ASSESSMENT OF PHYTOTOXIC ACTIVITY OF SOME SELECTED MEDICINAL PLANTS IN BANGLADESH

MD. MAMUN

REGISTRATION NO. 13-05705

DEPARTMENT OF AGRICULTURAL CHEMISTRY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA -1207

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MD. MAMUN

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Approved by:

Dr. Md. Sirajul Islam Khan	**Dr. Mohammed Ariful Islam**
Professor	**Professor**
Department of Agricultural Chemistry	Department of Agricultural Chemistry
SAU, Dhaka	SAU, Dhaka
Supervisor	**Co-Supervisor**

Prof. Dr. Md. Tazul Islam Chowdhury
Chairman
Department of Agricultural Chemistry

DEPARTMENT OF AGRICULTURAL CHEMISTRY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar,Dhaka-1207

CERTIFICATE

This is to certify that the thesis entitled **"ASSESSMENT OF PHYTOTOXIC ACTIVITY OF SOME SELECTED MEDICINAL PLANTS 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 bonafide research work carried out by **MD. MAMUN**, Registration No. **13-05705** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

June, 2020

Dhaka, Bangladesh

(Dr. Md. Sirajul Islam Khan)
Professor
Department of Agricultural Chemistry
SAU, Dhaka

Dedicated to
My
Beloved Parents

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The Author

ASSESSMENT OF PHYTOTOXIC ACTIVITY OF SOME SELECTED MEDICINAL PLANTS IN BANGLADESH

ABSTRACT

The study was carried out at the Laboratory of the Department of Agricultural Chemistry of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, during the period from December 2019 to March 2020. Investigation was conducted to evaluate the effect of five medicinal plant species (Henna, Gardenia, Bohera, Nagesshor and Tamarind) on phytotoxicity regarding germination and root and shoot growth of two test species (okra and barnyard grass). Five concentrations of each medicinal plant extract *viz*. P_0 (control; no extract), P_1 (0.01 g dry wt. eq. extract/mL), P_2 (0.03 g dry wt. eq. extract/mL), P_3 (0.1 g dry wt. eq. extract/mL) and P_4 (0.3 g dry wt. eq. extract/mL) were used for phytotoxicity test. The experiment was laid out in Completely Randomized Design (CRD) with three replications. Results showed that all the test species were found sensitive under extracts of all five medicinal plants. Results indicated that all concentrations of plant extract showed phytotoxic effect on okra and barnyard grass. Among all concentration of plant extract, P_4 (0.3 g dry wt. eq. extract/mL) showed highest phytotoxic effect on both okra and barnyard grass seeds. We found that Gardenia extract at P_4 (0.3 g dry wt. eq. extract/mL) concentration in okra seeds showed highest phytotoxic effect for seed germination (30%). Similarly, Henna extract at P_4 (0.3 g dry wt. eq. extract/mL) concentration in barnyard grass seeds showed highest phytotoxic effect for seed germination (30%). Again, in terms of root length of okra seeds, Bohera extract at P_4 (0.3 g dry wt. eq. extract/mL) showed highest phytotoxic effect and gave lowest root length (0.72 mm). Similarly, Bohera extract at P_4 (0.3 g dry wt. eq. extract/mL) concentration on barnyard grass seeds showed highest phytotoxic effect for shoot and root length (0.67 and 0.99 mm, respectively). The above results suggested that those medicinal plants may have phytotoxins and could be use as bioherbicide for successful crop production.

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ABBREVIATIONS AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSRI	=	Bangladesh Council of Scientific Research Institute
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan's Multiple Range Test
et al.,	=	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^2	=	Meter squares
ml	=	MiliLitre
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
var.	=	Variety
^oC	=	Degree Celceous
%	=	Percentage
NaOH	=	Sodium hydroxide
GM	=	Geometric mean
mg	=	Miligram
P	=	Phosphorus
K	=	Potassium
Ca	=	Calcium
L	=	Litre
µg	=	Microgram
USA	=	United States of America
WHO	=	World Health Organization

CHAPTER I

INTRODUCTION

Phytotoxicity or phytotoxic activity is the measure at which a chemical or compound becomes harmful or lethal to plants (Bulut and Demir, 2007.). Phytotoxicity is a significant issue regarding the potential for treating highly contaminated soil and groundwater. It is simply plant damage – a toxic effect – from something the plant was exposed to. Phytotoxicity is the capacity of a compound (such as a plant protection product) to cause temporary or long-lasting damage to plants. All plant parts have been shown to contain allelochemicals, but leaves and roots are the most important source. Medicinal plants have strong phytotoxic activity. There are 450-500 medicinal plants available in Bangladesh. Weeds pose a serious problem for crop production even more than other pests worldwide. In Asia and other continents, around 33-53% crop produce is damaged if weeds are not controlled from the crop fields. In spite of advances made in weed control measure, weeds continue to cause serious crop losses every year. The higher amount of herbicide application lead to an increase in production cost as well as severe environmental problems (Inderjit and Callaway, 2003).

Nowadays, weeds and invasive plant species cause a serious problem in agriculture worldwide. They grow fast and are well adapted and resistant to adverse climatic conditions. They compete with crops for nutrients, light, and water, interfere with crop growth, and finally, result in reduction of crop yield. Weeds could be controlled by mechanical, chemical, and biological methods. The use of synthetic herbicides or chemical control is more common because of its effectiveness. However, increased application of herbicides has lead to environmental pollution and human health problems (Singh *et al*., 2006).

Plants are one of the richest sources of organic compounds in the world. Allelopathic chemicals released from plants into the soil may inhibit growth, nutrient uptake, or germination in neighboring plants (Ayeni *et al*.,

1997 and Inderjit, 1996). For example, it was reported that there were several allelochemicals released from rye and sorghum that could inhibit other crops and weed (Schulz *et al*., 2013 and Weston *et al*., 2013). The advantage of allelochemicals is that they are renewable and easily degradable. Therefore, allelochemicals have received great attention recently as environmentally friendly and safer natural herbicides for weed control (Batish *et al*., 2001 and Khanh *et al*., 2007).

Use of herbicides (synthetic chemical) is an effective means to control weed infestation in crop field. But overuse of synthetic herbicides to control weeds lead to an increased risk of herbicide resistant weed biotypes (Heap, 2014) and harsh environmental pollutions (Aktar *et al*., 2009, Pell *et al*., 1998, Roger *et al*., 1994). Alternative weed management strategies that are ecofriendly and cost-effective are therefore a time demanding issue throughout the world. In this backdrop, phytotoxic plants might help in resolving the problems created by synthetic herbicides as they possess growth retarding substances.

To avoid the detrimental effects of herbicide, the researchers are now searching for novel natural plant products to develop bio-herbicides. Numerous weeds and crop plants have been reported to possess allelopathic substances in order to compete with neighbouring plant species.

The increasing interest on medicinal plants could be due to either (i) the easier screening process of phytotoxic plants from medicinal plants (Fujii *et al*., 2003) or (ii) the possibility to have more bioactive compounds in medicinal plants than other plants (Gilani *et al*., 2010). These phytotoxic plants could be used in several ways to control weeds (Piyatida and Kato-Noguchi, 2010).

The term allelopathy refers to the direct or indirect (adverse or beneficial) effect of one plant on the other as a result of producing and releasing metabolites to the environment (Baziar *et al*., 2014 and Peng, 2019). The phytochemicals that produces inhibitory or stimulatory impact on hostile or friendly plants are called allelochemicals and the phenomenon is called as

allelopathy while the toxicity to other plants is called as phytotoxicity (Lungu *et al*., 2011; Khan *et al*., 2012; Gilani *et al*., 2010).

Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. These biochemicals are known as allelochemicals release some phytotoxins into soil and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on germination and final yield of associated plants (Alam and Islam, 2002). These chemicals are largely classified as secondary plant metabolites such as alkaloids, isoprenoids, phenolics, flavonoids, terpanoids and gluconolates, etc. (Kruse *et al*., 2000). Allelopathic interactions are an important factor in determining species distribution and abundance within plant communities and are also thought to be important in the success of many invasive plants (Klejdus and Kuban, 1999). Allelochemicals present in all plant tissues includes leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollen and there release from the plant in the environment from major ecological process i.e. volatilization, leaching, root exudation and decomposition of plant resides (Kruse *et al*., 2000).

In conventional agriculture, the use of synthesis herbicides is still recognized as an effective tool to eliminate weeds and to promote the highest possible yield of crops (Norsworthy *et al*., 2012; Kniss, 2017). On the other hand, the overuse of synthetic herbicides negatively affects both the environment and human health, and increases the number of herbicide-resistant weeds (Aktar *et al*., 2009; Staley *et al*., 2015). Also, increasing consumer awareness of herbicide residues in production practices leads to increased demand for organic products or safer foods (McErlich and Boydston, 2013; Tal, 2018). To overcome these problems, reducing the reliance on synthetic herbicides and shifting to sustainable agriculture is needed. Organic farming is a feasible alternative agricultural practice that relies on an integrated natural-based system (Gomiero *et al*., 2011; IFOAM EU Group, 2016). In this direction, using natural plant

products and allelopathy for weed management is gaining attention (Singh *et al*., 2003). Additionally, using natural substances including plant extracts is considered safe and acceptable in farming practices (Verhoog *et al*., 2007; Jespersen *et al*., 2017).

Therefore, the study of phytotoxic effects of the medicinal plants on the test plant species would be useful, not only as a guide for organic culture, but also for rotation programming in medicinal plant production. Furthermore, the study was conducted with the following objectives:

1. To assess the phytotoxic effects of the selected medicinal plants on germination and growth of test plants
2. To evaluate the comparison of phytotoxic potential among five medicinal plants

CHAPTER II

REVIEW OF LITERATURE

Weed species are frequently considered to be competitive because they grow vigorously in crops and affect the crop yields. The possession of certain biological characteristics has the potential to predispose a species to exhibiting weediness. An ideal weed has the ability to show stronger and also interspecific competition via special mechanisms such as allelopathic processes (Mortimer, 1990). Allelopathic chemicals play an important role in determination of the persistence and abundance of the weed species in mixtures of the plants. Limited number of research works on phytotoxicity of medicinal plants have been conducted in different parts of the worlds but their findings have little relevance to the agro-ecological situation of Bangladesh. The present study has been undertaken to phytotoxicity of some selected medicinal plants available in Bangladesh. The relevant literatures available have been reviewed in this chapter.

2.1 Phytotoxicity of plants

Plants have biomechanisms to save from hostile plants and animals (e.g. insects). They produce certain metabolites intheir environments that not only save them from offensive organisms (plants, animals, microbes) but also eliminate the hostile one (Inderjit and Callaway. 2003; Yasmin *et al*, 2011). These metabolites are called phytochemicals and may be present in all of their parts. In hostile plants these phytochemicals induce chlorosis, wilting or death (Lungu *et al*., 2011). Some have stimulatory effect on non-hostile or friendly plants also (Yasmin *et al.,* 2011). The phytochemicals that produces inhibitory or stimulatory impact on hostile or friendly plants are called allelochemicals and the phenomenon is called as allelopathy while the toxicity to other plants is called as phytotoxicity (Lungu *et al*., 2011; Khan *et al.,* 2012; Gilani *et al.,* 2010).

Weed species are frequently considered to be competitive because they grow vigorously in crops and affect the crop yields. The possession of certain biological characteristics has the potential to predispose a species to exhibiting weediness. An ideal weed has the ability to show stronger and also interspecific competition via special mechanisms such as allelopathic processes (Mortimer, 1990). Allelopathic chemicals play an important role in determination of the persistence and abundance of the weed species in mixtures of the plants. The allelopathic effects of weeds on crops have been extensively studied since 1970 (Rice, 1979).

According to Rice, the modification of seed germination and plant growth is one of the obvious manifestations of allelopathy, and germination is one of the important tools for the study of allelopathy. Allelopathy is a mechanism in which chemicals produced by weed plants may increase or decrease the associated plant growth. Rice (1979) defined allelopathy as the effects of one plant (including microorganisms) on another plant via the release of chemicals into the environments. Allelopathy is an interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth (Harper, 1977; May and Ash, 1990). Allelopathy may also play an eminent role in the intraspecific and interspecific competition and may determine the type of interspecific association.

The plant may exhibit inhibitory or rarely stimulatory effects on germination and growth of other plants in the immediate vicinity. It offers potential for selective biological weed management through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or dead decomposing plant materials (Weston, 1996). The term allelopathy refers to biochemical interactions among the plants, including those mediated by microorganisms. This broad definition of allelopathy is appropriate as considerable research has indicated the involvement of microorganisms and lower plants in production of phytotoxins (Garlado and Chilton, 1992).

Exotic and native species in competition produce a large amount of toxins (Allelochemical substances) which effectively repel other species and thus their ability to invade the whole plant community is increased (Indergit and Dakshini, 1998). Many secondary metabolites acting as allelochemicals include alkaloids, phenols and terpenoids. Phenols are the most abundant substances under the field conditions that affect the seed germination, seedling growth, cell division and fungal activity (Lodhi, 1976).

Many laboratory techniques have been developed for the measurement and quantification of allelopathy without interfering the resource competition (Leather and Einhelling, 1986; Navarez and Olofsdotter, 1996; Kawaguchi *et al*., 1997). Large screenings of germ plasm collection require reliable test species. It is a common tradition that easily grown but sensitive reliable species like *Lemna minor,* Lettuce *(Lactuca sativa)* and radish *(Raphanus sativa)* seeds have been used as test plants in allelopathic studies (Putnam *et al*., 1983; Einhelling and Rasmussen 1978; Leather and Einhelling, 1986). This assay has a wide range of application in research towards the discovery of active principles in plants (Arzu and Camper, 2002).

Medicinal plants synthesize various biologically active compounds such as alkaloids, flavonoids, saponins, phenolics, tannins, essential oils, and other compounds and show wide range of biological activity (Puupponen-Pimia *et al*. 2001; Varsha *et al*. 2013; Vashist and Sharma 2013). Several secondary metabolites in plants can act as allelochemicals to other plants, stimulating or inhibiting their growth and development.

Fujii *et al.* (2003) evaluated the allelopathic activities of 239 medicinal species using the sandwich method and 223 species of them were found to inhibit the seeds germination, while 17 species were found to stimulate lettuce radicle growth. Fujii *et al.* (2004) reported the allelopathic effect from leaf mess leachates on lettuce seed germination and found inhibitory action determined by the sandwich method.

There are several reports on the bio-insecticidal and plant growth stimulating effects of plant extracts from certain herbal plants (Jbilou *et al*. 2008; Ma *et al*. 2012). Earlier, Angelini *et al*. (2003) found that essential oils of medicinal plants may pose inhibitory effects on growth of other plants by releasing allelopathic substances.

Inayatullah *et al*. (2007) evaluated seven methanol extracts from five different plant species *[Salvia nubicola* B. (Laminiaceae), *Acer oblongifolium* D. (Aceraceae), *Sorbaria tomentosa* L. (Rosaceae), *Hedera nepalensis* K. (Araliaceae), and *Artemisia fragrans* W. (Asteraceae)] for brine shrimp cytotoxicity, antitumor potato disc, and radish seed phytotoxicity activity. Four of the seven extracts revealed significant ED_{50} value ranging from 11.9 to 226.8 ppm. Inhibition of tumor formation ranged from 9 to 82.9% by all extracts in antitumor potato disc assay at three different concentrations tested (1000, 100, and 10 ppm). Growth inhibition was observed by all extracts in radish seed bioassay at high concentration (10,000 ppm). At low concentration (1000 ppm), three extracts from two plant species (leaf and flower extract of *S. nubicola*, stem extract of *S. nubicola*, and stem extract of *H. nepalensis*) presented stimulation of growth ranging from 3.5 to 43.2%. A positive correlation was observed in the results of three of the described assays.

Ali *et al*. (2010) reported that allelopathic compounds in plants such as polyphenols inhibit seed germination and plant growth. The composition of plant secondary metabolites is strongly affected by the endophytic microbes associated with the host plant (Brader *et al*. 2014; Chaparro *et al*. 2014; Hashem *et al*. 2016; Egamberdieva *et al*. 2016).

Gilani *et al*. (2010) carried out a study to identify significantly higher allelopathic species from Allelopathic screening of 81 medicinal plant species for future phytochemical analyses. For this purpose, sandwich method was used to test allelopathic potentials of leaf leachates of these plant species against lettuce seeds (*Lactuca sativa* L.). Two different concentrations of 10

mg and 50 mg of leaf leachates were used in the study. The radicle and hypocotyl growths were measured and compared with control treatments. It was observed that an endemic species *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* were the stronger phytotoxic plants as compared to the other test species. Based on the current screening, three potential medicinal plants are recommended for future bioassay guided isolation of allelochemicals and for genetic diversity studies. It would also be interesting to see correlation between genetic markers and isolated allelochemicals. Gilani *et al.* **(**2010**)** screened 81 Pakistani medicinal plants and found that plants with allelopathic potentials also have stimulatory effects side by side with inhibition.

Hussain *et al*. (2010) determined the cytotoxicity of the crude methanolic extracts of *Rumex hastatus, Rumex dentatus, Rumex nepalensis, Rheum australe, Polygonum persicaria and Polygonum plebejum* (Family *Polygonaceae)* against *Artemia salina* at 1000, 100 and 10 pg/ml. *R. hastatus, R. dentatus* and *R. nepalensis* and showed significant activity at a concentration of 1000 pg/ml against *Artemia salina. R. australe* showed low activity at 1000 pg/ml and no activity at 100 and 10 pg/ml. At concentration of 10 pg/ml, *R. australe* showed no activity. Similarly the phytotoxicity of the crude extracts of these six plants was determined against *Lemna minor.* All the plants except *R. hastatus* showed significant activity at a concentration of 1000 pg/ml. Moderate activity was shown by *R. australe, R. nepalensis* and *P. persicaria* at the concentration of 100 pg/ml. All the plants showed low phytotoxic activity at concentration of 10 pg/ml.

Khan *et al*. (2011) conducted a studies to cover the phytotoxic effects of the crude methanolic extracts of different parts of 13 medicinal plants *viz. Woodfordia fruiticosa, Adhatoda vasica, Chenopodium ambrosoides, Viburnum cotinifolium, Euphorbia hirta, Vitex negundo, Peganum harmala, Broussonetia papyrifera, Taraxacum officinale, Urtica dioica, Verbascum thapsus, Caryopteris grata* and *Mimosa rubicaulis* collected from different

localities of Margalla Hills on the germination of radish seeds to study the germination %, growth inhibition %, root shoot length, velocity of germination, biomass fresh weight, dry weight and moisture content (%) at two concentration levels. Germination velocity was decreased by the application of the extracts, however more pronounced effect was seen at 10 mg/ml concentration. Maximum decrease in germination velocity of radish seed was exhibited by methanolic extract of *W. fruticosa* (25.23) and minimum by *V. negundo* (39.06). Maximum inhibition of radish seed germination was caused by *B. papyrifera* (53.33%), *W. fruticosa* (52%), *V.thapsus* (48.89%) and minimum by *M. rubicaulis* (13.33%). At higher concentration that is, 10 mg/ml, the methanolic extract of *W. fruticosa* was most effective in decreasing the shoot fresh weight (0.15 gm), followed by *B. papyrifera* (0.29 gm), *Caryopteris grata* (0.39 gm), *U. dioica* (0.49 gm), *V.thapsus* (0.50 gm) and *P. harmala* (1.44 gm). The extract of *W. fruticosa* was more pronounced in decreasing the shoot dry weight (0.07 gm) followed by *B. papyrifera* (0.08 gm), *V. thapsus* (0.1 gm) and the least effective was the *M. rubicaulis* (0.21 gm). However at concentration 1 mg/ml, *T. officinale* exhibited maximum decrease in germination velocity (35.65) and maximum inhibition of seed germination was caused by methanolic extract of *U. dioica* (42.22%), followed by *V. thapsus* (40%). Lower concentrations of *V. negundo* and *T. officinale* exhibited similar effects on germination velocity. The intensity of decrease in moisture content at concentration 1mg/ml was lower than that at 10 mg/ml. Maximum reduction in seedling moisture content was also recorded at concentration 1 mg/ ml for *W. fruticosa* (83.1%), followed by *V. cotinifolium* (84.51%) and *B. papyrifera* (86.42%) and minimum for that of *V. thapsus* (90%). The *P. harmala* at low concentration (1 mg/ ml) promoted the growth rather showing the allelopathic effects. The phytotoxic activity of the selected medicinal plants on radish seed germination was dose dependent.

According to the host-endophyte coevolution hypothesis, chemical compounds synthesized by plants resemble those with the endophytic metabolites (Kumar

et al. 2012; Rai *et al*. 2014). The root-associated bacteria with antifungal activity were reported from medicinal plants *Matricaria chamomilla* L., and Calendula officinalis, known with antibacterial activity (Koberl *et al*. 2013). Goryluk *et al*. (2009) observed higher proportion of antagonistic endophytes associated with *Chelidonium majus* L., which has antimicrobial activity (Baker and Satish 2013).

Hameed *et al*. (2013) conducted a study and found that the methanolic extracts of the *Datura innoxia* Miller, *Solanum nigrum* Lin, *Solanum surattense* Burm. f, *Withania somnifera* L and *Withania coagulans* (Stocks) Dunal are more cytotoxic as compared to the acetone extracts of these plants except *W. coagulans.* Acetone extracts of *S. surattense* had very low activity as compared to other tested plants. Phytotoxic activity of the both extract had greater activity at 10 gg/ml as compared to at 100gg/ml except *W. somnifera* (methanolic) and *W. coagulans* (acetone) as well as 1000gg/ml activity which was low at the both solvent of the studied plants.

Plants of toxic nature can be used as natural herbicides or these phyto toxic components of interest can be extracted from the plants for developing new herbicides. A variety of plant's metabolites used to inhibit or stimulate the growth and development of other plants. These chemicals are called allelochemicals which exudated out, leached from different parts of plant or volatiled or plant residues present in the environment (Dastagir and Hussain, 2013).

The effect of allelochemicals is called allelopathy which is the effects of donor plant, on recipient plant by the discharge of chemicals in the environment. This activity influences the growth and development of other plants either by inhibiting or stimulating the bio-physiologic processes of recipient organism. These activities are concentration dependent and might inhibit the growth of one plant at one concentration and might stimulate the growth at other concentration. Such chemical are present in different plant parts in high

amounts at specific environmental conditions **(**Shinwari and Fujii, 2013). Environmental conditions are responsible for differences in concentration of these metabolites (Ncube *et al*., 2012).

Nekonam *et al.* (2014) also investigated medicinal plants which possess herbicidal properties against *Amaranthus retroflexus.* Zahedi and Ansari **(**Zahedi and Ansari, 2011) investigated allelopathic potential of mallow extract and leachates on crop. Use of medicinal plants having phytotoxic property possess significant role in weeds control. Plant residues of *Artemisia* reduces seed germination of *Agropyron repens* **(**Balicevic *et al*., 2015). Any part of the plant contained chemicals which might be allelochemicals, become part of the environment through root-exudates, leaching from upper plant parts or by decomposing of plant parts (Verma *et al*., 2012).

Islam and Kato-Noguchi (2014) investigated phytotoxic activity of *Ocimum tenuiflorum* (Lamiaceae) plant extracts against the germination and seedling growth of cress *(Lepidium sativum),* lettuce *(Lactuca sativa),* alfalfa *(Medicago sativa),* Italian ryegrass *(Lolium multiflorum),* barnyard grass *(Echinochloa crus-galli),* and timothy *(Phleumpratense)* at four different concentrations. The plant extracts at concentrations greater than 30 g dry weight equivalent extract mL^{-1} reduced significantly the total germination percent (GP), germination index (GI), germination energy (GE), speed of emergence (SE), seedling vigour index (SVI), and coefficient of the rate of germination (CRG) of all test species except barnyard grass and GP of lettuce. In contrast, time required for 50% germination (T_{50}) and mean germination time (MGT) were increased at the same or higher than this concentration. The increasing trend of T_{50} and MGT and the decreasing trend of other indices indicated a significant inhibition or delay of germination of the test species by *O. tenuiflorum* plant extracts and vice versa. In addition, the shoot and root growth of all test species were significantly inhibited by the extracts at concentrations greater than 10 g dry weight equivalent extract mL^{-1}. The I_{50} values for shoot and root growth were

ranged from 26 to 104 g dry weight equivalent extract mL^{-1}. Seedling growth was more sensitive to the extracts compared to seed germination. Results of this study suggest that *O. tenuiflorum* plant extracts have phytotoxic properties and thus contain phytotoxic substances. Isolation and characterization of those substances from this plant may act as a tool for new natural, biodegradable herbicide development to control weeds.

Nazir *et al*. (2014) conducted an investigation to evaluate the effect of three medicinal plant species on germination and radical- plumule growth of different test crops. All the test crops were found sensitive under leaf and root extracts of all three medicinal plant species except in *Centella asiatica* as it shows least/no effect and can therefore, cultivated with agriculture crops. The preference order of medicinal plant species on the basis of laboratory tests is suggested as: *Centella asiatica* > *Catharanthus roseus* > *Bryophyllum pinnatum* and the order of field crops preference is: *Vigna radiata* > *Zea mays* > *Cicer arietinum.* Therefore, it is concluded that the results obtained within the scope of our study yielded sufficient preliminary evidence for considerable allelopathic effects from *Bryophyllum pinnatum* and *Catharanthus roseus.* Our data support allelopathy as a substantial factor in the competitive ability of the medicinal plant species and field crops. Further focus may be on the study for population dynamical aspects to unravel the key traits underlying the establishment of sustained agriculture.

Parvez *et al*. (2014) investigated the phytotoxic effect of *Euphorbia granulata* Forssk using *Lemna minor* growth, radish seed germination and roots length determination. No lethality was recorded at 0.01 and 1.0mg/ml concentrations however, at 0.1mg/ml of plant extracts the number of fronds increased by 21%. The lower toxic concentration (LTC) and the upper toxic concentrations (UTC) determined were 5.0mg/ml and 55.0mg/ml respectively while the LC_{50} was 33.88mg/ml. In case of radish seeds, 92.7, 95.3 and 94% germination take place with negative control, 1.0mg/ml, and 7.5mg/ml concentrations while the

roots sizes at day five were 5.1, 5.4 and 5.5cm with negative control, 1.0 and 10mg/ml dose of extract respectively. The results indicated no inhibitory but a slight stimulatory effect of the plant extract.

Allelochemicals can alter the contents of plant growth hormones or make inequalities in many phyto-hormones, which hinders plant growth and development, for instance, with respect to germination of seed and sapling growth. Allelochemicals of phenolic nature can motivate IAA oxidase activity and obstruct the reaction of POD with IAA, unavoidable GA or IAA to effect endogenous hormone stages **(**Cheng and Cheng, 2015).

Medicinal plants like *Carum carvi* L., *Coriandrum sativum* and *Foeneculum vulgare* also inhibit the growth of weeds **(**Dikic, 2005). *Lavendula angustifolia, Mentha longifolia* and *M. piperrita* reduces germination of *Sorghum helapence* and *Rumex crispus* **(**Petrova *et al.,* 2015).

Nebo *et al*. (2015) evaluated the bioactivity profiles of four triterpenes (1-4) and six limonoids (5-10) from Meliaceae and Rutaceae. It was observed that Limonoids and triterpenes are the largest groups of secondary metabolites and have notable biological activities. Meliaceae and Rutaceae are known for their high diversity of metabolites, including limonoids, and are distinguished from other families due to the frequent occurrence of such compounds. The increased interest in crop protection associated with the diverse bioactivity of these compounds has made these families attractive in the search for new allelopathic compounds. The compounds (four triterpenes (1-4) and six limonoids (5-10) from Meliaceae and Rutaceae) were assessed in a wheat coleoptile bioassay and those that had the highest activities were tested on the standard target species *Lepidium sativum* (cress), *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato) and *Allium cepa* (onion). Limonoids showed phytotoxic activity and 5a,6,S,8a,12a- tetrahydro-28-norisotoonafolin (10) and gedunin (5) were the most active, with bioactivity levels similar to, and in some cases better than, those of the commercial herbicide Logran. The results

indicate that these products could also be allelochemicals involved in the ecological interactions of these plant species.

Ramalakshmi and Muthuchelian (2015) aimed to investigate the phytotoxicity of various organic extracts from the leaves of medicinal plant *Tabebuia rosea* (TR). The leaf powder of TR was extracted with different solvents such as petroleum ether, chloroform, ethyl acetate, acetone and aqueous ethanol. The phytotoxicity of plant extracts was assessed by using radish seeds. For root, shoot length inhibition and seed germination studies were carried out at 100; 1000; 10,000 ppm and 1000; 10,000 ppm respectively. All extracts of TR significantly inhibited root growth at 10,000 ppm. The relative germination rates were found to be higher in ethanol fractions of *T. rosea.* Thus the germination index of radish seeds showed higher activity for petroleum ether and ethanol fractions was observed for *T. rosea* extracts. Thus the TR plant leaves in general is said to possess significant allelopathic potential, which can be used for herbicide applications.

Germination of seeds and plant development is altered by allelopathy and therefore germination is important way for the learning of allelopathy. Allelopathy has a significant part in agriculture and disturbs the progress and magnitude of the crops by the exchange of chemicals among crops, weeds and trees. Allelochemicals secreted by unknown plants significantly affect the intrinsic plants regardless of native species secreted allelochemicals or not **(**Ramgunde and Chaturvedi, 2016**)**.

Martins *et al*. (2016) conducted a study to assess the possible antimicrobial activities of a popular crude aqueous extract. It was noticed that the use of plants or their parts as alternative therapies for disease is very common in many countries worldwide. Secondary metabolites present in the extracts of certain plants can inhibit or halt the development of certain pathogen species. The aerial parts of the Amazon tree *Bellucia grossularioides* (L) Triana (popularly known as Muuba or Angry-Jambo) were prepared according to folk

recommendations and tested against four microorganisms related to health concerns in three concentrations (20, 10 and 5 mg/ml). The results showed no antimicrobial potential against *Staphylococcus aureus***,** *Candida albicans* and *Candida krusei***,** which cause furunculosis and leukorrhea, respectively. Additionally, growth inhibition of the toxigenic fungus *Aspergillus parasiticus* was assayed *in vitro* and the results showed no inhibitory activity for any of the tested concentrations. These findings contradict the traditional knowledge and may assist the targeting of future therapeutics practices. However, an inhibitory effect was observed for all forms of the preparations and concentrations tested on the roots of *Allium cepa***,** indicating phytotoxic effects.

Rawat *et al*. (2016) conducted the present study aimed to determine the allelopathic effect of aqueous extract of above ground (AG) and below ground (BG) part of four medicinal plants (MAPs) viz., *Picrorhiza kurroa, Asperagus racemosus, Ocimum sanctum* and *Valeriana wallichii* on germination and seedling growth of some traditional food and oilseed crops. The aqueous extract of AG and BG part of the studied MAPs at 2 % significantly reduced the germination, plumule and radicle growth of the selected pulse and oil seed crops in bioassays. The results of the present experiments revealed that MAPs have inhibitory effect on food and oilseed crops owing an occurrence of allelochemicals.

Suksungworna *et al*. (2016) evaluated the extracts of wood, bark, and leaves of *Haldina cordifolia* for their phytotoxicity on seed germination, seedling growth, and root cell viability in two weeds *(Mimosa pigra* and *Cenchrus echina- tus)* and two crop plants *(Vigna radiata* and *Oryza sativa* cv. Khao Dawk Mali 105). Seeds were grown in petri dishes and treated with 5 ml of extracts at various concentrations: 0.5,1.0,5.0, and 10.0 mg/ml. The inhibitory effect on seed germination increased with increasing concentration of the extract treatment. Bark extract was the most toxic at the highest concentration, causing total inhibition of germination in all tested seeds except in *V. radiata*.

Low concentrations (0.5 and 1.0 mg/ml) of wood extract inhibited shoot and root growth in *C. echinatus* by 31.0%-56.0% and 67.0%-71.0%, respectively. Interestingly, it promoted root growth in *M. pigra* by 106.9%-108.8% (at low concentrations) and in *V. radiata* (at all concentrations) by 108.1%-108.9% (shoot) and 108.8%-120.1% (root). Bark extract inhibited seedling growth in all tested plants at different levels. Strong inhibition was found in roots of *O. sativa* (3.0%-4.0%). The result from Evans blue uptake study suggested that the *H. cordifolia* extract did not directly affect the root cell viability. Surprisingly, we found that *M. pigra* and *V. radiata* treated with the extracts at low concentrations had increasing number of lateral roots, suggesting that *H. cordifolia* extract could act as a plant growth regulator (PGR) and an herbicide at the same time, depending on concentration and target plant.

Shurigin *et al*. (2018) investigated The cultivable endophytic bacteria associated with two medicinal plants *Hypericum perforatum* L. and *Ziziphora capitata* L. contrasting with phytotoxic activity. The phytotoxic activity of plant extracts, and bacterial metabolites on seed germination and seedling growth of tomato were evaluated. In comparison to Z. *capitata*, the extract of *H. perforatum* contains a higher content of phenolic compounds. The crude extract of *H. perforatum* inhibited germination of seeds and seedling growth of tomato, whereas *Z. capitata* extracts only slightly reduced these parameters. Interestingly, almost half of the endophytes associated with *H. perforatum* had an inhibitory effect on plant growth, whereas rarely any plant inhibitory effect was found among isolates from Z. *capitata*. All bacterial isolates from *Z. capitata* were able to stimulate plant growth, by 35-80%. In contrast, only five isolates from *H. perforatum* caused significant improvement in plant growth (22-46%). The results showed that medicinal plants with higher phytotoxic activity were colonized with endophytic bacteria which inhibit plant growth and development. These findings indicate that plant phytochemical constituents and activity determine the physiological properties of their endophytes.

The endophytes that colonise inside plant tissues produce various metabolites and stimulate plant growth and protect host plant from soil borne pathogens (Egamberdieva *et al*. 2018). They produce various biological active metabolites including phytohormones, enzymes, antifungal compounds, and volatile organic compounds (Davis *et al*. 2013; Cho *et al*. 2015). Bioactive secondary metabolites produced by endophytes may also assist the plants in chemical defence (Ji *et al*. 2009).

Boonmee and Kato-Noguchi (2019) observed that medicinal plants are potential sources of secondary metabolites which may possess allelopathic properties. They carried out a study to investigate the allelopathic potential of 12 Thai medicinal plants against the growth of cress and barnyard grass. The 12 medicinal plant extracts showed a significant inhibition on the growth of those test plants. The inhibitory potentials depended on the extract concentration and test plant species. Comparing the average growth inhibition, the extracts from *Crateva adansonii* strongly inhibited the cress shoots and roots (96.0 and 95.6%), followed by *Phlogacanthuspulcherrimus* (93.1 and 93.4%), and *Cuscuta chinensis* (92.3 and 91.5%). Meanwhile, the barnyard grass shoots and roots were strongly inhibited by the extracts of *P. pulcherrimus* (88.2 and 97.6%), *C. chinensis* (73.7 and 82.3%), and *Acanthopanax trifoliatus* (73.0 and 94.3%). However, *P. pulcherrimus* extracts had a high allelopathic potential against both test plants, suggesting that *P. pulcherrimus* may be a potential candidate for purification of allelochemicals.

Begum *et al*. (2019) found that medicinal plants are best sources to treat various illnesses with them. So they might prove be a good source of developing novel herbicides. They conducted a study to investigate the potential inhibition effects of *Cucumis sativus, Portulaca oleracea, Malus baccata, Saxifraga flagillaris, Geranium wallichianum* and *Monotheca buxifolia* powdered material on germination of *Lactuca sativa* seeds. Sandwich method was used for determining phytotoxicity of these plants in terms of

radicle and plumule length of *Lactuca sativa* seeds with different amounts of 10, 20, and 40mg of powdered plant material. *Cucumis sativus* and *Monotheca buxifolia* were found the most phytotoxic among the selected medicinal plants at all concentration. The results can be sum off as *Monotheca buxifolia* > *Cucumis sativus* > *Malus baccata* > *Saxifraga flagillaris* > *Geranium wallichianum* > *Portulaca oleraceae.* From the results it can be assumed that the phyto-toxic effects of the aforementioned plants could be helpful in searching and development of new pharmaceuticals that can be used as positive sources for the development of new weedicides. New botanical insecticides which are basically obtained from plants, are very necessary to overcome the resisting population of insects and have minimum or low threats to the environment. The present investigation was undertaken to evaluate medicinal plants for allelopathic property to produce harmless fumigant or insecticides that must be operative, cheap and suitable to use.

Findura *et al*. (2020) conducted a study to evaluate the effects of two extraction methods and the effects of allelopathic aqueous extracts from twenty plants as seed dressing preparations on the number of germinating and infested seeds of cauliflower *(Brassica oleracea convarietas* L. *botrytis var. botrytis)* and observed that allelopathic plants can be widely used in bio-farming considering their potential role in the improvement of seed germination. Plant extracts (in the form of cold-soaked macerates and infusions) were used for seed dressing. The percentages of normally germinating, non-germinating, and pathogen-infested seeds were determined in a paper test. Of the 20 herbal plant species used in the study, the biopreparations extracted from *Zea mays* L. moles were the most effective as they evoked the most beneficial effects on both seed germination and reduction of infestation by microbial pathogens. The study also showed that infusions used for seed treatment were better at improving cauliflower seed germination than were macerates. This method of extract preparation probably enabled an increase both in the availability and activity of allelochemical compounds.

CHAPTER III

MATERIALS AND METHODS

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

3.1 Location of the experimental site

The experiment was conducted at the Department of Agricultural Chemistry Laboratory of Sher-e-Bangla Agricultural University, Dhaka during the period from April to March 2020. The site is 90.2°N and 23.50° E Latitude and at a altitude of 8.2 m from the sea level. Location of the experimental site is shown in Appendix I.

3.2 Planting materials used for experiment

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

1. Okra (Scientific name: *Abelmoschus esculentus*)
2. Barnyard grass (Scientific name: *Echinochloa crus-galli*)

Five medicinal plants were used to identify their phytoxicity with test crops which were as follows:

1. Henna (Mehedi pata) - Scientific name: *Lawsonia inermis*
2. Gardenia (Gondhoraj) - Scientific name: *Gardenia jasminoides*
3. Bohera - Scientific name: *Terminalia belerica*
4. Nagesshor or Ceylon ironwood - Scientific name**:** *Mesua ferrea*
5. Tamarind (Tetul) - Scientific name: *Tamarindus indica*

All plant materials were collected from the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.3 Treatments of the experiment

The following treatments were considered for the phytoxicity of the medicinal plants against test crops

1. P_0 = 0 (control without extract)
2. P_1 = 0.01 g dry wt. eq. extract/mL
3. P_2 = 0.03 g dry wt. eq. extract/mL
4. P_3 = 0.1 g dry wt. eq. extract/mL
5. P_4 = 0.3 g dry wt. eq. extract/mL

3.4 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.

3.5 Sample preparation

The whole plants (leaves, stems, and roots) of Henna, Gardenia, Bohera, Nagesshor and Tamarind were collected from the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The plants were then washed with tap water to remove the soil and other debris, dried under sun, 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.

3.6 Extraction procedure of medicinal plants

All collected sun dried samples were grounded separately in a mechanical grinder. A powder sample of 30g of each sample were weighed and extracted with 300 mL of 70% (v/v) aqueous methanol for 48 hours. After filtration using one layer of filter paper (number 2; Advantec Toyo Roshi Kaisha, Ltd., Tokyo, Japan), the residue was extracted again with the same volume of methanol for

24 hours and filtered. Two filtrates were mixed together and then evaporated with a rotary evaporator (Model: R-200) at 40°C.

3.7 Preparation of extract concentration of medicinal plants

A portion of the extract was diluted into small volume of methanol to prepare four assay concentrations 0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract mL^{-1} 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).

3.8 Experimental procedure

3.8.1 Germination

The effects of aqueous extracts ongermination 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 incubated in growth chamber at 25°C.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 Collection of data

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 measured with 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 (plumule) 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 (radicale) 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 (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

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

4.1 Effects of Henna extract on the germination of okra

Significant variation was found on germination percentage at different duration of okra seeds affected by different levels of extract of Henna (Figure 1 and Appendix II). Results revealed that the control treatment P_0 (no extract) gave the highest percentage of seed germination which was 86.67% at 24 hours of seed treatment (with water) and maximum 93.33% was at 36 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. But the maximum seed germination (73.33%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. But at 60 hours to germination test, the maximum seed germination (66.67%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. But the maximum seed germination (50.00%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. But at 36 hours to germination test, the maximum seed germination (33.33%) was found and it was fixed to 72 hours.

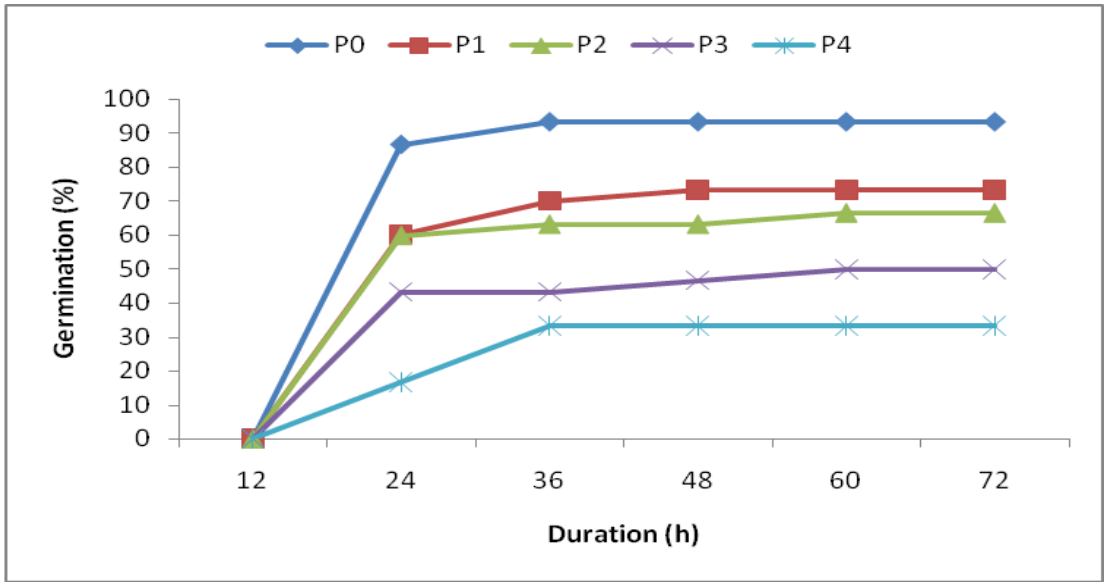

Figure 1. Impact of extracts obtained from Henna on the germination of okra ($LSD_{0.05}$ = NA, 2.43, 2.64, 3.12, 2.76 and 2.22 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.2 Effects of Gardenia extract on the germination of okra

Significant influence was recorded on germination percentage at different duration of okra seeds influenced by different levels of extract of Gardenia (Figure 2 and Appendix III). Results revealed that germination of okra seeds when treated with Gardenia extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of okra seed at all duration of germination test.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 86.67% seed germination was found from control treatment P_0 (no extract) and maximum 93.33% was at 36 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 60.00% seed

germination was found whereas the maximum seed germination (80.00%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 46.67% seed germination was found whereas at 60 hours to germination test, the maximum seed germination (56.67%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 33.33% seed germination was recorded whereas the maximum seed germination (40.00%) was found at 36 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 20.00% seed germination was found but at 48 hours to germination test, the maximum seed germination (30.00%) was found and it was fixed to 72 hours.

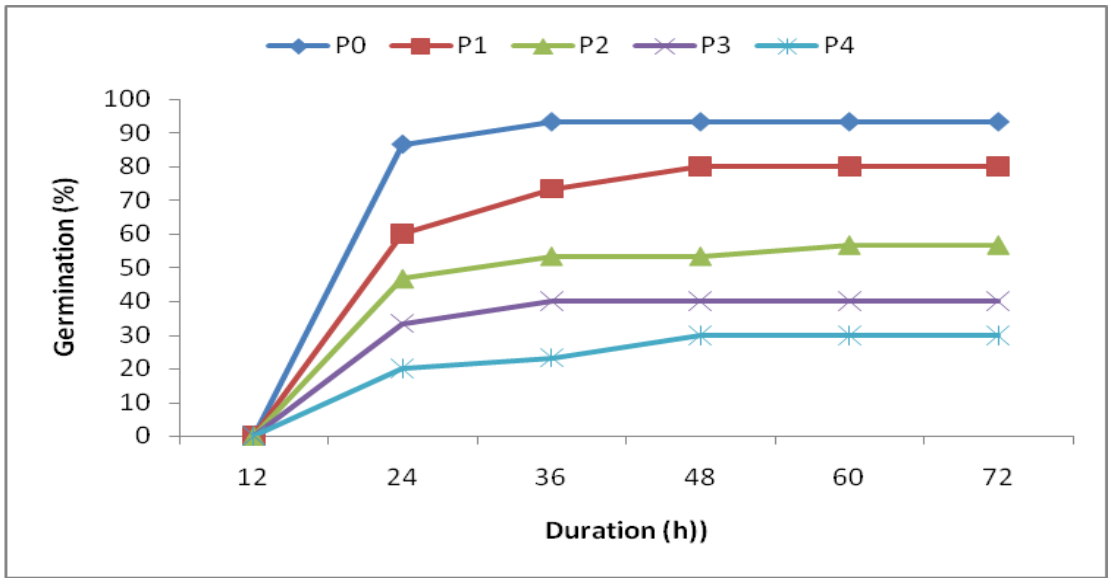

Figure 2. Impact of extracts obtained from Gardenia on the germination of okra ($LSD_{0.05}$ = NA, 3.12, 2.54, 3.76, 3.37 and 3.44 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.3 Effects of Bohera extract on the germination of okra

Different levels of Bohera extract showed significant variation on germination percentage at different duration of okra seeds (Figure 3 and Appendix IV). Results revealed that germination of okra seeds when treated with Bohera extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of okra seed.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 90.00% seed germination was found from control treatment P_0 (no extract) and maximum 93.33% was found at 48 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 70.00% seed germination was found whereas the maximum seed germination (81.00%) was found at 72 hours to seed germination test.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 61.67% seed germination was found whereas at 60 hours to germination test, the maximum seed germination (77.67%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 60.00% seed germination was recorded whereas the maximum seed germination (76.00%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 51.67% seed germination was found but at 48 hours to germination test, the maximum seed germination (69.67%) was found and it was fixed to 72 hours.

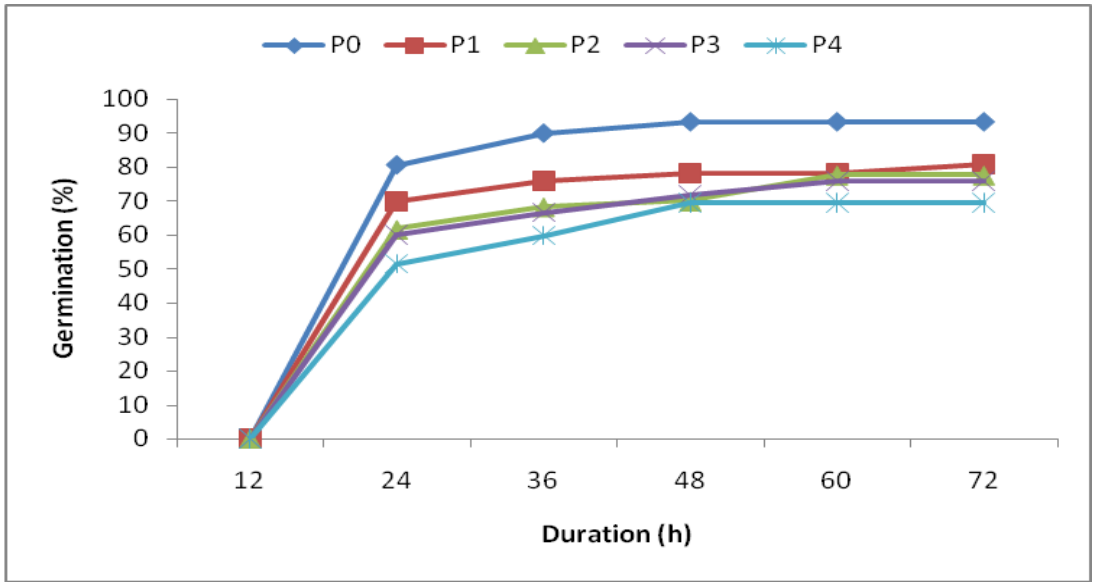

Figure 3. Impact of extracts obtained from Bohera on the germination of okra ($LSD_{0.05}$ = NA, 2.71, 3.24, 2.78, 1.92 and 2.64 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.4 Effects of Nagesshor extract on the germination of okra

There was a significant variation was found on germination percentage of okra seeds at different duration influenced by different levels of Nagesshor extract (Figure 4 and Appendix V). Results revealed that germination of okra seeds when treated with Nagesshor extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of okra seeds.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 83.33% seed germination was found from control treatment P_0 (no extract) and maximum 94.33% was at 36 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 71.67% seed

germination was found whereas the maximum seed germination (84.00%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 67.00% seed germination was found whereas at 48 hours to germination test, the maximum seed germination (82.33%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 57.33% seed germination was recorded whereas the maximum seed germination (69.67%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 43.00% seed germination was found but at 48 hours to germination test, the maximum seed germination (63.00%) was found and it was fixed to 72 hours.

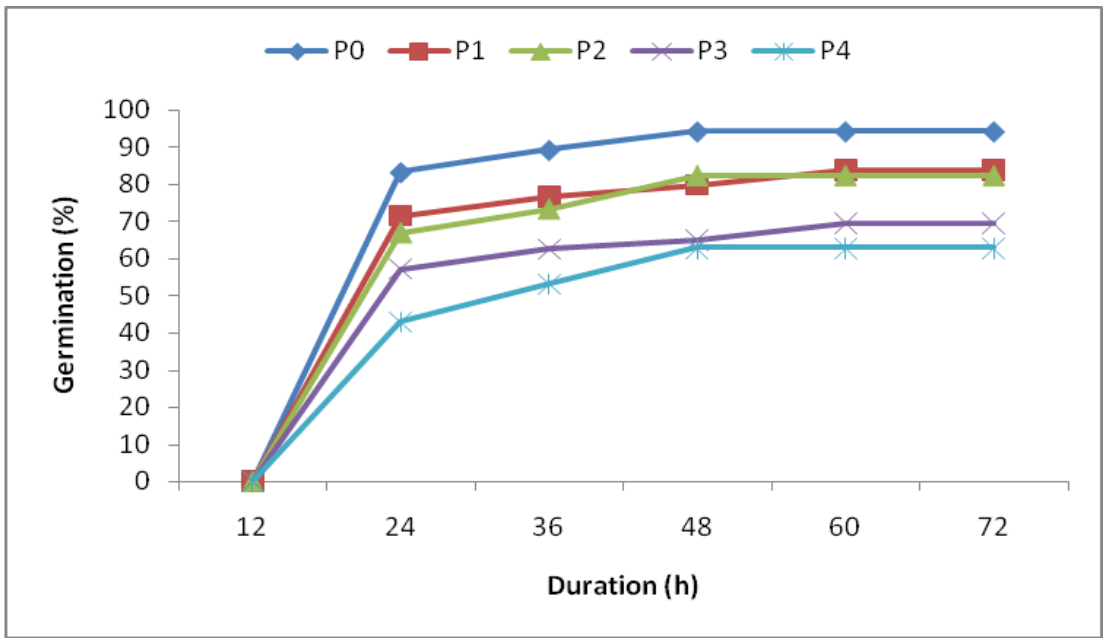

Figure 4. Impact of extracts obtained from Nagesshor on the germination of okra ($LSD_{0.05}$ = NA, 2.71, 2.05, 2.36, 1.87 and 1.64 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.5 Effects of Tamarind extract on the germination of okra

Germination percentage of okra seeds influenced significantly at different duration due to different levels of Tamarind extract (Figure 5 and Appendix VI). Results revealed that germination of okra seeds when treated with Tamarind extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of okra seed.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 82.67% seed germination was found from control treatment P_0 (no extract) and maximum 94.00% was found at 48 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 74.00% seed germination was found whereas the maximum seed germination (85.67%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 66.00% seed germination was found whereas at 60 hours to germination test, the maximum seed germination (80.00%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 62.67% seed germination was recorded whereas the maximum seed germination (75.33%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 55.00% seed germination was found but at 36 hours to germination test, the maximum seed germination (60.00%) was found and it was fixed to 72 hours.

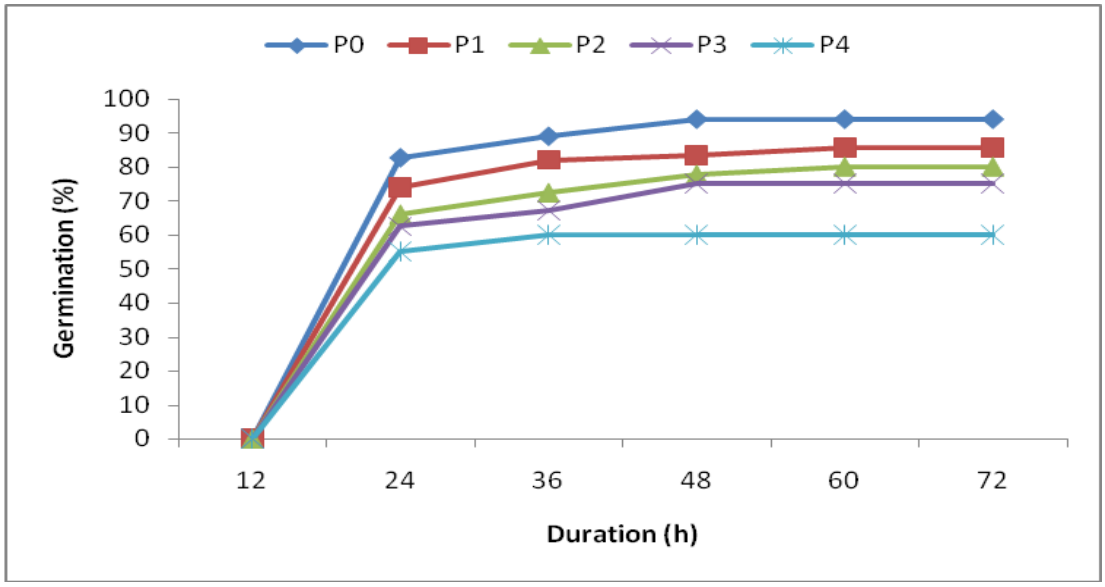

Figure 5. Impact of extracts obtained from Tamarind on the germination of okra ($LSD_{0.05}$ = NA, 1.74, 2.12, 2.07, 1.78 and 2.14 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.6 Effects of Henna extract on the germination of barnyard grass

Significant variation was observed on germination percentage at different duration of barnyard grass seeds influenced by different levels of Henna extract (Figure 6 and Appendix VII). Results revealed that germination of barnyard grass seeds with Henna extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of barnyard grass seed.

At 12 and 24 hours to seed germination test, no germination was found. It was recorded that the control treatment P_0 (no extract) gave the highest percentage of seed germination which was 88.67% at 36 hours of seed treatment (with water) and maximum 88.67% seed germination was fixed up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 and 24 hours to seed germination test. But the maximum seed germination

(66.67%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 and 24 hours to seed germination test. But at 36 hours to germination test, the maximum seed germination (46.67%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 and 24 hours to seed germination test. But the maximum seed germination (40.00%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 and 24 hours to seed germination test. But at 60 hours to germination test, the maximum seed germination (30.00%) was found and it was fixed to 72 hours.

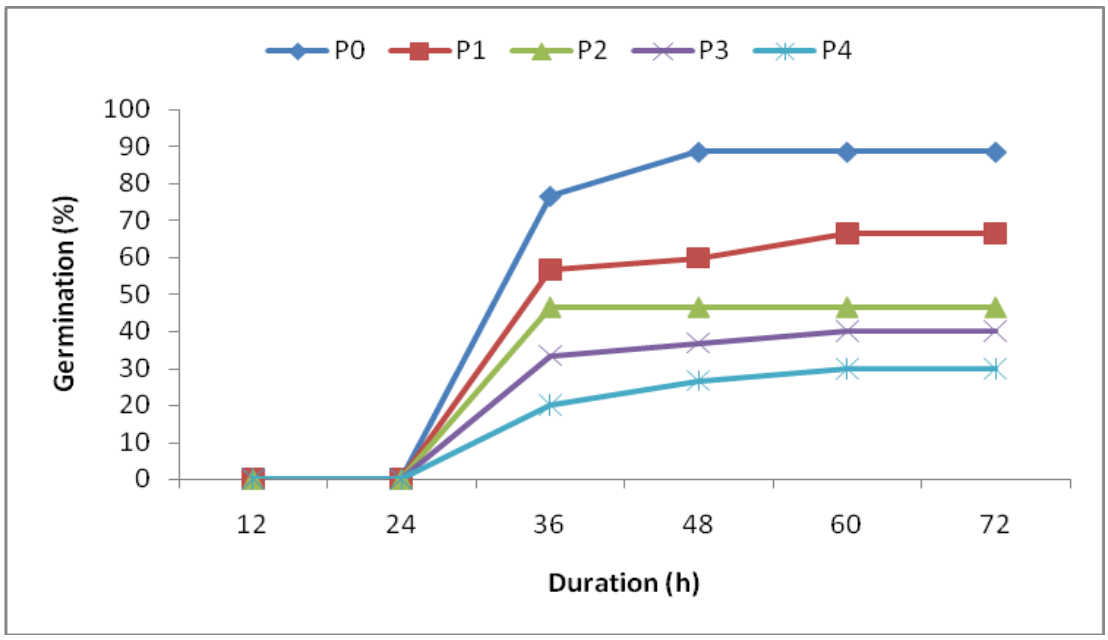

Figure 6. Impact of extracts obtained from Henna on the germination of barnyard grass ($LSD_{0.05}$ = NA, NA, 2.04, 3.15, 2.54 and 3.56 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.7 Effects of Gardenia extract on the germination of barnyard grass

Germination percentage of barnyard grass seeds influenced significantly at different duration by different levels of Gardenia extract (Figure 7 and Appendix VIII). Results revealed that germination of barnyard grass seeds when treated with Gardenia extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of barnyard grass seeds.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 68.00% seed germination was found from control treatment P_0 (no extract) and maximum 90.00% was at 60 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 30.00% seed germination was found whereas the maximum seed germination (70.00%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 18.00% seed germination was found whereas at 60 hours to germination test, the maximum seed germination (60.00%) was found and it was fixed to 72 hours.

At P_3 (0.1 g 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, 43.33% seed germination was recorded whereas the maximum seed germination (56.67%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g 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, 23.33% seed germination was found which was highest and it was fixed to 72 hours.

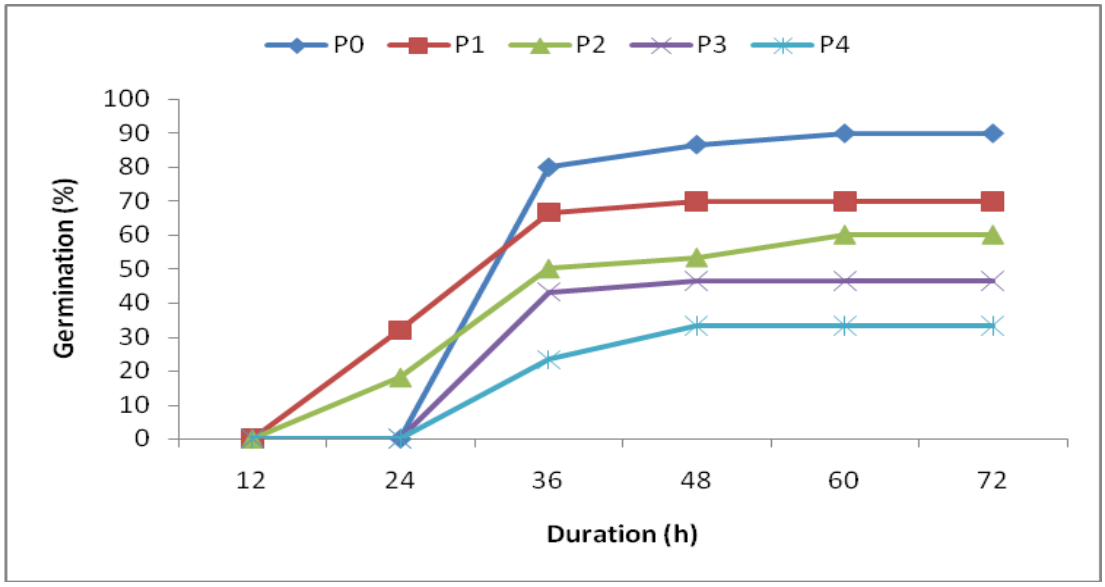

Figure 7. Impact of extracts obtained from Gardenia on the germination of barnyard grass ($LSD_{0.05}$ = NA, 3.24, 3.06, 4.31, 3.28 and 3.10 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.8 Effects of Bohera extract on the germination of barnyard grass

Different levels of Bohera extract showed significant variation on germination percentage of barnyard grass seeds at different duration (Figure 8 and Appendix IX). Results revealed that germination of barnyard grass seeds when treated with Bohera extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of barnyard grass seed.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 72.00% seed germination was found from control treatment P_0 (no extract) and maximum 93.67% was found at 60 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 42.00% seed

germination was found whereas the maximum seed germination (77.33%) was found at 60 hours to seed germination test and this result was continued up to 72 hours.

At P_2 (0.03 g 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% seed germination was found whereas at 60 hours to germination test, the maximum seed germination (75.33%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12and 24 hours to seed germination test. At 36 hours to germination test, 62.00% seed germination was recorded whereas the maximum seed germination (74.67%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12and 24 hours to seed germination test. At 36 hours to germination test, 51.67% seed germination was found but at 48 hours to germination test, the maximum seed germination (66.00%) was found and it was fixed to 72 hours.

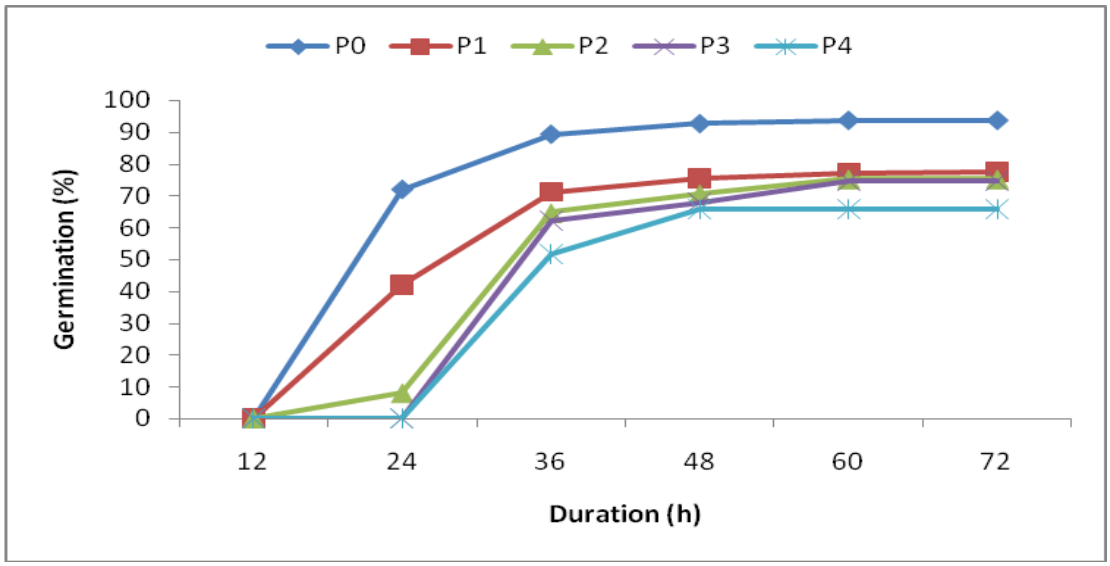

Figure 8. Impact of extracts obtained from Bohera extract on the germination of barnyard grass ($LSD_{0.05}$ = NA, 3.05, 3.10, 3.15, 2.85 and 3.71 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.9 Effects of Nagesshor extract on the germination of barnyard grass

At different duration, barnyard grass seeds showed significant variation on germination percentage of due different levels of extract of Nagesshor (Figure 9 and Appendix X). Results revealed that germination of barnyard grass seeds when treated with Nagesshor extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of barnyard grass seed.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 88.00% seed germination was found from control treatment P_0 (no extract) and maximum 94.33% was at 48 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 69.33% seed germination was found whereas the maximum seed germination (79.00%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 65.33% seed germination was found whereas at 48 hours to germination test, the maximum seed germination (77.67%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 59.33% seed germination was recorded whereas the maximum seed germination (73.67%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 55.00% seed germination was found but at 48 hours to germination test, the maximum seed germination (66.00%) was found and it was fixed to 72 hours.

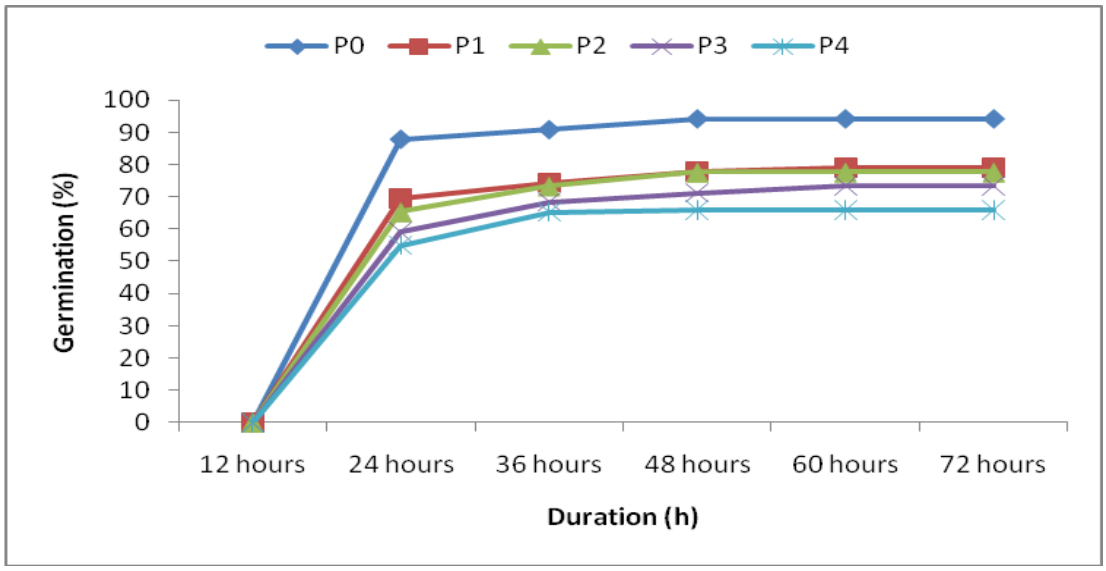

Figure 9. Impact of extracts obtained from Nagesshor on the germination of barnyard grass ($LSD_{0.05}$ = NA, 2.10, 1.81, 2.01, 2.53 and 2.01 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.10 Effects of Tamarind extract on the germination of barnyard grass

Different levels of Tamarind extract showed significant variation on germination percentage of barnyard grass seeds at different duration (Figure 10 and Appendix XI). Results revealed that germination of barnyard grass seeds when treated with Tamarind extract at P_4 (0.3 g dry wt. eq. extract/mL) showed lowest percent germination whereas control treatment P_0 (no extract) showed highest germination of barnyard grass seed.

It was found that at 12 hours to seed germination test, no germination was found from control treatment P_0 (no extract). At 24 hours to germination test, 76.33% seed germination was found from control treatment P_0 (no extract) and maximum 94.33% was found at 48 hours of seed treatment and also this result was continued up to 72 hours.

At P_1 (0.01 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 67.67% seed

germination was found whereas the maximum seed germination (81.33%) was found at 48 hours to seed germination test and it was fixed to 72 hours.

At P_2 (0.03 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 66.00% seed germination was found whereas at 60 hours to germination test, the maximum seed germination (78.33%) was found and it was fixed to 72 hours.

At P_3 (0.1 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 60.67% seed germination was recorded whereas the maximum seed germination (73.67%) was found at 60 hours to seed germination test and it was fixed to 72 hours.

At P_4 (0.3 g dry wt. eq. extract/mL) extract, no germination was found at 12 hours to seed germination test. At 24 hours to germination test, 56.67% seed germination was found but at 60 hours to germination test, the maximum seed germination (69.00%) was found and it was fixed to 72 hours.

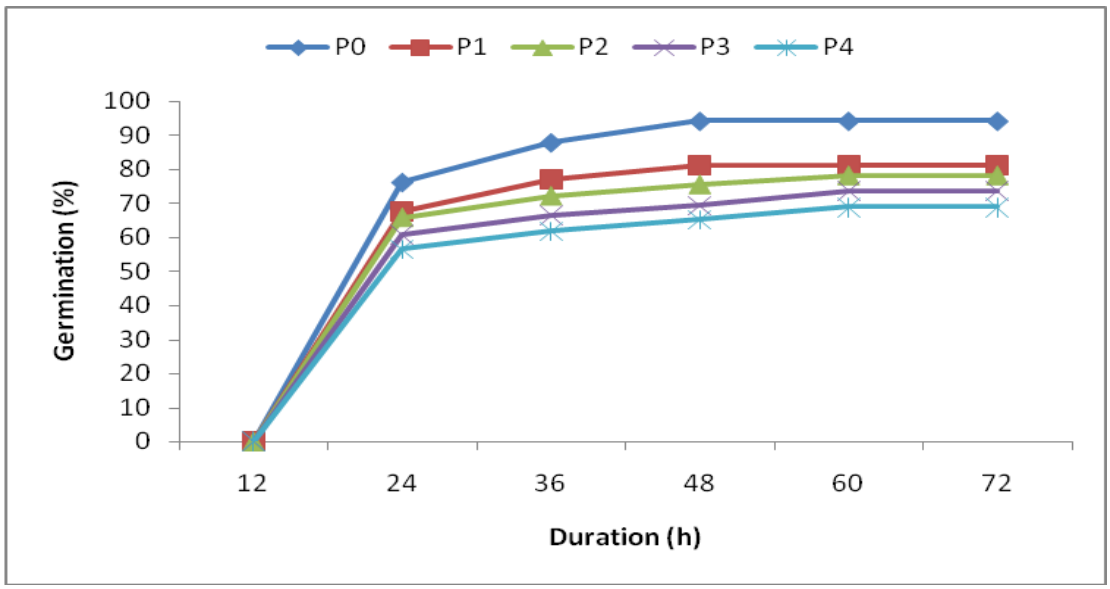

Figure 10. Impact of extracts obtained from Tamarind on the germination of barnyard grass ($LSD_{0.05}$ = NA, 1.76, 2.10, 2.04, 1.36 and 2.43 at 12, 24, 36, 48, 60 and 72 hours, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.11 Comparison on effect of medicinal plants extract on seed germination of okra

Figure 11 showed that the lowest germination percentage was found from P4 with Gardenia extract compared to other plant extracts whereas the Tamarind extract showed highest percentage of germination P_4 (0.3 g dry wt. eq. extract/mL) concentration. Similar trend was found for P_1 (0.01 g dry wt. eq. extract/mL), P_2 (0.03 g dry wt. eq. extract/mL) and P_3 (0.1 g dry wt. eq. extract/mL) extract. So, it can be mention that Gardenia showed highest phytotoxicity with okra seeds compared to other medicinal plants.

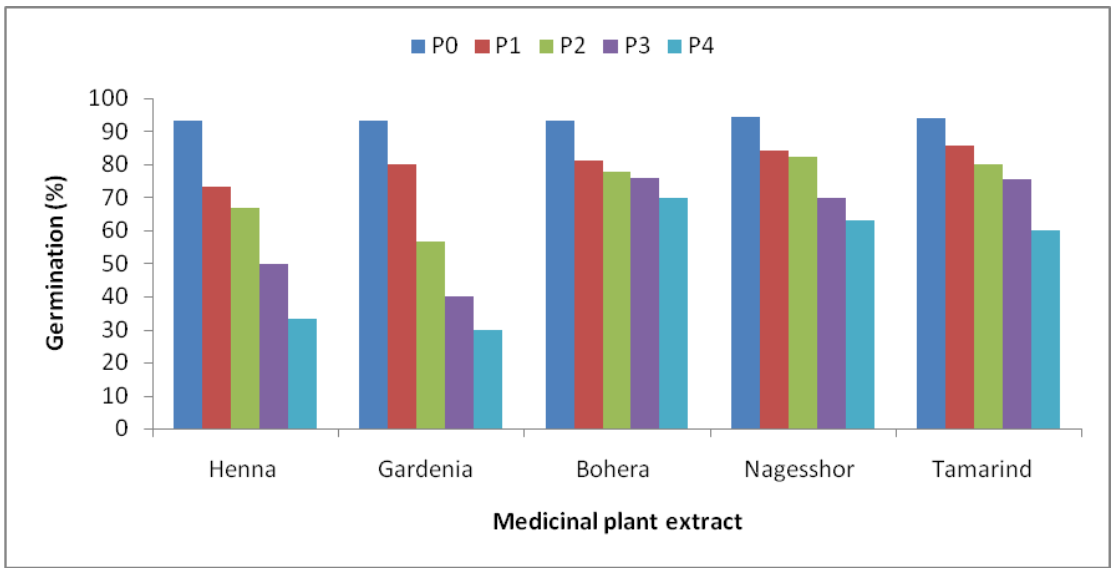

Figure 11. Germination percentage of okra affected by different extracts of medicinal plants

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.12 Comparison on effect of medicinal plants extract on seed germination of barnyard grass

Figure 12 showed that the lowest germination percentage was found from P4 with Gardenia extract compared to other plant extracts whereas Tamarind extract showed highest percentage of germination at P_4 (0.3 g dry wt. eq. extract/mL) concentration. Similar trend was found for P_1 (0.01 g dry wt. eq.

extract/mL), P_2 (0.03 g dry wt. eq. extract/mL) and P_3 (0.1 g dry wt. eq. extract/mL) extract. So, it can be mention that Gardenia showed highest phytotoxicity with barnyard grass seeds compared to other medicinal plants.

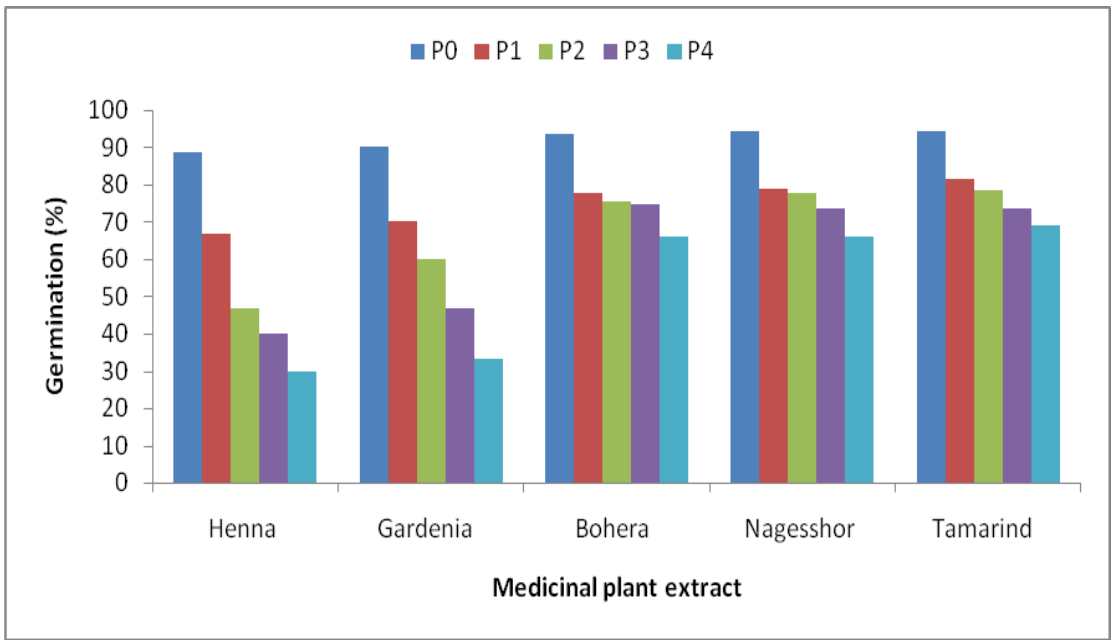

Figure 12. Germination percentage of barnyard grass affected by different extracts of medicinal plants

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.13 Effect of Henna extract on root length of okra

Significant variation was found on root length okra seeds affected by different levels of extract of Henna (Figure 13). Results indicated that the highest root length (5.10 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (2.20 mm). The lowest root length (0.85 mm) was observed from seed treatment with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was decreased with increasing of concentration and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

4.14 Effect of Gardenia extract on root length of okra

Significant variation was found on root length okra seeds affected by different levels of extract of Gardenia (Figure 13). Results indicated that the highest root length (5.40 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (2.28 mm). The lowest root length (0.79 mm) was observed from seed treatment with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment P_0 (no extract).

4.15 Effect Bohera on root length of okra

Significant variation was found on root length okra seeds affected by different levels of extract of Bohera (Figure 13). Results indicated that the highest root length (5.39 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (2.28 mm). The lowest root length (0.72 mm) was observed from seed treatment with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was decreased with increasing concentration of medicinal plants for seed treatment and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

4.16 Effect of Nagesshor on root length of okra

Significant variation was found on root length okra seeds affected by different levels of extract of Nagesshor (Figure 13). Results indicated that the highest root length (5.41 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (2.37 mm). The lowest root length (0.84 mm) was observed from seed treatment with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment P_0 (no extract).

4.17 Effect Tamarind extract on root length okra

Significant variation was found on root length okra seeds affected by different levels of extract of Tamarind (Figure 13). Results indicated that the highest root length (5.30 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (2.48 mm). The lowest root length (0.75 mm) was observed from seed treatment with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was decreased with increasing concentration of medicinal plants for seed treatment and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

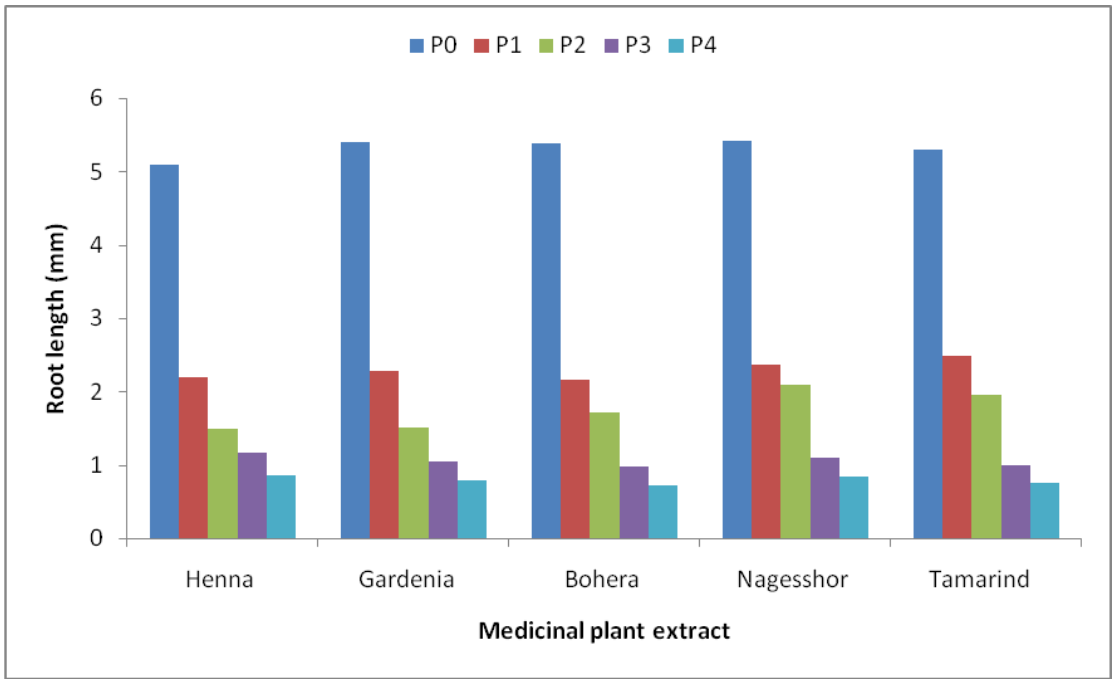

Figure 13. Root length (mm) of okra at 48 hours affected by different medicinal plants ($LSD_{0.05}$ = 0.12, 0.11, 0.21, 0.11 and 0.14 for Henna, Gardenia, Bohera, Nagesshor and Tamarind extract, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.18 Effect of Henna extract on shoot length of barnyard grass

Significant variation was found on shoot length barnyard grass seeds affected by different levels of extract of Henna (Figure 14). Results indicated that the highest shoot length (8.13 mm) was recorded from control treatment P_0 (no

extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (4.50 mm) and P_2 (0.03 g dry wt. eq. extract/mL) (2.50 mm). The lowest shoot length (0.80 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that shoot length was decreased with increasing of concentration and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

4.19 Effect of Gardenia extract on shoot length of barnyard grass

Significant variation was found on shoot length of barnyard grass seeds affected by different levels of extract of Gardenia (Figure 14). Results indicated that the highest shoot length (8.30 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (6.57 mm) and P_2 (0.03 g dry wt. eq. extract/mL) (4.17 mm). The lowest shoot length (0.97 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that shoot length was increased with decreasing of concentration and highest shoot length was found from control treatment P_0 (no extract).

4.20 Effect of Bohera extract on shoot length of barnyard grass

Significant variation was found on shoot length barnyard grass seeds affected by different levels of extract of Bohera (Figure 14). Results indicated that the highest shoot length (7.54 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (3.20 mm) and P_2 (0.03 g dry wt. eq. extract/mL) (2.08 mm). The lowest shoot length (0.67 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that shoot length was decreased with increasing of concentration and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

4.21 Effect of Nagesshor extract on shoot length of barnyard grass

Significant variation was found on shoot length of barnyard grass seeds affected by different levels of extract of Nagesshor (Figure 14). However, results indicated that the highest shoot length (7.70 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (3.69 mm) whereas the lowest shoot length (1.06 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that shoot length was increased with decreasing of concentration and highest shoot length was found from control treatment P_0 (no extract).

4.22 Effect of Tamarind extract on shoot length of barnyard grass

Significant variation was found on shoot length barnyard grass seeds affected by different levels of extract of Tamarind (Figure 14). However, it was found that the highest shoot length (7.71 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (3.70 mm) whereas the lowest shoot length (1.17 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that shoot length was decreased with increasing of concentration and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

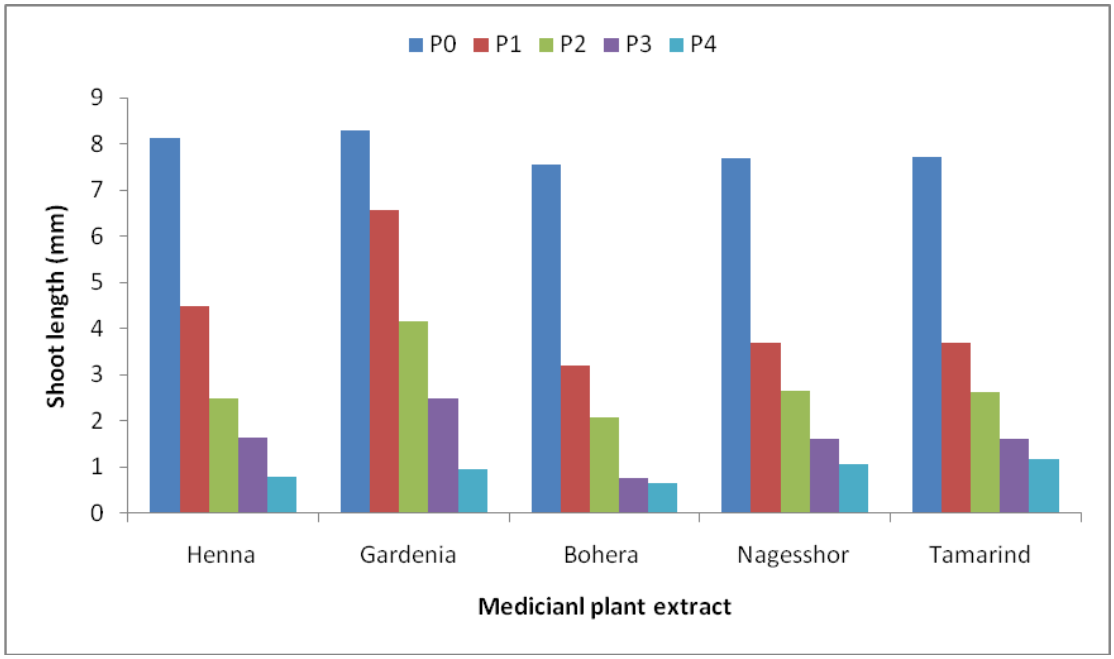

Figure 14. Shoot length (mm) of barnyard grass at 48 hours affected by different medicinal plants extract ($LSD_{0.05}$ = 0.13, 0.32, 0.17, 0.14 and 0.12 for Henna, Gardenia, Bohera, Nagesshor and Tamarind extract, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

4.23 Effect Henna extract on root length of barnyard grass

Significant variation was found on root length barnyard grass seeds affected by different levels of extract of Henna (Figure 15). Results indicated that the highest root length (14.10 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (6.90 mm) and P_2 (0.03 g dry wt. eq. extract/mL) (4.67 mm). The lowest root length (1.53 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was decreased with increasing of concentration and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

4.24 Effect of Gardenia extract on root length of barnyard grass

Significant variation was found on root length of barnyard grass seeds affected by different levels of extract of Gardenia (Figure 15). Results indicated that the highest root length (14.30 mm) was recorded from control treatment P_0 (no

extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (8.60 mm), P_2 (0.03 g dry wt. eq. extract/mL) (7.53 mm) and P_3 (0.1 g dry wt. eq. extract/mL) (4.30 mm). The lowest root length (1.97 mm) was observed from seed treatment with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment P_0 (no extract).

4.25 Effect of Bohera extract on root length of barnyard grass

Significant variation was found on root length barnyard grass seeds affected by different levels of extract of Bohera (Figure 15). Results indicated that the highest root length (12.21 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (6.74 mm) and P_2 (0.03 g dry wt. eq. extract/mL) (5.62 mm). The lowest root length (0.99 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was decreased with increasing of concentration and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

4.26 Effect of Nagesshor extract on root length of barnyard grass

Significant variation was found on root length of barnyard grass seeds affected by different levels of extract of Nagesshor (Figure 15). Results indicated that the highest root length (12.51 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (5.50 mm), P_2 (0.03 g dry wt. eq. extract/mL) (4.45 mm) and P_3 (0.1 g dry wt. eq. extract/mL) (2.42 mm). The lowest root length (2.12 mm) was observed from seed treatment with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was increased with decreasing of concentration and highest root length was found from control treatment P_0 (no extract).

4.27 Effect of Tamarind extract on root length of barnyard grass

Significant variation was found on root length barnyard grass seeds affected by different levels of extract of Tamarind (Figure 15). Results indicated that the highest root length (12.51 mm) was recorded from control treatment P_0 (no extract) followed by P_1 (0.01 g dry wt. eq. extract/mL) extract (5.45 mm) and P_2 (0.03 g dry wt. eq. extract/mL) (4.44 mm). The lowest root length (2.00 mm) was observed from seed treated with P_4 (0.3 g dry wt. eq. extract/mL). It was observed that root length was decreased with increasing of concentration and as a result P_4 (0.3 g dry wt. eq. extract/mL) extract gave lowest root length.

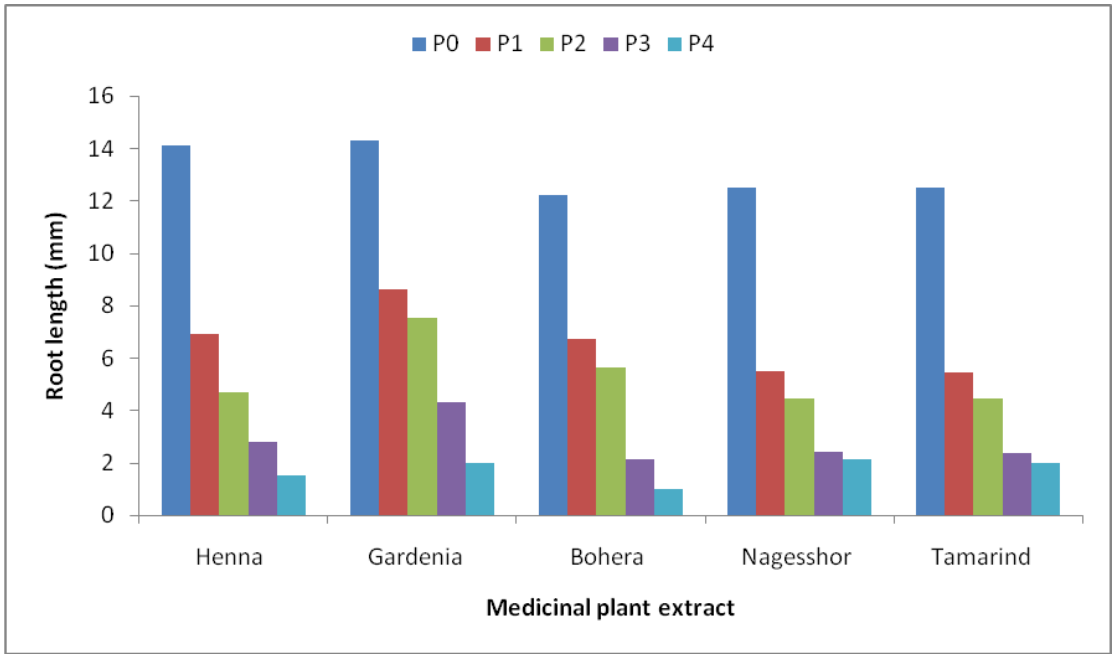

Figure 15. Root length (mm) of barnyard grass at 48 hours affected by different medicinal plants extract ($LSD_{0.05}$ = 0.15, 0.21, 0.17, NS and NS for Henna, Gardenia, Bohera, Nagesshor and Tamarind extract, respectively)

P_0 = 0 (control without extract), P_1 = 0.01 g dry wt. eq. extract/mL, P_2 = 0.03 g dry wt. eq. extract/mL, P_3 = 0.1 g dry wt. eq. extract/mL, P_4 = 0.3 g dry wt. eq. extract/mL

In all countries including Bangladesh, Pakistan and India, there is great reduction in crop yield due to weeds. The extent of losses caused by weeds was found to be more as compared to the insects and other diseases but their effects

are usually ignored. Weeds reduce productivity, because of completion for available natural resources such as sunlight, water and minerals etc. Also weeds might provide habitat for insects which damage the crops by eating them or spreading diseases. Weeds control through synthetic drugs has caused various human health problems and soil water pollution (Barkatullah et al., 2011). So weeds control through harmless means is indispensible, to increase yield of various crops and to protect environments.

The results of the Phytotoxicity of selected medicinal in present study indicated that seeds of okra and barnyard grass respond differentially to different medicinal plants (Figure 1-10). Seeds of okra and barnyard grass were susceptible to extracts of Henna, Gardenia, Bohera, Nagesshor and Tamarind with germination and shoot and root length.

Our finding is consistent with the results of a previous study by Bhatt and Todaria (1990) and they have reported that the leaf and bark extracts of Adina cordifolia (syn. *Haldina cordifolia*) reduce the germination rate in *Dolichos biflorus* and Glycine max. However, the degree of inhibition of *H. cordifolia* extract depends on the source of the extract and the species of target plants. This finding is in accordance with Huangfu *et al*. (2011) who have reported that the phytotoxicity of the extract of a weed yellowtop (*Flaveria bidentis*) was tissue dependent and species-specific. Therefore, application of these extracts from *H. cordifolia* in fields should be done with great care. *Xanthium strumarium* showed undistributed pattern of toxicity in results for hypocotyl growth. However, aqueous extracts of leaves and stems of *Xanthium strumarium* significantly reduced the germination, root and shoot lengths of corn, canola, sesame, lentils and chickpea (Shajie and Saffari, 2007).

Libralato *et al*. (2016) reported the Phytotoxicity of zerovalent iron on *Lepidium sativum*, *Sinapis alba* and *S. saccharatum*, their results showed bio stimulation effects as increased seedling length and high biomass. It is also reported that the chemicals of different part of the same plant can showed

phytotoxic, inhibitory or stimulatory effects on other recipient plants. Mehmoodzadeh *et al*. (2015) reported the allelopathic effect of *Cannabis sativa* shoot and root extract where shoot showed inhibitory effects while root gave stimulatory effects on seeds of *Lactuca sativa*.

Present study showed that at P_4 (0.3 g dry wt. eq. extract/mL) extract of all test medicinal plants showed least root length of okra and barnyard grass followed by shoot length of barnyard grass (Figure 1-10). Similar result was also observed by Naz and Bano (2014) who reported that leaf extracts of *Ricinus communis* and *L. camara* inhibit the growth of maize seedlings. Similar studies were also conducted by Algandaby and Salama (2016) where medicinal plants showed good phytotoxic or allelopathic effect. Devkota and Sherma (2014) also screened allelopathy of rhizome and leaves of justice adhatoda where they reported inhabited germination of wheat and pea seeds.

The present findings revealed that P_1 (0.01 g dry wt. eq. extract/mL), P_2 (0.03 g dry wt. eq. extract/mL), P_3 (0.1 g dry wt. eq. extract/mL) and P_4 (0.3 g dry wt. eq. extract/mL) extract showed reduceed shoot and root length of okra and barnyard grass and inhibited the germination. Macdonald *et al*. (2010) reported that *Ocimum gratissimum* flavonoids are phytotoxic. Jan *et al*. (2013) reported the presence of flavonoids in Monotheca buxifolia. Same results were reported by Saadullah *et al*. (2016).

These plants use in present study are commonly used for treating various illnesses but their allelopathic potentials are not reported yet. It is reported that medicinal plants can also be good sources of novel herbicides by inhibiting the growth seeds in vivo (Suwitchayanon *et al.,* 2017). It revealed from previous work that medicinal plants can produce inhibitory allelochemicals which reduces the germination of weeds (Sharma and Devkota, 2015).

Knox *et al*., (2010) reported the phytotoxic activity of *Cassia occidentalis*, *Rumex dentatus*, *Calotropis procera* and *Withania somnifera* against *Parthenium hysterophorus*. Javaid *et al*., (2009) repoted that phytotoxic activity

of aqueous extracts of two *Withania somnifera* and *Datura alba* root and shoot resulted in pronounced suppression in germination as well as seedling growth of target plant species. Application of aqueous extracts caused 68% reduction in germination, 62% in shoot length, 96% in root length and 68% in seedling biomass. Hussain *et al*., (2010) reported the phytotoxicity of the *R. hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* of the family Polygonaceae showed high effectiveness against *Lemna minor*.

CHAPTER V

SUMMARY AND CONCLUSION

In general observation, it was found that from the experiment, all plant extract showed higher phytotoxic effect on germination of seeds with higher concentration and gradually increased percent germination was found from gradually lower concentration of plant extract.

Considering germination percentage of okra seeds with Henna extract, lowest germination percentage (33.33%) and root length (0.85 mm) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) extract showed higher germination percentage (73.33%) and root length (2.20 mm) compared to seed germination 93.33% and root length 5.10 mm in control treatment P_0 (no extract).

In terms of germination percentage of okra seeds with Gardenia extract, lowest germination percentage (30.00%) and root length (0.79 mm) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) extract showed higher germination percentage (80.00%) and root length (2.28 mm) compared to seed germination 93.33% and root length 5.40 mm in control treatment P_0 (no extract).

For the germination percentage of okra seeds with Bohera extract, lowest germination percentage (69.67%) and root length (0.72 mm) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) extract showed higher germination percentage (81.00%) and root length (2.16 mm) compared to seed germination 93.33% and root length 5.39 mm in control treatment P_0 (no extract).

In terms of germination percentage of okra seeds with Nagesshor extract, lowest germination percentage (63.00%) and root length (0.84 mm) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq.

extract/mL) extract showed higher germination percentage (84.00%) and root length (2.37 mm) compared to seed germination 94.33% and root length 5.41 mm in control treatment P_0 (no extract).

For the germination percentage of okra seeds with Tamarind extract, lowest germination percentage (60.00%) and root length (0.75 mm) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) extract showed higher germination percentage (85.67%) and root length (2.48 mm) compared to seed germination 94.00% and root length 5.30 mm in control treatment P_0 (no extract).

In case of germination percentage of Barnyard grass seeds with Henna extract, lowest germination percentage (30.00%) and shoot and root length (0.80 and 1.53 mm, respectively) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) showed comparatively higher germination percentage (66.67%) and shoot and root length (4.50 and 6.90 mm, respectively) compared to highest seed germination 88.67%, shoot length 8.10 mm and root length 14.10 mm in control treatment P_0 (control; no extract).

Regarding germination percentage of Barnyard grass seeds with Gardenia extract, lowest germination percentage (33.33%) and shoot and root length (0.97 and 1.97 mm, respectively) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) showed comparatively higher germination percentage (70.00%) and shoot and root length (6.57 and 8.60 mm, respectively) compared to highest seed germination 90.00%, shoot length 8.30 mm and root length 14.30 mm in control treatment P_0 (control; no extract).

Regarding germination percentage of Barnyard grass seeds with Bohera extract, lowest germination percentage (66.00%) and shoot and root length (0.67 and 0.99 mm, respectively) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) showed comparatively higher germination percentage (77.67%) and shoot and root length (3.20 and 6.74 mm,

respectively) compared to highest seed germination 93.67%, shoot length 7.54 mm and root length 12.21 mm in control treatment P_0 (control; no extract).

Considering germination percentage of Barnyard grass seeds with Nagesshor extract, lowest germination percentage 66.00%) and shoot and root length (1.06 and 2.12 mm, respectively) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) showed comparatively higher germination percentage (79.00%) and shoot and root length (3.69 and 5.50 mm, respectively) compared to highest seed germination 94.33%, shoot length 7.70 mm and root length 12.51 mm in control treatment P_0 (control; no extract).

Regarding germination percentage of Barnyard grass seeds with Tamarind extract, lowest germination percentage (69.00%) and shoot and root length (1.17 and 2.00 mm, respectively) was found from P_4 (0.3 g dry wt. eq. extract/mL) extract whereas P_1 (0.01 g dry wt. eq. extract/mL) showed comparatively higher germination percentage (81.33%) and shoot and root length (3.70 and 5.45 mm, respectively) compared to highest seed germination 94.33%, shoot length 7.71 mm and root length 12.51 mm in control treatment P_0 (control; no extract).

From the above result it was observed that Gardenia extract at P_4 (0.3 g dry wt. eq. extract/mL) concentration in okra seeds showed highest phytotoxic effect for seed germination (30.00%) compared to all other concentration of all medicinal plants. Similarly, Henna extract at P_4 (0.3 g dry wt. eq. extract/mL) concentration in barnyard grass seeds showed highest phytotoxic effect for seed germination (30.00%) compared to all other concentration of all medicinal plants. Again, in terms of root length of okra seeds, Bohera extract at P_4 (0.3 g dry wt. eq. extract/mL) showed highest phytotoxic effect and gave lowest root length (0.72 mm) on okra compared to all other concentration of all medicinal plants. Similarly, Bohera extract at P_4 (0.3 g dry wt. eq. extract/mL) concentration on barnyard grass seeds showed highest phytotoxic effect for shoot and root length (0.67 and 0.99 mm, respectively). So, it can be concluded that Gardenia and

Bohera are more phytotoxic for okra seeds and Bohera and Henna are more phytotoxic for barnyard grass seeds.

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APPENDICES

Appendix I. Agro-Ecological Zone of Bangladesh showing the experimental location

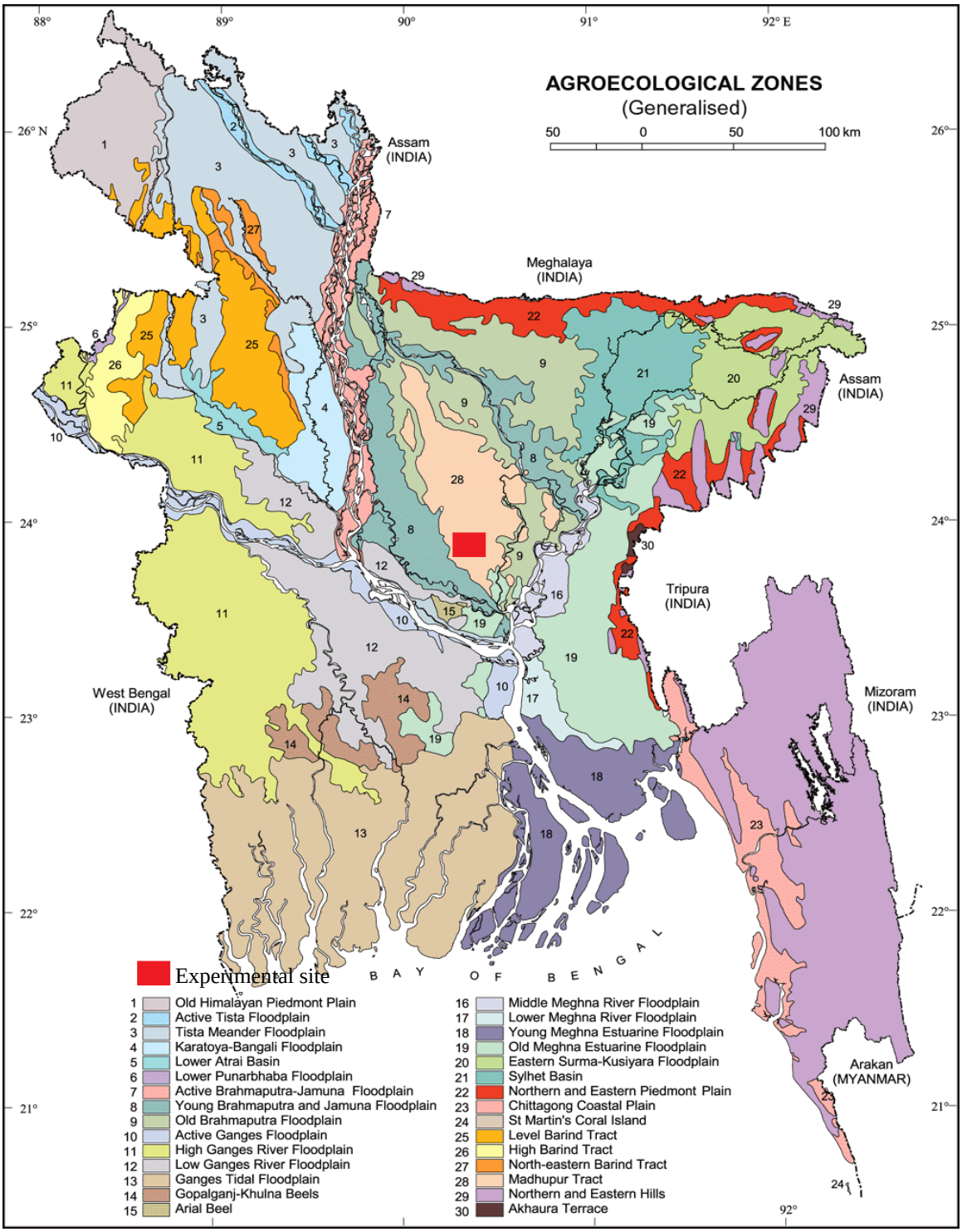

Fig. 16. Experimental site

Appendix II. Impact of Henna 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	-	0.124	0.245	0.212	0.014	0.133	0.001
Factor A	4	-	6.244**	8.312**	7.176**	12.32**	18.36**	1.03**
Error	8	-	0.112	0.436	1.044	0.632	0.711	0.002

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

Appendix III. Impact of Gardenia 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	-	0.014	0.036	0.105	0.033	0.107	0.001
Factor A	4	-	5.36**	7.21**	7.25**	10.91**	16.36**	1.01**
Error	8	-	0.014	0.132	0.101	0.024	0.155	0.003

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

Appendix IV. Impact of Bohera 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	-	0.207	0.144	0.083	0.071	0.042	0.002
Factor A	4	-	12.14**	14.26**	8.52**	9.37**	6.54**	0.61**
Error	8	-	0.105	0.084	0.104	0.077	0.068	0.003

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

Appendix V. Impact of Nagesshor 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	-	0.124	0.113	0.102	0.114	0.016	0.012
Factor A	4	-	16.35**	8.32**	11.24**	8.56**	9.47**	0.74**
Error	8	-	0.201	0.088	0.096	0.106	0.073	0.002

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

Appendix VI. Impact of Tamarind 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	-	0.003	0.015	0.009	0.104	0.035	0.003
Factor A	4	-	9.27**	10.56**	15.26**	7.42**	6.59**	0.46**
Error	8	-	0.092	0.137	0.069	0.044	0.101	0.002

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

Appendix VII. Impact of Henna extract on barnyard grass seeds germination and root 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	-	-	0.042	0.086	0.047	0.102	0.001	0.004
Factor A	4	-	-	11.27**	8.26**	6.29**	14.39**	0.48**	1.12**
Error	8	-	-	0.012	0.024	0.018	0.044	0.002	0.003

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

Appendix VIII. Impact of Gardenia extract on barnyard grass seeds germination and root 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	-	0.107	0.058	0.076	0.116	0.008	0.002	0.011
Factor A	4	-	8.27	10.14	8.36	11.27	9.76	0.83**	1.23**
Error	8	-	0.014	0.022	0.036	0.027	0.052	0.002	0.001

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

Appendix IX. Impact of Bohera extract on barnyard grass seeds germination and root 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	-	0.112	0.012	0.064	0.047	0.104	0.011	0.002
Factor A	4	-	8.47**	11.26**	7.24**	9.37**	14.07**	1.05**	0.60**
Error	8	-	0.103	0.009	0.014	0.117	0.022	0.001	0.001

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

Appendix X. Impact of Nagesshor extract on barnyard grass seeds germination and root 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	-	0.088	0.074	0.105	0.063	0.042	0.11	0.03
Factor A	4	-	14.32**	9.57**	6.48**	6.33**	7.41**	NS	1.04**
Error	8	-	0.007	0.014	0.102	0.206	0.011	0.001	0.004

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

Appendix XI. Impact of Tamarind extract on barnyard grass seeds germination and root 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	-	0.072	0.044	0.053	0.104	0.087	0.007	0.001
Factor A	4	-	15.36**	8.59**	10.27**	9.04**	7.52**	NS	1.014**
Error	8	-	0.103	0.074	0.048	0.092	0.028	0.002	0.003

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

Appendix XII. Some pictorial view of the experiment

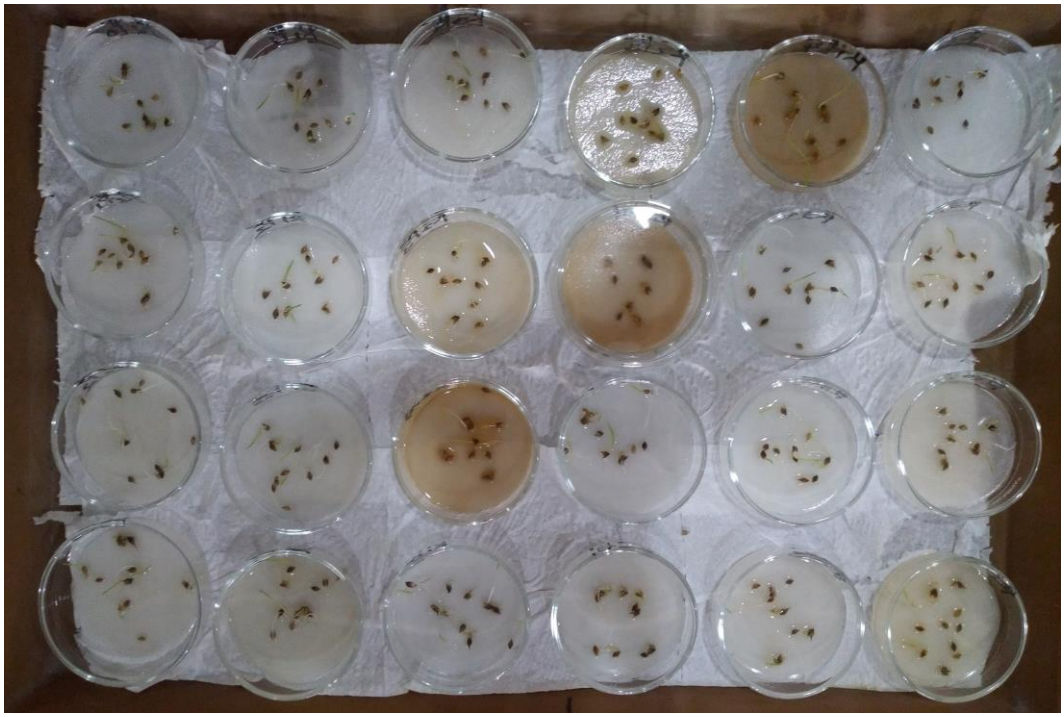

Plate 1. Sample set up of experiment with barnyard grass seeds

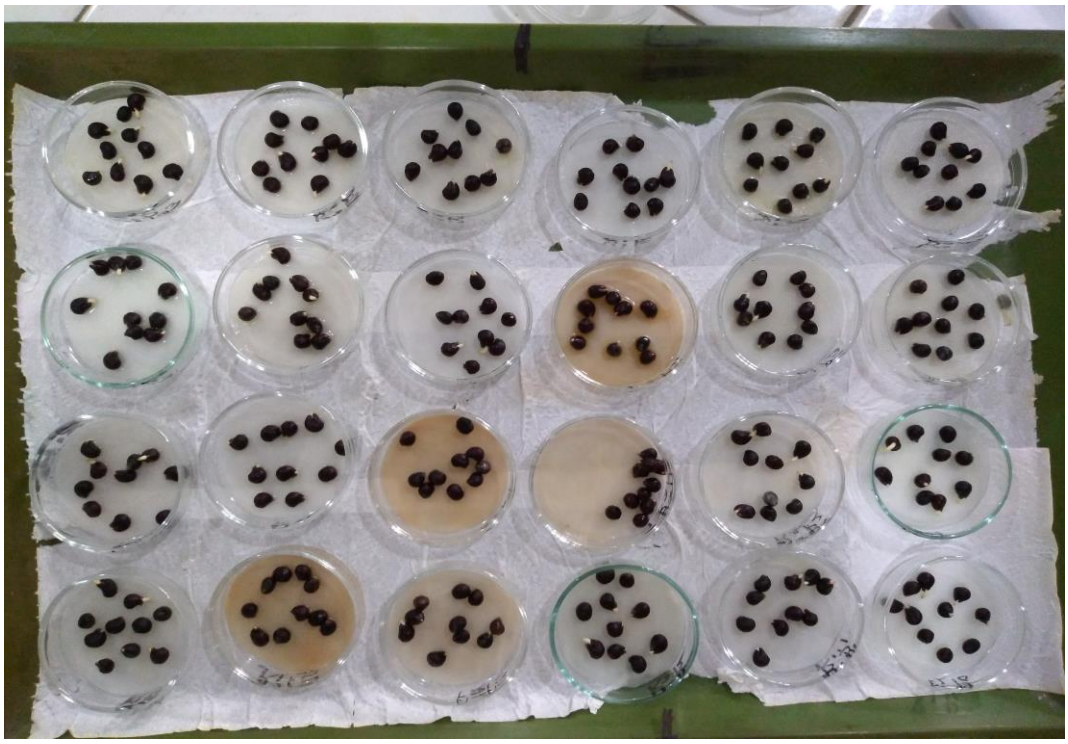

Plate 2. Sample set up of experiment with okra seed

Plate 3. Observation and data collection from barnyard grass seeds

Plate 4. Observation and data collection from okra seeds

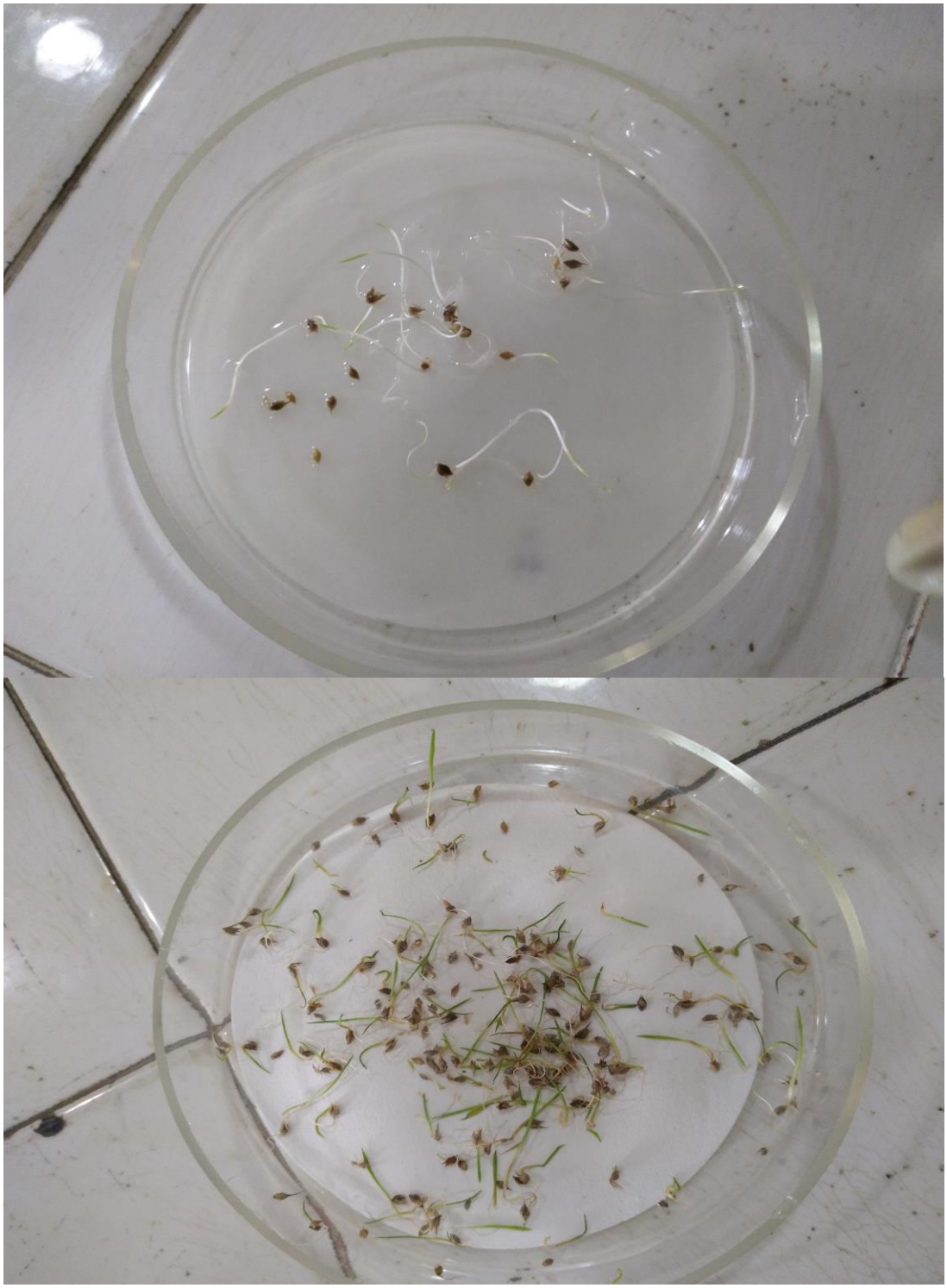

Plate 5. Sample of germinated seeds

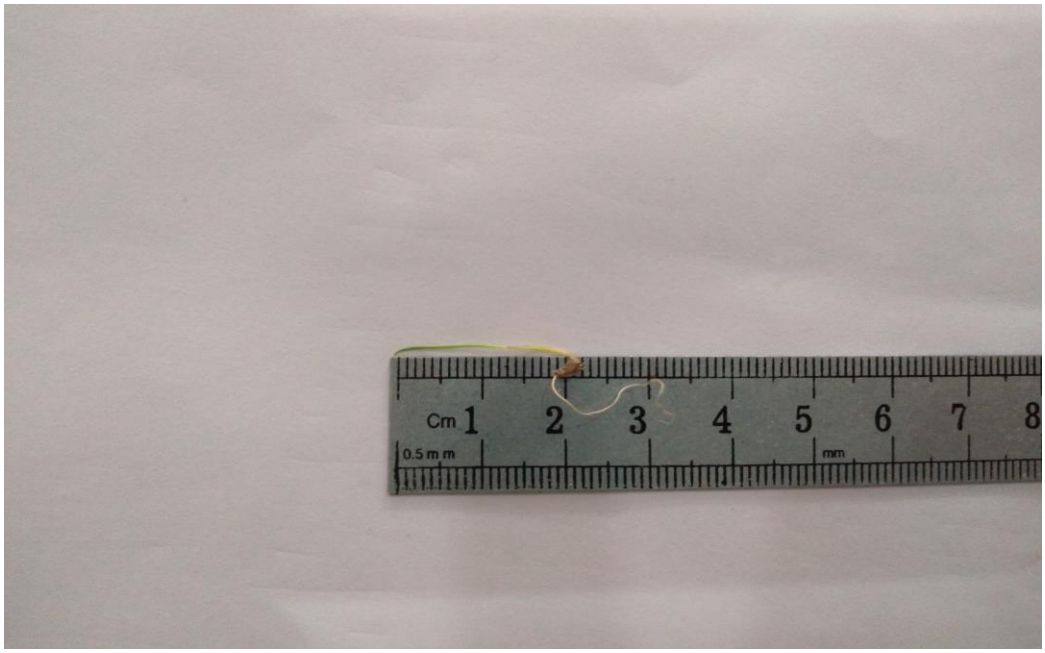

Plate 6. Sample data collection on shoot length

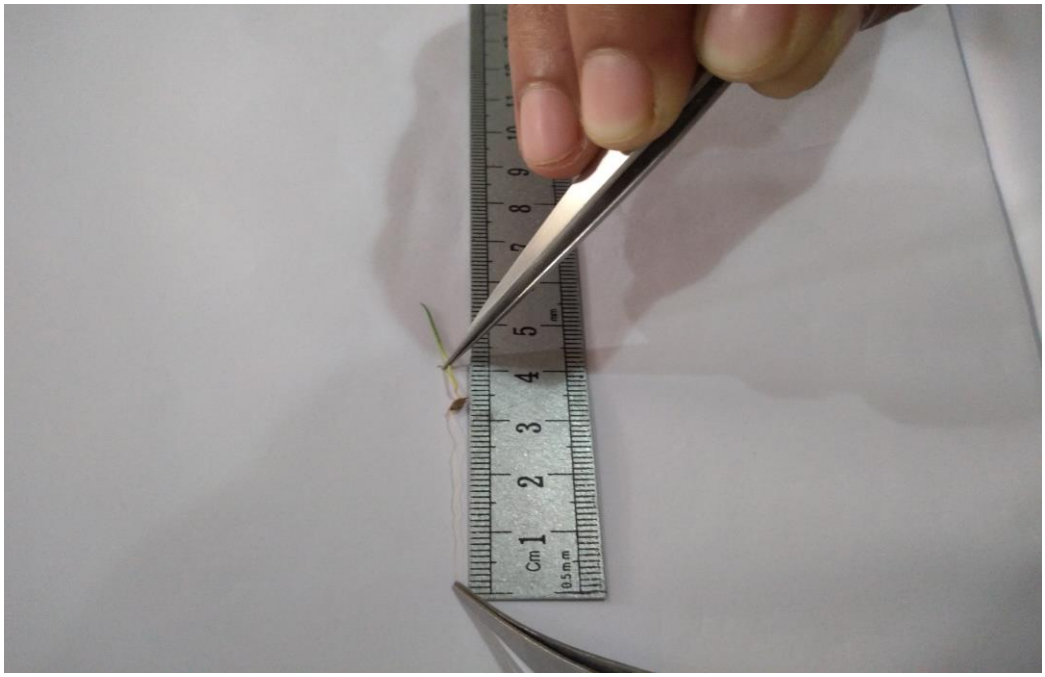

Plate 7. Sample data collection on root length