ALLELOPATHIC ACTIVITY OF SOME SELECTED MEDICINAL PLANTS ON BARNYARD GRASS (*Echinochloa crus-galli* (L.) P.Beauv.) AND OKRA (*Abelmoschus esculentus*)

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CERTIFICATE

This is to certify that the thesis entitled **“ALLELOPATHIC ACTIVITY OF SOME SELECTED MEDICINAL PLANTS ON BARNYARD GRASS (*Echinochloa crus-galli* (L.) P.Beauv) AND OKRA (*Abelmoschus esculentus*)”** 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 **SK. NYMUL ISLAM**, Registration No. **18-09220** 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.

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ALLELOPATHIC ACTIVITY OF SOME SELECTED MEDICINAL PLANTS ON BARNYARD GRASS (*Echinochloa crus-galli* (L.) P. Beauv) AND OKRA (*Abelmoschus esculentus*)

ABSTRACT

Medicinal plants enriched with many types of chemicals secondary metabolites which also act as allelochamicals. An experiment was conducted to evaluate the effect of allelochamicals of five medicinal plant species i.e. Asparagus (*Asparagus racemosus wild.*), Lebbeck (*Albizia lebbeck*), Devil's cotton (*Abroma augusta*), Scarlet gourd (*Coccinea cordifolia*), Spreading hogweed (*Boerhavia diffuse* linn.) on phytotoxicity regarding root and shoot growth of two test species (okra and barnyard grass). Five concentrations of each medicinal plant extract *viz*. T_0 (control; no extract), T_1 (0.01 mg dry wt. eq. extract/mL), T_2 (0.03 mg dry wt. eq. extract/mL), T_3 (0.1 mg dry wt. eq. extract/mL) and T_4 (0.3 mg dry wt. eq. extract/mL) were used for allelopathic 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 allelopathic effect on okra and barnyard grass. Among all concentration of plant extract, T_4 (0.3 mg dry wt. eq. extract/mL) showed highest allelopathic effect on both okra and barnyard grass seeds. In terms of root length of okra seeds, Spreading hogweed and Scarlet gourd extract at T_4 (0.3 mg dry wt. eq. extract/mL) showed highest allelopathic effect and gave lowest root length (0.533 mm). The Spreading hogweed (*Boerhavia diffuse* linn.) extract at T_4 (0.3 mg dry wt. eq. extract/mL) concentration on barnyard grass seeds showed highest phytotoxic effect for shoot and root length (1.9 mm and 0.93 mm, respectively). The above results suggested that those Scarlet gourd and Spreading hogweed may have allelochemicals and could be used as bioherbicide for successful crop production.

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ABBREVIATIONS AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSIR	=	Bangladesh Council of Scientific and Industrial Research
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	=	Milliliter
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

Allelopathy is defined as a delay of seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances (phytotoxins) or growing conditions (Chris Blok,2019). Allelopathy is the potentiality of a plant species to partially damage or kill another plant species through some chemical process. The terms phytotoxicity and allelopathy are about similar in action. Plants are one of the richest sources of organic compounds in the world. Allelopathy is described as direct or indirect, stimulatory or inhibitory action of one plant or microorgamism on another plant mediated by exuding chemicals into the environment (Rice, 1984; Brooks, 2008).

Allelopathic plants disperse some chemicals into the soil which interfere with nearby plants that may inhibit or stimulate plant growth, nutrient uptake or germination (Singh *et al.*, 2003). These chemicals, termed as allelochemicals, natural products or phytogrowth-inhibitors and these chemicals are a major factor in regulating the structure of plant communities (Smith and Martin, 1994; Aliotta *et al*., 2006). Allelochemicals present in all plant tissues such as leaves, roots, fruits, stem, flower including pollen and their 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). Medicinal plants contain large amount of various secondary metabolites (Azirak and Karaman, 2008; Mutlu and Atici, 2009). In Bangladesh perspective, there are so many medicinal plants in Bangladesh. There is a huge possibility having phytotoxic activity of medicinal plants on other plants. Medicinal plants contain high number of secondary metabolites or allelochamicals such as alkaloids, flavonoids, tannins, and some other phenolic compounds. Their activity varies with temperature, photoperiod, water and soils, during natural processes with its initial concentration, compound structure and mixed degree during functional processes with plant accessions, tissues and maturity within species (Shao-Lin *et al*., 2004). 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 effect of these chemicals is not limited to animal and human body alone but also on other plants. So, there is strong evidence that phytotoxic chemicals or natural plant products which are derived from higher plants/microbes can be ideal agrochemicals (Mattner, 2006; Soyler *et al*., 2012).

Weed is very harmful for our crop production and yield. In Bangladesh average 37.33% of crop produce is damaged if weeds are not controlled. Approximately 59665.70 million taka might be lost annually due to unrestricted growth of weeds in the country (Karim, 1998). The most effective way to control weeds for most farming operations is the application of synthetic herbicides in crop fields (Varshney *et al*. 2012). The allelochemicals can be used as bioherbicides in controlling weeds. Allelochamicals shows phytotoxicity effect to other monocot or dicot plants. For example, Researchers found that there were several phytotoxic chemicals 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, recently allelochemicals have received the attraction for being environmentally friendly and safer natural herbicides for weed control (Batish *et al*., 2001 and Khanh *et al*., 2007).

In this experiment, five medicinal plants are taken to observe the allelopathic effects or presence of allelochemicals on two test specimens- barnyard grass as monocotyledonous and okra as dicotyledonous plant. Asparagus is a perennial herb which belongs to family Asparagaceae. Asparagus have many types of secondary metabolites i.e., isoflavones, asparagamine, racemosol, polysaccharides (Mishra *et al.*, 2017). Lebbek is a perennial tree belongs to the family of Fabaceae. Devils cotton belongs to Malvaceae family. Scarlet gourd and Spreading hogweed are belong to the family of Cucurbitaceae and Nyctaginaceae respectively. All plants have secondary metabolites i.e., rotenoids, flavonoids, xanthones, alkaloids, abromin, sterol, β-sitosterol.

Overuse of synthetic herbicides controlling weeds lead to an increased risk of herbicide resistant of weed biotypes (Heap *et al*., 2014) and harsh environmental pollutions (Aktar *et al.,* 2009, Pell *et al*., 1998, Roger *et al.,* 1994). There are many types of synthetic herbicides for weed control that may cause serious damage the aqueous ecosystem by mixing pond or another water sources and also have some health issues. So, use of bio-herbicides can minimize the use of synthetic herbicides and also can control the weeds effectively. Researcher are being interested in phytotoxic activities of plant species for effective control of weed without using synthetic herbicides. Medicinal plants contain various types of phytotoxic chemicals which may affect on other harmful plant species such as weeds.

On the other hand, there could be some positive effect of allelochemicals as plant growth hormone. This might be effective stimulator for other plants in its growth and development. For example, Phenolic acids produced by plants are found to be among those compounds that act as osmoprotectants and antioxidants under stresses (Merkl, R. *et al*, 2010). In nature, phenolic acids are allelochemicals that are produced by the plants to protect from pathogens and mineral competition among plant species (Olofsdotter *et al*, 2002). Recently researchers have revealed that external applications of phenolic compounds help plants to cope with these environmental fluctuations (Lattanzio, *et al*, 2006).

Due to weeds, there is great reduction in crop yield in all countries including Bangladesh, Pakistan and India. 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 indispensable, to increase yield of various crops and to protect environments. So, Allelopathic plants are also a talking subject among researchers for weed control. Additionally, using natural substances including plant extracts is considered safe and acceptable in farming practices (Brandt, 2007; Verhoog *et al*., 2007; Jespersen *et al*., 2017).

Therefore, evaluating the phytotoxicity effect of medicinal plant would be a valuable finding for the ecological friendly agricultural practice and the positive effects on germination and development of medicinal plants selected in the research. Furthermore, the study is conducted for the following objectives-

1. To evaluate the allelopathic effect of five medicinal plants selected for the study on growth on the test specimens.
2. To estimate the potentiality as ecology friendly weed management.

CHAPTER II

Review of literature

Weed is a natural enemy of crop production. Weed competes with plants in terms of nutrition, population, growth and development. Our crop production seriously disturbed because of weed infestation in crop field every year. Along with the crop production loss, production cost also increasing due to the use of synthetic herbicides. The use of synthetic herbicides is elevating the risk of weed resistance, environmental pollution and hampering the ecosystem. Allelopathic chemicals play an important role in determination of the persistence and abundance of the weed species in mixtures of the plants. Medicinal plants are one of the best sources of allelopathic chemicals. Very few numbers of research work on phytotoxicity of medicinal plants have been conducted of the worlds but their findings have little relevance to the agro-ecological situation of Bangladesh. The present study has been undertaken to analysis the allelopathic effects of some selected medicinal plants available in Bangladesh. The relevant literatures available have been reviewed in this chapter.

Allelopathy of plants:

Allelopathy is an interference mechanism, by which live or dead plant materials release chemical substances that inhibit or stimulate the associated plant growth (Macias *et al*., 2003; Cheng and Cheng, 2016). The interactions caused by allelopathy are based primarily on the production of secondary chemicals by higher plants that produce a wide array of biochemical compounds that create biological changes (Reigosa and Pedrol, 2002; Hierro and Callaway, 2003; Heidarzade *et al*., 2010). Allelochemicals are released into the surrounding environment through a number of ways (Islam and Kato-Noguchi, 2013a). Plants produces biomechanisms to save themselves from harmful plants and animals (e.g., insects). They produce certain metabolites in the environments that save them from offensive organisms (plants, animals, microbes) and also eliminate the hostile one (Inderjit and Callaway. 2003; Yasmin *et al*, 2011). The phytochemicals that produce 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). The compounds, known as allelochemicals, are usually secondary plant products or waste products of the main metabolic pathways of plants and most of the compounds are derivative products of the shikimic acid and acetate pathway (RICE 1984). 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).

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 seediness. An ideal weed has the ability to show stronger and also interspecific competition via special mechanisms such as allelopathic processes (Mortimer, 1990). Weeds are the primary obstacle for rice production can reduce the rice yields from 44 to 96% in one crop season if the weeds are not control from the crop fields (Ampong-Nyarko and De Datta, 1991). Moreover, on an average 13-30% of crop reproduce is actually lost in the farmers' fields even after adopting conventional weed control techniques due to the weeds that grow after weed control (Swarbrick and Mercado, 1987; Mamun, 1990), though it may vary from country to country. Among the major weeds of rice, barnyard grass (Echinochloa crus-galli (L.) P.Beauv(L.) P. Beauv.) is the most notorious and dangerous one (Holm *et al*., 1977). The C4 photosynthetic system of barnyard grass facilitates it highest advantage under hot, arid and high light conditions (Patterson, 1985; Vidotto *et al*., 2007) along with higher water and nitrogen consume efficiency than C3 plants like rice (Ampong-Nyarko and De Datta, 1991). There is also another special characteristic, 'mimicry' with rice seedlings helps it to escape manual weeding (Barrett, 1983; Gibson *et al*., 2002). These above special characteristics help barnyard grass to be more competitive against rice and can reduce rice yields up to 100% (Ampong-Nyarko and De Datta, 1991). To control and destroy this notorious and dangerous weed from the rice fields on an average three million tonnes of herbicide has been used every year in almost all agricultural systems by the farmers (Stephenson, 2000). This over and uninterrupted use of synthetic herbicides in the rice field increase their resistance to some herbicides (Juliano *et al*., 2010; Beltran *et al*., 2012) and in the same time creates environmental hazards and pollution. Many researchers around the world show their keen interest on medicinal plants for searching new novel compounds as it may provide the clues to new and safe herbicide chemistry (Duke, 1986; Nimbal *et al*., 1996; Bhowmik and Inderjit, 2003; Li *et al*., 2009).

Recently, there is a growing effort in the sustainable agriculture, Focused on concerns of the adverse effects and extensive use of synthetic chemicals, such as cultivars with increased resistance to herbicides and soil & water contamination (Jinhu *et al*., 2012; Tigre *et al*., 2012). So, the interest in alternative compounds with phytotoxic properties has grown in recent decades, providing a promising field for the discovery of pesticides of natural origin that act directly on weeds and, most importantly, do not impose adverse effects on the environment or human health (Alves *et al*., 2003; Haig *et al*., 2009).

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-Pimiä *et al*. 2001; Varsha *et al*. 2013; Vashist and Sharma 2013). For the measurement and quantification of allelopathy without interfering the resource competition, many laboratory techniques have been developed (Leather and Einhelling, 1986; Navarezand-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 *et al*., 1985; Leather and Einhelling, 1986). This assay has a wide range of application in research towards the discovery of active principles in plants (Arzu *et al*., 2002).

There are so many researchers around the world who are now showing their keen interest on medicinal plants for searching new natural plant products. (Azizi, *et al*, 2006; Lin. D, *et al*, 2003; Lin. D. *et al*, 2004; Hun *et al*, 2008, Li, *et al*, 2008; Sodaeizadeh, H, *et al*, 2009). Islam and Kato-Noguchi (2014) found two reasons for this increasing interest: 1) the screening process is easier of phytotoxic plants from medicinal plants and 2) there is a possibility to have more bioactive compounds in medicinal plants than other plants.

Mardani *et al*. (2014) currently experimented the allelopathic potential of 83 Iranian medicinal plants and observed that more than 80% root growth of lettuce were suppressed by plant extracts of *Peganum harmala, Berberis vulgaris, Artemisia aucheri* and *Ferulago angulata*. Amini *et al*. (2016) investigated and evaluated the phytotoxic potential of 68 medicinal and wild plant species belong to 19 plant families grown in Iran. Among the examined plant species, stigma and style of *Crocus sativus*, leaves of *Artemisia kopetdaghensis, Mentha piperita, Zhumeria majdae, Frulago subvelutina*, flowers bud of *Eugenia caryophyllata*, flower of *Perovskia abrotanoides*, fruits of *Melia azedarach* and *Ruta graveolen* had the strongest phytotoxic effects on lettuce seedling growth.

Gilani *et al*. (2010) also evaluated the allelopathic potentiality of 81 medicinal plant species, collected from North West Frontier Province (NWFP) Pakistan. The research was conducted to find out the significantly higher allelopathic species for future phytochemical analyses. They used sandwich method to test allelopathic potentials of leaf leachates of these plant species and the selected test subject was lettuce seeds *(Lactuca sativa* L.). In that study, two different concentrations of 10 mg and 50 mg of leaf extracts were used. The radicle growth and hypocotyl growths were measured and comparison was made with control treatments. It was observed that an endemic species *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* showed stronger phytotoxicity effects as compared to the other test species. Based on the current study, those three potential medicinal plants are recommended for future bioassay guided isolation of allelochemicals.

They concluded and suggested that further researches could be interesting to see correlation between genetic markers and isolated allelochemicals.

Amir *et al.* (2011), conducted the research on phytotoxicity of 13 medicinal plant 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* on 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. Almost every plant extract shows negative effects on Germination velocity. Also, other parameters such as root & shoot growth was decreased by the application of the extracts, however more pronounced effect was seen at 10 mg/ml concentration.

Islam and Kato-Noguchi (2013) were investigated the Allelopathic potential of five Labiatae plant species on barnyard grass (*Echinochloa crusgalli*). Authors claimed that Plants belongs to the Labiatae family attracted the attention of many researchers in pharmacological interest because of their toxic potential and medicinal properties. However, there is very limited information available for the allelopathy of this family. The conducted research explores the allelopathic potential of the aqueous methanol extract of five Labiatae plants*: Leucas aspera L., Leonurus sibiricus L., Ocimum tenuiflorum L., Mentha sylvestris L. and Hyptis suaveolens L*. were tested against barnyard grass at four different concentrations (3, 10, 30 and 100 mg DW equivalent extract/mL). They found that root growth was more sensitive to the plant extracts than the coleoptile growth and the inhibitory activities were dependent on concentration. At that research, researchers found out that the concentration of 100 mg DW equivalent extract/mL, of *L. aspera* and *H. suaveolens* plant extracts strongly inhibited the seedling growth of barnyard grass. The seedling growth of barnyard grass was most susceptible to *L. aspera* plant followed by *H. suaveolens* to confirm 50% coleoptile and root growth inhibition, among the plant extracts. Researchers concluded that *L. aspera* and *H. suaveolens* have strong allelopathic potential and could be used as the good candidates for isolation and identification of allelochemicals to develop environment friendly new natural herbicides to control barnyard grass.

A.K.M Mominul Islam is a great researcher who experimenting the allelopathic activity of medicinal plants and also have many articles on it. One of those experiments is Allelopathy of Medicinal Plants: Current Status and Future Prospects in Weed Management conducting by Islam *et al.* (2018). This is a reviewed paper on allelopathic potentiality of medicinal plants. According to this experiment researcher found so many evidences of allelopathic potentiality of medicinal plants such as Fujii *et al*. (2003) carried out the first comprehensive experiments of allelopathic medicinal plants. They examined 387 Japanese medicinal plants and observed that a considerable number of those have growth inhibitory potential. Azizi *et al*. (2009) evaluated 56 aromatic medicinal plants of 22 families from Iran for their allelopathic potential and found 51 species inhibited the seedling growth of lettuce.

Islam *et al.* (2013) also investigated the allelopathic activity of *Litchi chinensis* Sonn., which is an important fruit plant of subtropical to tropical areas. The aqueous methanol extract of *L. chinensis* leaves at four different concentrations was experimented against the germination of lettuce (*Lactuca sativa* L.) and barnyard grass (*Echinochloa crus-galli (L.) P.Beauv*L. Beauv.). Also, the analysis conducted on the seedling growth of cress (*Lepidum sativum* L.), lettuce, alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.), Italian ryegrass (*Lolium multiflorum* Lam.) and barnyard grass. The extracts at concentrations ≥0.01 g dry weight equivalent (dry wt. eq.) extract mL^{-1} significantly inhibited the hypocotyl/coleoptile and root growth of all test plants. The inhibitory activities of the extracts were proportional to the concentrations of the extract. The hypocotyls growth of cress, lettuce and alfalfa, and the root growth of all test plant species were completely inhibited by the concentration 0.1 g dry wt. eq. extract mL^{-1} of the extracts. It is also observed that, at the same concentration, a complete inhibition of lettuce seed germination and a significant delay of germination on barnyard grass were occurred. The I_{50} values for the hypocotyl/coleoptile and root growth of test plants range from 0.002 to 0.079 g dry wt.

eq. extract mL^{-1}. The outcomes of the conducted experiment are *L. chinensis* leaf extracts possess allelopathic properties and contain allelopathic substances. Therefore, researchers found that the plant could be used as a candidate for isolation and identification of those secondary metabolite substances, which may explore the interactions of *L. chinensis* with other surrounding plants under natural settings.

Islam *et al.* (2018) have conducted many research on allelopathic potentiality. Among all, one of the researches was conducted to investigate the allelopathic properties of 55 medicinal plant species of Bangladesh representing 32 different families. Researcher took aqueous leaves or whole plant extract of those plant species. That diluted into four different concentrations viz., 1:5, 1:10, 1:15, 1:20 (w/v) and tested against the seedling growth of Raphanus sativus. A control (distilled water without extract) was also maintained in every cases. The aqueous extract of all these species disrupted both shoot and root length of R. sativus at concentration greater than 1:15 (w/v) with the exception of Delonix regia (Fabaceae) and Leucas aspera (Lamiaceae). The inhibitory activity was concentration dependent whereas root growth was more sensitive than their shoot. Among the plant species, *Citrus aurantifolia*, *Moringa oleifera*, *Annona muricata*, *Aegle marmelos*, *Cinnamomum tamala* and *Azadirachta indica* are completely inhibited the shoot and root growth of *R. sativus* at concentration 1:5 (w/v). Along with this six plant species, there are also 15 out of 49 medicinal plants showed that more than 95% shoot and root growth were inhibited at the same concentration. The least allelopathic potential plant was *Garcinia mangostana* (Clusiaceae) that showed on an average 36% growth inhibition. Based on these results they have concluded that among the tested plant species, C. aurantifolia, M. oleifera, A. muricata, A. marmelos, C. tamala, and A. indica are strongly allelopathic.

Azizi *et al.* (2016) published a research paper on the journal of ‘’International Journal of Horticultural Science and Technology” to investigate the allelopathic potential of 68 medicinal and wild plant species belong to 19 plant families grown in Iran. The results of that experiment show within all of the examined plants, stigma and style of *Crocus sativus*, leaves of *Artemisia kopetdaghensis*, *Mentha piperita*, *Zhumeria majdae*, *Frulago* *subvelutina*, flowers bud of *Eugenia caryophyllata*, flower of *Perovskia abrotanoides*, fruits of *Melia azedarach* and *Ruta graveolenhad* have strongest inhibitory effects on lettuce seedling growth. This is a matter of interest that by using of very low amount of plant extract (10 mg), more than 70% of growth inhibitory effects of these plants were observed. Additionally, the leaf of *Atriplex canescens* and the flower of Achillea millefolium had strongly inhibited the radicle growth (more than 75%). Here they have concluded that plants which have the inhibitory effects on growth and development of other plants, have the potentiality to be applied as biological herbicides.

Piyatida, P. and Kato-Noguchi, H. (2010) conducted the research on eleven species of Thai medicinal plants, namely *Rhinacanthus nasutus* (L.) Kurz, *Clitoria ternatea* L., *Mammea siamensis* Kosterm, *Centella asiatica* (L.) Urban, *Thunbergia lauriflolia* Linn., *Piper sarmentosum* Roxb., *Hibiscus sabdariffa* L., *Moringa oleifera* Lam., *Tinospora tuberculata* Beume, *Tiliacora triandra* (Colebr.) Diels and *Amomum krervanh* Pierre ex. Gagnep. were examined their allelopathic potentials against cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.) and crabgrass (*Digitaria sanguginalis* L.). The aqueous methanol extracts of these medicinal plants had inhibitory activity on all test plant species. They found that the aqueous methanol extracts of *H. sabdariffa* showed the highest growth inhibitory effect on cress and alfalfa seedlings and the extract obtained from *P. sarmentosum*, *R. nasutus* and *T. tuberculata* showed the highest allelopathic potential on lettuce, timothy and crabgrass seedlings, respectively. Inhibitory effects of these medicinal plants were dependent on test plant species. There may be a variation in result, from the different test plant species with different sensitivity to allelochemicals. However, four medicinal plants *H. sabdariffa*, *P. sarmentosum*, *R. nasutus* and *T. tuberculata* possessed high allelopathic potential and may be good candidates for isolation and identification of allelochemicals.

A.Reza Einodehi *et al.* (2006), performed a research on Allelopathic potential of tea (*Camellia sinensis* (L.) Kuntze) on germination and growth inhibitory effect on some spicies. In laboratory and greenhouse experiment was conducted in this research to determine the effects of tea (*Camellia sinensis* (L.) Kuntze) extracts (leaf, flower and fruit) at different concentrations on germination and growth of garden cress (*Lepidium sativum* L.), lettuce (Lactuca sativa L.), redroot pigweed (*Amaranthus retroflexus* L.) and golden foxtail (Setaria glauca (L.) P.Beauv.). the effects of dried residues of tea on leaf area and dry weight of redroot pigweed and golden foxtail were investigated. Concentrations ranged from 0, 2.5, 5 to 10 % for organ extracts and dried residues were tested in mixtures with perlite at rates of 0, 8, 16, 24 and 32 g kg-1. Results showed that all extracts of tea organs inhibit the garden cress and redroot pigweed germination with the exception of the leaf extract at the lowest concentration. The same was observed for lettuce and golden foxtail where germination was inhibited with the exception of leaf and fruit extracts at a concentration of 2.5 %.

S. Boonmee *et al.* (2018) recently conducted research to evaluate of phytotoxic activity of leaf and stem extracts and identification of a phytotoxic substance from *Caesalpinia mimosoides Lamk*. *Caesalpinia mimosoides* (Fabaceae) has been used as a folk medicine for long time. This is also reported to have pharmacological properties of *Caesalpinia mimosoides*. However, possibility of having phytotoxic potentiality of *C. mimosoides* has not yet been studied. So, the study on phytotoxic activity or allelopathic responce of *C. mimosoides* leaf and stem extracts was experimented against the growth of dicotyledonous and monocotyledonous plants. Inhibitory effects were examined on the growth of all the test plants by using aqueous methanol extracts of C. mimosoides leaves and stems. With the increase of concentrations of the extracts, the growth of the test plants decreased. They have found that the leaf extracts had more inhibitory effects than the stem extracts by comparing the concentrations required for 50% growth inhibition (I_{50} values). Thus, they separated the active substances in the leaf extracts using column chromatograph. They also isolated and identified the growth inhibitory substances as methyl gallate by using spectral analysis. Their study reports that they are the first isolator of methyl gallate in *C. mimosoides* leaves. At 10 mM concentration of Methyl gallate completely inhibited the growth of cress shoots and inhibited its roots by 4.7% of control growth. The shoots and roots of barnyard grass were inhibited by 84.2 and 1.7% of control growth, respectively at the same concentration. I_{50} values of methyl gallate for the growth of cress and barnyard grass ranged from 2.3 to 2.9 and 0.3 to 30.5 mM, respectively. They concluded at the result and suggestion that methyl gallate may have phytotoxic potentiality and could be responsible for the phytotoxic effects of *C. mimosoides.*

Aslani *et al.* (2014) experimented on phytotoxic interference of volatile organic compounds and water extracts of *Tinospora tuberculata Beumee* on growth of weeds in rice fields. *Tinospora tuberculata Beumee* is an aromatic shrub which have some pharmacological properties and belongs to the Menispermaceae family. They experimented *T. tuberculata* to find out the ability to inhibit growth of weeds in rice fields. In seedling bioassays, as the concentration of aqueous water extracts and volatile organic compounds increased, the rate of seed germination and the radicle and hypocotyl lengths of barnyard grass, rice and weedy rice seedlings were reduced compared to the control. In a glasshouse experiment, in the emergence and growth of weeds in rice fields also effectively reduced by the extract of *T. tuberculata* leaf powder. They also were analyzed the chemical compositions of extracts and volatiles. They analyzed 43 components in the leaf aqueous extract and 7 out of them examined as toxic compound and 5 of 15 compounds in the stem aqueous extract, are also found to be toxic compounds. They also identified eight main volatile organic compounds accounted for 97 and 95% of the whole fresh and dry leaf volatiles, respectively. They observed qualitative and quantitative differences in the compounds present in the dry and fresh leaf. Confocal laser scanning microscope images showed that the volatile emissions and aqueous extracts also inhibited the main root diameter, and the number and elongation of root hairs of tested weeds in rice fields. The results of this experiment suggests that *T. tuberculata* contains a significant source of plant growth inhibitors.

Xuan *et al*. (2005) demonstrated that extracts of allelopathic plant performed better than synthetic herbicides to control weeds. They observed that 80% total weed biomass of Japanese alfalfa variety (Rasen) were inhibited and also 81% rice yield were promoted when compared with the control (without any weed and fertilizer management). On the other hand, herbicide treatment suppressed 75% paddy weeds but increased rice yield by only 10%, and the ratio for hand weeding were about 70% and 25%, respectively. Xuan et al. (2005) also reported that through the incorporation of 1 - 2 ton per ha. of strong allelopathic plants, 70% - 80% weed reduction could be attained. Although the inhibitory activity of allelopathic plant biomass is dose-dependent, so they suggested not to exceed the limit above 2 ton per ha. The most important obstacle to use plant biomass is that it needs water for decomposition and incorporat with soil. Therefore, there is a difficulty to apply plant biomass in highland areas because of water disavailability. To overcome this problem many researchers currently focus on allelopathic substances rather than the plant itself.

Hong *et al.* (2004) experimented with ten allelopathic higher plants (*Ageratum conyzoides, Bidens pilosa, Blechnum orientale, Eupatorium canabium, Euphorbia hirta, Galactia pendula, Leucana glauca, Melia azedarach, Morus alba, and Tephrosia candida*) and found that 2 ton per ha application of the plant biomass of all the species at significantly reduced paddy weed growth and promoted the rice growth and yield. Among those species, highest potentiality was showed by *B. pilosa* and *T. candida* to reduce more than 80% weeds and to increase rice yields up to 20%. Many other examples are found in the literatures that are potential to control weeds (Fujii *et al*., 2003, Wu *et al*., 1999)

Sorgoleone is an isolated phytotoxic chemicals exudate from Sorghum root hair which has the characteristics as a potent bio-herbicide. It shows phytotoxicity to broadleaf and grass weeds at concentrations lower than 10 µM (Einhellig *et al*., 1992, Nimbal *et al.,* 1996). There is a significant result on inhibited growth of most of the small-seeded broadleaf weeds with application of post-emergent foliar application of Sorgoleone, at a similar concentration to labelled field rates of Atrazine (0.6 kg a.i. ha^{-1}), (Czarnota *et al*., 2001). Certain small-seeded weed species were showed toxic response with Pre-emergence soil applications (Weston *et al.* 2001). The allelochemicals founds in tomato such as tomatine and tomatidine also shows toxicity on the growth of weeds, pathogenic fungi at a satisfactory level (Hoagland, R.E. 2009).

There are several types of allelochemicals or plant secondary metabolites for examples, polyphenols, terpenoids, alkaloids, coumarins, tannins, flavonoids, steroids and quinines are involved in the phytotoxic activities of the allelopathic plants (Einhellig *et al.,* 1988, Kohli *et al*., 1997). Recently, a number of bio-chemical compounds extracted from higher plants, such as cineole, quinolinic acid, benzoxazinones and leptospermones, have been commercially applied in crop fields to control weeds such as Benzoxazinones and Quinolinic acid by BASF, Germany; Cineole as Cinmethylene by Shell, USA; Letospermones as Triketones by Zeneca, Letospermones as Mesotrione by Syngenta AG (Macías *et al.* 2007, Dayan *et al.*, 1999).

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 October 2020 to January 2021. The site is 90.2°N and 23.50° E Latitude and at 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 (*Abelmoschus esculentus*) and
2. Barnyard grass (*Echinochloa crus-galli* (L.) P.Beauv)

Five medicinal plants have been used to identify their allelopathic interaction with test crops which were as follows:

1. Shatamuli or Asparagus (*Asparagus racemosus wild.*)
2. Karoi or Lebbeck (*Albizia lebbeck*)
3. Ulatkambal or Devils Cotton (*Abroma augusta*)
4. Telakachu or Scarlet gourd (*Coccinea cordifolia*)
5. Punnarnarva or Spreading hogweed (*Boerhavia diffusa* linn.)

Plant samples of Asparagus, Lebbeck, Devils Cotton, Scarlet gourd & Spreading hogweed were collected from surroundings of 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. T_0 = 0 (control without extract)

2. T_1 = 0.01 mg dry wt. eq. extract/mL

3. T_2 = 0.03 mg dry wt. eq. extract/mL

4. T_3 = 0.1 mg dry wt. eq. extract/mL

5. T_4 = 0.3 mg 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 Microsoft Excel package.

3.5 Sample preparation

The plants part- leaves of Lebbeck (*Albizia lebbeck*), Asparagus (*Asparagus racemosus wild.*), Spreading hogweed (*Boerhavia diffuse* linn.), Devil's cotton (*Abroma augusta*), Scarlet gourd (*Coccinea cordifolia*) were washed with tap water to remove the soil and other debris, dried under sun for two days. After sun drying the samples were oven dried at 40°C temperature for 72 hours then the samples were cut and grinded to prepare as powder. Overall sample preparation process was conducted within five days. Okra (*Abelmoschus esculentus*) and barnyard grass *(Echinochloa crusgalli*) 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 grinded separately in a mechanical grinder. A powder sample of 5g of each sample were weighed with analytical balance (model: ATX224, SIMADZU, Tokyo, Japan) and extracted with 100 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 aqueous methanol for 24 hours and then filtered. Two filtrates were mixed together and then evaporated with a rotary evaporator (HAHNVAPOR, HS-2005S, HAHNSHIN S&T CO., LTD, Korea) 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 mg 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 Plant Growth

The effects of aqueous extracts on plant growth 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 (RGX-250E, Huanghua Faithful Instrument Co., Ltd, China) at 25°C. Moisture in the petri dishes was maintained by adding distilled water as required. The length of the root and shoot of the test specimens were measured after 96 hours.

3.9 Collection of data

The following parameters were collected for the present experiment

1. Shoot (plumule) length
2. Root (radical) length

3.10 Procedure of recording data

3.10.1 Shoot (plumule) length

After 96 hours of germination duration, shoot (plumule) length was recorded with a slide caliper carefully and was measured in mm.

3.10.2 Root (radical) length

After 96 hours of germination duration, shoot length was recorded with a slide caliper carefully and was measured in mm.

3.11 Statistical analysis

The collected data on various parameters were statically analyzed using Microsoft excel 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 seed shoot and/or root elongation of okra and barnyard grass are given below under some heading.

4.1 Effect of Asparagus (*Asparagus racemosus wild.*) on root length of Barnyard Grass

Significant variation was found on root length of barnyard grass seeds affected by different levels of extract of Asparagus (Figure 1). Results indicated that the highest root length (9.26 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (4.167 mm). The lowest root length (3.53 mm) was observed from seed treatment with T_4 (0.03 mg dry wt. eq. extract/mL). At the highest concentration T_4 (0.3 mg dry wt. eq. extract/mL) root length (3.53 mm) was significantly variable than control. It was observed that root length was decreased significantly from the lowest concentration T_1 (0.01 mg dry wt. eq. extract/mL) to the highest concentration T_4 (0.3 mg dry wt. eq. extract/mL). There is a related findings of S. H. Vargas *et al.* (2019) that the extract of *T. formosa* at higher concentrations, drastically reduce the dry biomass and length of shoots and root of lettuce.

4.2 Effect of Lebbeck (*Albizia lebbeck*) on root length of Barnyard Grass

Significant variation was found on root length Barnyard Grass seeds affected by different levels of extract of Lebbeck (*Albizia lebbeck*) (Figure 1). Results indicated that the highest root length (10.4 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (8.1 mm). The lowest root length (2.667 mm) was observed from both seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL) and T_3 (0.1 mg dry wt. eq. extract/mL). Lebbek may contain alkaloids which interferes with the growth of Barnyard grass. Varsha *et al*. (2013) also found similar type of results where alkaloids were responsible for the growth inhibition.

4.3 Effect of Devils cotton (*Abroma augusta*) extract on root length of Barnyard Grass

Root length growth of barnyard grass also have disturbed by the Devils cotton extract (Figure 1). The highest root length (13.3 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (10.833 mm). The lowest root length (1.567 mm) was observed with T_4 (0.03 mg dry wt. eq. extract/mL) treatment. At the highest concentration T_4, root length (1.567 mm) was significantly decreased to control treatment. A. Reza Einodehi (2006) have reported almost similar results that the radicle growth was affected at all test samples of *Camellia sinensis* used and at all extract concentrations.

4.4 Effect of Scarlet gourd (*Coccinea cordifolia*) extract on root length of Barnyard Grass

Scarlet gourd showed significant variation on root length of barnyard grass seeds at different concentration (Figure 1). According to result, the highest root length (15.267 mm) was recorded from control treatment T_0 (no extract). The lowest root length (3.433 mm) was observed from seed treatment with T_4 (0.03 mg dry wt. eq. extract/mL). At T_4 concentration, root length (3.433 mm) growth was greatly inhibited than control treatment. The scenario of growth inhibition was started at T_2 concentration significantly which partially supports the experiment of Islam and Kato-Noguchi (2013) conducted whereas the growth of barnyard grass had also suppressed by the medicinal plants due to presence of allelochemicals i.e., Phenolic compounds, Flavonoids etc.

4.5 Effect of Spreading hogweed (*Boerhavia diffusa* linn.) extract on root length of Barnyard Grass

Significant variation was recorded on root length of barnyard grass seeds affected by different levels of extract of Spreading hogweed (Figure 1). 16 mm root length was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (13.1 mm). The lowest root length (.933 mm) was observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL). The result of the experimented treatment in barnyard grass root length found gradual decreasing significantly with increase of the concentration of extract of Spreading hogweed extract. Muhammad Qasim *et al.* (2019) also experimented on medicinal plants and found almost similar result.

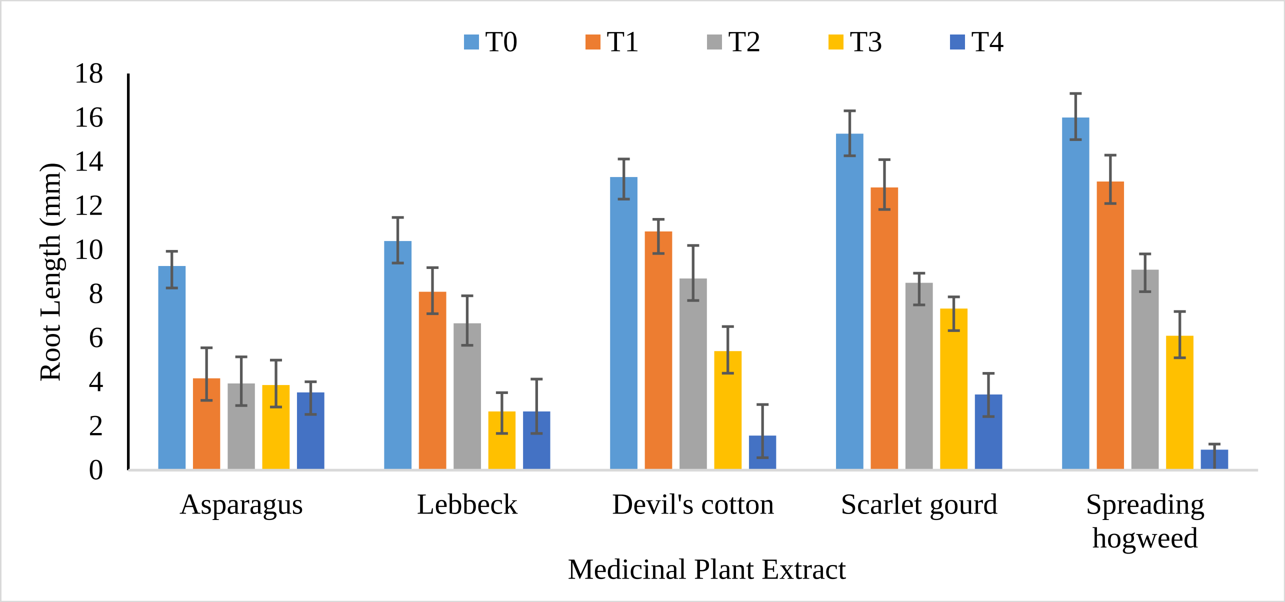

Figure 1. Root length (mm) of barnyard grass at 96 hours affected by different medicinal plants

T_0 = 0 (control without extract), T_1 = 0.01 mg dry wt. eq. extract/mL, T_2 = 0.03 mg dry wt. eq. extract/mL, T_3 = 0.1 mg dry wt. eq. extract/mL, T_4 = 0.3 mg dry wt. eq. extract/mL

4.6 Effect of Asparagus (*Asparagus racemosus wild.*) extract on shoot length of Barnyard Grass

Shoot length of Asparagus also significantly varied like root length on barnyard grass seeds affected by different levels of extract (Figure 2). The highest shoot length (9.1 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (7.433 mm) and T_2 (0.03 mg dry wt. eq. extract/mL) (5.3 mm). The lowest shoot length (3.16 mm) was observed from seed treated with T_4 (0.3 mg dry wt. eq. extract/mL). As a medicinal plant, Asparagus contains many secondary metabolites which may cause the result of shoot growth inhibition.

4.7 Effect of Lebbeck (*Albizia lebbeck*) extract on shoot length of Barnyard Grass

Variation was found on shoot length barnyard grass seeds affected by different levels of extract of Lebbeck (Figure 2). Results indicated that the highest root length (7.2 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (4.13 mm). The lowest root length (2.067 mm) was observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL) and the root length at the T_3 (0.1 mg dry wt. eq. extract/mL) was recorded 2.7 which was decreased gradually from T_0 (Control).

4.8 Effect of Devils cotton (*Abroma augusta*) extract on shoot length of Barnyard Grass

Results indicated that the 8.967 mm was recorded from control treatment T_0 (no extract) which was highest root length. T_1 (0.01 mg dry wt. eq. extract/mL) extract showed 5.7 mm. The lowest root length (1.867 mm) was observed from seed treatment T_4 (0.3 mg dry wt. eq. extract/mL) and the root length at the T_3 (0.1 mg dry wt. eq. extract/mL) was recorded 3.6 mm which was gradually and significantly decreased from T_0 (Control). Naz and Bano (2014) who reported that leaf extracts of *Ricinus communis* and *L. camara* inhibit the growth of maize seedlings which make resemblance with existing result that may be caused by the effect of volatile phenolic compounds or alkaloids.

4.9 Effect of Scarlet gourd (*Coccinea cordifolia*) extract on shoot length of Barnyard Grass

Significant variation was found on shoot length barnyard grass seeds affected by different levels of extract of Scarlet gourd (Figure 2). Control treatment T_0 (no extract) showed the highest root length (10.667 mm) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract and T_2 (0.03 mg dry wt. eq. extract/mL) extract (7.33 mm). The lowest root length (2.867 mm) was observed from seed treatment T_4 (0.3 mg dry wt. eq. extract/mL) which was significantly and gradually decreasing from T_0 (Control). Scarlet gourd is a very common and available medicinal plant which may have vast amount of secondary metabolites which are highly disturbing the shoot growth of barnyard grass. So, this plant have a chance to claim economic source of weed suppression by natural mean.

4.10 Effect of Spreading hogweed (*Boerhavia diffuse* linn.) extract on shoot length of Barnyard Grass

The variation was found on shoot length of barnyard grass seeds and the result was significant (Figure 2). Results indicated that the highest shoot length (7.9667 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (7.433 mm). The lowest shoot length 1.9 mm was observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL). At the result of highest concentration T_4 shoot length 1.9 mm was significantly decreased from T_0 (control) that may contain flavonoids can also be responsible as phytotoxins. Macdonald *et al*. (2010) reported that *Ocimum gratissimum* flavonoids are phytotoxic which supports the current result partially.

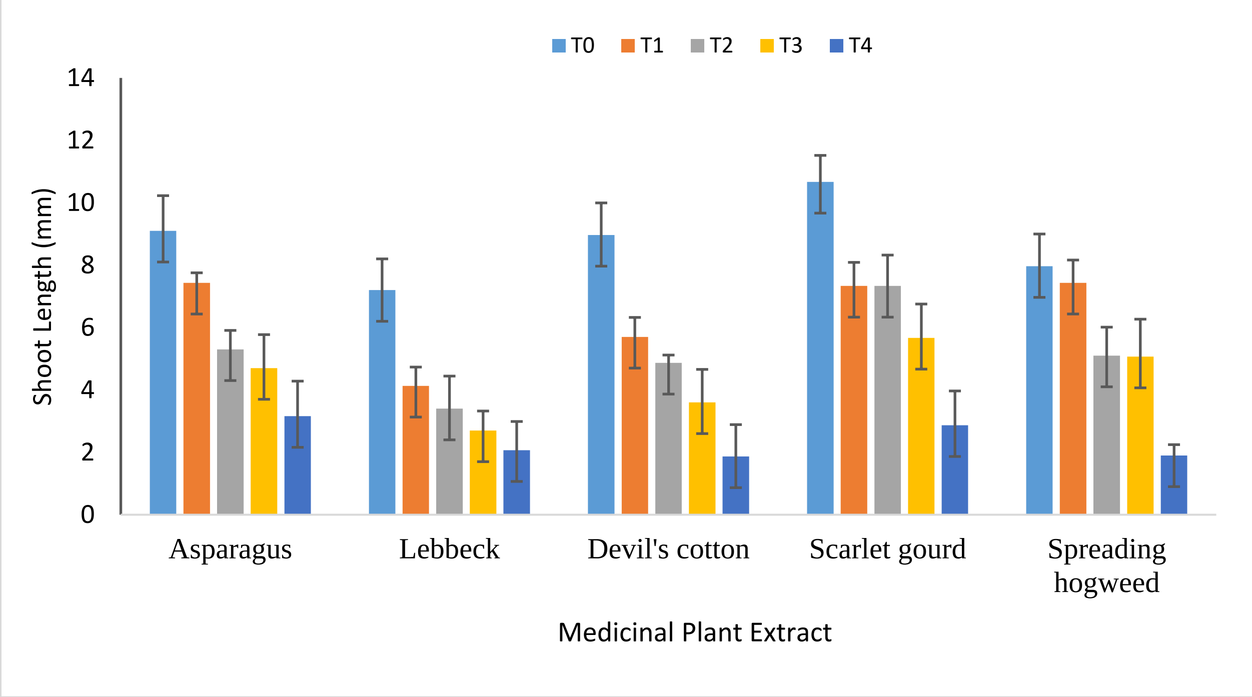

Figure 2. Shoot length (mm) of barnyard grass at 96 hours affected by different medicinal plants

T_0 = 0 (control without extract), T_1 = 0.01 mg dry wt. eq. extract/mL, T_2 = 0.03 mg dry wt. eq. extract/mL, T_3 = 0.1 mg dry wt. eq. extract/mL, T_4 = 0.3 mg dry wt. eq. extract/mL

4.11 **Effect of Asparagus (*Asparagus racemosus* wild.) extract on root length of Okra**

Asparagus (Asparagus racemosus wild.) made variation on root length Okra seeds in different levels of extract (Figure 3). Highest root length (10.533 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (7.667 mm). The lowest root length (2 mm) was observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL) and the root length at the T_3 (0.1 mg dry wt. eq. extract/mL) was recorded 3.767 mm which was also significantly decreasing from T_0 (Control). Jan *et al*. (2013) reported the presence of flavonoids in *Monotheca buxifolia*.

4.12 Effect of Lebbeck (*Albizia lebbeck*) extract on root length of Okra

Variation was found on root length of Okra seeds by different levels of extract concentration (Figure 3). Results showed that the highest root length (7.533 mm) was recorded from control treatment T_0 (no extract) followed by T_1 (0.01 mg dry wt. eq. extract/mL) extract (4.367 mm). The lowest root length (2.7 mm) was observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL) which was at decreasing rate from T_0 (Control). It was observed that root length was decreased with increasing of concentration.

4.13 Effect of Devils cotton (*Abroma augusta*) extract on root length of Okra

Significant variation was found on root length Okra seeds affected by different levels of extract of Devils cotton (*Abroma augusta*) (Figure 3). The highest root length 10.067 mm was recorded from control treatment T_0 (no extract) and the lowest root length was 2.167 mm observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL) which was significantly decreasing from T_0 (Control). The effect of treatment T_1 (0.01 mg dry wt. eq. extract/mL) extract showed the root length (5.767 mm) and followed by T_2 (0.03 mg dry wt. eq. extract/mL) extract (5.067mm). S. Boonmee *et al.* (2018) conducted research to evaluate of phytotoxic activity of leaf and stem extracts and identification of a phytotoxic substance from *Caesalpinia mimosoides* Lamk. Both results prove that the medicinal plants must contain allelochemicals which can suppress the dicotyledonous palnts.

4.14 Effect of Scarlet gourd (*Coccinea cordifolia*) extract on root length of Okra

The findings on Scarlet gourd showed significant variation on root length of Okra seeds at different levels of extract (Figure 3). Lowest root length (0.567 mm) was observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL) and highest root length (9.7 mm) was recorded from control treatment T_0 (no extract) which was significantly decreasing. The effect of treatment T_1 (0.01 mg dry wt. eq. extract/mL) extract showed the root length (7.167 mm) and followed by T_2 (0.03 mg dry wt. eq. extract/mL) extract (4.133mm). Scarlet gourd may contain alkaloids, sterols or many other phenolic compounds which interfere with the growth of dicotyledonous seeds. It would be a great finding to overcome dicotyledonous weed.

4.15 Effect of Spreading hogweed (*Boerhavia diffuse* linn.) extract on root length of Okra

Significant variation was found on root length Okra seeds by different treatment concentration of Spreading hogweed (Figure 3). 10.167 mm was recorded from control treatment T_0 (no extract) which was the highest root length and the lowest root length 0.533 mm was observed from seed treatment with T_4 (0.3 mg dry wt. eq. extract/mL) which was significantly and gradually decreasing from T_0 (Control). The effect of treatment T_1 (0.01 mg dry wt. eq. extract/mL) extract showed the root length (7.767 mm) and followed by T_2 (0.03 mg dry wt. eq. extract/mL) extract (5.93mm). Similar results were found by Algandaby and Salama (2016) where medicinal plants showed good phytotoxic or allelopathic effect. Spreading hogweed have many purposes as medicinal plant besides it can be found in agricultural lands. There is huge possibility to contain allelochemicallls in significant amount which may be useful in further research.

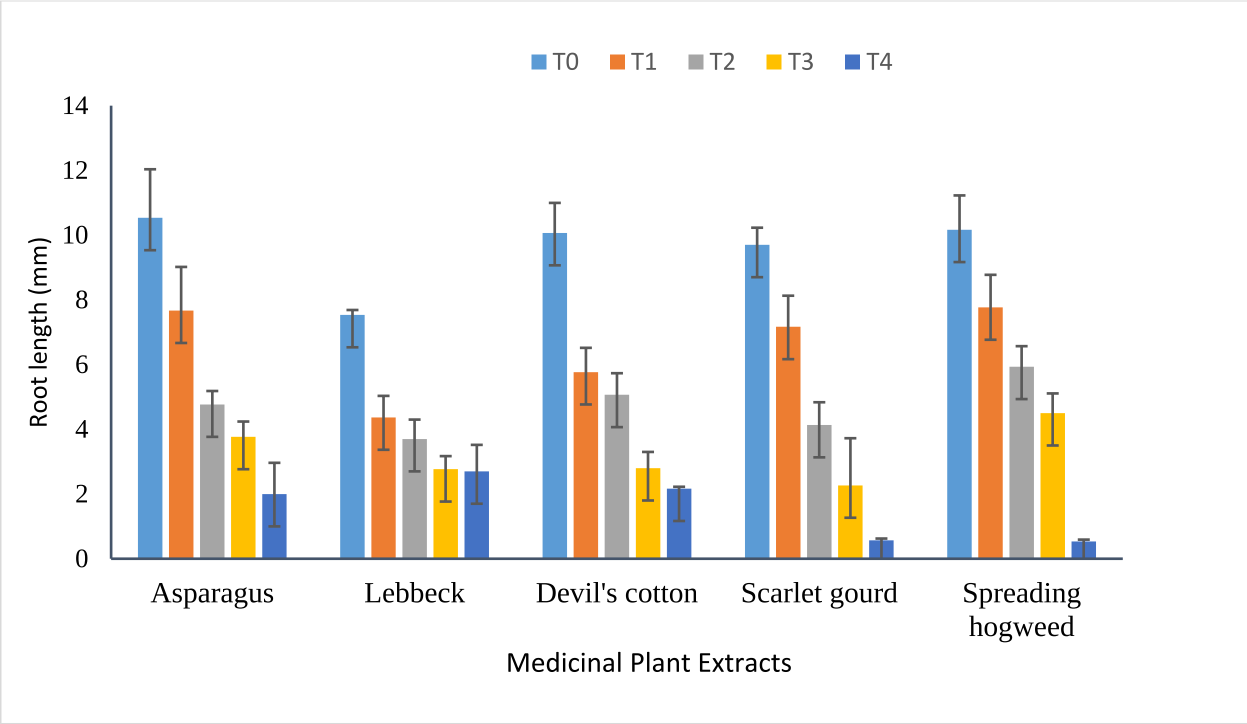

Figure 3. Root length (mm) of Okra at 96 hours affected by different medicinal plants

T_0 = 0 (control without extract), T_1 = 0.01 mg dry wt. eq. extract/mL, T_2 = 0.03 mg dry wt. eq. extract/mL, T_3 = 0.1 mg dry wt. eq. extract/mL, T_4 = 0.3 mg dry wt. eq. extract/mL.

CHAPTER V

SUMMARY AND CONCLUSION

In general observation, it was found that from the experiment, all plant extract showed higher allelopathic effect on growth of both monocotyledonous and dicotyledonous plant at higher concentration.

In terms of dicotyledonous plant, root of okra seeds showed susceptibility on spreading hogweed, scarlet gourd, and Devils cotton extracts in high extract concentration. scarlet gourd (*Coccinea cordifolia*) extract, T_4 (0.3 mg dry wt. eq. extract/mL) showed lowest root length (0.567 mm) whereas with Spreading hogweed (*Boerhavia diffuse* linn.) extract, lowest root length (0.533 mm) was found from T_4 (0.3 mg dry wt. eq. extract/mL) and 9.7mm was recorded at T_0 of scarlet gourd where and 10.167 mm root growth was recorded in control treatment T_0 of spreading hogweed. Asparagus, Lebbeck and Devil's cotton extract also have showed allelopathic activity on dicotyledonous species-Okra at high concentration but the inhibition rate are not significant like Spreading hogweed and Scarlet gourd.

In case of Barnyard grass seeds, Asparagus (*Asparagus racem*osus wild.) showed lowest shoot and root length (3.16 mm and 3.53mm, respectively) was found from T_4 (0.3 mg dry wt. eq. extract/mL) extract compared to highest shoot length 9.10 mm and root length 9.266 mm in control treatment T_0 (control; no extract), it was a huge declination. Barnyard grass with Devil's cotton extract, shoot length 8.967 mm and root length 13.3 mm recorded in control treatment T_0 and lowest shoot and root length (1.867 and 1.567 mm, respectively) was found from T_4 (0.3 mg dry wt. eq. extract/mL) extract. Considering shoot and root length of Barnyard grass seeds with Scarlet gourd (*Coccinea cordifolia*) extract, lowest root length 3.43 mm and shoot length 2.86 mm was found from extract T_4. The results showed significant result on Barnyard grass. Spreading hogweed extract also have showed significant result in root length and shoot length. All results indicate to have allelochamicals in all experimented medicinal plants.

So, it can be concluded that all those selected medicinal plants have phytotoxic properties but among them Spreading hogweed and Scarlet gourd and Devil's cotton showed much allelopathic effect for both okra seeds barnyard grass seeds. Further research should conduct for determination and isolation of allelochamicals of Spreading hogweed, Scarlet gourd and Devil's cotton plant species which could be used as natural herbicides.

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APPENDICES

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

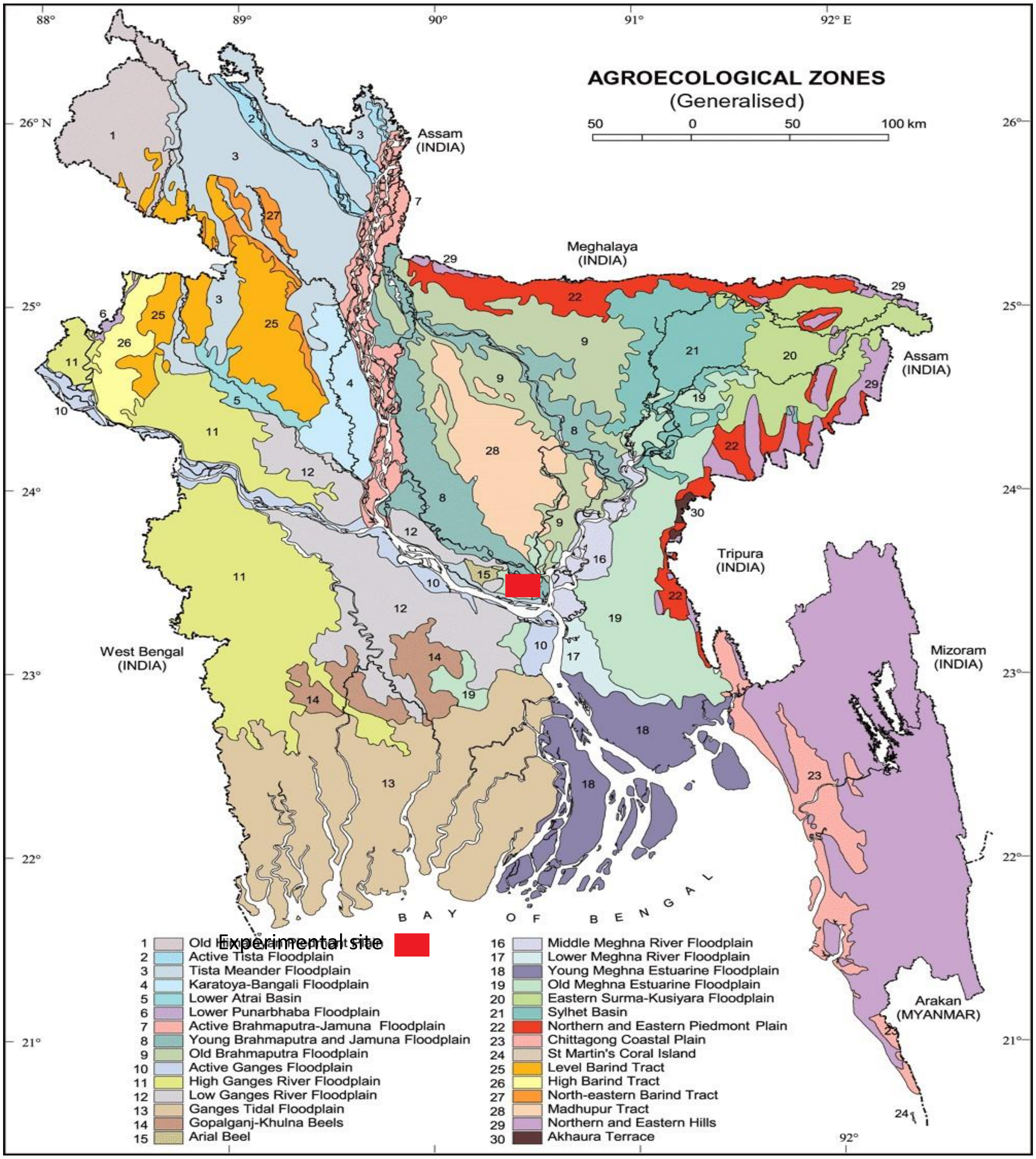
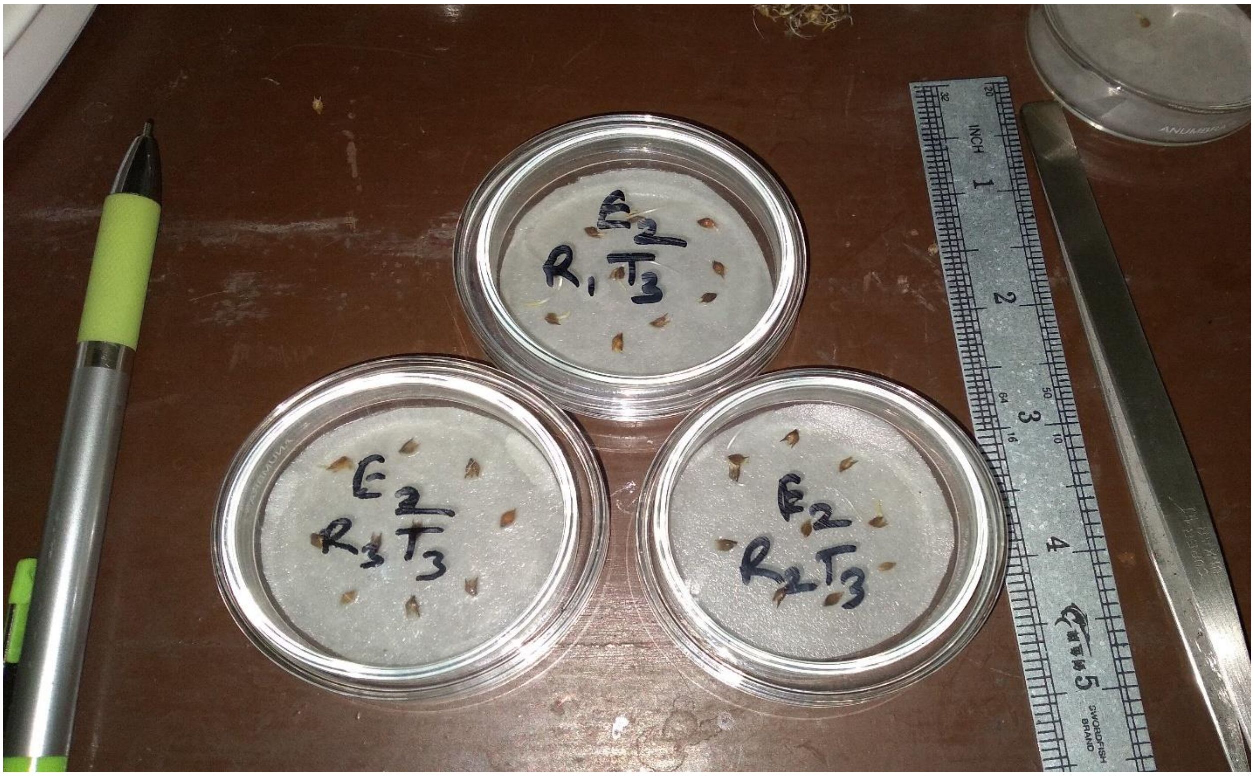


Fig. 04. Experimental site

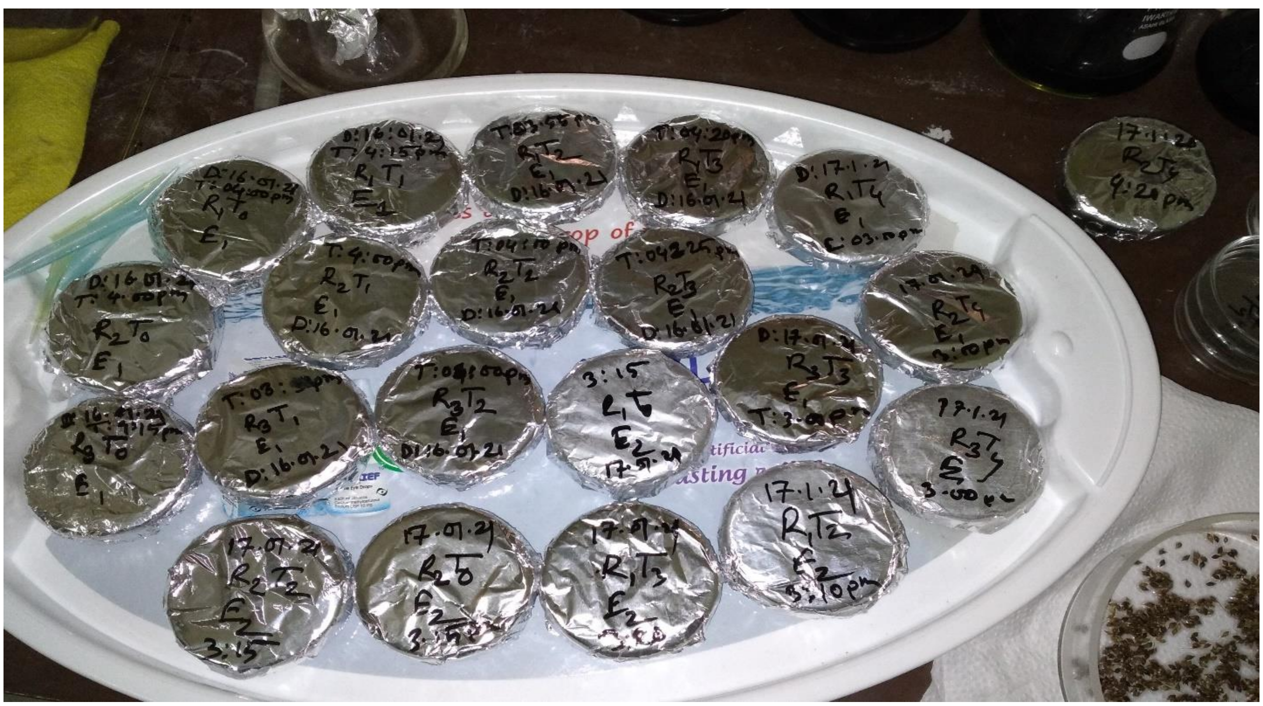
Apendex II: Some pictures of experimental work

Picture 1: Sample extraction using Rotary Evaporator

Picture 2: Sample extraction using Rotary Evaporator



Picture 3: Inhibited root and shoot growth on T_3 treatment of Lebbek.

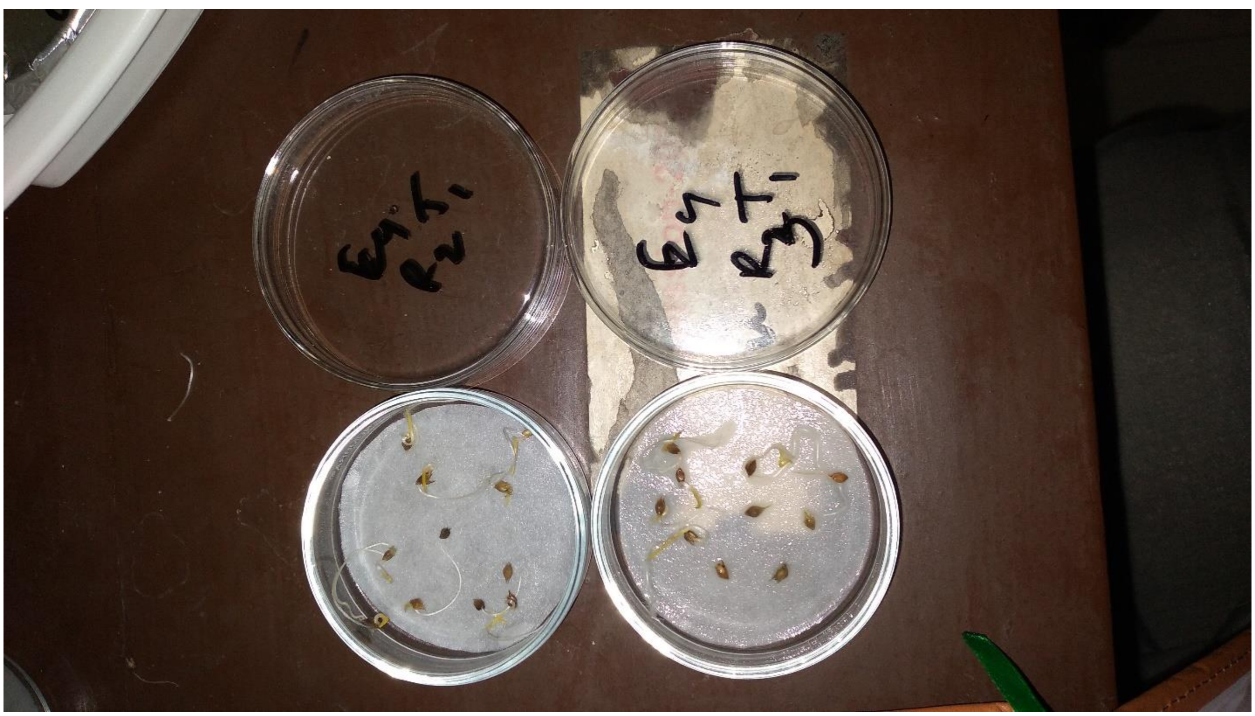


Picture 4: Replication of treatment of medicinal plant.

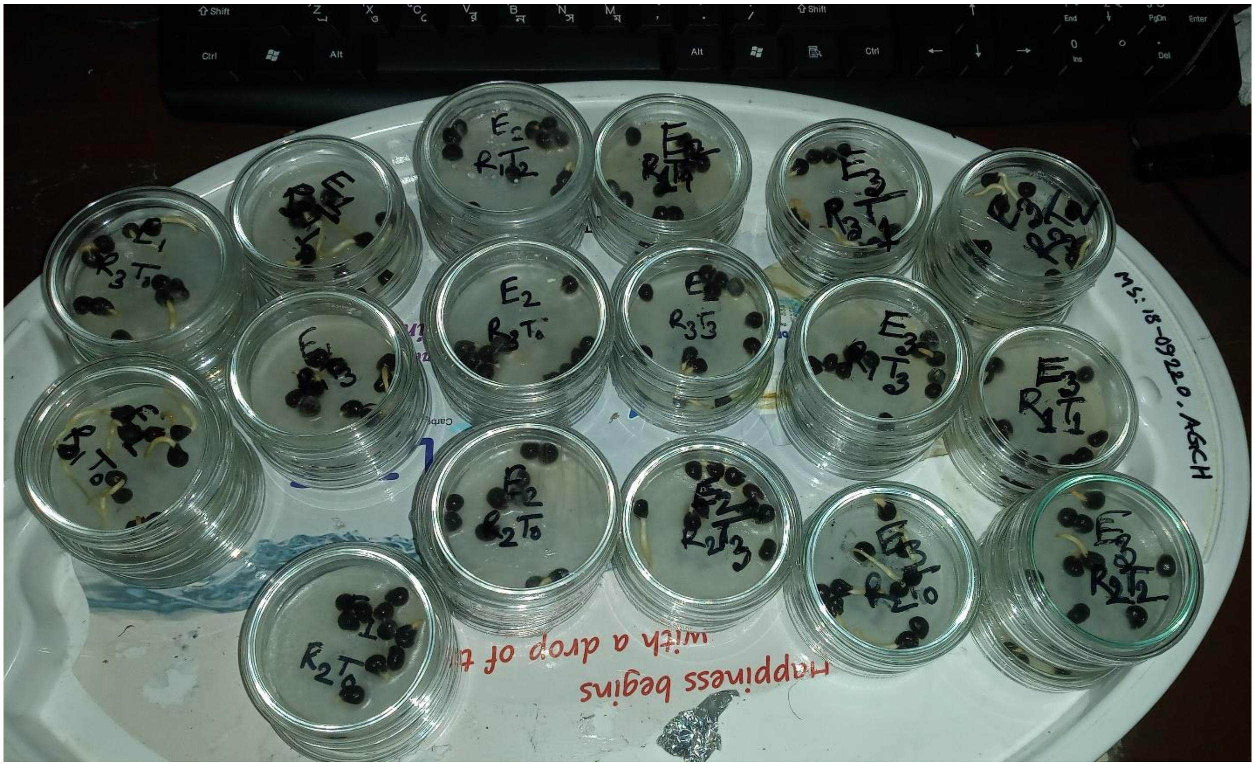
Picture 5: Effect of control treatment

Picture 6: Effect of T_4 treatment of Spreading hogweed extract

Picture 7: Effect of T_4 treatment of Scarlet gourd Sample on Barnyard grass.

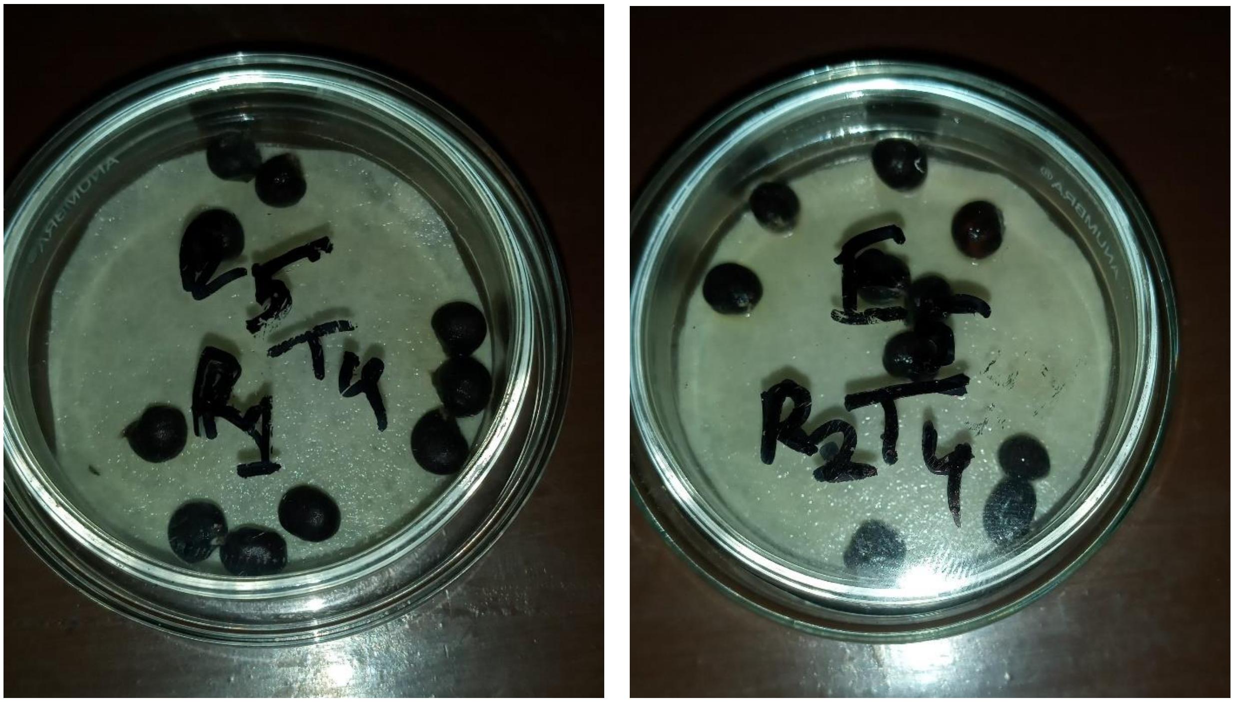


Picture 8: Effect of T_1 treatment of Scarlet gourd Sample on Barnyard grass.



Picture 9: Replication of treatments of medicinal plant extract on okra

Picture 10: Effect of T_4 treatment of Asparagus and Lebbeck



Picture 11: Effect of T_4 treatment of Spreading hogweed extract on okra seed