CHEMICAL INVESTIGATION ON SOME WEEDS OF RICE AND MUSTARD CROPS FOR JUSTIFICATION OF ALLELOPATHIC EFFECT

A Thesis

By

MD: ABU ZAFAR
Student no. 0405001
Session: 2004-2005
Semester: January-June, 2006

MASTER OF SCIENCE (M S)
IN
AGRICULTURAL CHEMISTRY

DEPARMENT OF
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Submitted to Department of Agricultural Chemistry and Biochemistry Hajee Mohammad Danesh Science and Technology University Dinajpur in Partial Fulfillment of the Requirements for the Degree of

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DEDICATED
TO MY
BELOVED MOTHER
AND
DEPARTED SOUL OF
MY FATHER

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ABSTRACT

The allelopathic effects of some weeds on germination and growth of rice and mustard seedlings with their chemical investigation were conducted in the laboratory.This study was performed on naturally occurring growth factors in aqueous extracts of some common weeds viz. Aryl (Leersia hexandra), Jaina (Fimbristylis miliacea), Sushnisak (Marsilea quadrifolia), Masurchana (Vicia hirsuta), Tita begun (Solanum nigrum) and Bankapi (Gnaphalium affine) with the attempt for chemical investigation on effective extracts. The boiled and unboiled extracts of all the weed species prepared from whole plant were used for treating seeds of rice and mustard along with water as control. Boiled and unboiled extracts of all the weed species significantly reduced and delayed germination of rice and mustard seeds compared with control. The effect of unboiled extract of Jaina showed the lowest germination (82.7 %) in rice seed. But the unboiled extract of Aryl mostly inhibited the root and shoot length of rice seedlings. The effect of unboiled extract of Tita begun showed the lowest germination (50.6 %) in mustard seed and the unboiled extract of Tita begun inhibited the root and shoot length of mustard seedlings. The root and shoot length of rice and mustard were reduced in presence of all the weed extract. The thin layer chromatography (TLC) examination of crude extract of Aryl (Leersia hexandra) showed four separate compounds at hexane: ethyl acetate (10:1, v/v) and the crude extract of Tita begun (Solanum nigrum) also detected four separate compounds at hexane: ethyl acetate (10:1, v/v).

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Chapter -1

INTRODUCTION

From the ancient of the world, the human being and animal life as a whole are dependent to a greater degree on the plant kingdom. The plant kingdom supplies us with food, fuel, fodder, shelter, wind breaker and provides raw material for clothing, medicine. They also provides acidic substance e.g. tartaric acid, oxalic acid, citric acid, celicilic acid, butyric acid and provides gum substance such as lignin, citin, pectin, latex and provides us dying substance e.g. khayer, tarmaric, jafran, mahedi, etc and provides us protienious substance e.g. mithonine, tryptophan, valine, isopropine, histidine, lysine which essential amino acid only geting from plant. Some plant supplies us mineral substance e.g. Ca, Mg, Fe, Co, Cl, K, Mn, Mo, Zn, S, N, P etc which is essential to build human & animal body. The vital part of the plant is carbohydrate (CHO) which contains galactose, maltose, starch, cellulose, sucrose, and fructose, supplies energy in human & animal body. Some plant use as a pesticide i.e. leaf of biskatali, neem, tobacco, datura, dust of chili, wood ash etc. We get lipid substance such as triacyl glycerol, phospholipid, fatty acid, wax etc. Some provides us oil substance, example mustard oil, soybean oil, sunflower oil, linseed oil, veranda oil and palm oil etc that contain energy, taste and protect the skin membrane, maintains the temperature of the body.

The people of developing country like ours cannot afford to use expensive chemotherapeutic drugs for economic reasons. But our country abounds with a vast majority of medicinal plants and herbs. The availability of medicinal plants demands the isolation, separation, purification and characterization of physiologically active principle which are actually useful for the treatment of diseases. The use of drugs obtained from the medicinal plants is not only geared up in our country alone but also over the most developed ones, In 1960 physicians prescribed the drugs in which 47% of drugs were from natural sources in the United States. Natural products are

found from plant which play important role for growth and developing in human body, among the natural products available in plant sources antibiotics, vitamins, alkaloid, hormones are important substances. Some plants and weeds are harmful to human, animals and neighbour crops due to their toxicity and inhibitory activity. Because they produce toxic compounds creating fatal diseases in human body, animals, and also suppress the neighbour plant and reduce the yield of crops. Some weeds and crops secrete toxic and inhibitory compounds which suppress the seed germination and seedling growth of neighbor weed or crops ultimately reduce the yield of the crops and control the weed growth, respectively.

Bangladesh is an under developing country. More than 80% people of this country are farmer and commonly their source of income is the production of agricultural crops. Among them, Rice and Wheat are the main cereal crops of Bangladesh. But globally, Rice is the second most important crop to wheat in terms of area. But in Bangladesh, rice is the first most important cereal crops. As food rice is important since it prove more calorie than any other cereals. About 40% of the world's populations consume rice as a major source of calorie (Banick, 1999). Among the major rice growing countries of the world, Bangladesh ranks third in respect of growing area and fourth in production (Huke and Huke, 1990). Here rice is cultivated in 10.26 million hectares having the average yield 1.79 ton/ha with the production of 19.9 million metric ton (BBS, 1999).

Mustard is the important oil seed crop of Bangladesh. Mustard cultivated in Rabi season of Bangladesh. Rice and Mustard are the major kharip and Rabi crops respectively being grown in association in Bangladesh. Literature study observed that in Kharip season of rice, these are invaded variety of weed species which reduce their productivity either by producing

biophysical disturbances to limited resources i.e., competition or by biochemical interactions with the crop plants i.e. allelopathy. On average yield losses due to weeds in rice and mustard have been estimated to be 10% and 30% respectively (BBS, 1999).

List of some important weed and plant species which secrete allelo-chemical:

Plant/weed species	Name of allelo-chemical
Parthenium hysterophorus	sesquiterpene lactones,
Lantana camara	parthenin,
Blumea lacera	parahydroxybenzoic acid,
Ageratum conyzoides	*p*- pcoumarica acid,
Calotropis gigantea	syringic acid,
Datura stramonium	ferulic acid,
Cyperus rotundus	Phenolic acids
Echinocloa colonu	such as caffeic acid,
Sorghum halepense	vanillic acid,
Casuarina equisetifolia	anisicacid,
Eucalyptus tereticornis	cholorogenic acid,
Leucaena leuceophala	*p*- anisic acid,
Oryza sativa	*p*-hydroxybenzoic acid etc.

Allelopathy is not a recent discovery. In 300 BC, Theophrastus realized that planting chickpeas made the soil unsuitable for many other plants. Earlier records of allelopathy date back before 300 BC when Democritus reported that weed could be controlled by using naturally occurring plant products and trees could be killed by treating their roots with a mixture of lupine flowers soaked in hemlock juice.

The world allelopathy is derived from the root words allelon "of each other" and pathos "to suffer". Allelopathy is a type of interference competition. Competition between two species occurs when a resource such as nutrients, water is in limited supply. Interference competition occurs when species directly affects the other competition that occurs among plants. Allelopathy involves a chemical inhibition of one species by another. Molecules produced into the environment and then development of neighboring plants. So, Allelopathy refers to any direct or indirect inhibitory effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment (Rice, 1974).

Allelopathic chemicals may be present in any part of a plant including the leaves, flowers, fruits, stems, roots, rhizomes and seeds. These toxins can affect a target species in a number of ways. For example, they may inhibit the target species nutrient uptake, or they may inhibit shoot or root growth. Some plant such as clover, have a symbiotic or mutually beneficial relationship with nitrogen fixing bacteria that provides nitrogen for the plants growth and allelopathic agent against clover might directly attack the symbiotic bacteria and destroy the plants source of usable nitrogen. Allelopathy is a phenomenon of chemical interaction and is probably of wide spread

significance in the functioning of natural communities. In plant-plant interactions, Allelopathy generally used to denoted the process by which plants release phytotoxic compounds (allelochemicals) to the environment, resulting in harmful beneficial effect on neighbouring plants. Utilization of the Allelopathic potential of plant for weed control is given great emphasis, because it would reduce the risk of environmental toxicity.

Since the major requisite of the allelopathy is an organic substance or allelochemical be. transferred from a donor plant to a recipient. Therefore, mode of transfer plays a great role in its toxicity and persistence. The donor plants generally store these allelochemical in the plant cells in a bound form, such as water-soluble glycosides, polymers including tannins, lignins and salts. Hence these chemicals are not toxic to the donor plants. Once the chemical from the donor plants are released into the environment, they may be either degraded or transformed into other forms, which affects the receiver plants and may also be toxic to host plants. Upon cleavage by plant enzyme or environmental stress, these toxic chemicals are release into environment from special glands on the stem or leaves.

First the terpinoids such as cineole and camphor are release to environment through volatilization. Then the water –borne phenolics and alkaloids are moved out by rainfall through leaching. Next, phytotoxic glycons such as phenolics are released during the decomposition of plant residues in soil. Finally many plants uptake nutrients with those release chemicals from the soil.

Because of simultaneous effect of two processes of interference it becomes difficult to isolate the effects of weeds on crops plants. The toxicity

of weed on crops through the phytotoxic exudates was first reported by De Candolle (1832). Molisch (1937) coined and detrimental biochemical interactions between microorgansms and plants, where as Rice (1974) defined it as the direct or indirect harmful effects one plant on another through the production of chemical substances that escape into the environment. Putnum and Duke (1978), Hussain (1983) and Rice (1984) emphasized the role of allelopathy in agrosystems.

Hussain (1980) demonstrated the allelopathic effects of *Euphorbia granulata.* Also . *Lolium multiflorum* exhibits allelopathy against the agricultural crops (Naqvi and Muller, 1975). Menges (1988) reported that phytol, vanillic acid, 3-methoxy 4-hydroxy nitrobenzene and 2, 6-dimethoxy benzoquinone were isolated from palmer amaranth which inhibited germination and growth of onion and wheat.

Allelopathy is defined as the direct influence from a chemical released from one plant on the development and another. Allelochemicals are secreted to the rhizosphere and suppress the growth of neighboring plant [Bais et al. 2004].

Sanchez et al (1973) indentified some of the allelopathy to toxins in yellow nutsedge (C*yperus esculatus*) as the hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid and p-coumaric acid which inhibit the germination and radicle elongation of crop plants. Bhowmick and Doll (1979 and 1983) reported that both water extract as well as residues of *Echinochloa crusgalli* inhibited the growth of radicle and coleoptile of maize soybean in pots and glass house and resulted in reduced yields both the crops under field condition.

Both living buckwheat and living buckwheat residue have an allelopathic effect on weed germination. Recently it was reported that aqueous extract of aerial part of buckwheat inhibited germination or seedling growth of lettuce and other weeds (Isojima et al. 2000). Iqbal et al (2002 and 2003) was reported that Buckwheat shoots produce and release a variety of bioactive chemicals, which is toxic to weed. There are reported that allelochemicals have advantageous effects in crop production. From above reports many scientist are reported that various weed extract have allelopathic effect to crop production. In view of this, above fact and attempt has been made to conduct this research work with the following **objectives**:

1. To find out the problem by a comprehensive study and survey of weed in rice and mustard field crops.
2. To measure the allelopathic effect of boiled and unboiled aqueous extract of different weeds on seed germination and growth of rice and mustard.
3. To isolate the enantiomeric compound from effective aqueous extracts.
4. To suggest the farmers our achievement.

Chapter -2

REVIEW OF LITERATURE

Sufficient information regarding the factors of weed with allelochemical is meager in the world literature. In this chapter, an approach has been made to review some available information related to the study. A short review of some pertinent available information is given below:

Bhatia et al. (1982) studied the allelopathic effects of some important weed on wheat that the fresh plant mass of *Avena ludoviciana* and *Phalaris minor* promoted the wheat seedling growth while dry straw glumes inhibited it. The other weeds which inhibited the growth of wheat seedling were *Chenopodium album, Lepidium sativa*, stem and root of *Silene conidea, Sonchus arvensis ,Amaranthus viridis* and *Celosia argentea*. Weeds which promoted the growth were *Melilotus indica*, leaves of *Silene conoidea* and *Rumex dentatus*. *Circium arvense* promoted the length of seedlings but reduced their dry weight.

An allelopathic effect of pakistani weed (*cynodon dactylon*) that is allelopathic potential was tested against wheat, barley and maize varieties sarhad white and sarhad yellow. The shoot and root litter or their aqueous extracts and soil collected from beneath *Cynodon dactylon* patches significantly reduced either the germination, early growth, biomass, moisture or chlorophyll contents of the susceptible species in various laboratory experiments and Paper chromatography indicated the presence of ferulic, p-coumaric, vanillic, p-hydroxybenzoic and syringic acids in the shoots and roots while gas chromatography revealed the last three and benzoic and caffeic acids reported Hussain et al (1988) .

Angiras et al. (1987) carried out an experiment to study the allelopathic effects of six weed and germination and growth of maize and soybean seedling in laboratory. They observed that the boiled and unboiled extracts of

Lantana, *Eupatorium*, *Cyperus*, *Ageratum*, Janson-Grass and *Echinochola* prepared from whole plant were used along with water as control. The boiled extract of weed did not affect the germination of maize and soybean. Whereas, the unboiled extract of *Echinochloa* reduced the germination of both crops. The boiled and unboiled extract of *Eupatorium* and *Agratum* and unboiled extract of *Echinochloa* delayed the germination of both the crops by two days. The leaf development of maize and soybean was significantly inhibited by boiled extract of *Eupatorium* and unboiled extract of *Echinochloa*. Boiled extract of jonson grass promoted the growth of radical and coleoptiles of both the crops. Where as boiled and unboiled extract of *Eupatorium* and *Ageratum* as well as unboiled extract of *Echinochloa* inhibited the growth of radical and coleoptiles.

Sanchez et al. (1973) indentified some of the allelopathic to toxin in yellow nutsedge (*Cyperus esculentus*) as the phydroxy-benzoic acid, vanillic acid, syringic acid, ferulic acid and P-coumaric acid which inhibit the germination and radical elongation of crop plants.

Abdul Wahab and Rice (1976) reported that some of the growth inhibitory chemicals identified in johnsgrass (*Sorghum helepense*) are chlorogenic acid, *p*-cumaric acid and *p*-hydroxybenzaldehyde which are capable of effecting the growth and development of other associate plants.

Bhowmick and Doll (1979 and 1983) reported that both water extracts as well as residues of *Echinochloa crusgalli* inhibited the growth of radical and coleoptiles of maize and soybean in pot and glass house and resulted in reduced yield both the crops under field conditions.

Tripathi et al. (1981) were reported that the strongest inhibitory effect of aqueous extracts of *Eupatorium adenophorum* on wheat seed germination, radical and plumule growth.

Lockerman and Putnam (1979 and 1981) reported that the most active cucumber accessions in field trials and found that interference caused by allelopathy and competition reduced weed densities.

Massentini et al. (1977) observed that the effects of 141 soybean (*Glycine max*) line on *Helminthis echioides* and *Alopecurus myosuroides* and found that two lines inhibited the growth of *Helminthis echioides* but none affected *Alopecurus myosuroides* growth. They also found that one soybean line promoted the growth of both weeds.

Leather (1983) found that several varieties of sunflower (*Helianthus annuus* L) were more allelopathic to broad leaf weeds than the native wide sunflower.

Smith and Martin (1994) studied the allelopathic characteristics of three cool season grass species in the forage Ecosystem that the purpose of this research was to determine the potential allelopathic influence of selected cool season grass species on species interseeded into the pasture ecosystem. Aqueous extracts of tall fescue (*Festuca arundinacea* schreb.), Italian ryegrass (*Lolium multiflorum* lam.), and little barley (*Critesion pusillum*) leaf and stem tissue harvested at the mature stage of plant development reduced seed germination and seedling growth of alfalfa (*Medicago sativa*) and Italian ryegrass. It was estimated that 2.8 and 2.5 g/L aqueous extracts mature tall fescue stem tissue resulted in a 50% reduction in alfalfa seed germination and seedling growth respectively. For the extracts from Italian ryegrass and little

barley, it was estimated that tissue concentration of near 5.0 g/L resulted in a 50% reduction in seed germination and seedling growth for the two bioassay species, leaf and stem tissue concentrations of the three grass > 7.0 g/L resulted in complete inhibition of alfalfa seedling growth.

Alsaadawi et al. (1985) conducted a screening experiment to examine the activity of sorghum root exudates of 100 cultivars to inhibit germination and seedling development of pigweed (*Amaranthus retroflexus*) in a sand culture medium. A high variability was observed in the ability of the test cultivars to alter seed germination and /or seedling growth of the weed. They found in 82% of the control reduction in seed germination in 25 cultivars. They also found 10 cultivars inhibiting *Amaranthus retroflexus* growth by more than 79% of control.

Chou (1990) found that rice (*Oryza sativa*) planted twice a year in a monoculture system reduced the second crop yield by about 25% in areas of poor water drainage. During the fallow period, rice stubble is left in the field after harvesting and incorporated into the soil for decomposition. During decomposition, great quantities of phytotoxins were produced resulting in the suppression of rice growth and subsequent grain yield.

Putnum et al. (1990) reported that have drilled rye into field plots during early October, over wintered and killed with glyphosate (N-phosphonomethyl glycine) when it attained about 5 metric tons / ha and evaluated its weed control capacity in vegetable and fruit cropping systems over a 10 year period. Their experiments indicated that living rye has strong interference ability against weeds, providing excellent weed control prior to annual crop establishment or provides soil cover during dormant period in perennial crops.

Barnes and Putnum (1983 and 1986) were reported that the glucosyl conjugate of 2, 4-dihydroxy-1, 4(2H) benzoxazin 3-one (GDIBOA) forms the aglycone (DIBOA) upon injury or death of the rye plant. DIBOA degrades to another active compound, 2(3H) benzoxazalinone (BOA). Both compounds are known to have strong inhibitory action on germination dicotyledons weed seedlings.

Barnes et al. (1986) reported that weed biomass in a cover crop of living rye was reduced 90% over unplanted control and even a mulch of 40 days old spring planted rye gave 69% reduction. Soybean and sunflowers planted without tillage into desiccated rye mulch gave over 90% reductions in the biomass of common lambsquarter (*Chenopodium album* L.), red root pigweed (*Amarenthus retroflexus* L.) and common ragweed (*Ambrosia artemisiifolia* L.) compared to tillage and no rye.

Alsaadawi et al. (1986) found that sorghum plants from seeds exposed to gamma rays have more inhibitory activity in their root exudates, aqueous extracts, and decaying residues against growth of *Amaranthus retroflexus*. Regarding genome enhancement, the possibility for exploiting allelopathy includes genetic manipulation of crops to increase their capability for weed control.

Menges (1988) conducted a study of allelopathic effect of palmer amaranth (*Ameranthus plmeri*) on seedling growth that palmer amaranth residue was incorporated into soil to determine its allelopathic effects on the seedling growth of grain sorghum (*Sorghum bicolor*) cabbage (*Brassica oleracea*) carrot (*Daucus carota*) and onion (*Allium cepa*) . Root and shoot growths were equally sensitive to the toxic effects of soil incorporated palmer

amaranth. Growth of "Grand Slam" cultivar of cabbage was 17 to 30% more Sensitive then the growth of "Sansibel" cabbage. Growth onion and carrot seedling was less inhibited then either cabbage or grain sorghum. Growth of grain sorghum root was severely inhibited 8000 and 16000 ppm of palmer amaranth in soil and was not affected by over drying other then lyophilization. Seedling growth was more severely inhibited by thyrsus and leaf tissues of palmer amaranth.

Patternson (1981) reported that the effect of extracted secondary metabolites from Johnson grass (*Sorghum helepense*) on germination, growth and development of various crops. Phytol, vanillin, 3-methoxy 4-hydroxy nitrobenzene and 2,6-dimethoxy benzoquinone were isolated from palmer amaranth which inhibited germination and growth of onion and wheat reported by Menges (1988).

Rahman et al. (1996) conducted an experiment occuring growth factors in aqueous extracts of some common weeds viz. Katanotey (*Amaranthus spinosus* L), Durba *Cynodon dactylon*), Lazzabati (*Mimosa pudica*), Mutha (*Cyperus rotundus)*, Shama (*Echinochloa crusgallai*), Baradudhia (*Euphorbia hirta*) and Haldemutha (*Cyperus esculentus*). Boiled and Unboiled extracts of katanotey, durba and lazzaboti reduced the germination early growth of rice while all the 7 weed species inhibited germination and growth of jute. The extracts of all weed delayed the germination of rice and jute seeds.

Rimando et al. (2001) conducted an experiment to study the several compounds from taichung native 1, an allelopathic rice have been identified by the bioassay guided isolation method. They were azelaic acid, *p*-cumaric acid, 1H indole carboxaldehyde, 1H indole 3-carboxylic acid, 1H indole 5-

carboxylic acid, and 1,2–benzenedicarboxylic acid bis (2-ethylhexyl) ester. Among the allelophatic substances identified, *p*-cumaric acid, known allelochemical inhibited the germination of lettuce (*Lactuca sativa* L.) seedling at 1 mM, but was active against barnyardgrass only at concentrations higher than 3 mM.

Isojima et al. (2000) were reported that aqueous extract of aerial part of buckwheat inhibited germination or seedling growth of lettuce and other weeds.

Kayode (2004) observed that the effect of 24 and 28-h *Colotropis procera* leaf extracts on the radicale and plumule growth of maize cultivars observation super I, II, III and IV were studied. Both extracts showed considerable inhibitory effect on radicale and plumule growth of the cultivars. The severity of inhibition increased with an increase in the duration of the extraction. The growth of observation super I was the least inhibited while the growth of observation super III was the most inhibited by the extracts when the growth and development of the redicale and plumule were compared to those of control .

Bansal and Singh (1986) conducted an experiment in root, shoot, leaf and flower extracts of *Phalaris minor*, *Lolium temulentum* and *Avena fatua* mixed with soil were studied for their effects on rice seedling growth. All *Phalaris minor* plant parts decreased rice root DW compared with the control (pure soil); leaf extracts stimulated shoot length and shoot DW/pot. Root extracts of *Lolium temulentum* were inhibitorier than shoots and leaves or inflorescences whereas in *Avena fatua*, shoot extracts were the inhibitoriest. Leaf extracts of *Avena fatua* has a similar stimulatory effect on rice shoot length and DW/pot as leaves of *Phalaris minor*.

✳ Sarma and Nathawat (1987) reported that seed of 4 crops were germinated in the presence of 50, 100 and 200 mg *Argemone maxicana* plant power /100 ml water. Increasing the amount of the power decreased percentage germination in *Raphnus sativus* and *Penisetum typhoides* but effects on germination of wheat and *Brasica campestris* var. sarson were inconsistent; Shoot length of the 4 species was decreased. Root length in wheat and *Brassica napus* var. *glauca* decreased with increasing amount of the power .Root length in *Penisetum americanum* decreased with up to 100 mg powder. Root length in *Raphanus sativus* increased with 50gm, was not affected with 100 mg and was decreased with 200mg.

Meissner, et al. (1989) reported that the growth pattern of young carrot, cucumber, lettuce, maize, squash, onion, radish, sunflower and tomato plants was affected when grown in *Cynodon dectylon* infested soil. Shoot growth on all the species was reduced. Reduction of plant height occurred in all species, except for sunflower, which was spindly in appearance.

Patil (2002) observed that the effects of *Casuarina equisetifolia* leaf leachate (1.0, 22.5 and 5%) on the germination and growth of groundnut, soybean and green gram were kept at room temperature for 11 days. Observation on germination percentage were recorded on the 6^{th} and 11^{th} day, whereas observations on shoot length, root length, shoot dry weight and root dry weight were recorded on the 11^{th} day. Seed germination was not significantly affected by 1.0 % leachate, but was reduced by higher leachate concentrations. Soybean treated with 5.0% leachate exhibited the highest reduction in seed germination (62.5%) and shoot growth (99.4%). The leachate at 1% stimulated root growth in green gram (19.64%) and groundnut

(6.14%) but inhibited root growth in soybean (18.08%). Further increase in leachate concentration adversely affected root growth in all crops.

Lazauska and Balinevichiute (1972) tested excretions from seeds of hairy vetch against seed germination and seedling growth of thirteen species of weeds. They found that seed germination and particularly seedling growth were markedly inhibited in many tests. Reported that germinating seeds of millet, wheat, oat, vetch, maize and buckwheat stimulated germination of crunch weed (*Brassica kaber* L) seeds, whereas germinating barley seeds inhibited germination and crunch weed seeds. She found that the compounds which stimulated or inhibited the germination and crunch weed seeds were produced during germination.

Overland (1966) and Peters (1968) reported that several other crop plants are allelopathic to various species of weeds. Sorghum (*Sorghum bicolor* L.) residues reduced the populations of common purslane (*Portulaca oleracea* L.) by 70% and smooth crabgrass (*Digitaria ischaemum* L.) by 98%. Residues of barley, oats, wheat, rye and sorghum were very effective in reducing weed populations in several vegetable crops.

Putnam and Defrank (1983) found that the addition and sunflower residues to the soil reduced the seed germination of *Amaranthus retroflexus* and percentage of inhibition increased with increased concentration of total phenolics in that sunflower residue.

Einhellig and Souza (1992) found that root exudates of sorghum reduced the growth of all weed seedlings tested: *Abutlon theophrasti, Datura stramonium, Amaranthus retroflexus, Setaria viridis, Digitaria sanguinalic and Echinochloa crusgalli*. They further stated that root exudates of *sorghum*

bicolor consist primarily of a dihydroquinone that is quickly oxidized to a p-benzoquinone named sorgoleone.

Schreiner et al. (1907) tested water extracts of the soil from wheat, oat, corn and cowpea fields on the growth of wheat. They found that these crops exude materials into the growing medium inhibiting the growth of crop plants.

Mc Calla and Duley (1984) reported that stubble mulch reduced the stand and growth of corn under some conditions. They found that soaking corn seeds in an aqueous extract of sweet clover for 24 hours reduced germination and growth of tops and roots. Alfalfa extracts had less depressing effects and wheat straw extracts either stimulated growth and had no effect.

Guenzi and Mc Calla (1962) showed that the water extracts and a number of crop residues inhibited the germination and growth of sorghum, corn and wheat in a laboratory experiment. They further reported that all residues contained water-soluble substances that depressed the growth.

Martin and Rademacher (1960) incorporated fresh rape (*Brassica napus* L.) root in soil and planted wheat seeds in this soil and similar soil without the roots. Growth Of the wheat seedling was inhibited at first, but after 4 days, it was stimulated.

Oleswzek and Zurzysta (1987) reported that wheat seed germination and seedling growth were suppressed by water and alcohol extracts of alfalfa roots. Medicagenic and glycosides were found to be the inhibitors.

Cheema et al. (1988) reported that wheat straw aqueous extract caused 15- 20% inhibition of germination of cotton, but stimulated shoot & roots growth and dry matter production. Extract of decomposing field residues of barley, rye, broad bean (*vicia faba*), wheat, vetch and sudan grass (*sorghum sudanense*) were found to be toxic to lettuce seedlings.

Chou and Lin (1976) studied the effects of decomposing rice residues in soil on the growth of rice plants. They found that aqueous extract of decomposing rice residues in soil inhibited radical growth of rice seedlings and growth of rice plants. Maximum toxicity occurred in the first month of decomposition and declined thereafter. Some toxicity persisted for four months. Five inhibitory phenolic acids were identified from decaying rice residues.

Irons and Burnside (1982) reported that when a 2% (w/w) mature sunflower leaves mixed with the soil reduced emergence, growth of soybean, sorghum and sunflower. The root exudates of sunflower inhibited sunflower emergence, height, fresh weight and dry weight. Aqueous extracts from fresh and dried roots and roots exudates of the Chinese cabbage inhibited growth of mustard.

Walker et al. (1989) conducted that decaying sweet potato plant residues incorporated in to the soil caused significantly inhibition of growth of sweet potato vine cutting and cowpea plants.

Yang and Futsuhara (1991) found that when soybean callus and cultured bottle, the allelopathic effect was so intense that the growth rates of the soybean, calli were reduced by more than 100-fold under many

experimental conditions. Further studies showed that the inhibitory effect was form volatile compounds which were produced by rice callus.

Lovett and Jessop (1982) studied a range of twelve crop plants (four cereals, fives legumes, and three oil seeds) and found that all produced chemicals, which significantly reduced the early growth of wheat under controlled and field conditions.

Hakim et al. (1991) found that maize dry matter accumulation reduced and tissue N concentration was increased in intercrop culture with wing bean (*Phosphocarpus tetragonolobus* L.). These results indicated that N stress did not caused the decreased growth of maize when grown with wing bean on maize be occurred.

Rice (1974) reported that secondary compounds are either byproducts of metabolism or waste products stored in the vacuoles to prevent deleterious effects on the producing plant. Compounds implicated as likely phytotoxic agents are phenolic acids, terpenoids, flavonoids, alkaloids, cynogenic glycosides, quinines and amino acid derivatives.

Le Tourneau et al. (1956) found that water extracts from 23 common weed and crop species inhibited germination and growth of wheat seedlings. Water extracts of corn and sorghum residues inhibited germination and growth of wheat.

Kossanel et al. (1977) found that root exudates of common lambsquarters in culture solutions retorted growth of radicals of corn. Water solutions in which common lambsquarters previously grew and water extracts of its roots also inhibited growth of corn roots.

Bhatia et al. (1982) studied the effects of common lambsquarters on wheat and found stimulating effect on the growth of wheat.

Datta and Ghosh (1982) studied the effects of nettle leaf goose foot (*Chenopodium murale* L.) on mustard (*Brassica juncea*) and found phytotoxicity effect of the growth of mustard. An oily residue having carboxyl and hydroxyl functions and a solid residue containing oxalic acid were probably involved in the phytotoxicity of this weed. Root exudates of *Phalaris minor* and *Chenopodium murale* decreased shoot and ear length and dry matter production of wheat.

Pope et al. (1984) reported that root exudates of *Portulaca olerecea* significantly reduced soybean height.

Dharmaraj et at. (1988) reported that root and shoot leachetes of *Amaranthus viridis* and *Portulaca oleracea* caused greatest reduction in sorghum seed germination.

Saleem and Fawusi (1983) found in greenhouse and laboratory experiments that aqueous extracts of the purple nutsedge completely inhibited root development and reduced growth of aerial part of rice crop.

Elmore (1985) reported that *Cyperus rotundus* allelopathically caused the crop losses of cotton, maize, soybean, sorghum, groundnut and tobacco.

Lopes et al. (1987) studied that the effects of aqueous extracts of shoots and roots of Cyprus on germination and seedling growth of rice. About 95% germination of rice seeds was obtained in all cases.

Alam et al. (1990) demonstrated that aqueous extract of fresh leaves at 0.05, 1.0, 1.5, and 2.0% (w/w) of purple nutsedge significantly reduced the present germination, shoot and root lengths of wheat crops. At the highest level of 2% extract, the shoot growth was reduced by 33% and root by 40%. The reductions in shoot and root growth may possibly be due to release of water-soluble compounds affecting the growth. Interference by yellow nutsedge (*Cyprus esqulentus* L.) markedly reduced crop yield.

Drost and Doll (1980) conducted that the foliage residues of yellow nutsedge were very inhibitory to root and shoot growth of corn and soybean.

Bansal and Singh (1986) reported that root, shoot; leaf and flower extracts of *Phalaris minor* mixes with soil were studied for their effect, on the rice seedling growth. All *Phalaris minor* plant parts decreased rice root dry weight compared with the control.

Kil and Lee (1987) reported that aqueous extracts of young green tops of *Chrysynthamum moriflorum* significantly inhibited the germination and seedling growth of six flowering plants: *Callistephus chinensis, Cosmos bipinnata, Tagetes electa, Petunia bybrida, Celosia cristata, Salvia splendens and Portuaca grandiflora*. Allelochemicals related to this phenomenon here salicylic, ferulic, vanillic, gallic and caffeic acids.

Chapter -3

MATERIALS AND METHODS

3.1. Experimental Site:

The experiments were conducted at Agricultural Chemistry and Biochemistry Laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during July to September, 2004 to study the effect of weed extracts on the germination and initial growth of rice and November, 2004 to March, 2005 for mustard seed germination and initial growth.

This chapter presents a brief description about the data for identification of rice and mustard field weeds, procedures of aqueous extracts of different weeds, experimental design, treatments under investigations, analytical methods involved in isolating the biological active compound in the samples.

3.2. Rice and Mustard Crop Field Weeds:

Rice was grown by direct seeded method as well as transplanted method. Weeds are more severe in the direct seeded crop than in the transplanted crop at the time of seed germination and canopy development of rice. The major weeds that normally infested rice crop are *Echinochloa crusgalli*, *Echinochloa colnum*, *Panicum spp*, *Setaria glaucea*, *Cyperus rotundus*, *Cyperus difformis*, *Fimbristylis miliacea*, *Cynodon dactylon*, *Digitaria spp*, *Leersia hexandra*, *Marsilea quadrifalia*, *Alternanthera philloxeroides* and *Alternanthera sessilis*, etc.

Mustard was grown generally in Rabi season. Losses caused by weeds in different oil seed crops are reported to be 23 to 70 percent in mustard (Ghosh and Mukhopadhyay, 1981).

Important weeds species observed in mustard field are given below: *Vica sativa, Vicia lirsuta, Solanum trovum, Solanum nigrum, Rumex maritinus, Gnapholium affine, Aeliotropium indicum* and *Chenopodium album.*

3.3. Selection and Collection of Weed Species from Rice and Mustard Crop Field.

Summer crop field weeds of rice and winter crop field weeds of mustard were collected randomly from selected farmer's crop field of Dinajpur district. The weed species which may be more harmful for germination of seed and due to their allelopathic effects were selected for the investigation.

For the studies on the allelopathic effect and their chemical investigation the weeds of rice crop field were as follows:

No.	Common name	English name	Scientific name
01.	Aryl	Swamp rice grass	*Leersia hexandra*
02.	Jaina	Jaina	*Fimbristylis miliacea*
03.	Sushnisak	Four leaved water clover	*Marsilea quadrifolia*

The weed species collected from the rice crop field for the allelopathic effect and their chemical investigation were given below:

No.	Common name	English name	Scientific name
01.	Masur chana	Common vetch	*Vicia hirsuta*
02.	Tita begun	Black night shade	*Solanum nigrum*
03.	Bankapi	Bankapi	*Gnaphalium affine*

3.4 Preparation of Aqueous Weed Extracts:

3.4.1. Weed Extracts for the Study of Rice Crop.

Aqueous extracts were prepared from fresh as well as dry leaves, stem and root of weed species. For this study, mature weeds species *viz*. Aryl, Jaina and Sushnisak were collected, air-dried at room temperature (25-30°C), and cut into smaller pieces and then it was made into powdered form using grinder machine and also sometimes mortar with pestle.

i) Preparation of Unboiled Extracts (UBE):

Exactly 100 g of each dried plant part was soaked in 250 ml of water for 72 hours at a room temperature (25 ±2°C). The aqueous slurry was filtered through Whatman filter paper No. 1 and the volume was made up to 500 ml. The filtrates of individual weed extracts were stored in 1000 ml reagent bottles and were used for treating the seeds of rice along with water treatment as a control and other comprehensive study.

ii) Preparation of Boiled Extracts (BE):

Exactly 100 g of each dried plant part along with necessary water were boiled individually at 90°C-100°C temperature for 2 (two) hours and the slurry was filtered through Whatman filter paper No.1. The filtrates volume of individual weed species were made up to 500 ml with distilled water and stored in 1000 ml reagent bottles for comprehensive study. This study was conducted at July to September, 2004.

3.4.2. Weed Extracts for the Study of Mustard Crop.

The weed of mustard crops i.e. masurchana, Titabegun and Bankopi were collected, washed, cleaned, dried and chopped in same way. After this, boiled and unboiled extracts were prepared and stored in 1000 ml reagent

bottles, the volume also made up 500 ml. This extracts were used for mustard seed germination and others comprehensive study. This study was conducted at November, 2004 to March, 2005.

3.5. Treatments:

3.5.1. Treatments for the study of rice seed germination

The individual stored weed extracts were investigated with the following sequential treatments:

a) Distilled water or control - T_1
b) Unboiled extracts of Aryl - T_2
c) Boiled extracts of Aryl - T_3
d) Unboiled extracts of Jaina - T_4
e) Boiled extracts of Jaina - T_5
f) Unboiled extracts of Sushnisak - T_6
g) Boiled extracts of Sushnisak - T_7

3.5.2. Treatments for the study of mustard seed germination

For the observations on mustard seedling growth, individual stored weed extracts were investigated with the following sequential treatments:

a) Distilled water or controlled - T_1
b) Unboiled extracts of Masurchana - T_2
c) Boiled extracts of Masurchana - T_3
d) Unboiled extracts of Tita begun - T_4
e) Boiled extracts of Tita begun - T_5
f) Unboiled extracts of Bankopi - T_6
g) Boiled extracts of Bankopi - T_7

3.5.3. Selection of the Variety of Rice and Mustard Seeds.

For the germination and development of seedling under investigation, variety BR 11 of rice was selected and collected from BADC, Dinajpur. The purity and germination percentages of seeds were tested and found 96%.

The var. Tori 7 of mustard was chosen and collected from BADC, Dinajpur. These seed were tested and found the purity and germination percentages were 97%.

3.5.4. Rice and Mustard Seeds Setup:

Petridish experiments were done for rice and mustard seeds for the observation of germination percentage, shoot and root growth and plant height. For this experiment, clean petridish with two (2) sheets of filter paper were used.

For the investigation of rice seed growth and development, fifteen (15) ml of each solution was kept in each petridish. In control, only distilled water was used. Then twenty five (25) seeds were kept in each petridish and each treatment was replicated three (3) times. The petridishes were kept in natural diffused light under laboratory conditions at 26 ± 2°C temperature after the inhibitor solutions of various dilutions were used as moistening agent using five (5) ml of water if necessary per day per petridish (Rahman et al. 1996).

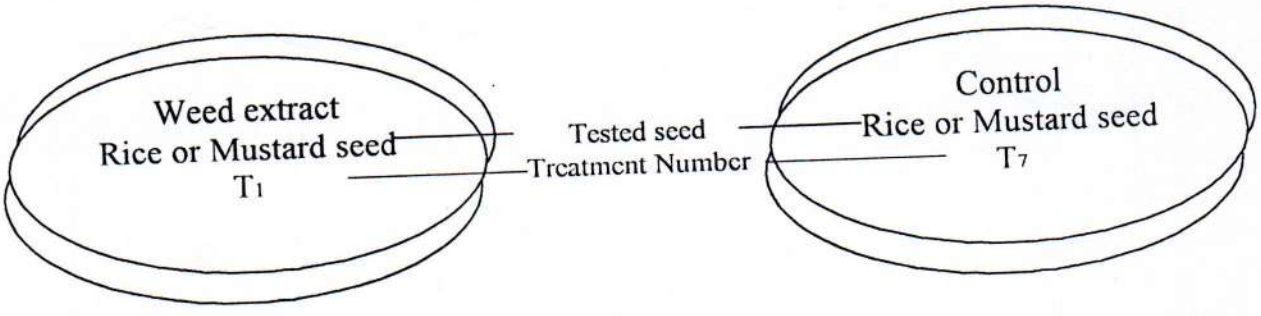

Figure 1: Labeled Petridishes.

Germination and all subsequent observations were recorded the number of days for starting and completion of germination. After seven (7) days of rice seed sowing, observations on number of opened leaves per plant, radicale and coleoptile were recorded. Effects of different treatments on morphology of seedlings were also recorded. The data on the analysis of variance and means were compared by the LSD test.

3.5.5. Technique for Root and Shoot Growth Measurement:

Number of 10 healthy seedling were taken from each replication of all treatment for growth of root and shoot length measurement. Each replications of individual treatment were averaged the root and shoot length and were measured individual treatment finally. The collected data were analyzed statistically and the differences between means were compared by using Duncan's New Multiple Range Test (DMRT).

3.6 Isolation of Crude Compounds from Aqueous Extracts:

Isolation of Crude Compounds from Effective Rice and Mustard Crop Field Weeds:

It was observed that both unboiled and boiled extracts of Aryl showed effective result in the germination of rice seeds. Similarly both unboiled and boiled extracts of Titabegun showed effective result in the germination of mustard seeds.

For the isolation of crude compounds from unboiled extracts, individual weed extract was taken in a separatory funnel. Then the equal amount of solvent as $CHCl_3$ was added to it and the resulting mixture was shaken for 15 minutes with an interval of times and it was kept for 30 minutes with a stand and clamp.

As a result, two layers were separated, *i.e.* the upper layer was aqueous layer and the lower layer was organic layer. The organic layer was separated and it was taken in a 500 ml conical flask. This process was continued for another two times using 100 ml $CHCl_3$ for each time and similarly the two layers were separated. After this, all chloroform layers was combined in a conical flask or beaker. Then the little amount of anhydrous sodium sulphate (Na_2SO_4) or anhydrous magnesium sulphate ($MgSO_4$) was added to it to remove excess amount of water. The organic layer was filtered through a filter paper. The filtrate was then evaporated using rotary thin film evaporator under reduced pressure. After complete evaporation of chloroform ($CHCl_3$), it gave crude extracts 0.9 g (by weight) of Aryl weed and similarly it gave also crude extracts 0.4 g (by weight) of Tita begun.

3.7. Examination of Crude Extracts or Crude Compounds by Thin Layer Chromatography (TLC)

3.7.1. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is one of the most important technique by which we are able to detect or identify the presence of number of compounds or number of components present in a crude extracts or crude compounds in which R_f value of each component was calculated by using this formulae.

$$R_f = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the solvent Front}}$$

Thin layer chromatography (TLC) was carried on glass plates coated with silicagel G type 60 (BDH, England).

3.7.2. Procedure for Preparation of TLC Plates

Method-1:

The glass plates (7.6 x 2.7 cm) were cleaned with strong soda water and completely freed from grease. These glass plates were then washed with distilled water and dried well in an oven. A tray made of steel approximately 60 cm long and 8 cm wide was laid on a bench with its short rim on the right. Twenty five (25) glass plates of equal thickness were placed on the tray. The coating material with appropriate amount of water (10 g of silica gel intimately mixed with 20 ml of distilled water) was vigorously shaken in a 100 ml stoppered conical flask to yield a homogeneous suspension. It was then transferred to the open spreader. The spreader was placed on the left end of the bench and held with right hand and then the spreader was drawn across the plates to the other end of the bench uniformly without applying much force.

The plates were left in position until their surfaces became completely dry and then activated by allowing then to stand over night when the coated plates were obtained for use.

Method-2:

Slurry was prepared by the slow addition with shaking 30 g of absorbent (silica gel) to 100 ml of dry chloroform in a wide racked capped bottle. A pair of microscopic slide were held together and dipped into the slurry, slowly with drawn and allowed to drain momentarily while held over the bottle. The slides were parted carefully and placed horizontally in a rack; it was then dried in sunlight /in oven at 30-40° C for 10-15 minutes (Furniss *et. al.*, 1989).

3.7.3. Procedure for TLC technique:

The compounds were dissolved in the appropriate solvents and the solutions of the compounds were then spotted with thin glass capillary tube at one end of the plates. The plates were then placed vertically with the spotted end downwards in a solvent tank. Crude extracts were checked by TLC by the following way:

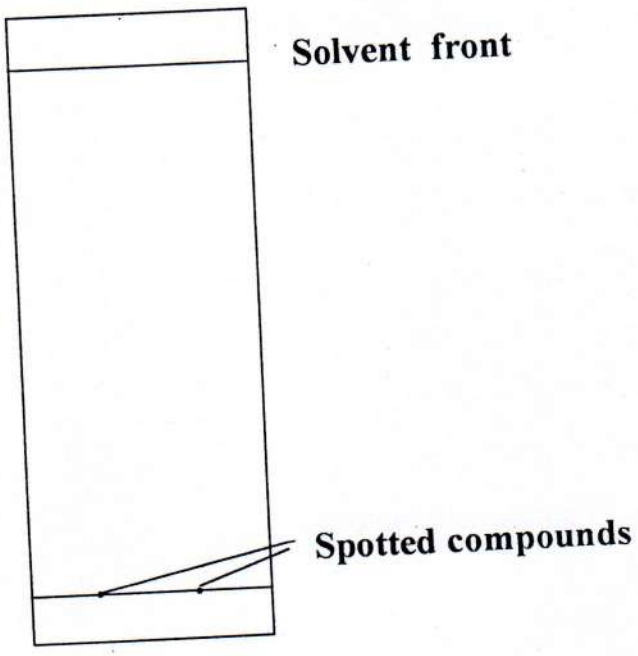

Figure 2: Thin Layer Chromatographic Plates.

3.7.4. Preparation of Solvent Tank:

A 250 ml wide mouth reagent bottle was used as solvent tank containing desired single solvent or mixture of solvent as the mobile phase, so that the spots did not immerse in the solvent. The plates were taken out and allowed to dry in air for 10-20 minutes. Subsequently the plates were developed in iodine vapour in an iodine tank. The polar and the non-polar solvent mixed it a certain ratio in a solvent tank as mobile phase.

3.7.5. Preparation of Iodine Tank:

A 250 ml wide mouth reagent bottle containing little amount of crystal iodine was used as iodine tank. After drying, the TLC plate from the solvent tank was then placed vertically with the spotted end down wards in the iodine tank. The desired compound reacts with iodine vapour forming a complex compound and was indicated as yellowish spot for each component.

Figure 3: An Iodine Tank

3.8. Purification of Crude Product by Column Chromatography.

3.8.1. Column Chromatography:

The crude products containing a mixture of compounds were separated individually by using column chromatography after preliminary TLC examination, where silica gel was used as stationary phase and solvent or mixture of solvent was treated as mobile phase.

3.8.2. Preparation of Column Chromatography

The column was prepared by slurry method, silica gel (70- 230 mesh, BDH England) being the stationary phase; the column was first washed well with washing mixture and then with distilled water and then dried with a drier. It was then rinsed with the solvent used in the preparation of silica gel slurry and again the column was dried and then fitted with a cotton plug at the bottom. The column was half filled with the appropriate solvent / eluent (non polar solvent-hexane) and the slurry was then poured into the column so that the packing was compact and uniform. Air bubble was avoided by making the column as quickly as possible. The crude extracts (40 times of crude product) were carefully placed on the surface of the column with glass dropper so that the surface of the column was not disturbed and then crystal silica gels were added. Again the little amount of cotton was placed at the top of surface of the column. The elution was continued until the desired fractions were eluted out. It was carefully marked that the slurry was always under the required solvent or solvent ratio.

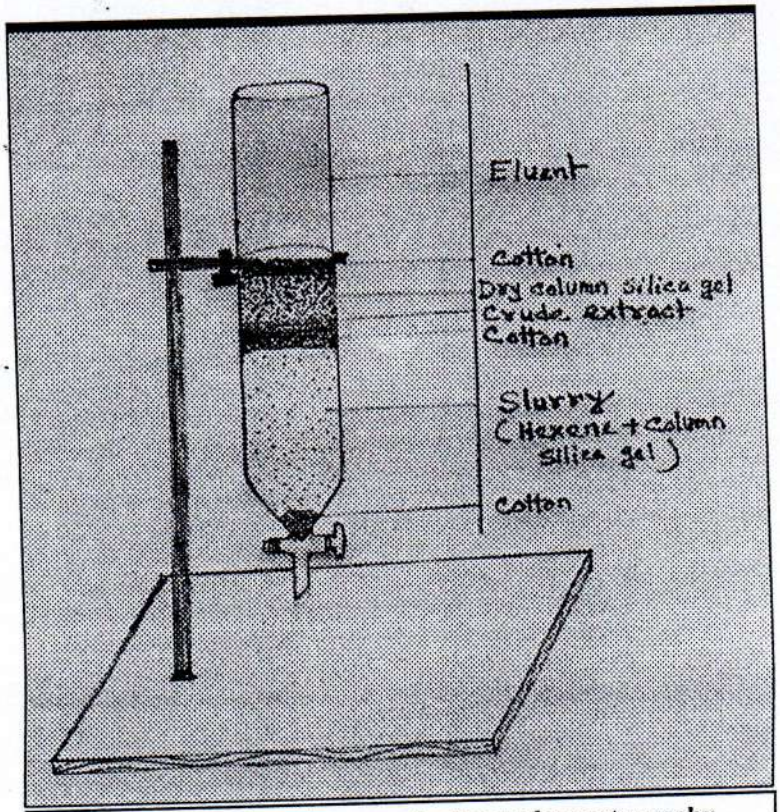

Figure 4: Preparation of a manual column chromatography

3.8.3. PREPARATION OF PREPARATIVE THIN LAYER CHROMATOGRAPHY:

Preparative thin layer chromatography was carried out on preparative glass plates coated with silica gel G type 60 (Merck). The glass plates were cleaned with strong soda water and made completely free from grease. The plates were then washed with distilled water and dried in an oven. Five glass plates (25 × 23 cm) were then placed on a bench made of steel and provided with arrangements so that the plates could be held very tightly. The slurry was made by vigorously shaking the coating materials with the required amount of water (silica gel 70 g evenly mixed with 140 ml of distilled water) in a 500 ml stoppered conical flask. The slurry was then transferred to the open spreader which was placed at one end on the bench in such a way that the coatings of thickness (0.50 mm) could be obtained. The spreader was then drawn across in the plates uniformly to the other end of the bench. The plates were then left in position until their surfaces become completely mat and were farther allowed to stand overnight when the plates were finely coated. Allowing them to stand for 48 hours at room temperature then activated them and then those were ready for use. Preparative thin layer chromatography was used to separate different components of a mixture after establishing the solvent system for TLC.

The solution was placed along a straight line vertically at right end to left of the plates by means of glass capillary. The solvent was then allowed to vertically in a large solvent (same ratio) tank containing the solvent used as the mobile phase so that the line containing the mixture stayed half inch above the solvent level in the tank. After the mobile phase had moved over appreciable distance, the plates were taken out and dried in air. The

appropriate zones corresponding to different R_f values were detected by exposing one side of the plate in iodine vapour with the rest of the plates surface covered by a clean glass plates. The relevant zone / zones were significantly indicated compound were cut out from the plates and extracted separately with appropriate solvent. The solvent was then removed under reduced pressure to get the desired compound.

Figure 5: A Preparative TLC Plates.

Chapter – 4

RESULTS AND DISCUSSION

4.1. Effect of Weed Extracts on Rice:

A petridish experiment for effect of weed extracts on rice was shown in the following figures:

Figure 6: Setup of the rice seed with extracts on petridish

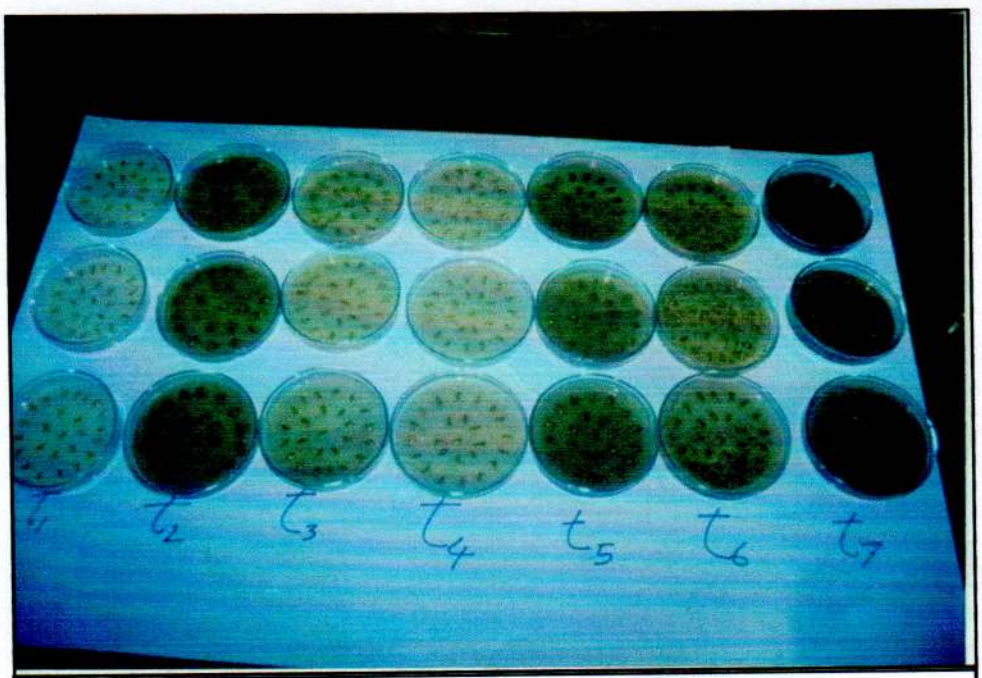

Figure 7: Initial germination of rice on petridish

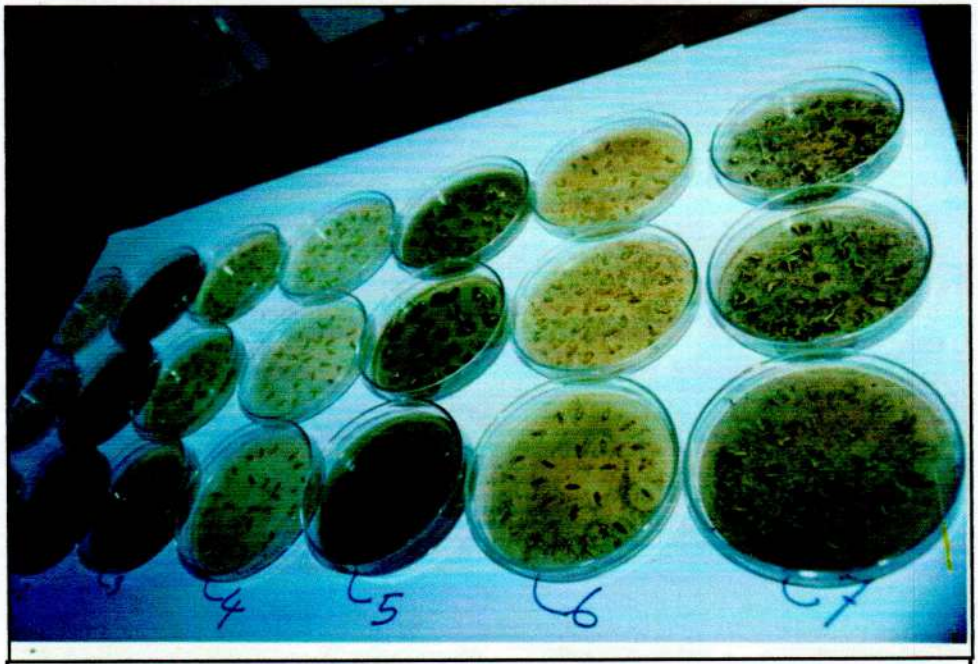

Figure 8: Rice seed germination after 3 days.

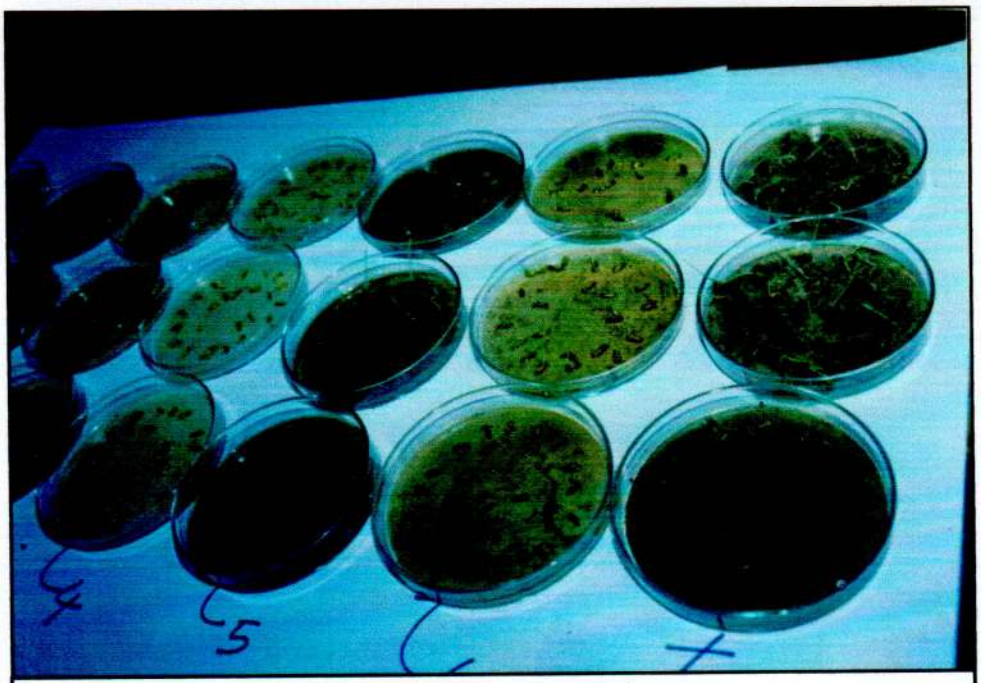

Figure 9: Rice seed germination after 4 days

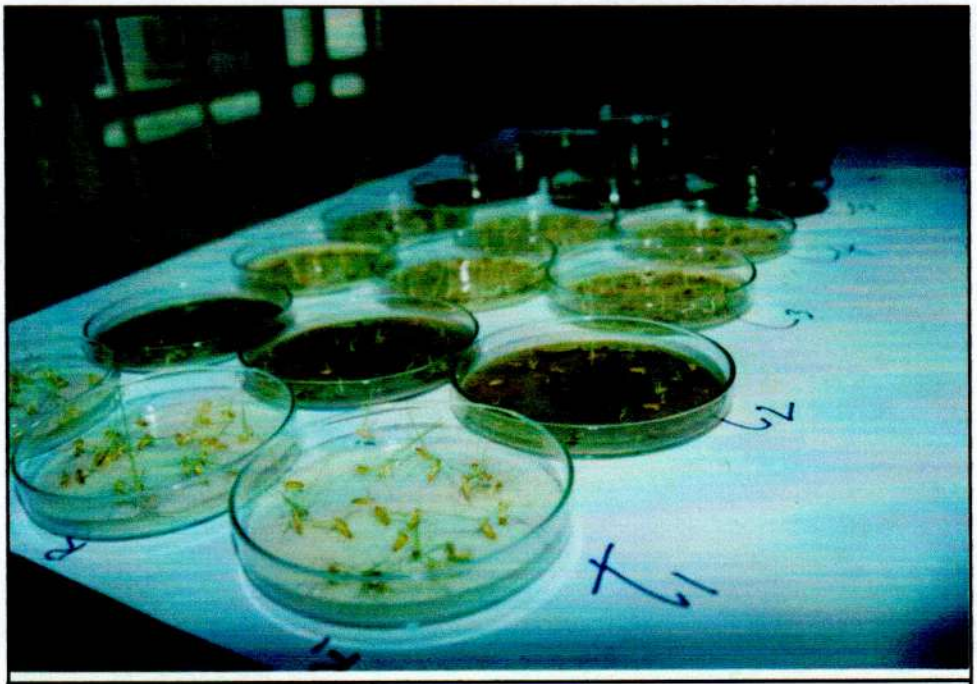

Figure 10: Rice seed germination after seven days

4.2. Germination of rice seed in different treatments

The boiled and unboiled extracts of different weed species was significantly inhibited the germination of rice seeds over control which was shown in petridish experiment (Table 1).

Table 1. Germination of rice seed in different treatments.

Day of germination	Average number from three replications	Treatments						
		T1	T2	T3	T4	T5	T6	T7
1st day		7.33	3	1	3	3	3	3
2nd day		19.66	4	14.33	14	8	10.33	16.33
3rd day		21.33	5.66	20.66	20.66	15.33	17.66	20
4th day		24	16	20.66	20.66	20.66	21.66	22.66
5th day		24	21.66	21	20.66	21.66	21.66	23
6th day		24	21.66	21	20.66	21.66	21.66	23
7th day		24	21.66	21	20.66	21.66	21.66	23

The effect of weed extracts on germination and growth of rice (Table 2) revealed that boiled extract of Aryl, Sushnisak and Jaina reduced the germination of rice significantly by 8.3, 8.4 and 9.6% respectively compared with control. Unboiled extract of the same weed species significantly also reduced the germination of rice seed by 9.7, 9.8 and 13.8%, respectively over control. Maximum germination (98%) was found in seeds treated with control and the minimum germination (82.7%) was recorded with unboiled extract treated seeds of Jaina. All the boiled and unboiled weed extracts significantly delayed germination of rice seed compared with the control. Root length of rice seedlings were significantly reduced by boiled extracts of weed species compared with the control. Among different weed extracts under study, root length decreased more (86%) by unboiled extracts of Aryl followed by unboiled extracts of Susnishak and boiled extracts of Aryl. The

lowest root length (0.33 cm) was observed in seeds treated unboiled extracts of Aryl and the highest root length (2.29 cm) was observed in seedlings treated with control. Shoot length of rice was also significantly decreased by boiled and unboiled weed extracts. The lowest shoot length (.73 cm) was measured by seed treatment with unboiled extract of Aryl and the highest shoot length (5.47 cm) was recorded over control treatment. This inhibitory activity of above mentioned weed extracts on the germination, shoot and root lengths of rice was observed in this experiment possibly due to the presence of some toxic compound / allelochemicals in the weed species under study. Similar result was also observed by Prasad and Srivastava (1991).

Table 2: Effect of weed extracts on germination and primary growth of rice.

Treatment	Germination (%)	Days to complete germination	Root length (cm)	Shoot length (cm)
Water (control)	96.0 a	4.0 c	2.29 a	5.47 a
Aryl (UBE)	86.6 cd	5.6 ab	0.33 e	0.73 d
Jaina (UBE)	82.7 d	5.6 ab	1.05 bcd	2.19 bcd
Sushnisak (UBE)	86.5 cd	6.3 a	0.60 de	1.91 cd
Aryl (BE)	88.0 bc	5.4 ab	0.86 cd	3.73 b
Jaina (BE)	86.7 cd	6.0 ab	1.34 b	3.57 b
Sushnisak (BE)	87.9 bc	6.0 ab	1.19 bc	2.53 bc
$S_{\bar{x}}$	1.42	0.42	0.14	0.49

(BE= Boiled extract, UBE= Unboiled extract). In a column, means followed by same letter(s) did not differ significantly at 5% level of probability.

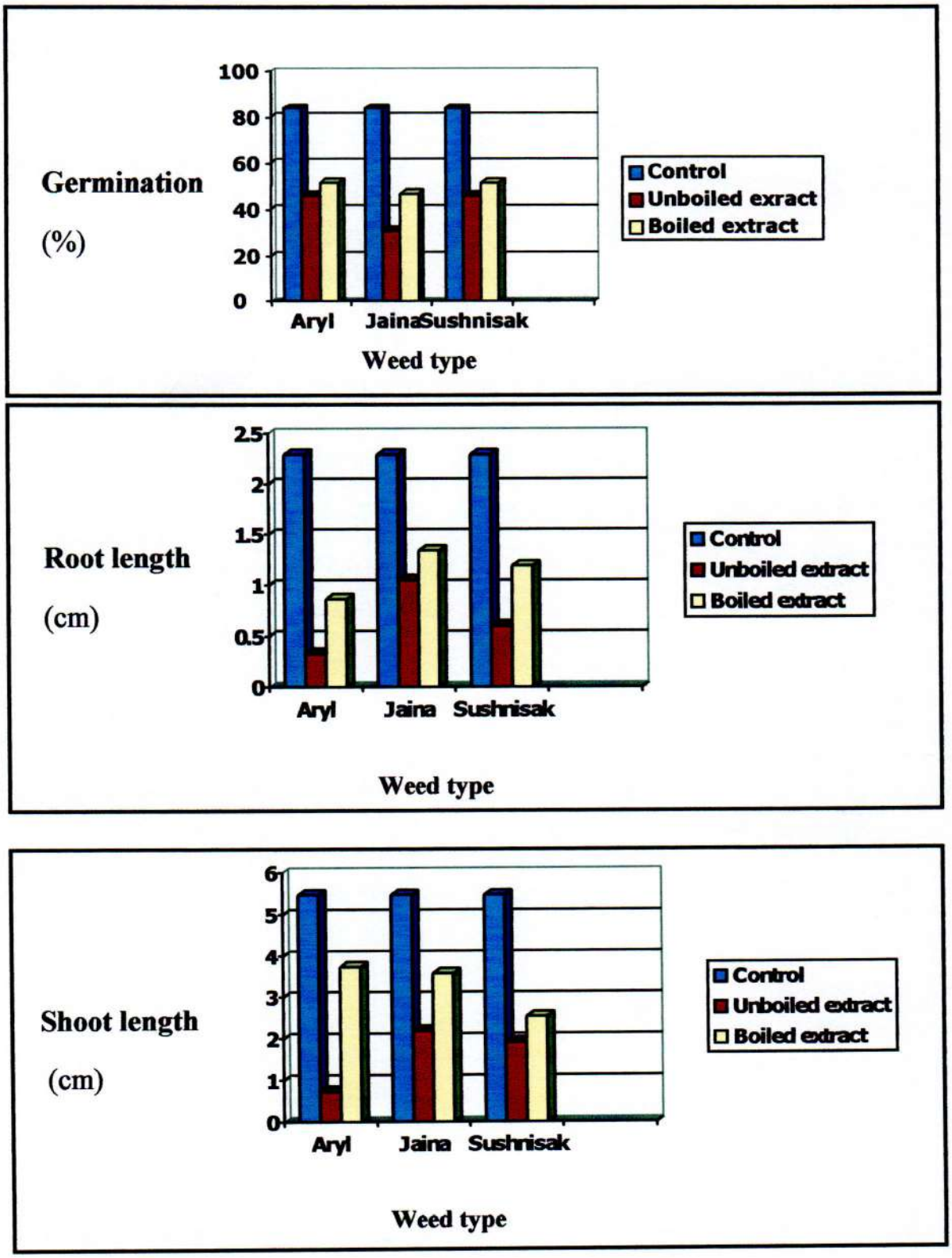

Figure 11: The Effect of individual weed extracts (unboiled and boiled) on germination, root and shoot lengths of rice seedling.

4.3. Effect of Weed Extracts on Mustard:

A petridish experiment for effect of weed extract on mustard was shown in the following figures:

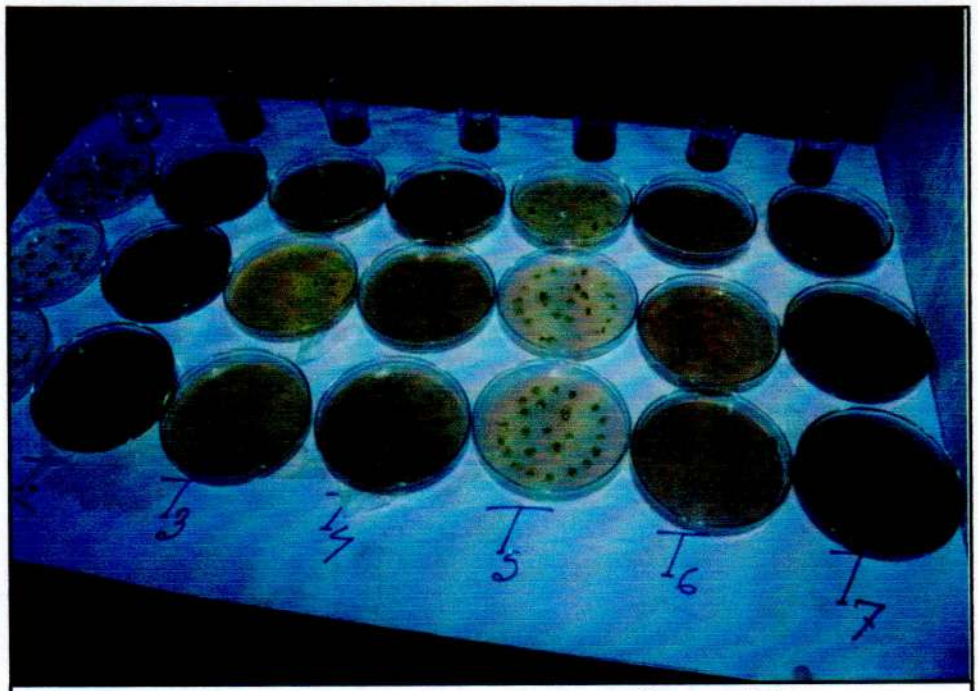

Figure 12: Mustard seed with extract on the petridish.

Figure 13: Initial mustard seed germination after two days.

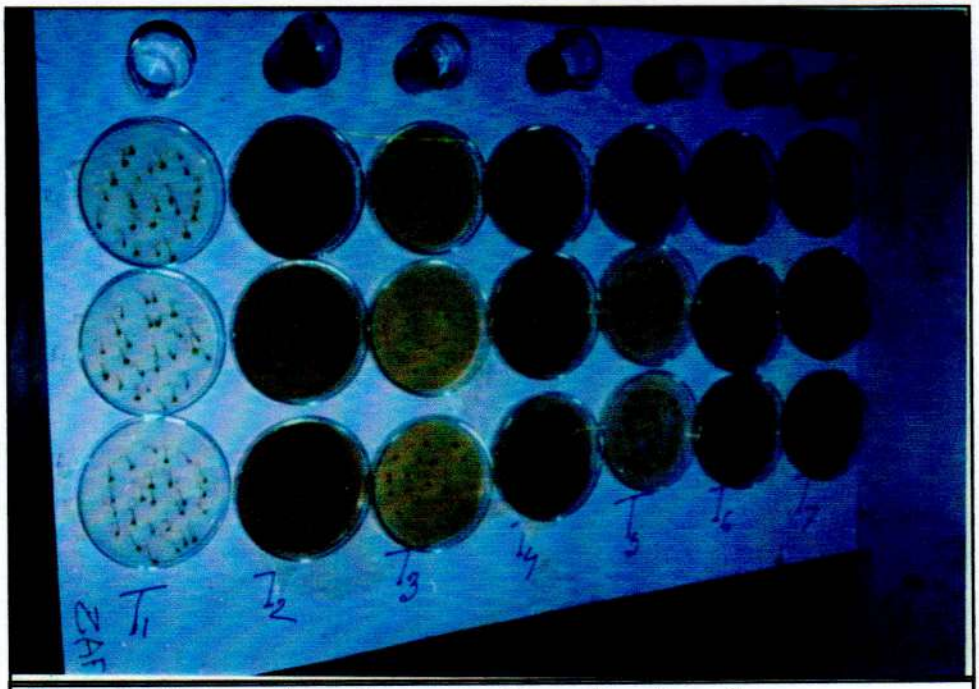

Figure14: Mustard seed germination after four days.

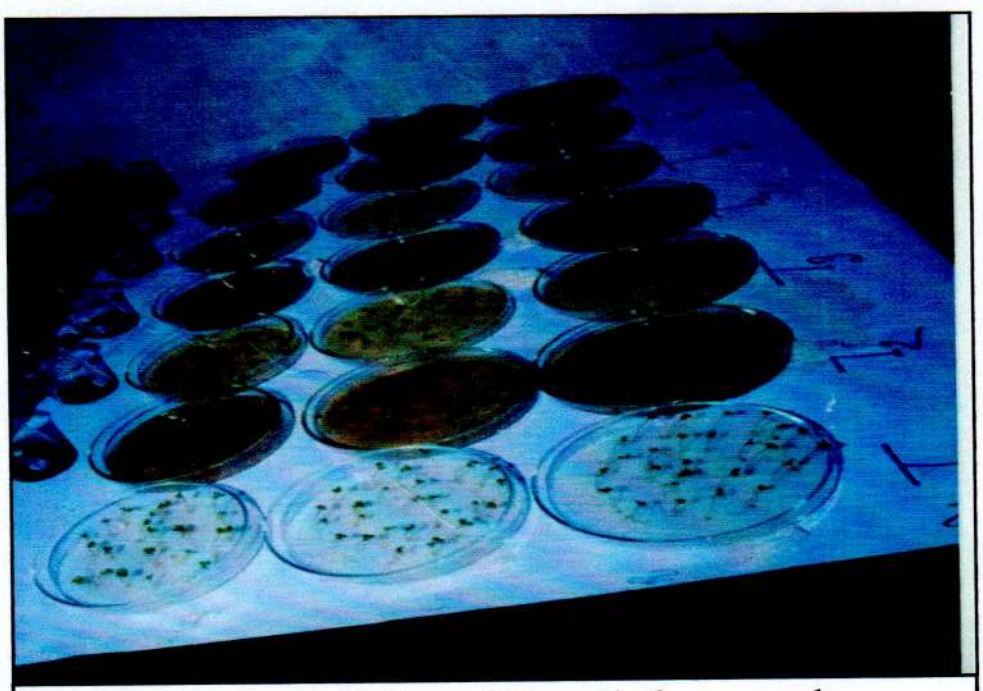

Figure 15: Seed germination of Mustard after seven days.

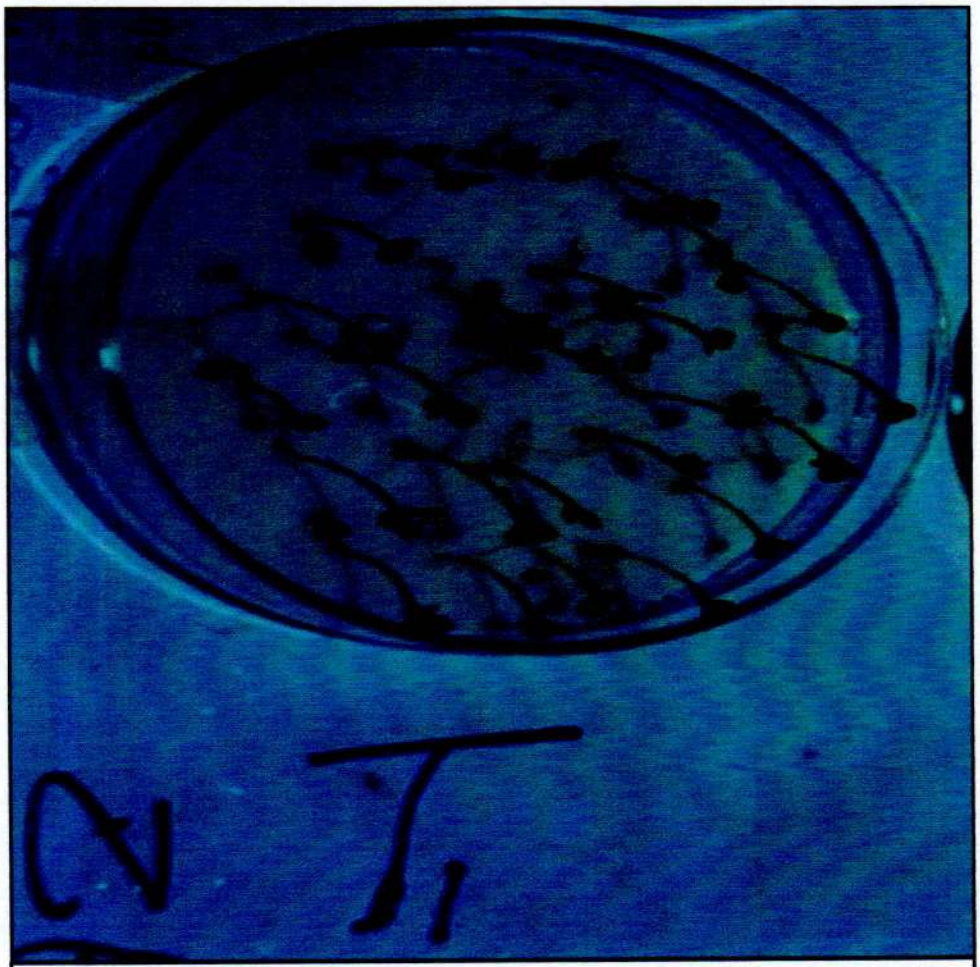

Figure 16: Control treatment (T_1) of Mustard after seven days.

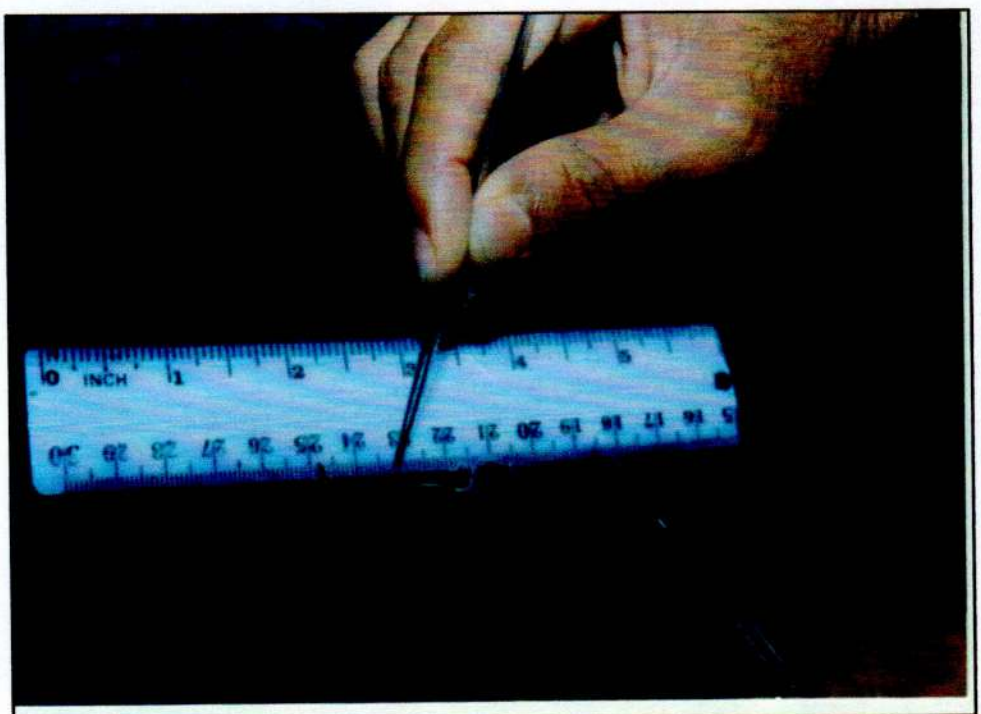

Figure 17: Measurement technique for root and shoot length of seedling.

4.4. Germination of Mustard seed in different treatments

The boiled and unboiled extracts of different weed species was significantly inhibited the germination of Mustard seeds over control which shown in petridish experiment (Table-3).

Table 3. Germination Number of Mustard seed in different treatments

Days of germination	*Average number of replications*	*Treatment*						
		T_1	T_2	T_3	T_4	T_5	T_6	T_7
1st Day		0	0	0	0	0	0	0
2nd Day		0	0	0	0	0	0	0
3rd Day		24.33	0	.66	0	3	0	0
4th Day		24.33	8.66	12.33	7	19.66	7.33	17.66
5th Day		24.33	15	17	9	21.33	9.66	21
6th Day		24.33	22	21.33	11	22.33	15.33	21
7th Day		24.33	22.66	21.33	12.66	23.00	17.33	22

Data in Table 4 showed that boiled and unboiled extracts of different weed species significantly inhibited the germination of mustard seeds over control. The highest germination (97.3%) was observed in seeds treated with control. Boiled extracts of weeds reduced germination by 5-12% over control and the lowest germination (85.3%) was observed in seeds with boiled extract of masurchana. The germination was decreased by 6-48% with unboiled weed extracts compared with control and the minimum germination (50.6%) was recorded with unboiled extract treated seeds of Tita begun.

The time required for complete germination significantly increased in weed extract treated seeds over control. Root length of mustard seedlings were significantly reduced by boiled and unboiled extracts of weed species

compared with control except in seeds treated with extracts of masurchana. The least root length (62 and 65 cm) were recorded in seedlings treated with unboiled and boiled extracts of tita begun followed by weed extracts of bankapi (0.79cm and 0.81cm) were recorded in seedlings treated with unboiled and boiled extracts of tita begun followed by weed extracts of bankapi (0.79 cm and .81 cm). Maximum root length (2.32 cm) was recorded over control. Shoot length of Mustard was also decreased by boiled and unboiled weed extracts except those of boiled extract of bankapi and unboiled extract of masurchana. The least shoot length (0.37 cm) was observed in seed treated with unboiled extract of tita begun and maximum shoot length (2.01 cm) showed over control.

Table 4: Effect of weed extracts on germination and primary growth of mustard

Treatment	Germination (%)	Days to complete germination	Root length (cm)	Shoot length (cm)
Water (control)	97.3 a	3.0 c	2.32 a	2.01 a
Masur chana(UBE)	90.6 b	6.6 a	2.01 a	1.71 ab
Tita begun(UBE)	50.6 f	6.0 ab	0.62 b	0.37 d
Bankapi(UBE)	69.3 e	5.3 b	0.81 b	1.09 c
Masur chana(BE)	85.3 d	5.0 b	1.87 a	1.47 bc
Tita begun (BE)	92.0 b	5.6 ab	0.65 b	1.35 bc
Ban kapi(BE)	88.0 c	6.0 ab	0.79 b	1.91 a
$S\text{-}_x$	0.82	0.31	0.14	0.11

(BE= Boiled extract, UBE= Unboiled extract)

In a column, means followed by same letter(s) did not differ significantly at 5% level of probability.

Similarly, the decreasing tendency of germination, root length and shoot length was also represented in graphical form

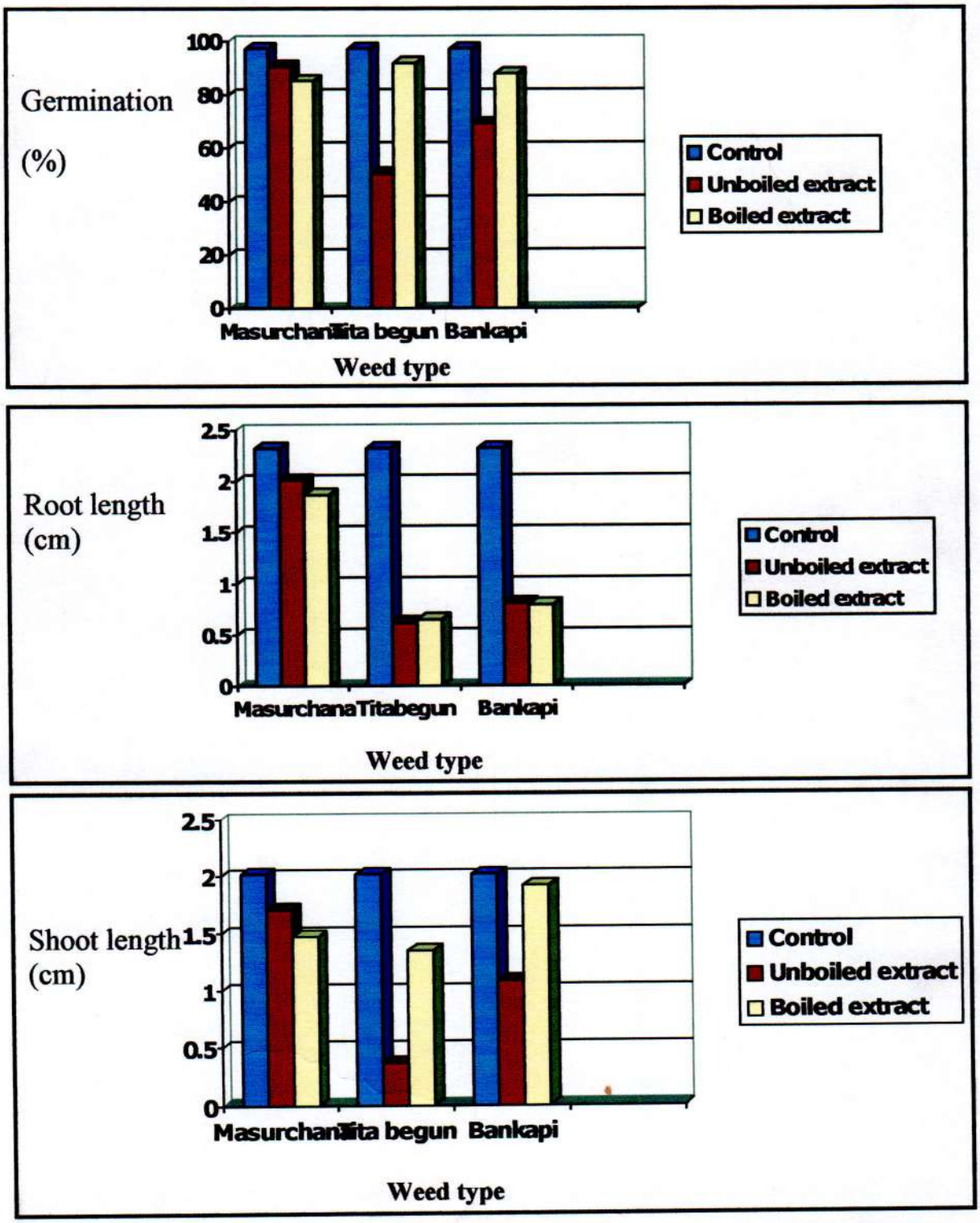

Figure 18: The Effect of individual weed extracts (unboiled & boiled) on germination, root and shoot lengths of mustard seedling.

The decreasing tendency of germination and also their root and shoot length in different type of extract treated seedlings might be due to the presence of some toxic compounds/components or allelochemicals. Similar results were observed by Mohant and Soni (1979) who reported that aqueous extract of Chandlai (*Amaranths*) significantly inhibited the growth of root and shoot of Jowar seedlings due to presence of growth regulating factors in it. Kulshrestha and Mohant (1973) reported that aqueous extract of Bapji *(Ocimum omericana)* had a phytotoxic effect on seed germination and seedling growth of Sarson. Angiras et al. (1988) observed the harmful effect of boiled extracts of white sedge and goat weed on chickpea (*Cicer arietinim* L).The results in this trial indicated that boiled and unboiled extracts of different weed species have inhibitory activity on germination and early growth of rice and mustard seedlings.

It is very interesting that the decreasing tendency of germination root and shoot length in aqueous extracts of Aryl (*Leersia haxendra*) in case of rice and titabegun (*Solanum nigrum*) in mustard is a great challenge for the farmer's of our country that why and which compound is responsible for this type of activity. For that I separated organic layer from both aqueous extract. Boiled and unboiled extract both showed decreasing tendency but I emphasized on boiled extract, compared with boiled extract because in case of boiled extract there is some tendency for decomposition of some valuable compounds due to high temperature.

The TLC (thin layer chromatography) of crude extract of Aryl (*Leersia hexandra*) was showed interestingly clear four compounds at hexane: ethyl acetate (10:1 v/v) as shown in Fig 20. This result suggested that it contained four distinct compounds, designated as $Aryl_1$, $Aryl_2$, $Aryl_3$ and $Aryl_4$,

much high comparison with other. Similarly TLC of crude extract of Tita begun (*Solanum nigrum*) was showed that it contained four compounds at hexane: ethyl acetate (10:1, v/v), respectively (Fig. 21). Figure 21 indicated that the intensity of the compound Tit_1 is too much high, indicating a major compound in comparison with Tit_2, Tit_3 and Tit_4. In both figures 20 and 21, the compounds were detected in iodine tank by calculating the R_f value using formula by the method of Furniss et al. (1989).

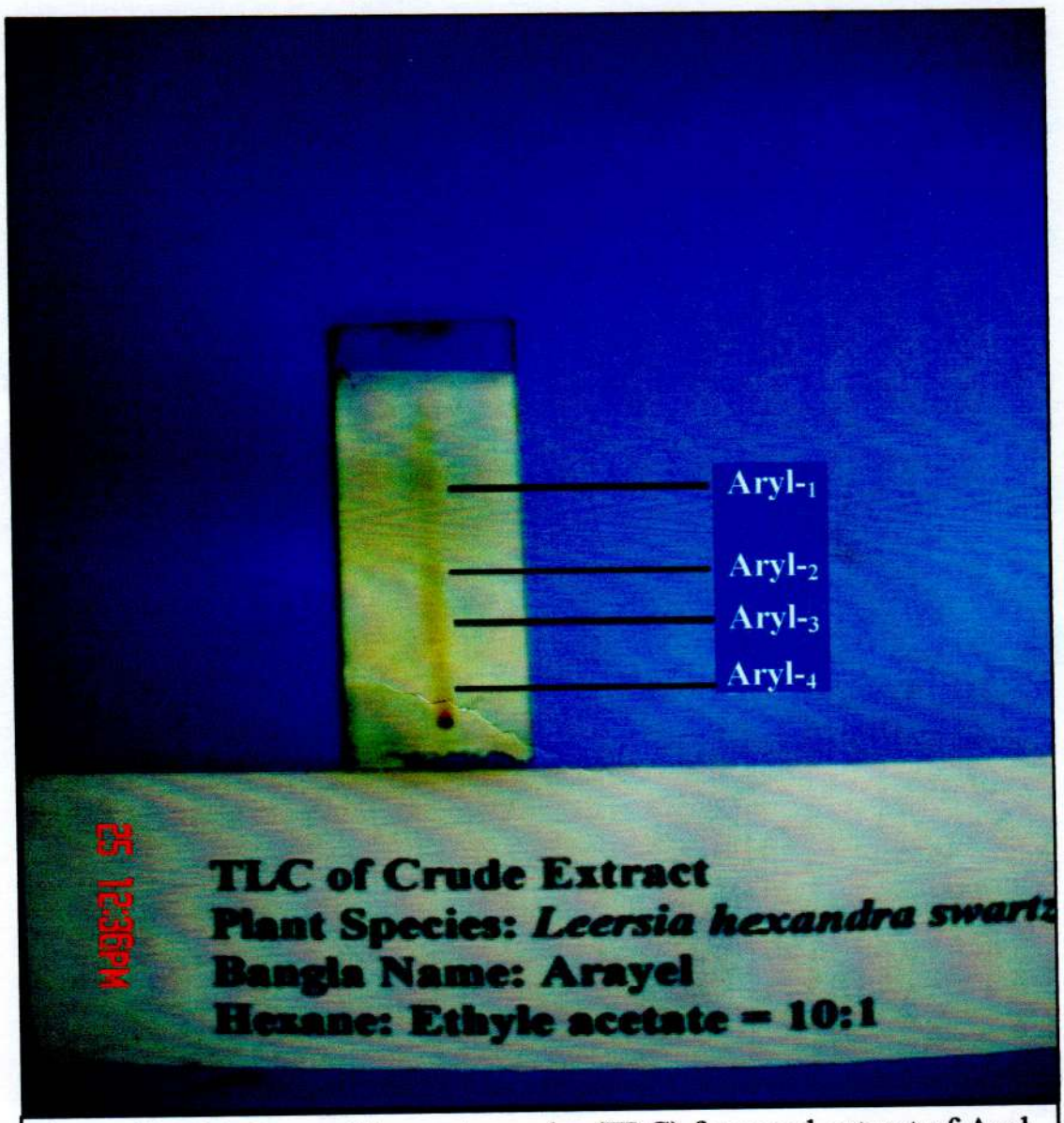

Figure 19: Thin Layer Chromatography (TLC) for weed extract of Aryl.

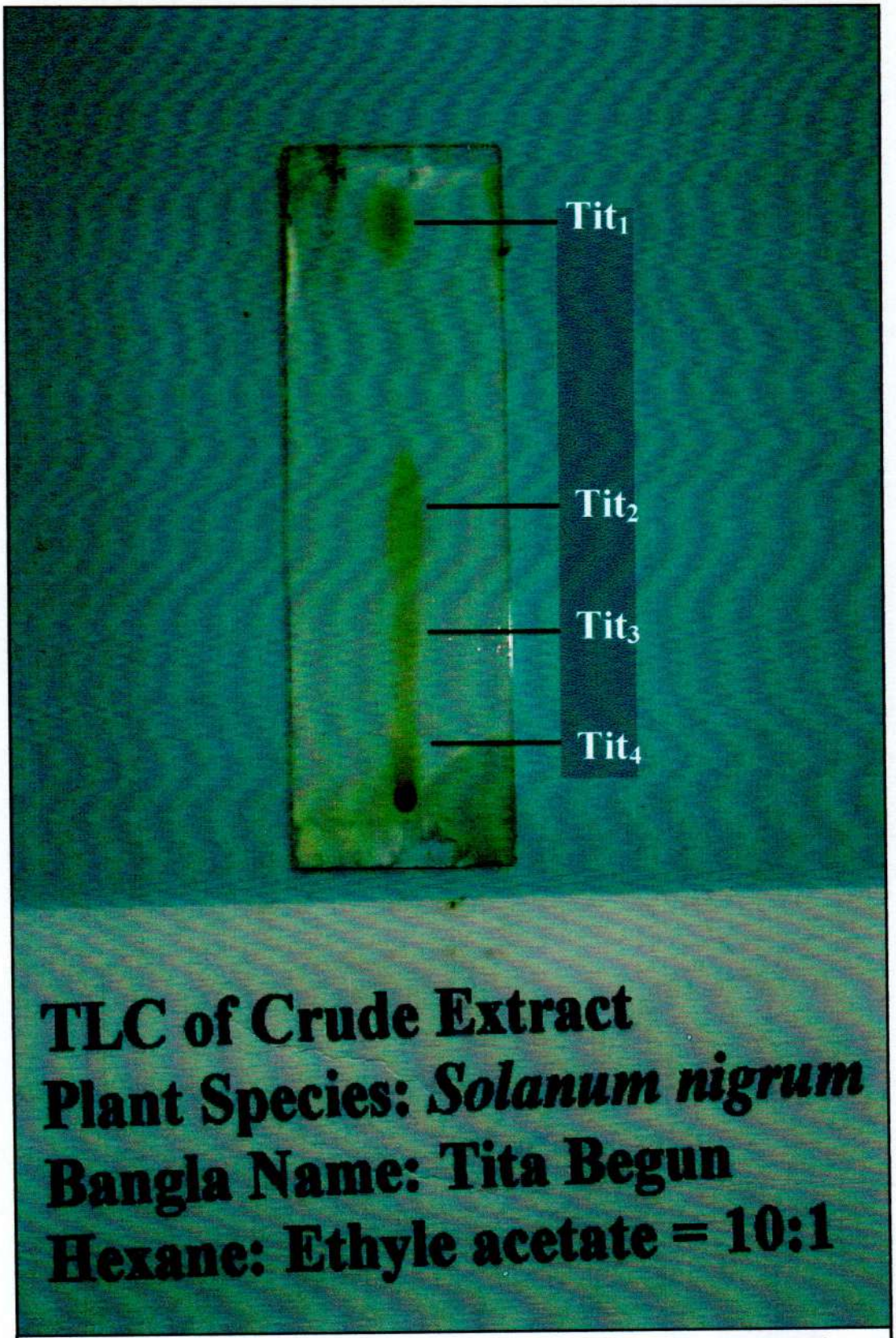

Figure 20: Thin Layer Chromatography (TLC) for weed extract of Titabegun.

Table 5: R_f values of detected component of different extracts.

Weed species	Ratio of Hexane and ethyl acetate	Detected component	R_f value (cm.)
Aryl	10:1	Aryl $_1$/C_1	0.852
		Aryl $_2$/C_2	0.672
		Aryl $_3$/C_3	0.409
		Aryl $_4$/C_4	0.098
	9:1	Aryl $_1$/C_1	0.918
		Aryl $_2$/C_2	0.803
		Aryl $_3$/C_3	0.540
		Aryl $_4$/C_4	0.114
	8:1	Aryl $_1$/C_1	0.920
		Aryl $_2$/C_2	0.825
		Aryl $_3$/C_3	0.571
		Aryl $_4$/C_4	0.111
Tita begun	10:1	Tit $_1$/C_1	0.909
		Tit $_2$/C_2	0.469
		Tit $_3$/C_3	0.303
		Tit$_4$/C_4	0.075
	9:1	Tit $_1$/C_1	0.893
		Tit $_2$/C_2	0.439
		Tit $_3$/C_3	0.272
		Tit$_4$/C_4	0.090
	8:1	Tit $_1$/C_1	0.921
		Tit $_2$/C_2	0.437
		Tit $_3$/C_3	0.328
		Tit$_4$/C_4	0.125

Compounds showing the higher R_f value indicate most non-polar compound and compounds/components showing least R_f value indicate most polar compound. In case of Aryl, we designed four components as Aryl $_1$, Aryl $_2$, Aryl $_3$ and Aryl $_4$ according to their polarity. Similarly for Tita begun, we designed as Tit_1, Tit_2, Tit_3 and Tit_4 as four components according to their order of polarity.

Then the crude extract of Aryl (*Leersia hexandra*) was under went for column chromatography selecting with hexane: ethyl acetate (50:1, 30:1, 20:1 & 10:1, v/v) respectively and were collected the following fractions like $Aryl_1$ (9-12), $Aryl_2$ (15-20), $Aryl_3$ (25-35) and $Aryl_4$ (37- upwards). TLC examinations of the above fractions unfortunately were indicated that all of them contained little-bit tailing type impurities. So, it needs further purification by micro-column or preparative TLC. After further purification of the above component, the structure of them will be determined by ^{1}H-NMR, ^{13}C-NMR, IR and mass spectroscopy studies, which will be reported in due course. If possible, further experiment on germination of respective seeds will be done using purified compounds by other worker of our group, which will be also reported later on.

Chapter -5

SUMMARY

A piece of research work was conducted at the Agricultural Chemistry and Bio-Chemistry laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur from July, 2004 to March, 2005 with a view to chemical investigation on some weeds of rice and mustard crop for justification of allelopathic effect. The experiment consisted of 7 treatments of weeds viz. control (T_1), unboiled extract of Aryl (T_2), boiled extract of Aryl (T_3), unboiled extract of Jaina (T_4), boiled extract of Jaina (T_5), unboiled extract of Shusnisak (T_6), boiled extract of Shusnisak (T_7) for rice and also 7 treatments of weeds viz. Control (T_1), unboiled extract of Masurchna (T_2), boiled extract of Masurchana (T_3), unboiled extract of Tita begun (T_4), boiled extract of Tita begun (T_5), unboiled extract of Bankapi (T_6) and boiled extract of Bankapi (T_7) for Mustard crop.

The boiled and unboiled extracts of Aryl (*Leersia hexandra*) for rice showed a strong inhibitory activity (lowest germination-86.6%, lowest root length-0.33 cm, and shoot length-0.73 cm) possibly due to the presence of some allelochemicals in the mentioned extract.

Similarly, the boiled and unboiled extracts of Tita begun (*Solanum nigrum*) also showed a strong inhibitory activity (lowest germination-50.6%, lowest root length- 0.62 cm and shoot length-0.37cm) possibly due to the presence of some toxic compounds / allelochemicals in the above extract.

TLC of crude extract of Aryl showed four distinct compounds as $Aryl_1$, $Aryl_2$, $Aryl_3$ and $Aryl_4$. Similarly extracts of Titabegun was also exhibited four distinct compounds designated as Tit_1, Tit_2, Tit_3 and Tit_4, respectively.

Chapter -6

CONCLUSION

This study indicated that weed extracts have harmful effects on germination & growth of rice and mustard seeds. The strongest inhibitory activity was observed on the aqueous extracts of Aryl (*Leersia hexandra)* during rice seed germination, seedling growth. Similarly the strongest inhibitory effect was also observed on aqueous extracts of *Solanum nigrum* (Tita begun) during mustard seed germination, seedling growth. Because all type of weed extracts may contain toxic compounds or allelochemicals, which may responsible for this type of inhibitory activity.

Therefore it is better to suggest the farmer to remove the Aryl (*Leersia hexandra*) and Tita begun (*Solanum nigrum*) rapidly during germination of rice and mustard seeds, respectively.

Chapter -7

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