluate the levels of phytotoxicity against Okra and Barnyard grass

## CHAPTER-2

### REVIEW OF LITERATURE

Phytotoxicity is a toxic effect by a compound on plant growth. Such damage may be caused by a wide variety of compounds, including trace metals, salinity, pesticides, phytotoxins or allelochemicals.

Allolli and Narayanareddy (2000) described that leaf extract of coriander inhibited seed germination and reduced root and shoot length of cucumber and maximum inhibition was observed in higher concentrations of extract. Alam and Islam (2002) reported that plants produce chemicals, which interfere with other plants and affect seed germination and seedling growth. These chemicals have harmful effects on crops in the eco-system resulting in the reduction and delayed germination, seedling mortality and reduction in growth and yield (McWhorter, 1984; Herro and Callaway, 2003).

Anjum and Bajwa (2008) while studying the phytotoxicity influence of the sunflower on some weeds indicated the strong suppressive potential of this plant on some growth and physiological parameters of the tested plants. Phytochemicals emancipated as residues, exudates and leaches by many plants from leaves, stem, roots, fruit and seeds reported to interfere with growth of other plants (Asgharipour and Armin, 2010). These chemicals products mainly affect plants at seed emergence and seedling levels (Alam and Islam, 2002; Hussain *et al.*, 2007; Mohamadi and Rajaie, 2009; Naseem *et al.*, 2009).

Arshad and Frankenberger (1998) found that phytochemicals have shown far-reaching effects on the growth and development of plants even at low concentration. A number of laboratory-based experiments have focused on the effects of leaf sap, volatile compounds, foliage decomposition and root exudation on seed germination and the early growth stages of various receptor species (Molina *et al.*, 1991; Lisanework and Michelsen, 1993; Fang *et al.*, 2009). This is in agreement with past studies which found that seedlings planted in Eucalyptus plantations were affected by allelopathic chemicals from volatilization, leaching.

Asgharipour and Armin (2010) observed that phytochemicals might affect both crop and weeds when found together. The crop was distress directly or indirectly by the phytochemicals and lead to either stimulation or inhibition of growth.

Asgharipour and Armin (2010) reported that the study the phytotoxic of plant organs extract at seed germination and seedling growth stages was beneficial for it is difficult to separate the phytochemicals effects from that of competition among crop and phytotoxic plants. The phytochemicals sometimes have positive effects on sorghum growth. For example, *Moringa oleifera* leaf extracts enhanced germination of sorghum by 29% (Phiri, 2010). The similar type of germination pomotary behavior was also observed in extract of *Cassia angustifolia* (Hussain *et al.*, 2007).

Chatiyanon *et al.* (2012) reported that the water and methanol extract of the leaves of *H. suaveolens* has phytotoxic effects on the germination and seedling growth of *Pennisetum setosum* (Swartz.) L.C. Rich and Mimosa invisa Mart. Similar findings were also reported by Kapoor (2011) who worked with dry leaf residue of *H. suaveolens* and observed inhibitory activity on the growth and physiological parameters of *Parthenium hysterophorus* L.

Chung *et al.* (2001) assessed the phytotoxic potential of 44 rice cultivars (*Oryza sativa* L.) on barnyard grass. All 44 cultivars exhibited marked differences in the inhibition of barnyard grass growth and development.

Colquhoun (2006) evaluated that phytotoxic compounds, often considered plant- produced herbicides, can inhibit growth of nearby plants. These compounds could be an alternative weed management strategy for crop production and can offer environmental benefits.

Crawley (1997) reported that the phytotoxic effects of eucalyptus are also due to inhibition of some physiological process such as nutrient uptake, cell division, synthesis of carbohydrates, proteins and nucleic acids and phosphorylation pathways. These inhibitory effects can mediate by phenolic compounds. Therefore, these phenolic compounds reduce seed germination and growth of seedlings.

Dhima *et al.* (2009) indicated that green manure of aromatic plants, such as anise, dill, oregano or lacy phacelia could be used for the suppression of barnyard grass and some broad leaf weeds in maize which consequently minimize herbicide usage.

Djanaguiraman *et al.* (2005) observed that the leaf leachate of *E. globulus* inhibited germination and growth of rice, sorghum and black gram. Moreover, the extract of *E. globulus* inhibited germination and seedling growth of green gram and cowpea.

Djanaguiraman *et al.* (2005) who found that seedling dry matter of rice, sorghum and black gram significantly reduced by leaf leachate of *E. globulus* and highest inhibition was observed in highest concentration. Moreover, dry weights of broad bean and maize reduced by leaf-litter water extract of *E. rostrata*. Fresh and dry weights of three wheat cultivars decreased in response to aqueous eucalyptus extract. Many polyphenols have catechol groups and at higher concentrations can chelate divalent or trivalent metal ions. Therefore, they inhibit ion uptake and thus cause reduction in seedling dry matter.

Einhelling and Leather (1988) studied that there are about 400,000 secondary metabolites in plants with allelopathic activities (Swain, 1977), of which only a few have been examined. The rest of the compounds, might contain very promising growth inhibitors are still unknown. Since about 12.5% of the total plants species of the world are considered as medicinal plants (Wakdikar, 2004), therefore, they could be served as important candidates for phytopathic research. Isolation and characterization of that unknown phytochemicals from spices plants might provide the chemical basis for new natural herbicides developments.

El-khawas and Shehata (2005) found that leaf extract of *E. globulus* inhibited germination of maize and kidney-bean. The phytopathic effect of extract from *E. camaldulensis* was tested on tomato; the extract significantly inhibited germination and growth of this plant. Fujii (2001) assessed 53 cover crop plant species (including 26 leguminous, 19 graminaceous, and 8 others) for their allelopathic activity. It was found that leguminous cover crops such as hairy vetch and velvet bean, and graminaceous cover crops, such as oat (*Avena sativa* L.) and rye (*Secale cereale* L.) as well as certain cultivars of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) showed high phytopathic potential. *Nerium oleander* extract was reported to have rutin, quercetin (flavonoids),

oleandrin, neriine (cardiac glycosides), rosagenin, folinerin, neritaloside and other compounds (Rajyalakshmi *et al.*, 2011).

Ghorbani *et al.* (2008) studied that aqueous extracts of sunflower reduced germination and mean daily germination.

Jeffersona and Pennacchio (2003) carried out an investigation and revealed that phytochemicals may reduce weed competition with crops by delaying weed germination. Aqueous extracts of leaves have notably inhibited seed germination of sorghum with application of *Parthenium hysterophorus* (Murthy *et al.*, 1995), *Ipomoea cornea* (Jadhav *et al.*, 1997) *Commelina benghalensis* and *Cyperus rotundus* (Channappagoudar *et al.*, 2003) and *Eucalyptus camaldulensis* (Mohamadi and Rajaie, 2009).

Kaur *et al.* (2011) showed that the leaf volatile oils of *E. tereticornis* caused a significant reduction in early seedling growth and vigor, respiration and photosynthetic pigments of *Amaranthus viridis*.

Kaur *et al.* (2012) revealed that the leaf extracts of *E. globulus* had varying degrees of phytotoxicity against *S. nigrum*. Methanolic and ethyl acetate extracts had maximum inhibitory effects while aqueous extract had least inhibition. Solubility of phytochemicals is one of the major factors determining their phytotoxicity.

Mohamadi and Rajaie (2009) showed that the effects of phytochemicals on seeds germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage or organelles. The extract concentrations of phytochemical will reduce sorghum seeds germination and ultimately results in reduction in yield. These results are in agreement with those of Singh *et al.* (1992), Nandal *et al.* (1999 a, b) and Patel *et al.* (2002) who all observed reduction in germination percentage with extract/leachates application to wheat seed.

Naseem *et al.* (2009) studied that today; phytotoxicity is recognized as appropriate potential technology to control weeds using chemicals released from decomposed plant parts of various species.

Saberi *et al.* (2013) showed that leaf extracts of *E. globulus* had phytotoxic effects on germination and seedling growth of *S. nigrum* seeds. The phytotoxicity of volatile oils and extracts of different Eucalyptus species has been reported against many weedy species.

Salam and Kato-Noguchi (2010) also reported that roots were more sensitive to the phytochemicals than hypocotyls/coleoptiles because the roots are the first organ to absorb phytochemicals from the environment. Whereas, Nishida *et al.* (2005) stated that the permeability of phytochemicals into root tissue is higher than the shoot tissue.

Singh *et al.* (2005) found that seed germination seedling length of ragweed parthenium decreased with increasing concentration of cardamom oils from 0.002 to 0.005 g mL<sup>-1</sup>.

Akter *et al.* (2018) observed that *C. longa* extracts with curcuminoids are able to inhibit the germination and growth of *Bidens Pilosa*.

Chapius-Lardy *et al.* (2002) observed that several phenolic compounds such as caffeic, coumaric, gallic, gentisic, hydroxybenzoic, syringic, ferulic and vanillic acids have been identified in the leaves and under store soil of eucalyptus plantations and methanol and aqueous leaf extracts of black pepper that have phytopathic potential. These compounds have been reported to cause clogging of stomata, enhanced electrolyte leakage and impairment of photosynthetic and energy machinery. It has been reported that total phenolic content of methanolic leaf extracts of three eucalyptus hybrids was higher than aqueous leaf extracts. Therefore, the higher inhibitory effect of methanol extract can be due to higher amounts of phenolic compounds.

Khan *et al.* (2011) found that garlic root exudates played an important role in inhibiting the growth of pepper blight mycelia.

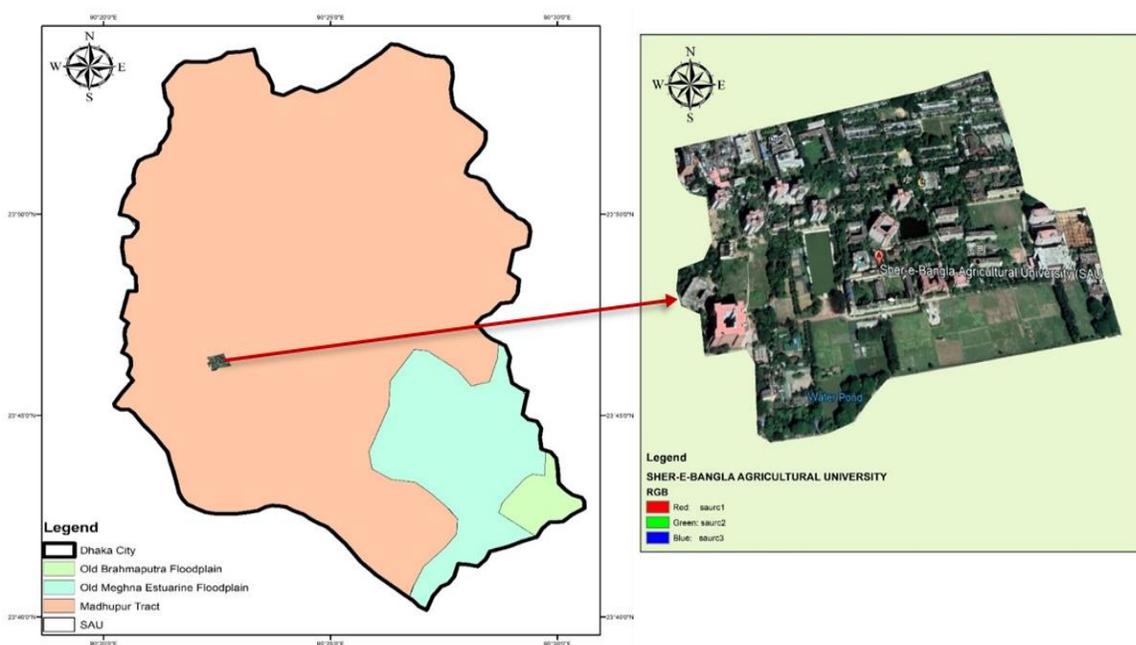
## CHAPTER-3

### MATERIALS AND METHODS

The materials and methods that were used for conducting the experiment have been presented in this chapter.

#### 3.1 Experimental site

The experiment was conducted at the Department of Agricultural Chemistry Laboratory of Sher-e-Bangla Agricultural University, Dhaka during the period from October 2019 to September 2020. The site is 90.2°N and 23.50° E Latitude and at an altitude of 8.2 m from the sea level. Location of the experimental site is shown in following figure.



**Figure 1:** Study Area Map

#### 3.2 Collection of seed samples and plant materials

Five spices plants were used to identify their phytotoxicity with test crops which were as follows:

1. Clove
2. Black paper

3. Bay leaf
4. Cinnamon and
5. Cardamom

Two test crops were considered for the present study which as follow:

1. Okra
2. Barnyard grass

All plant materials were collected from different nursery in Dhaka, Bangladesh.

### **3.3 Sample preparation**

The leaves of the clove, black paper, bay leaf, cinnamon and cardamom plants were collected from different nursery around the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The plants were then washed with tap water to remove the soil and other debris, oven dried and kept at 2°C until extraction. Okra (*Abelmoschus esculentus*) and barnyard grass (*Echinochloa crus-galli* L.) were selected as test plant species. Those species were chosen on the basis of their (i) growth patterns, (ii) sensitivity to phytotoxic extracts, and (iii) weedy characteristics.

### **3.4 Extraction procedure**

1 g of each samples were taken in a several conical flask and added 80 mL methanol 20 mL distilled water. Then stirred and was covered by aluminium foil for keeping it 48 hours. The extract was filter through one layer of filter paper (number 2; Advantec Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The residue was re-extracted with 100mL of methanol for 24 h and filtered. The two filtrates were combined and evaporated to dryness using rotary evaporator at 40°C.

### **3.5 Preparation of extract concentration of spices plant**

A portion of the extract was diluted into required volume of methanol to prepare four assay concentrations 0.01, 0.03, 0.1 and 0.3 mg dry weight equivalent extract mL<sup>-1</sup> and then was added to a sheet of filter paper (number 2) in 28 mm Petri dishes. The methanol was evaporated in a draft chamber followed by adding 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethyl-enesorbitan- monolaurate (Tween 20: a nontoxic surfactant for germination and growth of all test plants).

### **3.6 Treatments of the experiment**

The following treatments were considered for the phytotoxicity of the spices plants against test crops

1. E0 = 0 (control without extract)
2. E1 = 0.01 mg dry wt. eq. extract/mL
3. E2 = 0.03 mg dry wt. eq. extract/mL
4. E3 = 0.1 mg dry wt. eq. extract/mL
5. E4 = 0.3 mg dry wt. eq. extract/mL

### **3.7 Experimental design**

The one factors experiment was laid out in the Completely Randomized Design (CRD) with three replications. The collected data on various parameters were statistically analyzed using MSTAT-C statistical package.

### **3.8 Experimental procedure**

#### **3.8.1 Germination**

The effects of aqueous extracts on germination were tested by placing 10 seeds of each test crop (Okra and Barnyard grass) in petri dishes (three replicates) containing three layers of filter paper saturated with the aqueous extracts. A separate control series was set up using distilled water. The Petri dishes were then placed in room temperature. Moisture in the petri dishes was maintained by adding aqueous extracts or distilled water as required. The numbers of seeds germinated were counted every 12 hours for 3 days (72 hours) (the time when no further seeds germinated) and was considered when the radical emerge by rupturing the seed coat.

#### **3.8.2 Growth**

For the growth determination seeds (Okra and Barnyard grass) are placing according to the same procedure described in the previous section. Then the length of the shoot and root are measured after 48 hours of seeds placing.

### **3.9 Data collection heads**

The following parameters were collected for the present experiment

- 1) Germination percentage

- 2) Shoot (plumule) length
- 3) Root (radical) length

### **3.10 Procedure of recording data**

#### **3.10.1 Germination percentage**

Germination percentage was recorded at 12, 24, 36, 48, 60 and 72 hours of germination test. Germination percentage was calculated using the following formula:

$$\% \text{ Germination} = \frac{\text{Number of germinated seeds}}{\text{Total seeds placed in the petri dish}} \times 100$$

#### **3.10.2 Shoot length**

After 48 hours of germination duration, shoot (plumule) length was recorded with a slide calipers carefully and was measured in mm.

#### **3.10.3 Root length**

After 48 hours of germination duration, shoot (radicle) length was recorded with a slide calipers carefully and was measured in mm.

### **3.11 Statistical analysis**

The collected data on various parameters were statically analyzed using MSTAT-C package program. The mean for all the treatment was calculated and analysis of variances of all the characters were performed by F-variance test. The significant of difference between the pairs of treatment means was evaluated by the least significant difference (LSD) test at 5% and at 1% levels of probability.

## CHAPTER – 4

### RESULTS AND DISCUSSION

The effect of extracts on seeds germination and shoot and root elongation of okra and barnyard grass were given below under some heading.

#### **4.1 Effect different spices plant extracts on the germination of barnyard grass**

##### **4.1.1 Effects of clove extract on the germination of barnyard grass**

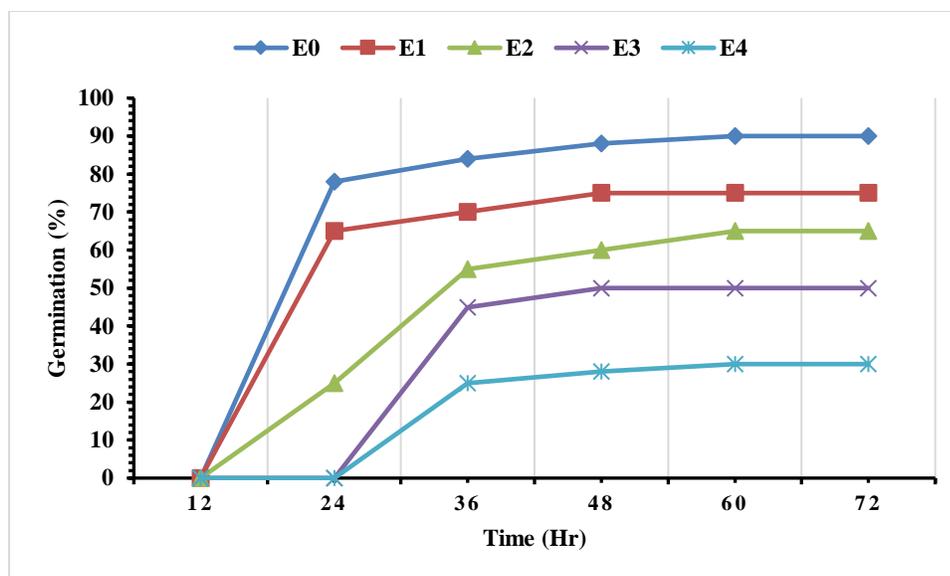
Statistically significant variation was recorded for the germination at different duration of barnyard grass seeds affected by different concentrations of extract of clove (Figure 2). The extract of clove at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of barnyard grass at 24 hours compared to control.

It was found that the control treatment E0 (no extract) gave the highest seed germination which was 90.20% at 60 hours and maximum 90.20% seed germination was fixed up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. But the maximum germination value (74.89%) was found at 60 hour and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, the highest germination value (50.00%) was observed at 60 hours and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 and 24 hours to seed germination test. But the highest germination value (30.35%) was found at 60 hours and it was fixed to 72 hours. These result suggested that clove extract had phytotoxic properties in comparison to control. The concentration dependent inhibitory activity by phytotoxic plant extracts was also reported by Nasir *et al.* (2005).



**Figure 2:** Impact of extracts obtained from Clove on the germination of barnyard grass

E0 = 0 (control without extract), E1 = 0.01 mg dry wt. eq. extract/mL, E2 = 0.03 mg dry wt. eq. extract/mL, E3 = 0.1 mg dry wt. eq. extract/mL, E4 = 0.3 mg dry wt. eq. extract/mL

#### 4.1.2 Effects of Black paper extract on the germination of barnyard grass

Statistically significant variation was recorded for the germination at different duration of barnyard grass seeds affected by different concentrations of extract of Black paper (Figure 3). The extract of Black paper at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of barnyard grass compared to control which is similar with the findings by Bharath *et al.* (2014).

No germination was found from control treatment E0 (no extract) at 12 hours to 24 hours. At 36 hours, 74.00% germination was observed from control treatment E0 (no extract) and highest 80.00% was at 48 hours and also this result was continued up to 72 hours.

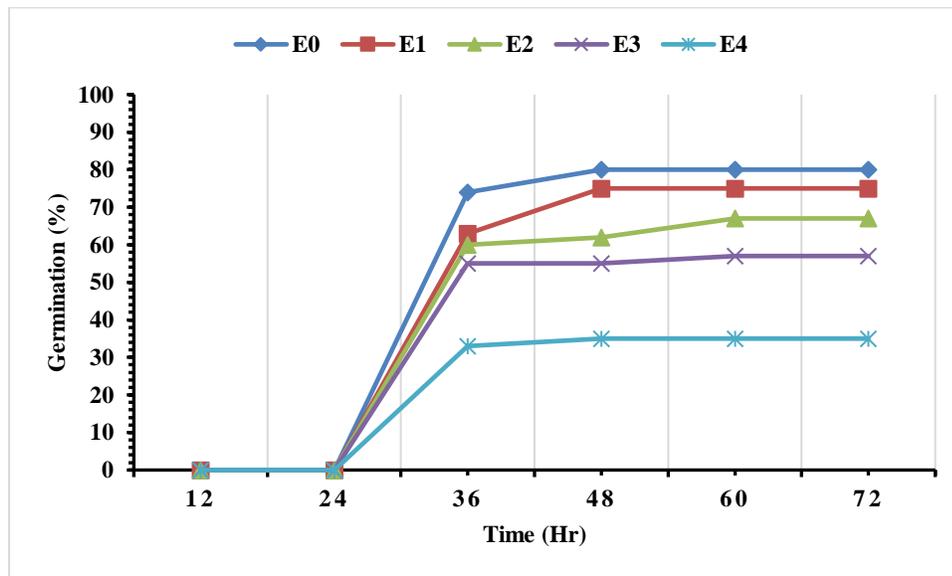
At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to 24 hours seed germination test. 63.00% seed germination value was found at 36 hours

whereas the highest germination value (75.00%) was observed at 48 hours to seed germination test and it was fixed to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to 24 hours seed germination test. 60.00% seed germination value was observed at 36 hours whereas at 60 hours to germination test, the highest seed germination value (67.00%) was found and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was found at 12 and 24 hours to seed germination test. At 36 hours to germination test, 54.80% germination value was showed whereas the highest germination value (56.67%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 and 24 hours to seed germination test. At 48 hours to germination test, 35.33% seed germination value was found which was highest and it was fixed to 72 hours.



**Figure 3:** Impact of extracts obtained from Black paper on the germination of barnyard grass

#### **4.1.3 Effects of Bay leaf extract on the germination of barnyard grass**

Statistically significant variation was recorded for the germination at different duration of barnyard grass seeds affected by different concentrations of extract of Bay leaf (Figure 4). The extract of bay leaf at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of barnyard grass compared to control. The decreasing of germination percentage was proportional to the extract concentrations (Batlang and Shushu, 2007)

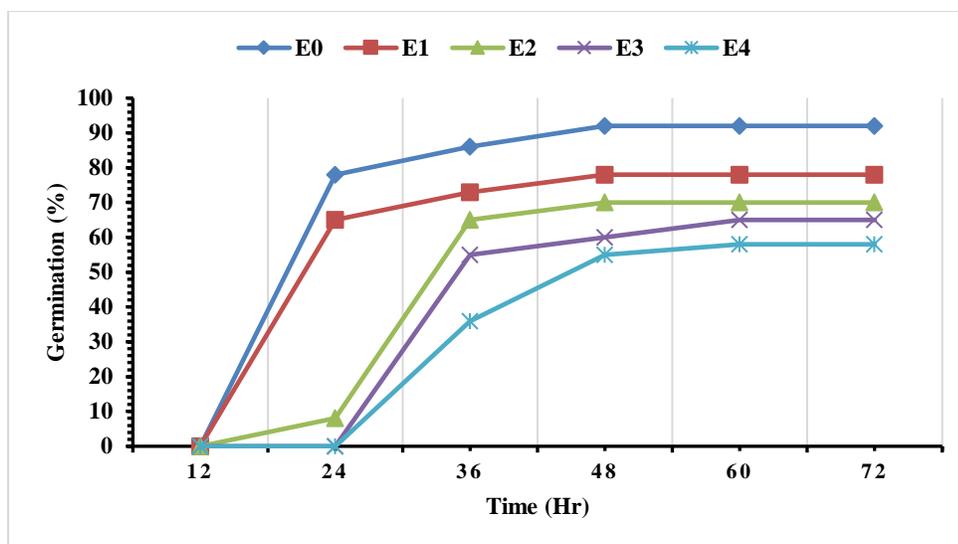
No germination was found from control treatment E0 (no extract) at 12 hours to seed germination test. At 24 hours to germination test, 78.00% germination value was observed from control treatment E0 (no extract) and highest 92.00% was observed at 60 hours of seed treatment and was continued up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 65.00% seed germination value was found whereas the highest germination value 75.10% was observed at 60 hours to seed germination test and this result was continued up to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 8.00% germination value was observed whereas at 60 hours to germination test, the maximum germination value 70.20% was observed and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 and 24 hours to seed germination test. At 36 hours to germination test, 55.00% germination value was observed whereas the highest germination value 64.67% was observed at 60 hours to seed germination test and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, no germination was found at 12 and 24 hours to seed germination test. At 36 hours to germination test, 36.12% germination value was recorded but at 60 hours to germination test, the highest germination value 58.10% was found and it was fixed to 72 hours.



**Figure 4:** Impact of extracts obtained from Bay leaf on the germination of barnyard grass

#### 4.1.4 Effects of Cinnamon extract on the germination of barnyard grass

Statistically significant variation was recorded for the germination at different period of barnyard grass seeds affected by different concentrations of extract of Cinnamon (Figure 5). The extract of cinnamon at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of barnyard grass compared to control (Shajie and Saffari, 2007).

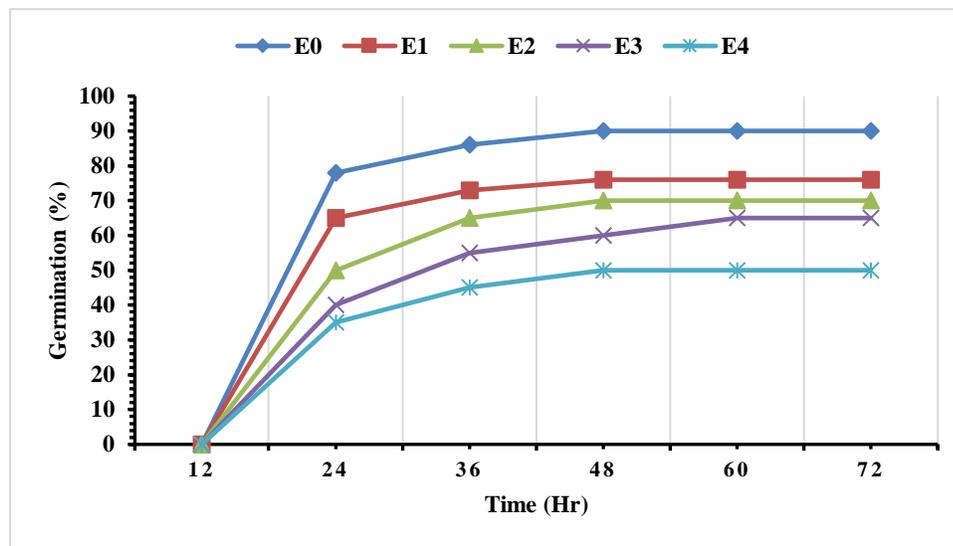
No germination was found from control treatment E0 (no extract) at 12 hours to seed germination test. At 24 hours to germination test, 78.00% germination value was found from control treatment E0 (no extract) and highest 90.33% was at 48 hours of seed treatment and also this result was continued up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. At 24 hours to germination test, 65.35% seed germination value was found whereas the maximum germination value 76.00% was found at 48 hours and it was fixed to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. At 24 hours, 50.33% seed germination value was observed whereas at 48 hours to germination test, the maximum seed germination value 70.00% was found and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours seed germination test. At 36 hours to germination test, 55.23% seed germination value was recorded whereas the maximum germination value (65.37%) was found at 60 hours and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 36 hours to germination test, 45.20% seed germination was observed but at 48 hours to germination test, the maximum germination value (50.00%) was found and it was fixed to 72 hours.



**Figure 5:** Impact of extracts obtained from Cinnamon extract on the germination of barnyard grass

#### 4.1.5 Effects of Cardamom extract on the germination of barnyard grass

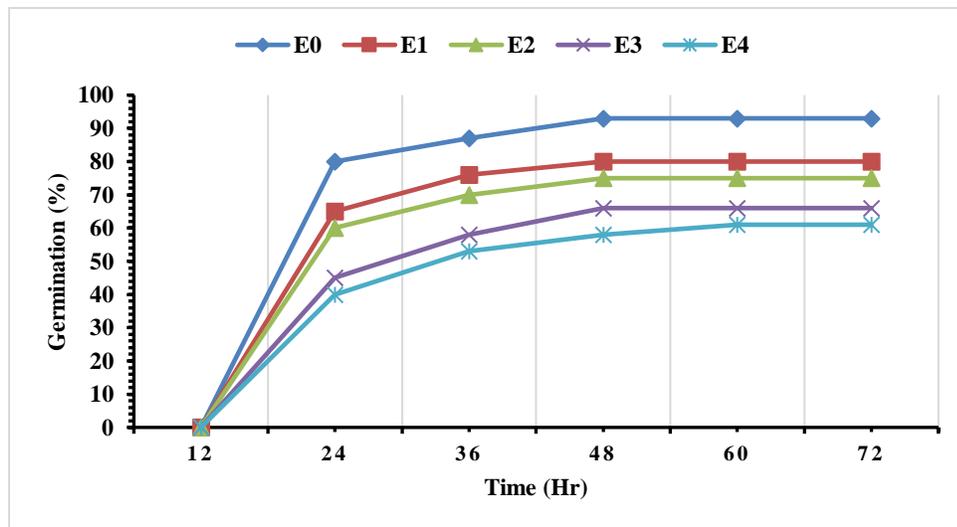
Statistically significant variation was recorded for the germination at different duration of barnyard grass seeds affected by different concentrations of extract of cardamom (Figure 6). The extract of cardamom at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of barnyard grass compared to control. It was found that at 12 hours to seed germination test, no germination was found from control treatment E0 (no extract). At 24 hours to germination test, 80.33% germination value was observed from control treatment E0 (no extract) and highest 93.33% was observed at 48 hours and was continued up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours, 65.37% seed germination value was observed whereas the highest germination value (80.33%) was observed at 48 hours to seed germination test and it was fixed to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours, 60.00% seed germination value was observed whereas at 48 hours to germination test, the highest germination value (75.33%) was observed and fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 45.44% seed germination was recorded whereas the highest germination value 66.20% was found at 48 hours and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, 39.67% seed germination value was observed at 24 hours but at 60 hours to germination test, the maximum germination value 61.00% was observed and it was fixed to 72 hours. The result indicated that increasing concentration level which was decreasing germination value (*Mahmoodzadeh et al.*, 2015)



**Figure 6:** Impact of extracts obtained from Cardamom on the germination of barnyard grass

## **4.2 Effect different spices plant extract on the germination of okra**

### **4.2.1 Effects of Clove extract on the germination of okra**

Statistically significant variation was recorded for the germination percentage at different duration of okra seeds affected by different concentrations of extract of clove (Figure 7). The extract of clove at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of okra compared to control treatment which is compatible with the findings by Han *et al.* (2008).

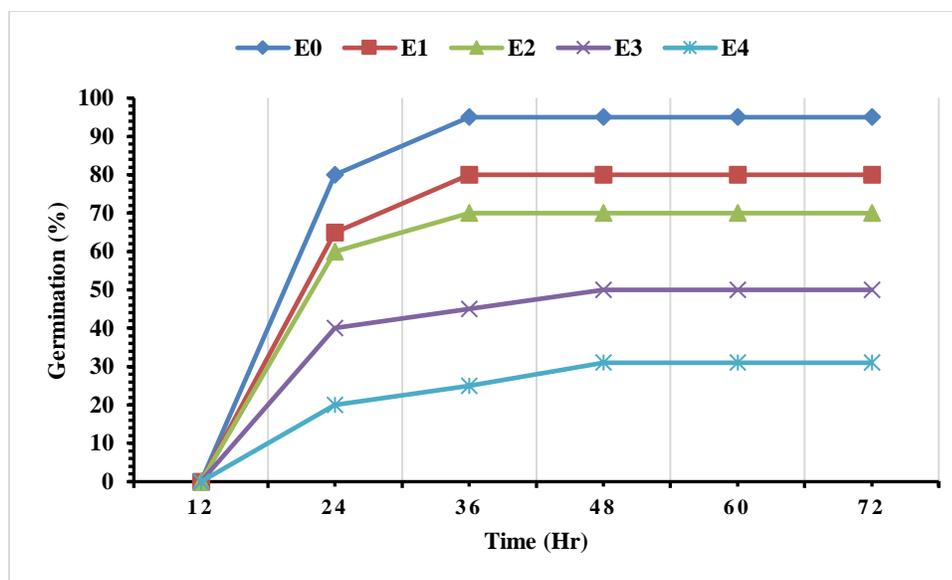
It was found that the control treatment E0 (control) gave the highest percentage of germination value which was 80.40% at 24 hours of seed treatment (with water) and maximum 95.33% was at 36 hours and was continued up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. But the maximum seed germination value (80.33%) was observed at 36 hours to seed germination test and it was fixed to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. But the highest seed germination value (72.67%) was found at 36 hours and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. But the highest germination value (50.15%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, the highest seed germination value (31.33%) was found 48 hours and it was fixed to 72 hours.



**Figure 7:** Impact of extracts obtained from Clove on the germination of okra

#### 4.2.2 Effects of Black paper extract on the germination of okra

Statistically significant variation was recorded for the germination percentage at different duration of okra seeds affected by different levels of extract of Bay leaf (Figure 8). The extract of black paper at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of okra compared to control treatment.

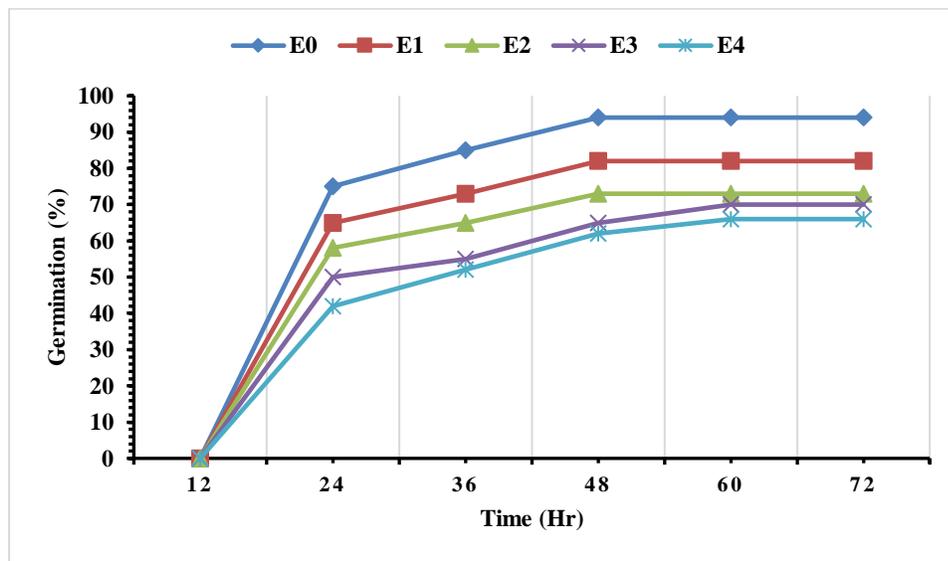
No germination was found from control treatment E0 (no extract) at 12 hours to seed germination. At 24 hours to germination test, 75.00% seed germination value was observed from control treatment E0 (no extract) and highest 94.33% was found at 48 hours and also this result was continued up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours, 65.00% seed germination value was observed whereas the highest germination value 82.00% was recorded at 48 hours to seed germination test.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. At 24 hours to germination test, 57.87% seed germination value was recorded whereas at 48 hours to germination test, the highest germination value (73.33%) was recorded and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. At 24 hours, 50.00% seed germination value was recorded whereas the highest seed germination value (70.00%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, 42.37% seed germination value was recorded at 24 hours but at 60 hours to germination test, the maximum seed germination (65.38%) was found and it was fixed to 72 hours. The result showed that the extract of black paper had phytotoxic influence on okra (Oyerinde *et al.*, 2009)



**Figure 8:** Impact of extracts obtained from Black paper on the germination of okra

#### 4.2.3 Effects of Bay Leaf Extract on the Germination of Okra

Statistically significant variation was recorded for the germination percentage at different duration of okra seeds affected by different concentrations of extract of bay leaf (Figure 9). The extract of bay leaf at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of okra compared to control treatment (Tawaha and turk, 2003).

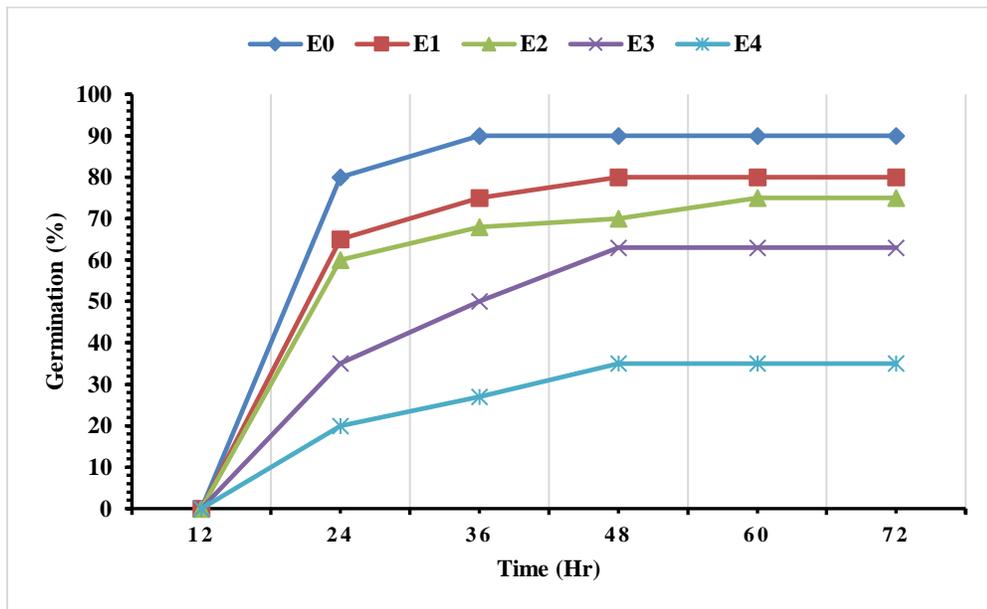
It was found that at 12 hours to seed germination test, no germination was observed from control treatment E0 (no extract). At 24 hours to germination test, 80.37% seed germination value was found and highest germination value was 90.33% at 36 hours and also this result was continued up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 65.00% seed germination was found whereas the highest germination value (80.00%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 59.67% seed germination value was found whereas at 60 hours to germination test, the highest germination value (74.67%) was found and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours, 35.33% seed germination was showed whereas the highest seed germination value (63.00%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, 20.00% seed germination was found at 24 hours but the highest seed germination value (35.00%) was found at 48 hours and it was fixed to 72 hours.



**Figure 9:** Impact of extracts obtained from Bay leaf on the germination of okra

#### **4.2.4 Effects of Cinnamon extract on the germination of okra**

Statistically Significant variation was recorded for the germination percentage at different duration of okra seeds affected by different concentrations of extract of cardamom (Figure 10). The extract of cinnamon at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of okra compared to control treatment (Hang *et al.*, 2008)

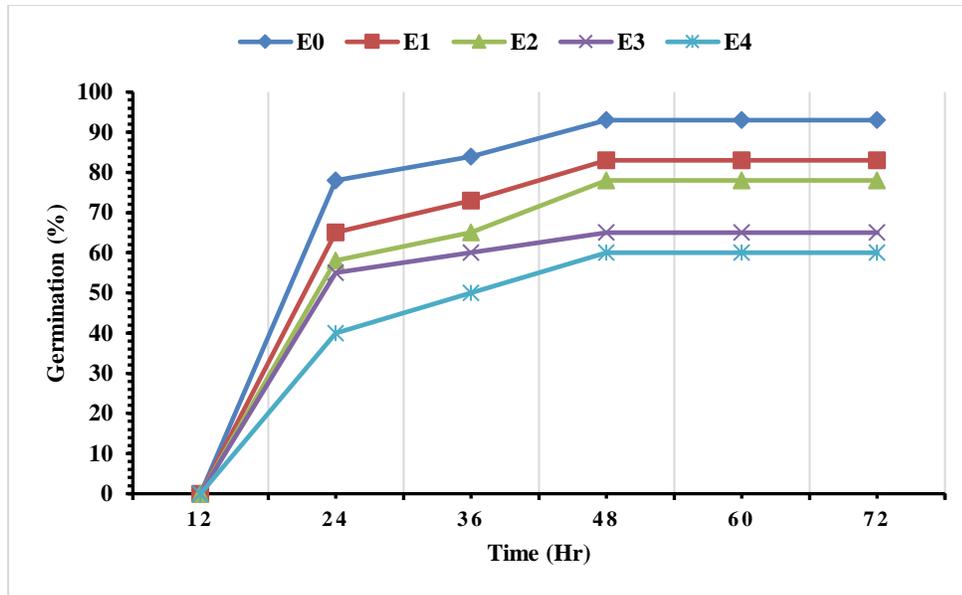
No germination was found from control treatment E0 (no extract) at 12 hours to seed germination test. At 24 hours to germination test, 77.77% seed germination was observed from control treatment E0 (no extract) and maximum 93.00% was found at 48 hours of seed treatment and also this result was continued up to 72 hours.

At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. At 24 hours, 65.00% seed germination was found whereas the highest germination value (83.37%) was observed at 48 hours to seed germination test and it was fixed to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. At 24 hours, 58.00% seed germination was observed whereas at 48 hours to germination test, the highest germination value (78.00%) was observed and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was observed at 12 hours to seed germination test. At 24 hours, 55.37% seed germination value was recorded whereas the highest germination value (65.33%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, 40.00% seed germination value was observed at 24 hours but at 48 hours to germination test, the highest germination value (60.00%) was found and it was fixed to 72 hours.



**Figure 10:** Impact of extracts obtained from Cinnamon on the germination of okra

#### 4.2.5 Effects of Cardamom extract on the germination of okra

Statistically significant variation was recorded for the germination percentage at different duration of okra seeds affected by different concentrations of extract of Cardamom (Figure 11). The extract of cardamom at the 0.3 mg dry wt. eq. extract/mL concentration resulted in significantly lower value of germination of okra compared to control treatment. The different concentration extract was significantly influenced germination percentage which is compatible with the findings by Pirzad *et al.*, (2010).

No germination was found from control treatment E0 (no extract) at 12 hours to seed germination test. At 24 hours to germination test, 82.33% seed germination value was found and highest 94.33% was at 36 hours and also this result was continued up to 72 hours.

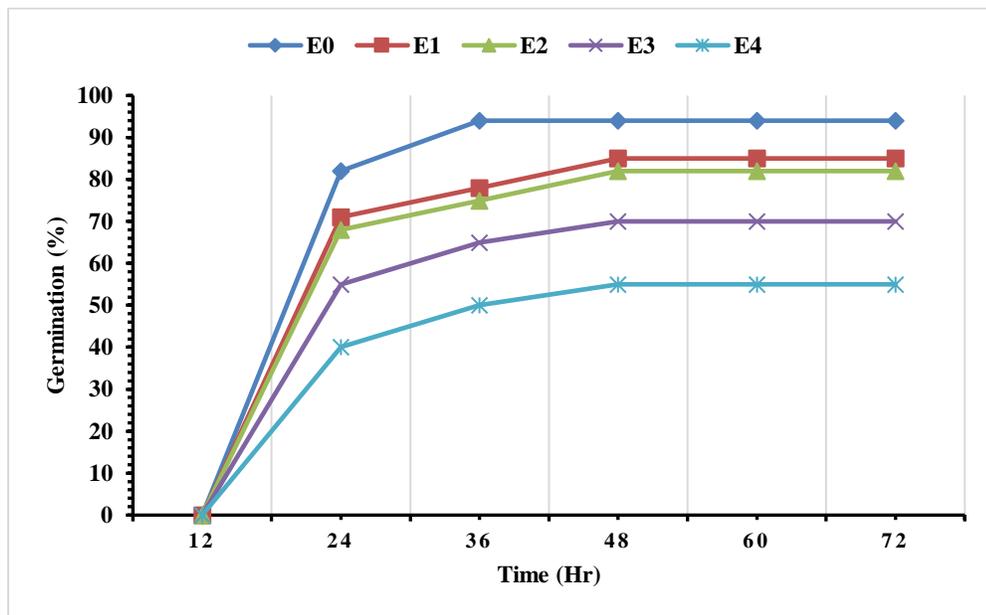
At E1 (0.01 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 70.67% seed germination value was observed whereas the highest germination value (84.53%) was found at 85 hours to seed germination test and it was fixed to 72 hours.

At E2 (0.03 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 68.00% seed germination value

was observed whereas the highest germination value (82.33%) was found at 48 hours and it was fixed to 72 hours.

At E3 (0.1 mg dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 55.33% seed germination value was observed whereas the highest germination value (69.67%) was observed at 48 hours to seed germination test and it was fixed to 72 hours.

At E4 (0.3 mg dry wt. eq. extract/mL) extract, 40.00% seed germination value was found at 24 hours but at 48 hours to germination test, the highest seed germination value (55.00%) was found and it was fixed to 72 hours.

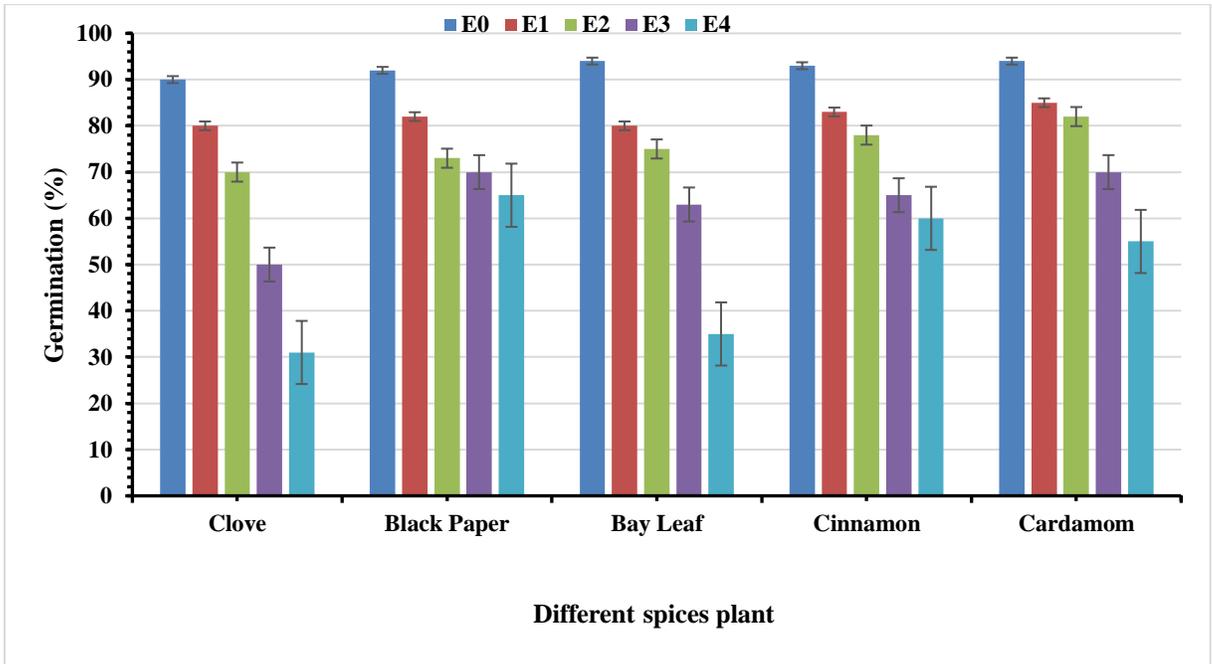


**Figure 11:** Impact of extracts obtained from Cardamom on the germination of okra

#### **4.3 Comparison on effect of spices plant extract on seed germination of barnyard grass**

Figure 12 showed that the lowest germination percentage was observed from at 0.3 mg dry wt. eq. extract/mL concentration with Clove extract compared to other spices plant extracts whereas Cardamom extract showed highest percentage of germination at E4 (0.3 mg dry wt. eq. extract/mL) concentration. Similar trend was found for E1 (0.01 mg dry wt. eq. extract/mL), E2 (0.03 mg dry wt. eq. extract/mL) and E3 (0.1 mg dry wt. eq.

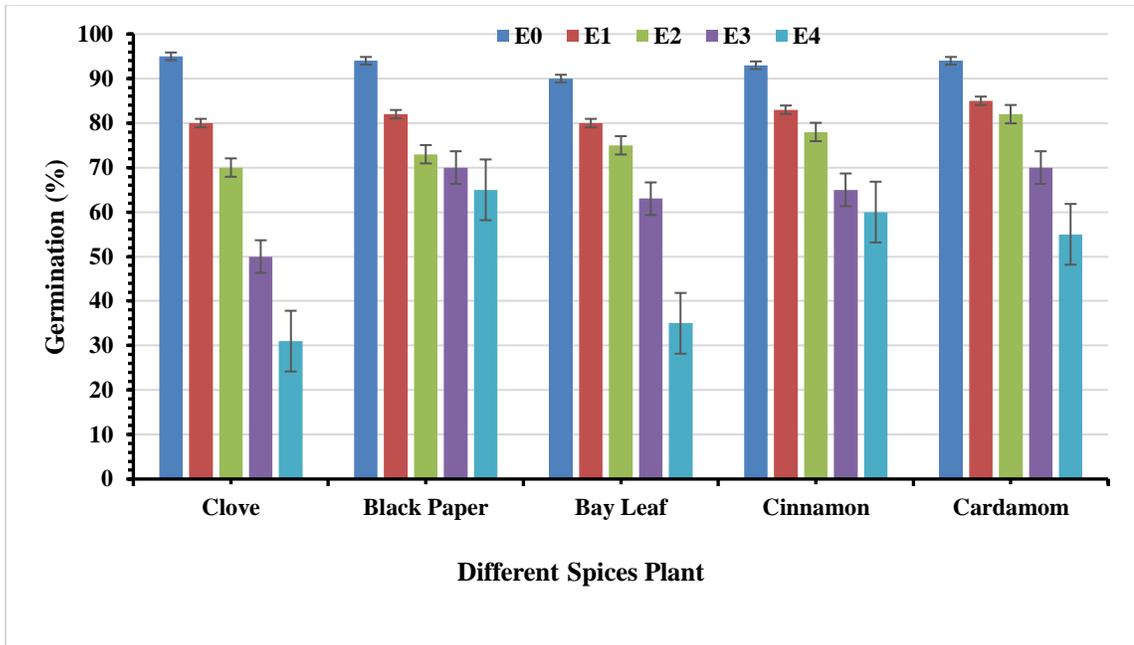
extract/mL) concentration. So, it can be mention that Clove showed highest phytotoxicity with barnyard grass compared to other spices plant.



**Figure 12:** Germination percentage of barnyard grass affected by different extracts of spices plant

#### 4.4 Comparison on effect of spices plant extract on seed germination of okra

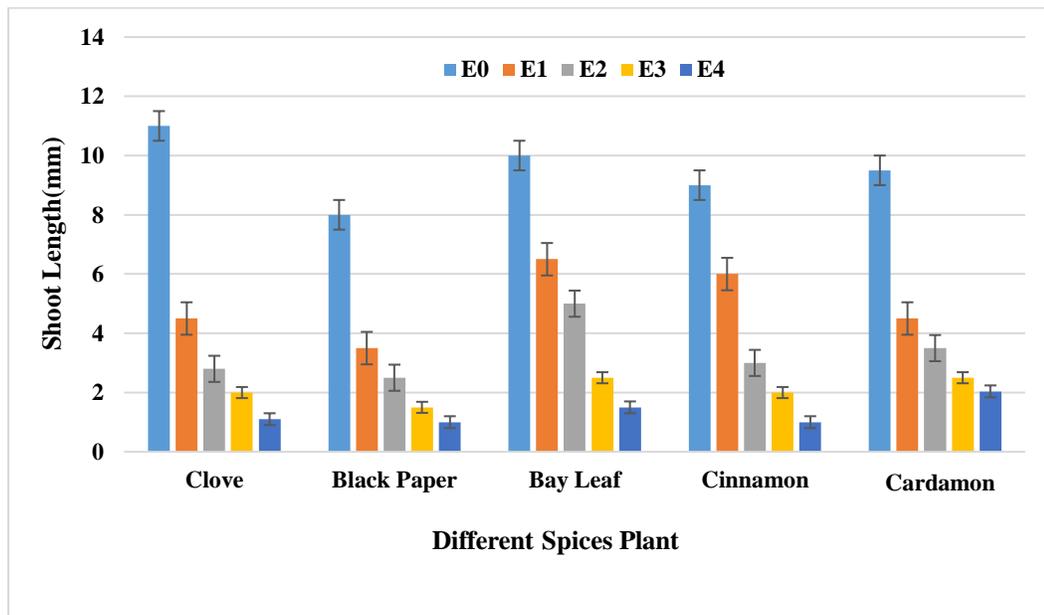
Figure 13 showed that the lowest germination percentage was found from at 0.3 mg dry wt. eq. extract/mL concentration with Clove extract compared to other plant extracts whereas the Cardamom extract showed highest percentage of germination E4 (0.3 mg dry wt. eq. extract/mL) concentration. Similar trend was found for E1 (0.01 mg dry wt. eq. extract/mL), E2 (0.03 mg dry wt. eq. extract/mL) and E3 (0.1 mg dry wt. eq. extract/mL) extract. So, it can be mention that Clove showed highest phytotoxicity with okra compared to other spices plant.



**Figure 13:** Germination percentage of okra affected by different extracts of spices plant

#### 4.5 Effect of different spices plant extract on shoot length of barnyard grass

The average marked difference on the length of shoot produced by barnyard grass was recorded and the effect of different spices extract on shoot length of barnyard grass indicated considerable differences due to different treatment concentrations were given below.



**Figure 14:** Shoot length (mm) of barnyard grass at 48 hours affected by different spices plant extract

#### **4.5.1 Effect of clove extract on shoot length of barnyard grass**

Statistically significant variation was recorded for the shoot length barnyard grass seed affected by different concentrations of extract of Clove (Figure 14). Results indicated that the highest shoot length (7.5 mm) was observed from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (4.50 mm). The lowest shoot length (1.1 mm) was observed at the 0.3 mg dry wt. eq. extract/mL concentration. It was observed that shoot length was decreased with increasing of concentration and as a result lowest root length gave at E4 (0.3 mg dry wt. eq. extract/mL) extract which is compatible with the finding by Liu and chen (2011)

#### **4.5.2 Effect of Black paper extract on shoot length of barnyard grass**

Statistically significant variation was recorded for the shoot length of barnyard grass seed affected by different concentrations of extract of Black paper (Figure 14). Results indicated that the highest shoot length (3.2 mm) was recorded from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (2.41 mm). The lowest shoot length (0.82 mm) was observed from seed treated with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that shoot length was increased with decreasing of concentration and highest shoot length was found from control treatment E0 (no extract). This result was similar to the findings by Iqbal *et al.* (2014).

#### **4.5.3 Effect of Bay leaf extract on shoot length of barnyard grass**

Statistically significant variation was recorded for the shoot length barnyard grass seed affected by different concentrations of extract of Bay leaf (Figure 14). Results indicated that the highest shoot length (10.20 mm) was found from control treatment E0 (no extract) followed by 0.01 mg dry wt. eq. extract/mL concentration (6.50 mm). The lowest shoot length (1 mm) was observed from seed treated with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that shoot length was decreased with increasing of concentration and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

#### **4.5.4 Effect of Cinnamon extract on shoot length of barnyard grass**

Statistically significant variation was recorded for the shoot length of barnyard grass seed affected by different concentrations of extract of Cinnamon (Figure 14). Results

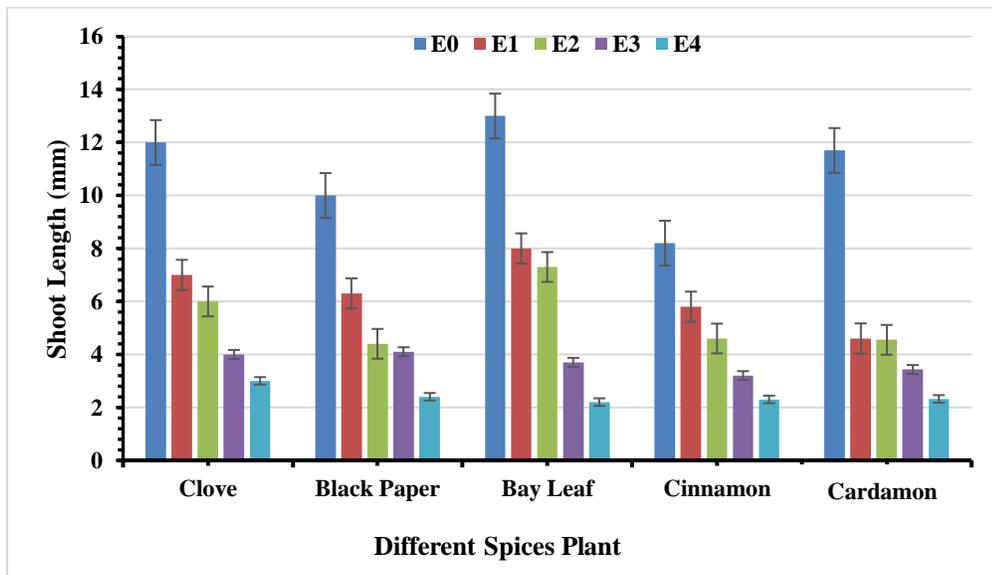
showed that the highest shoot length (3.30 mm) was found on control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (2.23 mm) whereas the lowest shoot length (0.75 mm) was observed from seed treated with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that shoot length was decreased with increasing of concentration and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

#### 4.5.5 Effect of Cardamom extract on shoot length of barnyard grass

Non-significant variation was recorded for the shoot length barnyard grass seeds affected by different concentrations of extract of Cardamom (Figure 14). However, it was found that the highest shoot length (2.19 mm) was showed from control treatment E0 (no extract) followed by 0.01 mg dry wt. eq. extract/mL concentration (2.17 mm) whereas the lowest shoot length (2.10 mm) was showed from seed treated with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that shoot length was decreased with increasing of concentration and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

#### 4.6 Effect different spices plant extract on root length of barnyard grass

Barnyard grass under study showed variations their root length. Considerable significant differences of root length were indicating among treatments studied given below.



**Figure 15:** Root length (mm) of barnyard grass at 48 hours affected by different spices plant extract

#### **4.6.1 Effect of Clove extract on root length of barnyard grass**

Statistically significant variation was recorded for the root length barnyard grass seeds affected by different concentrations of extract of clove (Figure 15). Results indicated that the highest root length (12.10 mm) was found from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (7.19 mm). The minimum root length (2.23 mm) was found from seed treated with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was decreased with increasing of concentration and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length. The inhibition of growth influenced by the concentration. This result was similar to the findings by Jan *et al.* (2013).

#### **4.6.2 Effect of Black paper extract on root length of barnyard grass**

Statistically significant variation was recorded for the root length of barnyard grass seeds affected by different concentrations of extract of Black paper (Figure 15). Results showed that the highest root length (4.10 mm) was found from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (3.36 mm). The lowest root length (1.47 mm) was observed from seed treatment with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment E0 (no extract).

#### **4.6.3 Effect of Bay leaf extract on root length of barnyard grass**

Statistically significant variation was recorded for the root length barnyard grass seeds affected by different concentrations of extract of Bay leaf (Figure 15). Results indicated that the highest root length (17.52 mm) was recorded from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (12.64 mm). The lowest root length (1.12 mm) was observed from seed treated with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was decreased with increasing of concentration and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

#### **4.6.4 Effect of Cinnamon extract on root length of barnyard grass**

Statistically significant variation was recorded for the root length of barnyard grass seeds affected by different concentrations of extract of Cinnamon (Figure 15). Results

indicated that the highest root length (4.21 mm) was showed from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (3.80 mm). The lowest root length (1.32 mm) was observed from seed treatment with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment E0 (no extract).

#### 4.6.5 Effect of Cardamom extract on root length of barnyard grass

Non-significant variation was recorded for the root length barnyard grass seeds affected by different concentrations of extract of cardamom (Figure 15). Results indicated that the highest root length (2.71 mm) was showed from control treatment which was statistically identical with 0.01 mg dry wt. eq. extract/mL concentration extract (2.62 mm). The lowest root length (2.37 mm) was observed from seed treated with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was decreased with increasing of concentration and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

#### 4.7 Effect different spices plant extract on root length of okra

Okra under study showed variations their root length. Considerable significant differences of root length were indicating among treatments studied given below.

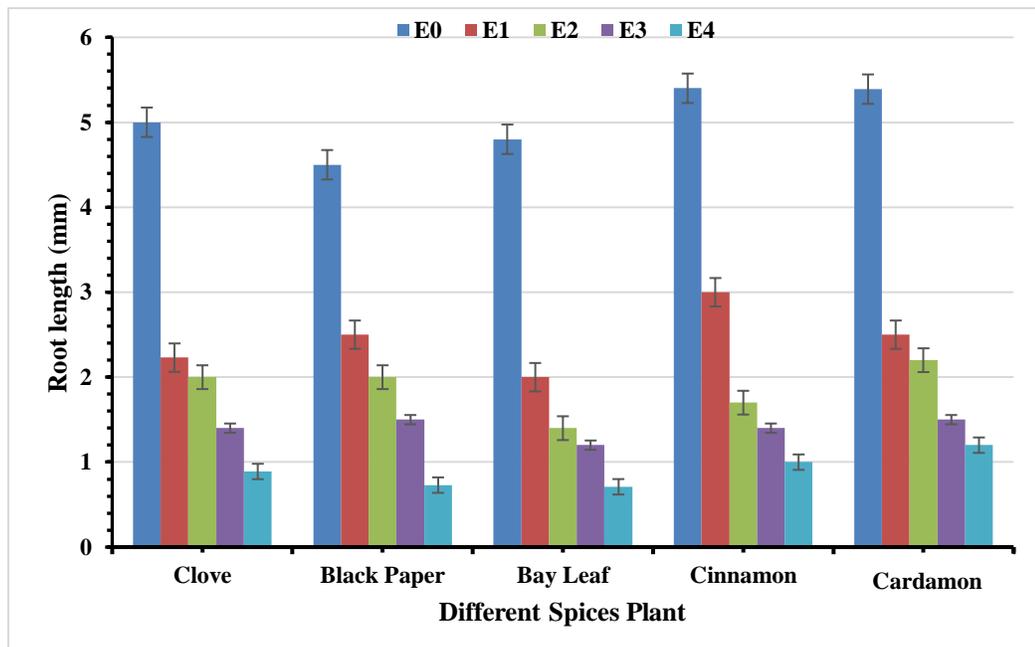


Figure 16: Root length (mm) of okra at 48 hours affected by different spices plant

#### **4.7.1 Effect of Clove extract on root length of okra**

Statistically significant variation was recorded for the root length okra seeds affected by different concentrations of extract of clove (Figure 16). Results indicated that the highest root length (5.00 mm) was indicated from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (2.23 mm). The lowest root length (0.89 mm) was observed from seed treatment with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was decreased with increasing of concentration and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

#### **4.7.2 Effect of Black paper extract on root length of okra**

Statistically significant variation was recorded for the root length okra seeds affected by different concentrations of extract of Black paper (Figure 16). Results indicated that the highest root length (4.5 mm) was indicated from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (2.5 mm). The lowest root length (0.73 mm) was observed from seed treatment with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment E0 (no extract).

#### **4.7.3 Effect Bay leaf on root length of okra**

Statistically significant variation was recorded for the root length okra seeds affected by different concentrations of extract of Bay leaf (Figure 16). Results indicated that the highest root length (4.8 mm) was found from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (2.20 mm). The lowest root length (0.71 mm) was observed from seed treatment with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was decreased with increasing concentration of spices plants for seed treatment and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

#### **4.7.4 Effect of Cinnamon on root length of okra**

Statistically significant variation was recorded for the root length okra seeds affected by different levels of extract of Cinnamon (Figure 16). Results indicated that the highest root length (5.40 mm) was showed from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (3 mm). The lowest root length (1 mm) was observed from

seed treatment with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment E0 (no extract).

#### **4.7.5 Effect Cardamom extract on root length okra**

Statistically significant variation was recorded for the root length okra seeds affected by different concentrations of extract of Cardamom (Figure 16). Results indicated that the highest root length (5.39 mm) was showed from control treatment followed by 0.01 mg dry wt. eq. extract/mL concentration (2.5 mm). The lowest root length (1.2 mm) was observed from seed treatment with 0.3 mg dry wt. eq. extract/mL concentration. It was observed that root length was decreased with increasing concentration of spices plants for seed treatment and as a result E4 (0.3 mg dry wt. eq. extract/mL) extract gave lowest root length.

## CHAPTER – 5

### SUMMARY AND CONCLUSION

The extracts of different spices plant at different concentration affected the germination of test plants. The phytotoxic effects of spices plant extracts on seed germination depended on their concentrations, the inhibition was stronger at the higher concentrations. The germination percentage values of test plants were recorded maximum at control conditions and was decreased upon applying at 0.01, 0.03, 0.1 and 0.3 mg dry wt. eq. extract/mL concentrations respectively. The minimum inhibitory activity was observed with control treatment. As the treatment concentration value increased, the germination percentage value of the plants were decreased in consistent and the least value of germination percentage was found from 0.3 mg dry wt. eq. extract/mL .From the above result, the delay of germination and reduction in germination percentage of all test plants were more pronounced with Clove extract at E4 (0.3 mg dry wt. eq. extract/mL) concentration which showed highest phytotoxic effect for seed germination compared to all other concentration of all spices plant. All the growth of test plants seedling were adversely affected by the application of the extracts of spices plant at different concentration. The inhibition of root and shoot growth of the barnyard seeds were more pronounced with cinnamon at E4 (0.3 mg dry wt. eq. extract/mL) concentration. Similarly, the inhibition of root growth of the okra seeds were more pronounced with bay leaf extract at E4 (0.3 mg dry wt. eq. extract/mL) concentration. Extracts of spices plant were more phytotoxic at higher concentration as compared to lower concentration.

In this study, extracts of spices plant (clove, Black paper, Bay leaf, Cinnamon and cardamom) effect on all test plant, reduced seed germination and seedling growth. The phytotoxic potential of 0.3 mg dry wt. eq. extract/mL concentration germination and seedling growth of all test plants were more as compared to .01 mg dry wt. eq. extract/mL concentration and spice plant species.

So, it can be concluded that Clove and Bay leaf are more phytotoxic for okra seeds. On the other hand, Clove and Cinnamon are more phytotoxic for barnyard grass seeds. Therefore, it is possible to use these extracts as a component for production of bio-herbicides. However further works are needed for greenhouse and field test and chemical characterization of these extracts.

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## APPENDICES

### Appendix I: Effect of Clove extract on barnyard grass seeds germination and root-shoot length

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root and shoot length (mm) at 48 hours |        |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|--------|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours | Shoot   | Root   |
| Replication          | 2                  | -                              | 0.145    | 0.057    | 0.075    | 0.116    | 0.007    | 0.003   | 0.010  |
| Treatment            | 4                  | -                              | 8.25     | 10.13    | 8.36     | 11.27    | 9.75     | 0.82**  | 1.22** |
| Error                | 8                  | -                              | 0.013    | 0.021    | 0.036    | 0.027    | 0.052    | 0.002   | 0.001  |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

### Appendix II: Effect of Black paper extract on barnyard grass seeds germination and root-shoot length

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root and shoot length (mm) at 48 hours |       |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|-------|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours | Shoot   | Root  |
| Replication          | 2                  | -                              | -        | 0.035    | 0.087    | 0.048    | 0.101    | 0.001   | 0.003 |
| Treatment            | 4                  | -                              | -        | 11.15*   | 8.27*    | 6.28*    | 14.38**  | 0.47**  | 1.10* |
| Error                | 8                  | -                              | -        | 0.013    | 0.024    | 0.018    | 0.044    | 0.002   | 0.003 |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix III: Effect of Bay leaf extract on barnyard grass seeds germination and root-shoot length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root and shoot length (mm) at 48 hours |       |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|-------|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours | Shoot   | Root  |
| Replication          | 2                  | -                              | 0.113    | 0.011    | 0.064    | 0.047    | 0.104    | 0.011   | 0.003 |
| Treatment            | 4                  | -                              | 8.46**   | 11.25**  | 7.24**   | 9.37**   | 14.06*   | 1.05*   | 0.62* |
| Error                | 8                  | -                              | 0.102    | 0.008    | 0.015    | 0.117    | 0.022    | 0.001   | 0.002 |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix IV: Effect of Cinnamon extract on barnyard grass seeds germination and root-shoot length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root and shoot length (mm) at 48 hours |        |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|--------|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours | Shoot   | Root   |
| Replication          | 2                  | -                              | 0.085    | 0.073    | 0.108    | 0.063    | 0.042    | 0.12  | 0.033  |
| Treatment            | 4                  | -                              | 14.28**  | 9.59**   | 6.49**   | 6.33**   | 7.41**   | 0.45**  | 1.05** |
| Error                | 8                  | -                              | 0.007    | 0.014    | 0.102    | 0.206    | 0.011    | 0.001   | 0.003  |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix V: Effect of Cardamom extract on barnyard grass seeds germination and root-shoot length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root and shoot length (mm) at 48 hours |         |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|---------|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours | Shoot   | Root    |
| Replication          | 2                  | -                              | 0.074    | 0.043    | 0.054    | 0.104    | 0.086    | 0.006   | 0.001   |
| Treatment            | 4                  | -                              | 15.37**  | 8.57**   | 10.27**  | 9.04**   | 7.52**   | NS  | 1.011** |
| Error                | 8                  | -                              | 0.104    | 0.073    | 0.049    | 0.092    | 0.029    | 0.002   | 0.003   |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix VI: Effect of Clove extract on okra seeds germination and root length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root length (mm) at 48 hours |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours |   |
| Replication          | 2                  | -                              | 0.122    | 0.247    | 0.212    | 0.014    | 0.133    | 0.001                                       |
| Treatment            | 4                  | -                              | 6.241*   | 8.314*   | 7.176*   | 12.32**  | 18.36**  | 1.04**                                      |
| Error                | 8                  | -                              | 0.113    | 0.435    | 1.044    | 0.632    | 0.711    | 0.003                                       |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix VII: Effect of Black paper extract on okra seeds germination and root length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root length (mm) at 48 hours |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours |   |
| Replication          | 2                  | -                              | 0.010    | 0.038    | 0.107    | 0.033    | 0.107    | 0.001                                       |
| Treatment            | 4                  | -                              | 5.31*    | 7.24*    | 7.28*    | 10.90**  | 16.37**  | 1.02**                                      |
| Error                | 8                  | -                              | 0.015    | 0.131    | 0.10     | 0.024    | 0.155    | 0.003                                       |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix VIII: Effect of Bay leaf extract on okra seeds germination and root length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root length (mm) at 48 hours |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours |   |
| Replication          | 2                  | -                              | 0.205    | 0.146    | 0.083    | 0.071    | 0.042    | 0.002                                       |
| Treatment            | 4                  | -                              | 12.10**  | 14.29**  | 8.52*    | 9.37*    | 6.54*    | 0.62**                                      |
| Error                | 8                  | -                              | 0.106    | 0.083    | 0.104    | 0.076    | 0.069    | 0.003                                       |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix IX: Effect of Cinnamon extract on okra seeds germination and root length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root length (mm) at 48 hours |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours |   |
| Replication          | 2                  | -                              | 0.122    | 0.114    | 0.104    | 0.114    | 0.016    | 0.013                                       |
| Treatment            | 4                  | -                              | 16.31**  | 8.34**   | 11.28**  | 8.56**   | 9.47**   | 0.75**                                      |
| Error                | 8                  | -                              | 0.202    | 0.088    | 0.095    | 0.106    | 0.073    | 0.002                                       |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

**Appendix X: Effect of Cardamom extract on okra seeds germination and root length**

| Sources of variation | Degrees of freedom | Mean square of Germination (%) |          |          |          |          |          | Mean square of Root length (mm) at 48 hours |
|----------------------|--------------------|--------------------------------|----------|----------|----------|----------|----------|---|
|                      |                    | 12 hours                       | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours |   |
| Replication          | 2                  | -                              | 0.002    | 0.017    | 0.009    | 0.104    | 0.035    | 0.003                                       |
| Treatment            | 4                  | -                              | 9.25*    | 10.59**  | 15.26**  | 7.42*    | 6.59**   | 0.46**                                      |
| Error                | 8                  | -                              | 0.093    | 0.136    | 0.069    | 0.044    | 0.101    | 0.002                                       |

NS = Non-significant \* = Significant at 5% level \*\* = Significant at 1% level

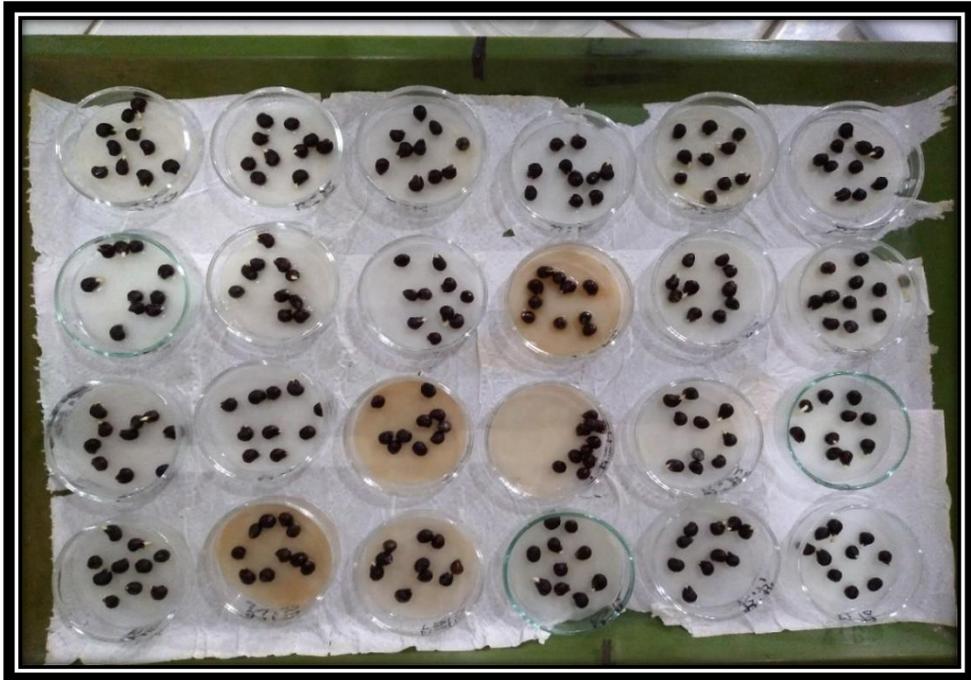
**Appendix XI: Some pictorial view related to the experiment**



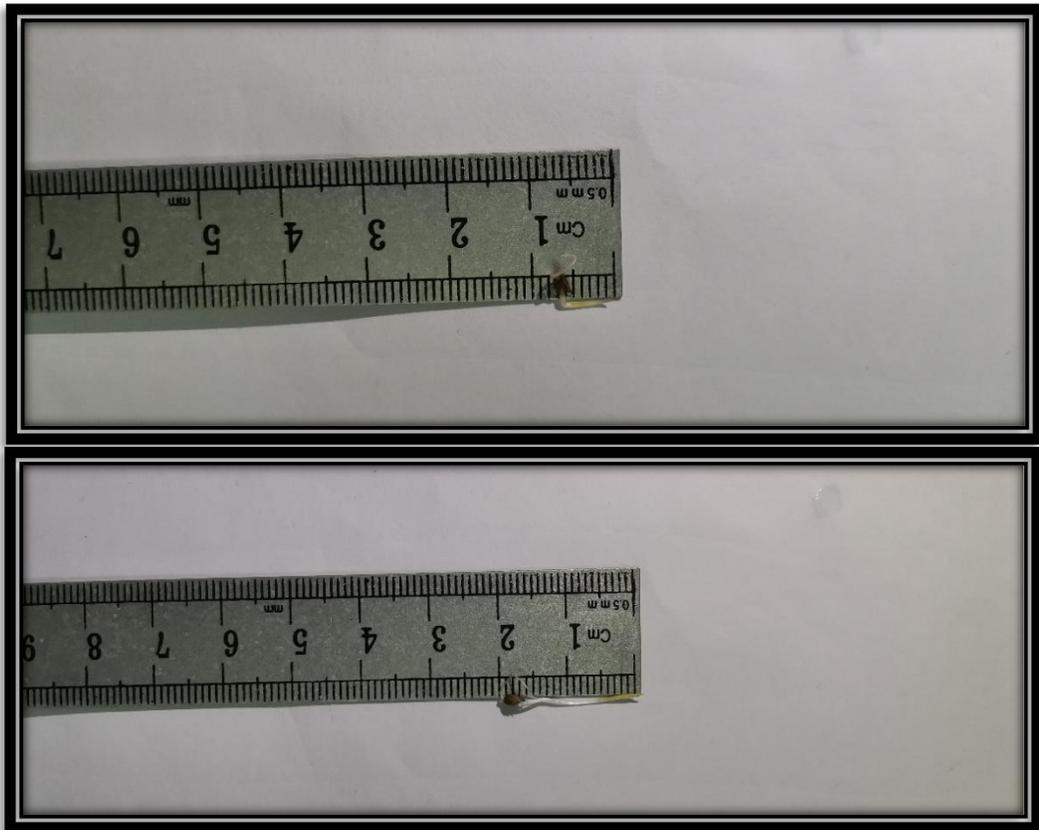
*Plate 1: Applying of extract on petri dish*



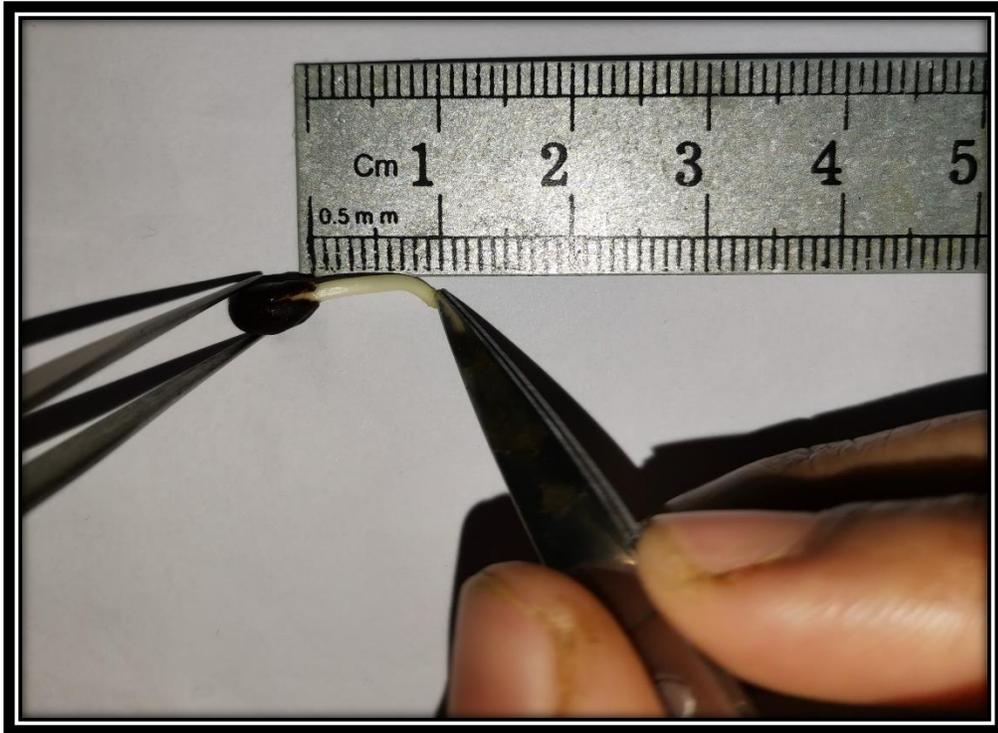
*Plate 2: Barnyard grass seeds set up of experiment*



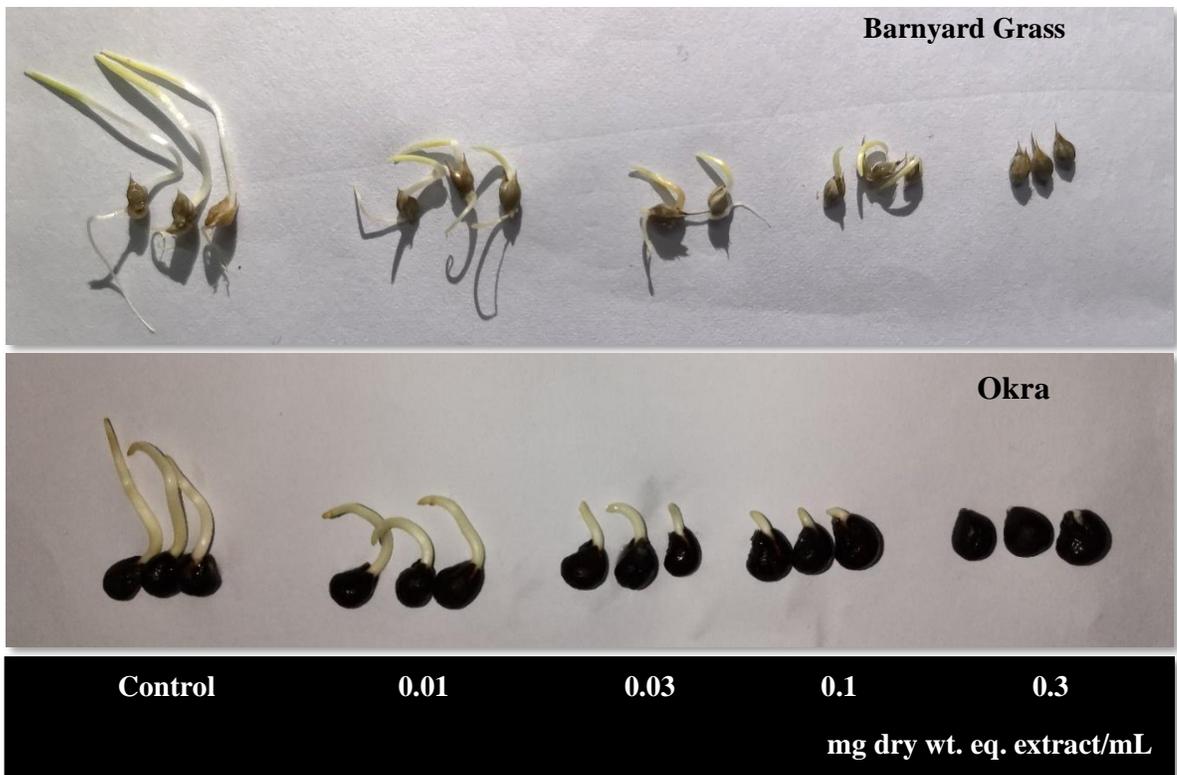
*Plate 3: Okra seeds set up of experiment*



*Plate 4: Data collection on shoot length of barnyard grass seed*



*Plate 5: Data collection on root length of okra seed*



*Plate 6: Effect of spices plant extracts on the seedling growth of the test plant species*