GROWTH AND PHYSIOLOGY OF *Isachne miliacea* ROTH. IN DIFFERENT SOIL TYPES AND ITS SENSITIVITY TO COMMON HERBICIDES

By

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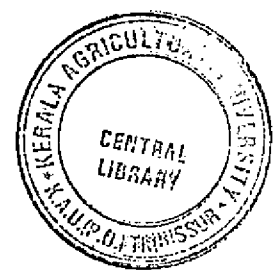
(2013-11-190)



THESIS

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Department of Plant physiology

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DECLARATION

I hereby declare that the thesis entitled **"Growth and physiology of *Isachne miliacea* Roth. in different soil types and its sensitivity to common herbicides"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that thesis entitled **"Growth and physiology of *Isachne miliacea* Roth. in different soil types and its sensitivity to common herbicides"** is a bonafide record of research work done independently by **Mrs. Suada A.P (2012-11-200)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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We, the undersigned members of the advisory committee of **Mrs. Suada A. P. (2013-11-190)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Physiology**, agree that the thesis entitled **"Growth and physiology of *Isachne miliacea* Roth. in different soil types and its sensitivity to common herbicides"** may be submitted by **Mrs. Suada A. P. (2013-11-190)**, in partial fulfillment of the requirement for the degree.

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Introduction

1. INTRODUCTION

Rice is a major crop of Kerala, occupying an area of 1.97 lakh ha with an annual production of 5.08 lakh tonnes (DES, 2014). Weeds are serious pests that damage most of the crops and is an everlasting problem for our agriculture. *Isachne miliacea,* has been reported to be a predominant weed in rice fields of Kerala both in *kharif* (KAU, 1988), and *rabi* seasons (KAU, 1990). Varghese (1996) has reported that *Isachne miliacea* alone can cause up to 61 per cent reduction in the production of rice in the Onattukara region. Currently there are reports that this weed becoming a serious problems in other rice growing regions of the state. The weed belongs to the family Poaceae. Locally it is known as 'Chovverippullu', 'Naringa', 'Njammal', or 'Changalipullu'.

Understanding the biology of the weed is essential for devising effective weed control measures. Environmental factors have a profound influence on the growth and phenology of weeds. Hodges (1991) proved that phenological development is controlled by temperature and photoperiod. Deen *et al.* (1998) reported that temperature and photoperiod were the major environmental variables influencing phenological development of common ragweed grown under controlled conditions. In the opinion of Gulshan *et al.* (2012), soil texture and depth of sowing play an important role in the emergence of various seeds of different species. Influence of soil factors on germination of seeds is based on transmittance of light through the soil, which depends on particle size, moisture content, particle colour and presence of organic matter (Tester and Morris, 2005).

Herbicide is considered as the most practical, effective and economical means of weed management in rice (De Datta, 1981). Continuous use of herbicides with the same mode of action has led to the development of resistance to herbicides including acetyl-CoA carboxylase (ACCase) inhibitor, acetolactate synthase (ALS) inhibitor, and photosystem II (PS II) inhibitor in weeds (Holt *et al.,* 1993). Rotational use of Pretilachlor and Butachlor is reported to be effective in controlling sedges in rice

(Ramachandra *et al.,* 2008). According to Rahman *et al.* (2011), site-specific mode of action of herbicides is responsible for variation in the sensitivity of weeds.

The sensitivity of a species to herbicides can be tested using bioassay tests. These tests are based on the response of the species to the specific herbicides (Horowitz, 1976). Blacklow and Pheloung (1991) stated that biological assays provide practical information on the response of the plant to the herbicide. Zhang *et al.* (2012) used whole plant bioassay techniques with weed species for herbicide dose-response studies and resistance diagnosis.

Weed shift has become a common phenomenon in the rice ecosystems of Kerala. Reduced tillage, long term no-till and continous use of herbicide lead to shift in weed flora (Durai *et al.,* 2012). Hence the tillage and herbicide programmes adopted in rice ecosystems of the state might have imparted selection pressure to the weed communities, which resulted in weed population shift. Weed shifts observed in the ecosystem can be attributed to the opportunistic germination, habit, fecundity and competitive ability of the weed together with the natural resistance of the weed to the newer herbicides which are more specific in action. These factors might have contributed to the fast spread of *I. miliacea* in the rice ecosystems of Kerala. The natural resistance of the species to the currently popular herbicides of rice may be one factor contributing to the shift. The prostrate nature of *I. miliacea* and its ability to germinate from both seeds and stem cuttings might be the factors that contribute to its dominance in the field. The weed escapes attention in a mature rice field due to its prostrate nature. Information on the sensitivity of the weed to new herbicides is also meagre. Hence a study was proposed with the following objectives:

1) To understand the phenology, growth habits and factors affecting propagation of *I. miliacea.*
2) To check the sensitivity of the weed to the herbicides commonly used in the state.

Review of Literature

2. REVIEW OF LITERATURE

Rice weed community appears as a complex ecological entity. About 350 plant species have been reported as weeds of rice, of which grasses are ranked as first, followed by sedges and broadleaf weeds (Holm *et al.,* 2003). Despite the drastic intervention required for land preparation, rice fields can be colonized by aquatic, semi-aquatic and terrestrial weeds (Fernando, 1980). Weeds have been identified as one of the most important constraints to rice production (KAU, 1988).

Weed succession and distribution pattern in rice fields are governed by spatio-temporal aspects, water management and cultural practices (Azmi and Baki, 2002). The repeated use of a particular herbicide greatly influences weed species dominance and composition. A noxious weed *Echinochloa crusgalli* was found to be dominant in plots repeatedly applied with 2, 4-D amine (Azmi and Baki, 2006).

Extensive use of herbicides has been reported to promote shifts in the weed population (De Datta and Baltazar, 2006). Broadleaved *Monochoria vaginalis* became dominant when propanil, benthiocarb, pretilachlor, quinclorac and fenoxaprop ethyl were used repeatedly. Weed species replace one another through succession and vary considerably in composition and species dominance from one rice ecosystem to another (Kosaka *et al.*, 2006; Juraimi *et al.*, 2011).

Isachne miliacea was found to be a predominant weed in rice fields of Onattukara, both during *kharif* (KAU, 1988) and *rabi* (KAU, 1990). Moody (1989) has described different species of *Isachne* in rice fields of South East Asia. Among the various species studied, *Isachne globosa* and *Isachne miliacea* were the most serious weeds in dry seeded and transplanted rice The weed belongs to the family Poacae, Locally it is known as 'Chovverippullu', 'Naringa', 'Njammal', or 'Changalipullu'.Varghese (1996) has reported that none of the weed control measures could check the residual build-up of *Isachne miliacea* in *kharif* and it alone can contribute up to 61 % reduction in the production of rice in the Onattukara region

2.1 Yield loss in rice due to weeds

Weed is a major yield limiting factor in rice culture and yield losses are numerous. Kuan *et al.* (2000) reported that rice yield loss due to weeds ranged from 5 to 72%. In Malaysia, the estimated average rice yield loss is between 10 to 35%, and yield losses by grasses, broad-leaved weeds and sedges are 41, 28 and 10%, respectively (Azmi, 2001).

Azmi and Baki (2001) estimated that the yield loss caused by grasses (mainly *Echinochloa crusgalli*), broadleaved weeds and sedges was 41, 28 and 10%, respectively.

Weedy rice at 35% infestation can cause total yield loss of about 60%, and under serious infestation, yield loss of 74% has been recorded in direct seeded rice (Azmi and Abdullah, 2003).

Weeds are estimated to cause rice yield losses of 35% in the tropics (Oerke and Dehne, 2004). In China, rice yield reduction caused by weeds is 10-20% (Zhang, 2005), while in India, yield losses due to weeds ranged from 32-83% (Savary *et al.*, 2006).

Rice yield losses due to weeds were estimated at 70-80% in "Aus" rice (early summer), 30-40% in transplanted Aman rice (late summer) and 22-36% in "Boro" rice (winter rice) (BRRI, 2006).

In direct wet sown fields of Pattambi (Kerala), Nair *et al.* (1974) reported *Echinochloa crusgalli, Cyperus* sp., *Fimbristylis miliacea and Monochoria vaginalis* as the major weeds. Sreedevi and Thomas (1993) suggested that sedges and broad leaved weeds constituted the major part of weed flora in direct sown puddled rice in Kerala, with few grasses.

The predominant weed species at Tirupati, in Andhra Pradesh were *Echinochloa crus-galli, Cynodon dactylon, Cyperus iria, Cyperus rotundus, Eclipta*

alba, Ammannia baccifera, Phyllanthus niruri and *Commelina bengalensis* (Subramanyam *et al.*, 2009).

Subramanian *et al.* (2006) reported that weed flora of the experimental field were composite in nature, consisting of grasses such as *Echinochloa colonum, E. crusgalli* and *Cynodon dactylon*; sedges such as *Cyperus rotundus, C. difformis* and *C. iria* ; broad leaved weeds such as *Eclipta alba, Ammania baccifera, Phyllanthus niruri* and *Ludwigia parviflora.*

Globally, actual yield losses due to pests have been estimated almost 40%, of which weeds caused the highest loss (32%) (Rao *et al.*, 2007).Yield losses are largely dependent on the season, weed species, weed density, rice cultivars, growth rate, management practices and rice ecosystem. Weedy rice cannot be harvested and it reduces yield because it matures earlier than cultivated rice, shatters and lodges easily (Azmi and Rezaul, 2008).

Water regimes in rice fields might determine the extent of yield loss due to weed competition. On an average, rice yield loss due to weed ranges from 15 to 20%, but in severe cases the yield loss may exceed 50% (Hasanuzzaman *et al.*, 2009) or even 100% (Jayadeva *et al.*, 2011). Yield loss depends on several factors like weed species, degree of infestation, rice ecosystem, growing season, rice cultivar, management practices and so on.

2.2 Biology and phenology of weeds

Knowledge about the biology, physiology, propagation and germination would help in better management of weeds. Weeds in general exhibit early germination, rapid growth, deeper and more spreading roots, more tillers, longer leaves, numerous flowers and seeds (Donald, 2003). Another advantage of weed is its greater phenotypic plasticity than crops, helping in adjusting with changing environmental factors such as temperature, light and water potentials (Pigliucci, 2003)

Poacae family has more than eighty species reported as weeds of rice fields. Members of Cyperaceae rank next in abundance with more than fifty species observed as weeds in rice fields. Other families with ten or more sp. reported as weeds of rice include Alismatacae, Astraceae, Fabacae and Scorphulaceae (Smith, 2003). Most of the rice weeds have C_4 photosynthetic pathway capable of thriving in tropics with high light and temperature and limited moisture (Patterson, 2005).

Weeds are classified into three broad groups based on lifespan: annuals, biennials and perennials. In each group there are both broad leaf weeds and grasses (Rao, 2000).

Annuals complete their life cycle in a year or less. They propagate by seeds. Normally, they are considered easy to control, but they are very persistent because of abundance of seeds which continue to germinate and grow fast even under unfavourable conditions. Biennials have a lifespan of two years. The first year's growth may be purely vegetative and this is known as rosette stage. The tap root is often fleshy and serves as a food-storage organ. During the second year, a flower stalk arises from the crown; this is known as bolting stage. After producing seed, the plant dies. Perennials live for more than two years and some of them live almost indefinitely. They propagate by seeds and underground storage organs like rhizomes, stolon, bulbs, tuber (Jensen, 1991).

Echinochloa crusgalli is an annual, widespread, tuft-forming, grass weed of warmer regions with fibrous, rather shallow roots, up to 60 mm, Propagation is by seeds, produced in great quantities (40,000 seeds per plant) (Nirmal and Jeyarajah, 1992).

Eleusine indica is an annual, branching at the base, 30-60 cm tall, the culms ascending or prostrate, smooth, compressed; leaf-sheaths smooth, blades linear, flat or folded, 3-8 mm wide. Two to six spikes, digitate, sessile, 4-15 cm long, with usually one inserted lower on the culm, the rachis prominently flattened with the spikelets

loosely imbricate. It has a particularly tough root system and is hard to pull out (Waterhouse, 1994).

The stems of *Digitaria ciliaris* produce runners which grow along the ground or grow obliquely at first, turning upwards at the ends (*ie.* either decumbent or ascending), and the stem growing in a looping fashion. Stem can be branched or unbranched. The inflorescence is a cluster of 4-9 fine spikes in a finger-like arrangement at the top of an erect 30-60 cm stalk (Murphy *et al.*, 2004).

Cyperus difformis is an annual, fibrous and with reddish roots; up to 100cm tall. The stem is tufted, smooth and erect, triangular and 2-3mm thick; slightly winged. The leaves are three to four basal; sheaths united at base, lower ones straw-coloured to brown; blades flaccid and linear, 15-45 cm long. Inflorescence umbellate and subtended by two leaf-like bracts; rays 1-5 cm long, some with long peduncle, some without stalk; spikelets numerous, crowded in masses about 2-5 mm long, each spikelet composed of 10-30 flowers. Fruit is a brownish nut, elliptical to slightly obovate, about 0.6mm long and lightly pitted (Holm *et al*, 1997).

Fimbristylis milacea is a herb, less than 50 cm or 50–150 cm, annual or perennial. Rhizomes present or absent. Stems are smooth, round or triangular or quadrangular, erect, solid. Leaves are of two types -large (more than 2 cm long/wide), smooth on upper surface, hairy on lower surface, sessile, simple, narrow, alternate, in a rosette or evenly distributed on stem, margin entire, apex acute, base truncate, parallel-veined, leaf sheath present. Leaves may be reduced to leaf sheaths; leaf blades often have grooves. Ligules present or absent, hairy or not hairy, non-membranous. Flowers are bisexual, grouped together in an inflorescence, terminal. Inflorescence is a spike, few flowered. Flowers are clustered, sessile, and small (less than 2 cm). Fruit is a nut. Bracts are leaf-like, with bristles or scale-like. It is one of the most serious weeds of rice (Holm *et al.*, 1997).

Cyperus iria is an annual herb, or occasionally perennial, with fibrous roots, 15-75 yellowish red roots; 10-70 cm tall. Stem is sharply 3 angled, tufted, smooth, 5-80 cm high. Leaf- basal, rough to touch in upper part, linear, flaccid, with gradually tapering point and 3-8 mm wide; sheath reddish or purplish brown, enveloping the stem at base. Inflorescence- simple or compound umbel composed of numerous erect-spreading 3-10 mm long flattened spikelets. Fruit is three-angled, 1.0-1.5 mm nut with slightly concave sides, and shiny dark brown to black (Venkitesan and Satyakumar, 1999).

The sedge *Scirpus maritimus* is occasional in brackish water or on the shore. Plant grows up to 120 cm tall. Spikelets are 10-20 mm long. It can be identified by egg-shaped spikelets. Stems are sharply 3-sided, sometimes even winged. It is much leafier with long leaves grooved above and keeled below. Inflorescence is often stalked clusters of spikelets. They have an upright leaf-like bract which appears like a continuation of the stem beyond the inflorescence, as well as two or three smaller bracts at an angle to the stem (Goldblatt and Manning, 2000).

Cyperus rotundus is a weed that grows straight, with roots extended. Growth of weeds is 15 to 20 centimeters in height. The stems are straight, no presence of branches, and shiny, pyramidal shape with inflatable trunks. A series of branches spread out, straight, white and massive. The eight primary leaf rays are five centimeters in length having five to ten spikelets containing 10 to 40 flowers in reddish brown colour. The fruit it bears is egg shaped with three sides, 1.5 millimetre wide (Meena *et al.*, 2010).

2.2.2 Influence of nutrient content and pH of soil on growth and development of plants

The amount of nutrients in the soil affects the rate of growth of plants. The plant enjoys nutrient-rich soil, but when it finds itself in a nutrient-poor soil, though it may grow, the growth however will be retarded (Robinson, 2006). High level of nutrients

in the soil causes contamination and in agriculture generally, optimal water supply as well as nutrient conditions are usually chosen for an optimal plants growth (Mackova *et al.,* 2009).

Nutrient availability and absorption by plants are regarded as the greatest driver of plant growth. Plants growth depends on nature of the soil, available nutrients, prevailing environmental conditions and pH (Kirmani *et al.,* 2011). Depending on its location, soils are made up of some combination of particles which include; sand, silt, clay, and organic matter. Soil texture and pH determines nutrient availability to plants (Parry, 2011).

In turf grass, when most plants experience a Ca deficiency, the first visible symptom is a cessation of shoot and root growth, and the symptom is most apparent at the growing points. (Hull *et al.*, 1997). Wheet (2005) reported that potassium affects the biochemical functions of the plants, which include cell division and resistance to diseases.

Phosphorus (P) is a naturally occurring element in the environment that can be found in all living organisms as well as in the soil. It is an important component of a lot of physiological processes related to energy utilization in both plants and animals (Daniels *et al.,* 2008).

When micronutrients are available in larger concentrations, they are regarded toxic (Al-Yemeni and Hashim, 2008). For example, excess microelements cause stunted growth, upset minerals, and affects membrane structure and permeability and uptake of mineral nutrients. However, high tolerance of plants to some micronutrient toxicity is based on reduced metal uptake or increased internal sequestration in the plant genotypes (Garg and Aggarwal, 2010).

Nutrient behavior in soils and ability of plants to absorb them depend on the organic matter content of the soil as well as the pH (Okoye *et al.,* 2008). This behavior varies according to the nature of the nutrients, the physico-chemical properties of soil and the plant species (Tuzen, 2008).

According to Hamlin (2001), the pH of the soil influences the availability of the elements for plant uptake. The presence of *Chromolaena odorata* in soil has been implicated in the shift of soil pH towards alkalinity (Jebril and Yahaya, 2010).

2.2.3 Potential roles of dehydrogenase enzyme in maintaining soil health

There are a lot of enzymes in the soil environment, such as oxidoreductases, hydrolases, isomerases, lyases and ligases. Each of them play key biochemical functions in the overall process of material and energy conversion, soil dehydrogenases (DHA) are the major representatives of the oxidoreductase enzymes class (Gu *et al.*, 2009). Dehydrogenases play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.*, 2012) Among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity (Salazar *et al.*, 2011), because they occur intracellular in all living microbial cells (Yuan & Yue, 2012). Moreover, they are tightly linked with microbial oxidoreduction processes (Moeskops *et al.*, 2012).

Many specific dehydrogenases transfer hydrogen to either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (Subhani *et al.*, 2001). The overall DHA of a soil depends on the activities of various dehydrogenases, which are fundamental part of the enzyme system of all living microorganisms, like enzymes of the respiratory metabolism, the citrate cycle, and N metabolism. Thus, DHA serves as an indicator of the microbiological redox-systems and could be considered a good and adequate measure of microbial oxidative activities in soil (Brzezinska *et al.*, 2001). Therefore, DHA reflects metabolic ability of the soil and its activity is considered to be proportional to the biomass of the microorganisms in soil. However, the relationship between an individual biochemical property of soil DHA and the total microbial activity is not always obvious, especially in the case of complex

systems like soils, where the microorganisms and processes involved in the degradation of the organic compounds are highly diverse (Salazar *et al.*, 2011).

2.3 Environmental influence on weed propagation

Many weeds spread vegetatively from stem cuttings, underground stolons and axillary buds. Weeds like *Commelina benghalensis* propagate by means of stem cuttings (Budd *et al.*, 1999). *Paspalum distichum* spreads by creeping underground stolons whereas *Cynodon dactylon* can propagate by rhizomes or auxillary buds (Reissig *et al.*, 1999).

Studying the dormancy and germination behaviour of weed seeds in soil will help in better management of the weed. Dormancy is usually considered to be the failure of a seed to germinate under conditions normally available for the germination and growth of seedlings. The ability of the seeds to survive as a dormant tissue for long periods and germinate at the right time is a very important survival advantage of weeds. Seeds of *Scriptus articulates* possessed internal dormancy and required an after ripening period of four months to germinate (Datta and Roy, 1997).

The requirement of oxygen for breaking dormancy and for initiation of germination varies with weed species. According to Smith and Fox (1999) fewer weeds emerged when the soil was flooded. At field capacity or soil saturation all weed species emerged readily.

Twenty five species of weeds exhibited definite periodicity in germination in a particular season and at a particular point of time (Mani and Singh, 1999). Light requirement is a principal factor due to by which seed germination is restricted to the proximity of soil surface (Woolley and Stoller, 1999, Karssen, 2000).

Even under optimal condition only a part of the seeds germinate at a time while the rest remaines in the earth's seed bank (Elgey and Duke, 2002). It was also found

that in the field, weed seeds alternatively lost and acquired dormancy and displayed seasonal rhythmic germinability during their periods of persistence in the soil.

Echinochloa crusgalli germinated under anaerobic and aerobic conditions (Kennedy *et al.,* 2002). Most weed seeds have a single temperature optimum for germination. Temperature strongly influenced the breaking of both primary and secondary dormancy especially the onset and breaking of secondary dormancy (Elgey, 2005). They also found that allelochemicals like coumarin inhibited germination of seeds. Nitrite or gibberellic acid stimulated germination of *Lactuca sativa.* Nitrite broke dormancy of *Oryza sativa.*

Some weed species have the ability to produce seeds between intervals of normal disturbance associated with a crop situation. *Avena fatua* (wild oats) germinate at the same time the wheat crop is sown and shatter their mature seeds before crop harvest. Many weeds can produce a large number of viable seeds even after having been cut soon after flowering. A few weed species produce seeds through apomixis i.e. without fertilization. Weeds such as ferns reproduce by spores rather than seeds (Rao, 2000).

2.3.1 Effect of depth of burial on propagation

In general, weed seeds buried near soil surface loose viability more rapidly than seeds buried more deeply (Toole, 1986). Roberts and Dawkins (1987) found that in the absence of reseeding weed seeds in cultivated soil were reduced in number by about 25 % per year.

Germination of seeds varied with depth of burial. High soil temperature also favoured loss of seed viability (Schafer and Chilcote, 1990). In fact secondary dormancy is induced by burial. Emergence of seeds of *Commelina benghalensis* were 19.5, 9.8, 2.5, 1.0 and 0.5 % for seeds buried at zero, two, four, six, and eight centimetre depth respectively (Budd *et al.,* 1997).

Burial of stem cuttings of *Commelina* (Budd *et al.*, 1999) and *Paspalum distichum* below two centimetre depth failed to regenerate but longer segments sustained for longer periods (Reissig *et al.*, 1999).

Most of the weed seedlings emerge from the top 0.5– 2 cm soil layer, but some weed species (e.g. *Mimosa invisa* and *Echinochloa crusgalli*) can emerge from 8-cm burial depth also (Mohler, 2006). Benvenuti and Macchia (2008) reported that the seeds of various plant species required light and oxygen for maximum germination.

Good seed depth of 1.5 to 2.0 inches or even deeper is recommended in dry conditions to ensure good moisture availability for successful seed germination (Al-Kaisi, 2009). Jun *et al.* (2010) determined the effects of sand burial on seed germination and seedling emergence of ten *Calligonum* L. species and suggested that the deeper the seeds in sand, the lower and slower their germination and seedling emergence.

Weed species with small seed size generally germinate better from the soil surface. The ability of seedlings to emerge from deeper depths depends on the energy reserves in the seeds; larger weed seeds with greater reserves can support seedling emergence from deep depths better than small weed seeds (Chauhan and Johnson, 2010).

In the opinion of Gulshan (2012) soil texture and depth of sowing play an important role in the emergence of various seeds of different species.

2.3.2 Effect of soil moisture level on propagation

Flooding is an important component of weed management in rice. In irrigated and flooded systems, the environment in which weed seeds have to germinate is characterized by the existence of low oxygen concentrations. Differential responses between rice and weeds to flooding could be an important component of weed management for the direct-seeded rice crop as rice is tolerant to flooding, but many weeds are not. However, the timing, duration and depth of flooding are critical in

suppressing germination and growth of a number of weed species. (Ismail and Hossain, 1995).

Smith and Fox (1999) and Vamadevan *et al.* (1994) reported that the biomass and number of weeds decreased with increase in water depth so much that maximum weed count and weight was noticed under the treatment of field capacity to saturation point

Bhan (2003) also observed that weed emergence was maximum at field capacity, the emergence reduced at 15 cm submergence. Gill *et al.* (2005) reported that five to six centimetre standing water for four weeks significantly reduced seedling emergence of *Echinochloa crusgalli* than at three centimetre water depth.

Rao and Nagamani (2010) proved that early and continuous, but shallow (2–4 cm) flooding may help to suppress the emergence and growth of *Cyperus iria, Fimbristylis miliacea, Leptochloa chinensis* and similar weed species.

2.3.3 Effect of light on propagation

Hendrix (1995) conducted an experiment and found that in *Ischaemum rugosum* greater rates of dry matter accumulation were observed in plants of open condition than in those shaded at 70% and 50%.

Marenco and Reis (1997) proved that in Wrinkled grass vegetative growth was greater in shaded than in unshaded plants.

Influence of soil factor on germination of seeds is based on transmittance of light through the soil, which depends on factors like particle size, moisture content, particle colour and presence of organic matter (Tester and Morris, 2005).

2.3.4 Effect of temperature on propagation

Fifty per cent of seeds from three other species of columnar cacti native to México, *Pachycereus hollianus*., *Cephalocereus chrysacanthus* and *Neobuxbaumia tetetzo* germinated at temperatures between 15 and 30 °C as well as at extreme temperatures of 10 and 40 °C, although showing low percentages of germination (Rojas *et al.*, 1998).

Lower temperatures led to lower germination in *Pachycereus hollianus* seeds (Flores and Briones, 2001).

Socolowski (2010) proved that, the highest percentage germination in *Cereus* species was between 25 and 30 °C. The minimum temperature was between 15 and 20 °C and the maximum between 35 and 40 °C.

2.5 Herbicides for weed management in paddy field

For the last few decades, herbicides have been tremendous contributors to agriculture. In large scale rice farming, herbicide based weed management has become the smartest and most viable option due to scarcity and high wages of labour (Singh *et al.*, 2006)

Though hand weeding is effective, it is tedious, time consuming and labour intensive. Due to the high cost of labour and inadequate availability at the peak period and unfavourable working conditions, it has become inevitable to use chemicals for weed control. The efficiency of herbicide depends upon the type of rice culture and the nature of weed species present. Identification of suitable herbicides for different systems is needed. Effectiveness of herbicides depend on so many factors such as water management, soil fertility, nature of rice variety and weed species and their growth pattern (Anwar *et al.*, 2012).

Works at IRRI, Philippines proved that glyphosate at 2.0 kg ha^{-1} effectively controlled *Paspalum paspalodes*. Trials at IRRI gave good weed control with pendimethalin 0.75, pretilachlor 0.50 and oxyfluorfen 0.25, thiobencarb 1.0, butachlor 0.75 kg ha^{-1} (IRRI, 1979). *Paspalum distichum* was controlled by applying glyphosate at 2.0 or 2.4 kg ha^{-1} (Corbetta *et al.,* 1979). Knyr *et al.,* (1985) found that the application of 2.4 kg ha^{-1} of glyphosate to fallow plots or 0.6 and 2.0 kg ha^{-1} to pre-sowing rice gave effective control of *Echinochloa* and showed no adverse effect on crop growth and paddy yield. They also found that glyphosate was degraded in soil within ten days of application. Singh *et al.* (2004) and Kim *et al.* (2003) have earlier reported the effectiveness of fenoxaprop p-ethyl against *Leptochloa.* Saini (2003) noticed reduction of annual grasses by the application of cyhalofop-butyl in wet seeded rice.

Application of 0.15 kg oxyfluorfen or 1.0 kg thiobencarb per hectare or 1.5 kg butachlor or 0.3 kg anilofos and 0.8 kg 2, 4 – D ethyl ester gave more than 3 t ha^{-1} of grain yields (Kumar and Gautam, 1986). Two hand weedings on 30^{th} and 50^{th} day increased the yields in the above treatments to 4 t ha^{-1}. Mishra *et al.* (1988) got better control with 0.1 and 0.15 kg oxyfluorfen 1.0 kg butachlor and 1.0 and 1.4 kg ha^{-1} of thiobencarb. But broadleaf weed control was poor with anilofos (arozin) at 0.3 and 0.4 kg ha^{-1}.

Continuous use of herbicides with the same mode of action has led to the development of resistance to herbicides including acetyl-CoA carboxylase (ACCase) inhibitors, acetolactate synthase (ALS) inhibitors, and photosystem II (PS II) inhibitors in weeds (Holt *et al.,* 1993).

Application of different pre-emergence herbicides including thiobencarb, pendimethalin, butachlor, oxadiazon and nitrofen has been found to control weed satisfactorily in direct seeded rice (Moorthy and Manna, 1993; Pellerin and Webster, 2004). Application of wider spectrum of chemicals could help delay the development of herbicide resistance in weed community (De Datta and Baltazar, 2006; Anwar *et al.*,

2012). Herbicides in mixture or year to year sequence of products having different modes of action might contribute to sustainable weed management (Valvarde *et al.*, 2000).

Many researchers working on weed management in direct seeded rice opined that herbicide may be considered to be a viable alternative/supplement to hand weeding (Kumar *et al.*, 2008; Mahajan *et al.*, 2009; Pacanoski and Glatkova, 2009; Chauhan and Johnson, 2011; Anwar *et al.*, 2012). In Malaysia, incidences of weed resistance to sulfonylurea, phenoxy and molinate compounds have been reported by many researchers (Watanabe *et al.*, 1997; Baki and Azmi, 2001). Therefore, it is a must to use herbicides judiciously.

Despite some undesirable side-effects no viable alternative is presently available to shift the chemical dependence for weed management in rice. Among the post emergence herbicides, ethoxysulfuron, cyhalofop-butyl, pretilachlor, chlorimuron, metsulfuron, bispyribac sodium and penoxsulam effectively controlled weeds in direct seeded rice (Mann *et al.*, 2007; Singh *et al.*, 2006; Mahajan *et al.*, 2009; Juraimi *et al.*, 2010).

2.5.1 Seed bioassay

Blacklow and Pheloung (1991) stated that the biological assays provide practical information on the response of the plant to the herbicide. Bioassays where either shoot or root length is used as the growth parameter to discriminate between resistant (R) and susceptible (S) biotypes exposed to herbicide solutions, have already been developed to screen resistance within populations. Petri- dish assays were developed to assess resistance to dinitroaniline herbicide in *Setaria viridis* (Beckie *et al.*, 2001), and ACCase-inhibiting herbicides in *Sorghum halepense* (Smeda *et al.*, 2001), *Avena fatua* (Murray *et al.*, 2001) and *Alopecurus myosuroides* and *Lolium* spp. (Letouze and Gasquez, 2002).

A seed-bioassay was developed by Tal *et al.* (2005) to study the resistance of grass weed populations from different regions to acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicides. He also used the technique to study the resistance of *Lolium rigidum, Phalaris minor* and *Alopecurus myosuroides* to diclofop, fenoxaprop-P and clodinafop, and compared the results obtained for seed bioassay with whole-plant trial and enzyme assay. The discriminating concentrations at which the differences between the resistant and suseceptable biotypes was evident for *L. rigidum, P. minor* and *A. myosuroides* were 6.0, 8.0 and 0.06 mg/l, for diclofop, fenoxaprop-P and clodinafop, respectively.

Kaundun *et al.* (2014) proved that seed bioassay method is very simple, quick and, cost- effective, it allows determination of glyphosate resistance in weeds prior to field application. It thus offers the opportunity for an informed choice of herbicides for effective weed control.

2.5.2 Whole plant bioassay

Whole-plant bioassay techniques has been successfully used to study the sensitivity of herbicides to different weed species (Tal *et al.*, 2005). Osuna *et al.* (2002) used this method to compare the responses between *Echinochloa oryzoides* and *Cyperus difformis* to bispyribac-sodium and bensulfuron-methyl. He detected the involvement of cytochrome P-450 monooxygenases in *E. oryzoides* resistance to bensulfuron-methyl. He used cytochrome P-450 inhibitors like piperonyl butoxide and malathion for confirmation.

Nine *Monochoria vaginalis* accessions from Chonnam province, Korea were tested for resistance to the sulfonylurea herbicide, imazosulfuron, in whole-plant response bioassay. The resistance of the species to imazosulfuron was confirmed by the test (Tal *et al.*, 2005).

To study the resistance of red rice accessions to imazethapyr, 130 red rice accessions collected from 26 rice-growing counties in Arkansas, USA, were tested for tolerance to imazethapyr using seed and whole-plant response bioassay techniques. The red rice accessions were compared with imazethapyr-resistant (Clearfield) rice cultivars (CL121, CL161 and CL-XL8) and conventional rice cultivars (Kuk *et al.*, 2008).

Zhang *et al.* (2012) used whole plant bioassay techniques with weed species for herbicide dose-response and resistance diagnosis.

Material and Methods

3. MATERIALS AND METHODS

The present study entitled 'Growth and physiology of *Isachne miliacea* Roth. in different soil types and its sensitivity to common herbicides' was conducted in the Department of Plant Physiology, College of Horticulture, Vellanikkara, during the year 2014 - 2015. The details of materials used and methods adopted are presented in this chapter.

3.1 General details

3.1.1 Location

The study was conducted at the College of Horticulture, Vellanikkara. The geographical co-ordinates of the location of the College are $10^0$32' N and $76^0$16 E with an altitude of 22.5 m above Mean Sea Level (MSL).

3.2 Experimental details

3.2.1 Experiment I.

Phenology and morphology of *Isachne miliacea* (Pot culture)

Growth and phenology of *Isachne miliacea* were studied in pot culture with soil collected from five different rice growing regions *viz.* Onattukara, Kole, Kuttanad, Pokkali and Palakkad. Ten kg soil each was filled in plastic boxes of size 50 x 25 cm and five replications were maintained for each soil type. Ten seeds were sown in each box and the germination was noted. At two leaf stage when the seedlings were identifiable, a single seedling was retained in each box and the excess seedlings were removed. It was irrigated regularly, the different phenophases and the morphological attributes were observed at fifteen days interval.

Design: CRD, No of treatments – 5, No. of replications – 5

3.2.1.1 Observations

Phenological observations

3.2.1.1.1 Phenophases of plant

The phenophases of the plant for different soil types were noted as below:

i) Days to seed germination: The number of days counted from the date of sowing to the date of germination in each replication and the values expressed as days for seed germination.

ii) Days to tillering: The number of days taken from sowing to first tillering stage was recorded in each replication and the values expressed as days for tillering.

iii) Days to flowering: The number of days taken from sowing to panicle emergence was counted in each replication and the values expressed as days to flowering.

iv) Days to seed formation: The number of days taken from sowing to the appearance of globular seeds at the lower rachis was recorded in each replication and the values expressed as days for seed formation.

v) Days to seed maturation: The number of days taken from sowing to the date of seed maturation (seed colour turned from green to gray) was counted in each replication and the values expressed as days for seed maturation.

Morphological observations

3.2.1.1.2 Total shoot length of weed

From each replication three tillers were selected randomly and tagged to measure the shoot length of the plant. The shoot length was measured from ground level to tip of longest leaf of plant and expressed in centimetres at 15 days interval.

3.2.1.1.3 Number of leaves

The total number of leaves present on the plant was counted and recorded at 15 days interval.

3.2.1.1.4 Internodal length

From each replication three tillers were selected randomly and tagged to measure the internodal length of the plant. The length between two successive nodes was measured and expressed in centimetres at 15 days interval.

3.2.1.1.5 Number of panicles

The total number of panicles present on the plant was counted and recorded at 15 days interval.

3.2.1.1.6 Number of seeds produced

From each replication five panicles were selected randomly and seeds were counted. The mean value of total number of seeds produced per panicle from 45th day till maturity was recorded.

3.2.1.1.7 Fresh weight

The weeds were gently pulled out at 75 DAS and washed to remove loose soil, wiped gently with soft paper towel to remove free surface moisture, weighed immediately and the weight expressed in grams.

3.2.1.1.8 Dry weight

Five plants were selected from each replication and air dried for six hours and then in hot air oven maintained at 60°C for 48 h. They were then cooled in a desiccator for 45 minutes. The dry weight of plants was recorded in grams.

Biochemical observations

3.2.1.1.9 Chlorophyll content

Chlorophyll a, chlorophyll b and total chlorophyll content in leaves were estimated in the youngest fully expanded leaves by adopting Hiscox and Israelstam (1979) method and expressed as milligram g^{-1} fresh weight of plant tissue and the calculation was done by the following formulae.

Chlorophyll 'a' = [(12.7 x A663) – (2.69 x A645)] x V/1000 x W

Chlorophyll 'b'= [(22.9 x A645) – (4.68 x A663)] x V/1000 x W

Total chlorophyll = [(20.2 x A645) + (8.02 x A663)] x V/1000 x W

where,

A = Absorption at given wavelength

V = Total volume of sample in extraction medium

W = Weight of sample

Characteristics of different soil types

3.2.1.1.10 Soil pH

The pH of the soils was determined in a 1:2.5 soil water suspension, potentiometrically using a pH meter (Jackson, 1958). For this different soil types were dried and powderd. Ten gram of each soil sample was sieved through 2 mm sieve and placed in a 50 ml beaker. Twenty five ml distilled water was added to this and stirred for five minutes. After half an hour the pH of the supernatent solution was read using a pH meter (Mettler Toledo).

3.2.1.1.11 Electrical conductivity (EC) (dS/m)

Electrical conductivity of five different soil types was estimated in the supernatant liquid of the soil water suspension (1:2.5) used for pH estimation with the help of a conductivity meter (Model- Eutech CON 510) and expressed in dS/m (Jackson, 1958).

3.2.1.1.12 Organic carbon (%)

Organic carbon of the soil was estimated by wet digestion method (Walkley and Black, 1947).

3.2.1.1.13 Available phosphorus (kg/ha)

Available phosphorus in each soil samples were extracted using Bray No.1 reagent (Bray and Kurtz, 1945) and estimated colorimetrically by reduced molybdate and expressed as kg/ha.

3.2.1.1.14 Available potassium (kg/ha)

Available potassium in each soil sample were extracted using neutral normal ammonium acetate and its content in the extract was estimated by flame photometry (Jackson, 1958) (Model: Elico CL365) and expressed as kg/ha.

3.2.1.1.15. Available calcium and magnesium (mg/kg)

Available calcium and magnesium in each soil sample were extracted using neutral normal ammonium acetate and its content in the extract was estimated using Perkin Elmer Atomic Absorption Spectrophotometer (Model: Analyst 400) and expressed as Available Ca/Mg (mg kg^{-1} soil) = µg Ca/Mg mL^{-1} of the aliquot x 5

3.2.1.1.16 Available sulphur (mg/kg)

Available sulphur in each soil samples were extracted using Massoumi and Cornfield method (Massoumi and Cornfield, 1963) and expressed as mg/kg.

Amount of sulphur (mg kg^{-1} soil) = Concentration from the instrument x 25/10 x 50/10

3.2.1.1.17 Available iron, manganese, zinc and copper (mg/kg)

Available micronutrients in each soil sample were extracted using 0.1M HCl (Sims and Johnson, 1991). Four gram soil with 40 ml of 0.1M HCl was shaken for five minutes. It was filtered through Whatmann No.1 filter paper and the filtrate was collected and analysed for Fe, Cu, Mn and Zn using Atomic Absorption Spectrophotometer (Model: Perkin Elmer Analyst 400) and expressed as mg/kg.

3.2.1.1.18 Dehydrogenase enzyme activity of soil

One gram air dried soil was taken in an air- tight screw capped test tube. Added 0.2 ml of three per cent TTC (2, 3, 5- Triphenyl Tetrazolium Chloride) solution in each of the tubes to saturate the soil and 0.5 ml of one per cent glucose solution was added in each tube. The bottom of the tube was sealed with a tape to drive out the trapped oxygen and to form a water seal on the surface of the soil. The tubes were then incubated at 28 ± 0.5°C for 24 hr. After that 10 ml methanol was added and incubated for another six hrs. The clear pink supernatent content was filtered and decanted and read in a spectrophotometer at wavelength of 485 nm. The dehydrogenase activity was expressed as microgram of Triphenyl formazan (TPF) formed per gram of soil per hour (µg TPF g^{-1} soil h^{-1}).

3.2.2 Experiment II

Propagation studies

Propagation studies of *Isachne miliacea* was conducted using both seeds and stem cuttings. The influence of depth of burial, moisture level of soil, ambient temperature and light on the germination and growth of the weed was studied.

3.2.2.1 Depth of burial (Pot culture)

To study the effect of depth of burial on germination, 25 pots were half filled with soil and 25 seeds each were placed on the soil surface. The pots (five numbers for each treatment) were then filled with soil up to a height of 0 cm, 2 cm, 4 cm, 6 cm and 10 cm respectively and irrigated on alternate days. The germination per cent and dry weight were calculated after 15 days.

The experiment was repeated using single node stem cutting (2 cm).The establishment frequency and dry weight were calculated after 15 days.

Design: CRD, No of treatments - 5, No. of replications – 5

3.2.2.2 Effect of light (Pot culture)

To study the effect of light on germination shade nets which allowed 50% and 25 % light infiltration imparting 50% and 75% shade respectively were chosen for the study. 3x 1 m sized nets were tied and ten pots each were placed under the nets. The control consisted of ten pots placed in open condition. Twenty five seeds each were sown in the pots and the germination per cent noted after one week and dry weight was estimated for each set after 60 days.

The experiment was repeated with single node stem cuttings and establishment frequency was calculated after one week and dry weight was estimated for each set after 60 days.

Design: CRD, No. of treatments 3, No. of replication 10

3.2.2.3 Moisture level (Pot culture)

Thirty pots were filled with soil and 20 seeds were sown in each pot. Ten pots each were maintained in submerged condition (flooded, watered up to 2 cm level), at field capacity (saturated water condition) and under dry condition (no water)

throughout the growing period. The germination per cent, growth of the weed, and dry weight were estimated after 15 days. The experiment was repeated using single node stem cutting and establishment frequency and dry weight were calculated after 15 days.

Design: CRD. , No. of treatments – 3, No. of replication - 10

3.2.2.4 Effect of temperature (Laboratory study)

To study the effect of temperature on seed germination, 25 seeds each in four replications were placed in an incubator (BOD – ROTEK) at 15° C, 20° C, 25° C, 30° C and 40° C for 48 hr and the germination per cent was noted.

Design: CRD, No. of treatments – 5, No. of replications 5 Petri plates in each replication

3.2.2.2 Observations recorded

3.2.2.2.1 Germination per cent / Establishment frequency

For seed propagation germination per cent was calculated by the following formula,

Germination per cent = Number of seeds germinated / Total number of seeds x 100.

For vegetative propagation establishment frequency was calculated by the following formula,

Establishment frequency = Number of stem cuttings established / Total number of stem cuttings x 100

3.2.2.2.2 Dry weight

Plants were air dried first for six hours and kept in hot air oven maintained at 60°C for 48 h, then cooled in a desiccator for 45 minutes and the dry weight of plants was recorded in grams.

3.2.3 Experiment III

Herbicides sensitivity of weeds using bioassay techniques

The sensitivity of *Isachne miliacea* to pre- emergence, post emergence and non-traditional rice herbicides were tested using bioassay techniques.

3.2.3.1 Seed bioassay using pre emergence herbicides

Table 1. The recommended dose and quantity of pre emergence herbicides

Sl. No.	Herbicide	Recommended dose (kg ai/ha)	Quantity used in petriplate (ml)
1.	Butachlor	1.25	0.008
2.	Pretilachlor	0.50-0.75	0.004
3.	Oxyflourfen	0.15	0.0009
4.	Pendimethalin	1- 1.50	0.008

Design: CRD, No. of treatments - 4, No. of replication - 4

The study was conducted in the month of May, so that the dormancy period was over. Twenty five seeds of *Isachne miliacea* were used in each replication. Three sets of experiments were conducted in petriplates of area 63.5 cm^2, using Top of paper method (TP) and Between paper method (BP) described by ISTA (1996). In the first set, herbicide soaked filter paper was placed at the bottom of petri plates and seeds were placed on it (TP method). In the second set, the herbicide soaked filter paper was

placed on top of the seeds and another water soaked filter paper was placed at the bottom (BP method I). In the third set, seeds were placed in between two filter paper soaked with herbicides (BP method II). Germination count was taken on the fifth and tenth day and expressed in percentage.

3.2.3.2 Whole plant bioassay using post emergence herbicides and non-traditional rice herbicides

Table 2. The recommended dose and quantity of post emergence herbicides

Sl. no.	Herbicide	Recommended dose (g ai/ha)	Quantity used in 0.16 sq.m area (g)
1.	Bispyribac sodium	25	0.0004
2.	Pyrazosulfuron ethyl	35	0.0005
3.	Azimsulfuron	35	0.0005
4.	Penoxsulam	25	0.0004
5.	Cyhalofop butyl	800 ml	0.013 ml
6.	Fenoxaprop p- ethyl	60	0.0001

Design: CRD. No. of treatments: 6 No. of replications: 4

Table 3. The recommended dose and quantity of non-traditional rice herbicides

Sl. No.	Herbicide	Recommended dose (kg ai/ha)	Quantity used in 0.16 sq.m (ml)
1.	Glyphosate	2	0.03
2.	Diuron	3	0.04
3.	Paraquat	3	0.04
4.	Glufosinate ammonium	2	0.03

Design: CRD No. of treatments – 4 No. of replication – 4

To test the efficacy of herbicides on *Isachne miliacea*, the weed was grown in mud pots of diameter 0.18 m. For each treatment four pots were arranged in an area of 0.16 sq.m and the water required for covering this area was calibrated using a hand sprayer. The quantity of herbicides required to cover 0.16 sq.m area was calculated. The weeds were allowed to spread in the pots. The herbicide was sprayed when the weeds were in the vegetative phase using a hand sprayer. Plants were classified as susceptible, moderately resistant and resistant to herbicides based on phytotoxicity symptoms (leaf scorching, leaf curling, tip burn, yellowing, dead plants and regrowth) 15 days after spraying.

3.3 Statistical analysis

Data were analysed using MSTATC package. Analysis of variance was done for each parameter under observation and critical difference (CD) computed.

Plate 1: *Isachne miliacea* Roth.

Plate 2: Infestation of *I. miliacea* in paddy field

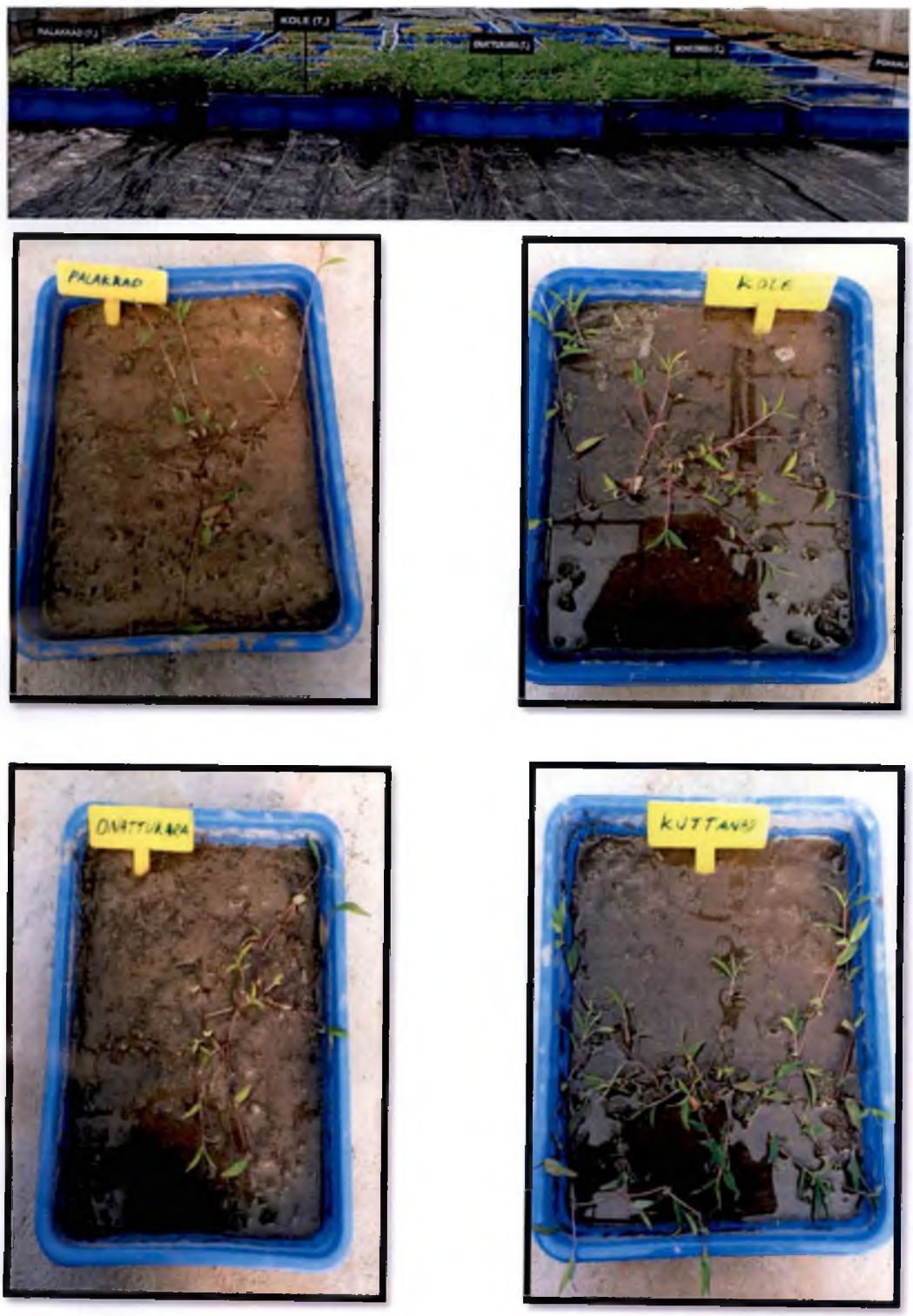


Plate 3: *I. miliacea* grown in different soil types

Plate 4: Testing of herbicide sensitivity using whole plant bioassay on *I. miliacea*

Plate 5: Spraying of post emergence herbicides on *I. miliacea* plant

Results

4. RESULTS

Results of growth, phenology, propagation and herbicidal sensitivity of *I. miliacea* are presented in this chapter. The data recorded were tabulated and analysed statistically. The results obtained are presented below. The mean data are presented in the relevant tables.

4.1 Experiment I (Phenology and morphology of *Isachne miliacea*)

The mean data of phenological observations recorded at 15 days interval during the growth period of the weed are given in Table 4 to 13.

An attempt has been made to study the physiology and growth habits of the weed in the different rice growing soils of Kerala *viz.*, Onattukara (T_1), Kole (T_2), Kuttanad (T_3) Palakkad (T_4) and Pokkali (T_5). Distinct variation was observed in the phenotypic characters of the plant.

4.1.1 Phenological observations

Table 4. Phenophases (Days) of *I. miliacea* grown in different soil types

Sl. no.	Phenophases	Onattukara	Kole	Kuttanad	Palakkad
1	Days to seed germination	8 – 10	8-12	5-8	12-15
2	Days to tillering	15-19	18-21	13-17	20-25
3	Days to flowering	36-52	38-55	28-60	41-57
4	Days to seed formation	40-62	42-65	33-68	50-62
5	Days to seed maturation	54-70	55-68	40-74	56-70

Soil type had an influence on the phenology of *I. miliacea*. Seed of *I. miliacea* grown in different soil types showed variation in days to seed germination. Due to high acidity and salinity seeds sown in Pokkali soils did not germinate. In Kuttanad soil seeds germinated within 5 to 8 days, while in Onattukara soil and Kole land soil germination started on the 8th day. Seed sown in Palakkad soil started germination by the 12th day.

Days to tillering also varied with the soil type. Earlier tillering was observed in seed sown in Kuttanad soil (13-17 days). Seedlings from Onattukara soil started tillering by 15-19 days, while those from Kole land soil reached this stage only by 18-21 days. Seedlings from Palakkad soil took 20-25 days for tillering.

Seedlings in Kuttanad soil started flowering by the 28th day and continued up to 60 days. In Onattukara and Kole land soil flowering started after 35th day and continued up to 55th day. Seedlings from Palakkad soil started flowering after 40 days and continued up to 57th day.

Seed formation and maturation varied with soil type. The plants dried after seed maturation. In Kuttanad soil the seed maturation phase started from the 33rd day and extended up to 68 days. The plant completed its life cycle by 74th day. In the plants grown in Onattukara and Kole land soils, seed formation was seen only after 40 days and by the 70th day the plants dried off. In Palakkad soils the seed formation was further delayed and was seen only by the 50th day and the plants lasted up to 70 days.

4.1.2 Morphological observations

4.1.2.1 Total shoot length of *I. miliacea*

Table 5. Total shoot length (cm) of *I. miliacea* in different soil types

Soil type	15 DAS	30 DAS	45 DAS	60 DAS	Maturity stage
Onattukara	14.00	27.60	34.40	39.80	43.00
Kole	13.00	28.40	37.60	38.00	40.00
Kuttanad	7.40	12.80	21.00	21.80	22.50
Palakkad	10.60	19.40	25.10	27.40	31.00
C.D	**1.98**	**13.03**	**3.74**	**3.23**	**3.84**
C.V (%)	**2.45**	**8.36**	**9.45**	**7.59**	**8.02**

There was significant difference between treatments in the total shoot length of *I. miliacea* (Table 5). Length gradually increased from 15 DAS to maturity stage in every treatment. Highest spreading was noted in the plants grown in Onattukara soil (14 cm), it was on par with plants from Kole land soil (13 cm).

Among the treatments, length varied from 15 DAS to maturity stage in the range of 14.00 cm to 43.00 cm, 13.00 cm to 40.00 cm, 7.40 cm to 22.50 cm and 10.60 cm to 31.00 cm in *I. miliacea* grown in Onattukara, Kole, Kuttanad and Palakkad soils respectively, indicating that the spread of the weed was more in Onattukara and Kole land soils.

4.1.2.2 Total leaf number per plant

Table 6. Leaf number per plant of *I. miliacea* in different soil types

Soil type	15 DAS	30 DAS	45 DAS	60 DAS	Maturity stage
Onattukara	8.60 (3.01)	38.60 (6.24)	54.00 (7.38)	65.80 (8.14)	70.80 (8.19)
Kole	4.40 (2.20)	31.60 (5.65)	37.00 (6.12)	41.20 (6.45)	41.80 (6.48)
Kuttanad	12.00 (3.53)	47.80 (6.94)	65.20 (8.10)	73.00 (8.57)	77.00 (9.04)
Palakkad	4.40 (2.20)	30.20 (5.53)	35.40 (5.98)	41.60 (6.48)	46.60 (7.07)
C.D	**0.30**	**0.47**	**0.33**	**0.23**	**0.27**
C.V (%)	**17.21**	**11.61**	**3.93**	**2.44**	**2.48**

[Figures in brackets are square root transformed values]

The number of leaves per plant of *I. miliacea* (Table 6) at 15 DAS to maturity stage was significantly higher in the plants grown in Kuttanad soil [12.00 to 77.00], followed by the plants grown in Onattukara soil [8.60 to 70.80], while *I. miliacea* grown in Kole [4.40 to 41.20] and Palakkad [4.40 to 46.60] soils were on par. It was observed that from15 DAS to 30 DAS, there was drastic increase in number of leaves in every treatment.

4.1.2.3 Internodal length

Table 7. Internodal length (cm) of *I. miliacea* in different soil types

Soil type	15 DAS	30 DAS	45 DAS	60 DAS	Maturity stage
Onattukara	3.00	5.76	5.78	6.58	7.35
Kole	3.20	6.16	6.18	6.28	6.74
Kuttanad	1.70	3.08	4.18	4.64	5.28
Palakkad	3.70	7.12	8.04	8.60	9.20
C.D	**0.75**	**0.57**	**0.12**	**0.33**	**0.38**
C.V (%)	**19.08**	**7.80**	**6.70**	**3.78**	**3.75**

Internodal length of *I. miliacea* varied significant with soil type (Table 7). Plants from Kuttanad soil had the lowest internodal length throughout the growth intervals [1.70cm to 5.28 cm]. *I. miliacea* in Palakkad soil had significantly higher internodal length than those in other treatments [3.70 cm to 9.20 cm], while the plants from Kole land soil [3.20 cm to 6.74 cm] and those from Onattukara soil [3.00 cm to 7.35 cm] were on par. It was noted that during 15 DAS to 30 DAS, there was comparatively higher increase in internodal length. By the 45th day the plants entered the reproductive phase and during this phase the increase in the internodal length was meager.

4.1.2.4 Panicle number per plant

Table 8. Panicle number per plant of *I. miliacea* in different soil types

Soil type	45 DAS	60 DAS	Maturity stage
Onattukara	1.36	3.80	4.50
Kole	1.36	4.40	4.80
Kuttanad	3.20	7.20	10.0
Palakkad	1.29	2.40	2.90
C.D	**0.26**	**0.17**	**0.19**
C.V (%)	**13.11**	**6.30**	**9.75**

Panicle production started by the 45th day onwards in *I. miliacea* and the number was significantly different in different soil types (Table 8). Plants grown in Kuttanad soil had significantly higher panicle number than all other treatments. The panicle number per plant in all other soil types were on par at 45 DAS, but during maturity stage the panicle number was significantly lower in plants on Palakkad soil.

4.1.2.5 Seed number per panicle

Table 9. Seed number per panicle of *I. miliacea* in different soil types

Soil type	45 DAS	60 DAS	Maturity stage
Onattukara	31.00	51.20	62.00
Kole	32.20	52.80	64.50
Kuttanad	41.00	66.40	76.80
Palakkad	24.60	33.40	45.50
C.D	**0.26**	**0.17**	**0.19**
C.V (%)	**4.80**	**3.88**	**9.75**

The number of seeds per panicle in *I. miliacea* showed highly significant difference among the treatments (Table. 9); it was noted that, seed production was comparatively higher in plants from Kuttanad soil at 45 DAS to maturity stage (41.00

-76.80). The seed production per panicle in all other soil types was on par during 45 DAS, but during maturity phase the seed production was significantly lower in plants from Palakkad soil.

4.1.2.6. Fresh weight and dry weight

Table 10. Fresh weight and dry weight of *I. miliacea* at 75 DAS

Soil type	Fresh weight (g)	Dry weight (g)
Onattukara	122.00	17.00
Kole	130.00	21.42
Kuttanad	182.00	26.14
Palakkad	73.30	9.08
C.D	**29.80**	**18.74**
C.V (%)	**11.99**	**6.28**

There was significant difference between treatments in the fresh weight and dry weight of *I. miliacea* at 75 DAS (Table 10). The fresh weight and dry weight were the highest in the plants grown in Kuttanad soil (182.00 g and 26.14 g), followed by the plants from Kole land soil (130.00 g and 21.42 g) and Onattukara soil (122.00 g and 17.00 g). Lowest value was recorded for plants grown in Palakkad soil (73.30 g and 9.08 g).

4.1.3 Biochemical observations

4.1.3.1 Chlorophyll content

Table 11. Chlorophyll (mg g^{-1} fresh weight) content of *I. miliacea* in different soil types

DAS	Chlorophyll Content (mg/g)	Onattukara	Kole	Kuttanad	Palakkad	C.D	C.V (%)
15 DAS	Chl. A	0.84	1.38	0.78	1.21	**0.06**	**4.16**
	Chl. B	0.45	0.89	0.30	0.78	**0.06**	**5.93**
	Total Chl.	1.30	2.28	1.09	1.99	**0.09**	**3.54**
30 DAS	Chl. A	0.92	1.47	0.86	1.29	**0.11**	**8.32**
	Chl. B	0.58	1.14	0.36	0.85	**0.08**	**8.78**
	Total Chl.	1.50	2.61	1.22	2.14	**0.14**	**6.26**
45 DAS	Chl. A	1.11	2.18	0.88	1.45	**0.08**	**9.63**
	Chl. B	0.70	1.26	0.34	0.12	**0.11**	**4.53**
	Total Chl.	1.82	3.45	1.29	2.61	**0.11**	**6.26**
60 DAS	Chl. A	1.15	1.87	0.90	1.67	**0.08**	**5.16**
	Chl. B	0.70	1.21	0.41	0.91	**0.06**	**5.99**
	Total Chl.	1.84	3.09	1.21	2.59	**0.11**	**6.26**
Maturity stage	Chl. A	1.10	1.52	1.00	1.73	**0.07**	**5.09**
	Chl. B	0.80	1.19	0.46	0.96	**0.09**	**5.91**
	Total Chl.	1.82	2.71	1.27	2.58	**0.13**	**6.25**

In the case of chlorophyll content (Table 11), there was significant difference between the treatments. *I. miliacea* grown in Kole land soil had highest amount of chlorophyll 'a', chlorophyll 'b' and total chlorophyll throughout the growth period, it was in the range of 1.38, 0.89, and 2.28 mg g^{-1} fr.wt. respectively at 15 DAS; and 1.52, 1.19, 2.71 mg g^{-1} fr.wt. respectively at maturity stage. Chlorophyll content was significantly lower in the plants grown in Kuttanad soil, it was in the range of 0.78, 0.30 and 1.09 mg g^{-1} fr.wt. , and 1.00, 0.46 and 1.27 mg g^{-1} fr.wt. (Chlorophyll 'a', chlorophyll 'b' and total chlorophyll) at 15 DAS and maturity stage respectively.

4.1.3.3 Soil analysis

Table 12. Characteristics of different soil types

Parameters	Palakkad	Kuttanad	Kole	Onattukara	Pokkali
pH	6.2	5.0	5.5	5.0	3.6
Electrical conductivity (EC) (dS/m)	0.73	2.06	0.16	0.10	4.46
Macro nutrients					
Organic carbon (%)	0.60	3.50	2.07	0.98	0.68
Available Phosphorus (kg/ha)	70.28	372.00	91.36	96.00	135.16
Available Potassium (kg/ha)	270.00	310.24	135.20	122.32	468.80
Available Calcium (mg/kg)	95.00	280.25	376.00	184.00	84.20
Available Magnesium (mg/kg)	55.00	41.00	81.00	43.00	78.50
Available Sulphur (mg/kg)	105.97	247.26	127.00	133.25	555.50
Micronutrients					
Copper (mg/kg)	5.66	3.65	2.97	5.43	5.15
Iron (mg/kg)	12.00	9.31	17.90	10.18	8.59
Zinc (mg/kg)	1.36	2.99	1.73	1.63	1.00
Manganese (mg/kg)	1.76	10.00	6.60	6.55	13.90
Boron (mg/kg)	0.21	0.59	1.98	0.25	4.10

The soil characteristics were estimated and variation was observed in pH, EC, macro and micro nutrient status of the soil types (Table 12).

Pokkali soils were found to be highly acidic with pH level of 3.6, while in Kuttanad, Kole land and Onattukara soils were recorded the pH range recorded was between 5 .0 and 5.5. Palakkad soil had a pH value of 6.2.

The EC indicates the amount of soluble ions in soil, highest value was in Pokkali soil (4.46 dS/m) followed by Kuttanad soil (2.06 dS/m), Palakkad soil (0.73 dS/m), Kole land soil (0.16 dS/m) and Onattukara soil (0.10 dS/m).

Organic carbon content was highest for Kuttanad soil (3.50 %) followed by Kole (2.07 %),Onattukara (0.98 %), Pokkali (0.68 %) and Palakkad (0.60%) soils.

Available phosphorus was found to be high in Kuttanad soil (372.00 kg/ha) followed by Pokkali (135.16 kg/ha), Onattukara (96.00 kg/ha), Kole (91.36 kg/ha) and Palakkad (70.28 kg/ha) soils.

Available pottasium was high for the Pokkali soil (468.80 kg/ha) followed by Kuttanad (310.24 kg/ha), Palakkad (270.00 kg/ha), Kole (135.20 kg/ha) and Onattukara (122.32 kg/ha) soils.

Available calcium was high for Kole land soil (376.00 mg/kg) followed by Kuttanad (280.25 mg/kg), Onattukara (184.00 mg/kg), Palakkad (95.00 mg/kg) and Pokkali (84.20 mg/kg) soils.

Kole land soil was found to have the highest amount of available magnesium (81.00 mg/kg) followed by Pokkali (78.50 mg/kg), Palakkad (55.00 mg/kg), Onattukara (43.00 mg/kg) and Kuttanad (41.00 mg/kg) soils.

Pokkali soils were found to be high in available sulphur (555.50 mg/kg) followed by Kuttanad (247.26 mg/kg), Onattukara (133.25 mg/kg), Kole (127.00 mg/kg) and Palakkad (105.97 mg/kg) soils.

The amount of copper was almost similar in the soils of Palakkad (5.66 mg/kg), Onattukara (5.43 mg/kg) and Pokkali (5.15 mg/kg) followed by Kuttanad (3.65 mg/kg) and Kole (2.97 mg/kg) soils.

The amount of iron was high in Kole land soil (17.90 mg/kg), followed by Palakkad (12.00 mg/kg), Onattukara (10.18 mg/kg) Kuttanad (9.31 mg/kg) and Pokkali (8.59 mg/kg) soils.

Kuttanad soil recorded highest amount of zinc (2.99 mg/kg) followed by Kole (1.73 mg/kg), Onattukara (1.63 mg/kg), Palakkad (1.36 mg/kg) and Pokkali (1.00 mg/kg) soils.

The amount of manganese was high from Pokkali soil (13.90 mg/kg) followed by Kuttanad (10.00 mg/kg), Kole (6.60 mg/kg), Onattukara (6.55 mg/kg) and Palakkad (1.76 mg/kg) soils.

Pokkali soil was found to have the highest amount of boron (4.10 mg/kg) followed by Kole (1.98 mg/kg), Kuttanad (0.59 mg/kg), Onattukara (0.25 mg/kg) and Palakkad (0.21 mg/kg) soils.

4.1.3.1 Dehydrogenase activity

Table 13. Dehydrogenase activity of different soil types

Soil type	Dehydrogenase activity (µg TPF /h/g)
Onattukara	0.006
Kole	0.007
Kuttanad	0.011
Palakkad	0.004
Pokkali	0.003
C.D	**0.003**
C.V (%)	**18.94**

The dehydrogenase enzyme activity of the different soil types were studied and the results showed that there was significant variation in the enzyme activity for the different soil types (Table 13). Kuttanad soil had the highest dehydrogenase activity (0.011 µg TPF /h/g). While the activity of Kole land and Onattukara soils were on par. The activity of Palakkad (0.004 µg TPF /h/g) and Pokkali (0.003 µg TPF /h/g) soils were lowest and on par.

4.2 Experiment II Propagation of *I. miliacea*

Propagation of *I. miliacea* using both seeds and stem cuttings were studied and the factors influencing propagation such as depth of burial, moisture level, temperature and light were observed. The results are given in table 14 to 20.

4.2.1 Depth of burial (seed propagation)

Table. 14 Effect of depth of burial on seed propagation of *I. miliacea*

Treatment	Germination per cent	Dry weight at 15 DAS (g)
Surface	100.00	6.80
2 cm	42.00	4.47
4 cm	22.00	2.69
6 cm	15.00	1.60
10 cm	0.00	0.00
C.D	**12.02**	**0.51**
C.V (%)	**18.23**	**13.05**

The pot culture experiment showed that germination of *I. miliacea* in different sowing depths significantly influenced the germination per cent and biomass of the plants (Table. 14).When the seeds were on the soil surface all the seeds germinated (100%) while only 42 % of the seeds germinated when placed at a depth of 2 cm. The seeds placed at 5cm depth had 22 %, germination, followed by 6 cm depth (15%). None of the seeds placed at 10 cm depth germinated. It was concluded that when the depth increased, the germination per cent reduced significantly. Depth of burial had a

significant effect on the dry weight production of the plant as the plant biomass reduced significantly with depth. Seeds in the soil surface had the highest dry weight of 6.80 g followed by 2 cm depth (4.47 g), 4 cm depth (2.69 g) and 6cm depth (1.60 g).

4.2.2 Depth of burial (vegetative propagation)

Table 15. Effect of depth of burial on vegetative propagation (stem cutting) of *I. miliacea*

Treatment	Establishment frequency	Dry weight at 15 DAP (g)
Surface	100.00	4.87
2 cm	32.46	2.22
4 cm	23.47	1.78
6 cm	7.76	1.45
10 cm	0.00	0.00
C.D	**11.20**	**0.30**
C.V (%)	**15.53**	**10.55**

The experiment was repeated in the same way with stem cutting to understand the effect of depth of burial in the vegetative propagation potential of the plant (Table.15). The highest establishment frequency was noted for the cuttings on the surface (100%).When the stem cutting were placed at 2 cm depth, there was significant reduction in establishment frequency (32.46%), which further reduced when the depth increased to 4 cm (23.47%), followed by 6 cm depth (7.76 %). None of the stem cuttings placed at 10 cm depth could establish.

The biomass (g) production potential of the plant also varied significantly with depth. It was significantly higher on soil surface (4.87 g), followed by 2 cm depth (2.22 g), 4 cm depth (1.78 g) and 6 cm depth (1.45 g).

4.2.3 Effect of light (seed propagation)

Table 16. Effect of light on seed propagation of *I. miliacea*

Treatment	Germination per cent	Dry weight at 60 DAS (g)
Open	100.00	18.57
50 % shade	32.00	8.15
75% shade	14.00	4.17
C.D	**9.47**	**0.81**
C.V (%)	**19.00**	**18.14**

The study showed that light has a significant influence on the germination of *I. miliacea* (Table. 16). Pots placed in open condition had the highest germination (100%). When they are placed under 50 % shade the germination per cent was only 32 % and the treatment under 75 % shade net showed only 14 % germination.

The dry weight was also significantly higher in open condition (18.57 g), followed by 50% shade (8.15 g) and 75 % shade (4.17 g).

4.2.4 Effect of light (vegetative propagation)

Table 17. Effect of light on vegetative propagation (stem cutting) of *Isachne miliacea*

Treatment	Establishment frequency	Dry weight at 60 DAP (g)
Open	100.00	9.86
50 % shade	20.00	4.53
75% shade	7.50	2.41
C.D	**1.14**	**0.09**
C.V (%)	**16.08**	**4.32**

Similar experiment was also conducted using vegetative propagule (stem cutting) both in open condition and under shade net (Table. 17). There was significant

variation in both establishment frequency and dry weight production. In open condition there was higher establishment frequency (100%) and dry weight production (9.86 g), followed by 50 % shade (20 % and 4.53 g) and in 75 % shade (7.50% and 2.41 g).

4.2.5 Effect of moisture (seed propagation)

Table. 18 Effect of moisture on seed propagation of *I. miliacea*

Treatment	Germination per cent	Dry weight at 15 DAS (g)
Dry condition	0.00	0.00
Field capacity	80.00	3.73
Flooded condition	0.00	0.00

The pot culture experiment showed that there was significant difference in the growth of *I. miliacea* under dry condition, field capacity and in flooded condition (Table. 18). It was also recorded that there was higher germination per cent (80%) and dry weight (3.73 g) at field capacity as it was the ideal condition for germination, while in the case of dry condition and flooded condition the seeds did not germinate. Lack of water and excess water both inhibited the germination of *I. miliacea*.

4.2.6 Effect of moisture (vegetative propagation)

Table. 19 Effect of moisture on vegetative propagation (stem cutting) of *I. miliacea*

Treatment	Establishment frequency	Dry weight at 15 DAP (g)
Dry condition	0.00	0.00
Field capacity	20.00	1.52
Flooded condition	70.00	2.84

Propagation potential of stem cutting of *I. miliacea* was also studied under different moisture regimes and the results were different from seed propagation (Table.

19). Here flooded condition gave more establishment frequency (70%) than field capacity (20 %). And in dry condition there was no establishment.

4.2.7 Effect of temperature

Table 20. Effect of temperature on seed propagation of *I. miliacea*

Treatment (°C)	Germination per cent
15	22
20	56
25	72
30	54
40	20
C.D	**1.20**
C.V (%)	**12.48**

The effect of temperature on germination of *I. miliacea* seeds was studied under laboratory condition in petriplates. The result indicated that temperature had significant influence on germination of the seeds (Table 20). Highest germination was observed at 25°C (72 %) followed by 20 °C (56%), 30 °C (54 %). The lowest germination of 20 % and 22 % were observed at 40 °C and 15 °C respectively

4.3 Experiment III. Herbicides sensitivity on weeds using bioassay techniques

Table 21. Seed bioassay using pre emergence herbicides

Seed germination per cent				
Sl. no.	**Herbicides**	**Top paper TP**	**Between paper BP I**	**Between paper BP II**
1.	**Control (T_1)**	47.5 [e]	42.5 [d]	50.0 [b]
2.	**Pretilachlor (T_2)**	7.5 [b]	5.0 [b]	0.0 [a]
3.	**Oxyfluorfen (T_3)**	10.0 [c]	0.0 [a]	0.0 [a]
4	**Pendimethalin (T_4)**	22.5 [d]	32.5 [c]	0.0 [a]
5	**Butachlor (T_5)**	0.0 [a]	0.0 [a]	0.0 [a]

[Statistical analysis done using Friedman test, the alphabets denote the effectiveness of the herbicide within the column]

Three different techniques were used for evaluating the bio efficacy of herbicides on germinating seeds of *I. miliacea*. All the herbicides tested gave excellent control in BP method II. The seed of *I. miliacea* showed zero germination for all the four herbicides tested by this method. Butachlor gave the best result in all the three methods. Oxyfluorfen inhibited seed germination in both BP methods I and II. Pretilachlor was the next best herbicide here only 5% germination was observed for BP method I. Pendimethaline alone gave better results (22.5 %) for TP method rather than BP method I (32.5 %).

Table. 22 Whole plant bioassay using post emergence herbicides

Sl. no.	Herbicides	Mode of Action	2 DAS	4 DAS	8 DAS	15 DAS
1.	Bispyribac sodium	Inhibition of ALS	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted, Regrowth
2.	Pyrazosulfur on ethyl	Inhibition of ALS	No discolouration	No discolouration	Leaf discolouration	Not wilted
3.	Azimsulfuron	Inhibition of ALS	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted
4.	Penoxsulam	Inhibition of ALS	No discolouration	Leaf scorching	Reddish colour at the tip of leaves, wilting started	Regrowth observed
5.	Cyhalofop butyl	Inhibition of ACCase	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted
6.	Fenoxaprop p- ethyl	Inhibition of ACCase	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted

[DAS – Days After Spraying, ALS - Aceto Lactate synthase, ACCase - Acetyl CoA Carboxylase

To understand the bioefficacy of post emergence herbicides on the control of *I. miliacea*, six post emergence herbicides, which are currently popular for rice weed control were tested. Among these the best results were obtained for azimsulfuron (ALS- inhibiting herbicides), cyhalofop butyl and fenoxaprop p- ethyl (ACCase inhibiting herbicides-) which showed visible symptoms (leaf discolouration) by 4th DAS and the leaves turned reddish in colour by the 8th DAS and completely wilted by 15th day. In the case of pyrazosulfuron ethyl (ALS inhibitor) there was no effect on *I. miliacea*. Penoxsulam and bispyribac sodium (ALS- inhibiting herbicides), sprayed *I. miliacea* plants showed wilting symptoms on 8 DAS, but regrowth was observed 2 weeks later.

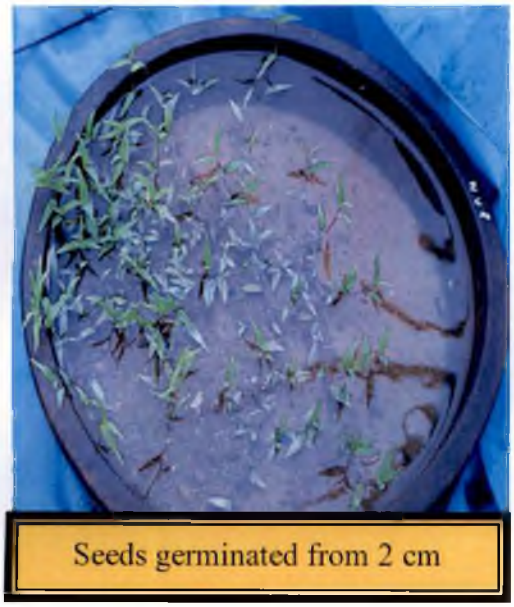
Table 23. Whole plant bioassay using non-traditional rice herbicides

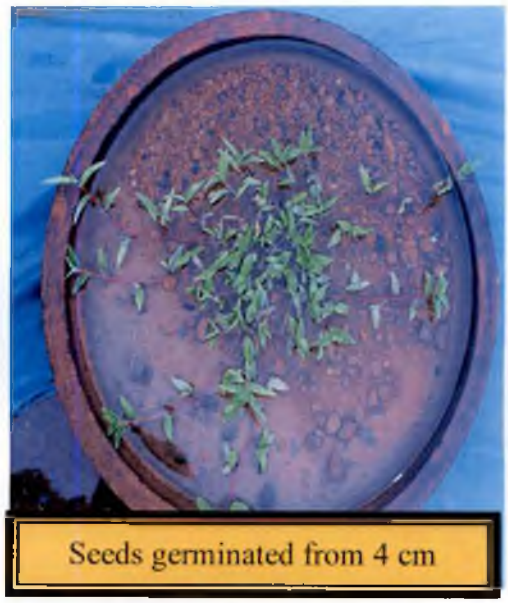
Sl. no	Herbicides	Mode of Action	2 DAS	4 DAS	8 DAS	15 DAS
1.	Glyphosate	Inhibition of amino acids	Leaf discolouration	Leaf tip and shoot tip burned	Wilting started	Permanently wilted
2.	Diuron	Inhibition of Photosystem-I	Leaf discolouration	Leaf scorching	Wilting started	Permanently wilted
3.	Paraquat	Inhibition of Photosystem-I	Wilting Started	Permanently wilted	Permanently wilted	Permanently wilted
4.	Glufosinate ammonium	Inhibition of glutamine synthetase	Leaf scorching	Leaf chlorosis	Leaf necrosis, wilting started	Permanently wilted

[DAS – Days After Spraying]

Non-traditional rice herbicides were the most effective herbicides for the control of *I. miliacea.* Leaf discolouration started by the 2nd day in glyphosate and diuron. By the 4th day, leaf showed scorched symptoms. By the 8th day wilting started and by the 15th day they were completely wilted. Paraquat being a contact herbicide, wilting started on 2nd day itself, and on 4th day the plant dried completely.

Glufosinate ammonium sprayed *I. miliacea* plants showed leaf scorching on 2 DAS, leaf scorching on 4 DAS, leaf necrosis and wilting symptoms on 8 DAS and permanently wilted on 15 DAS.





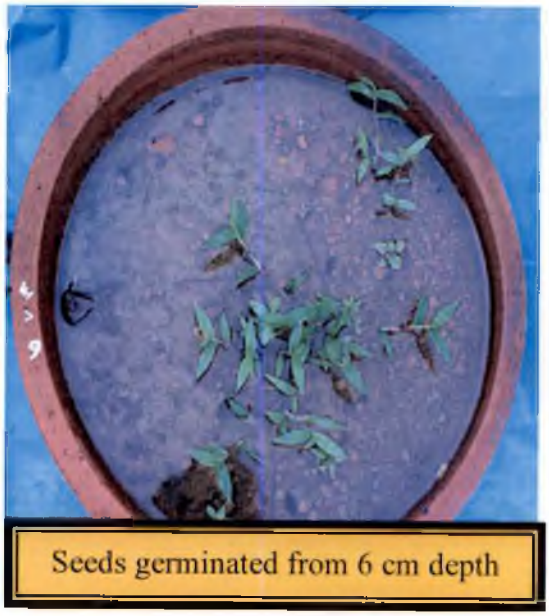


Plate 6: Effect of depth of burial on seed propagation of *I. miliacea*

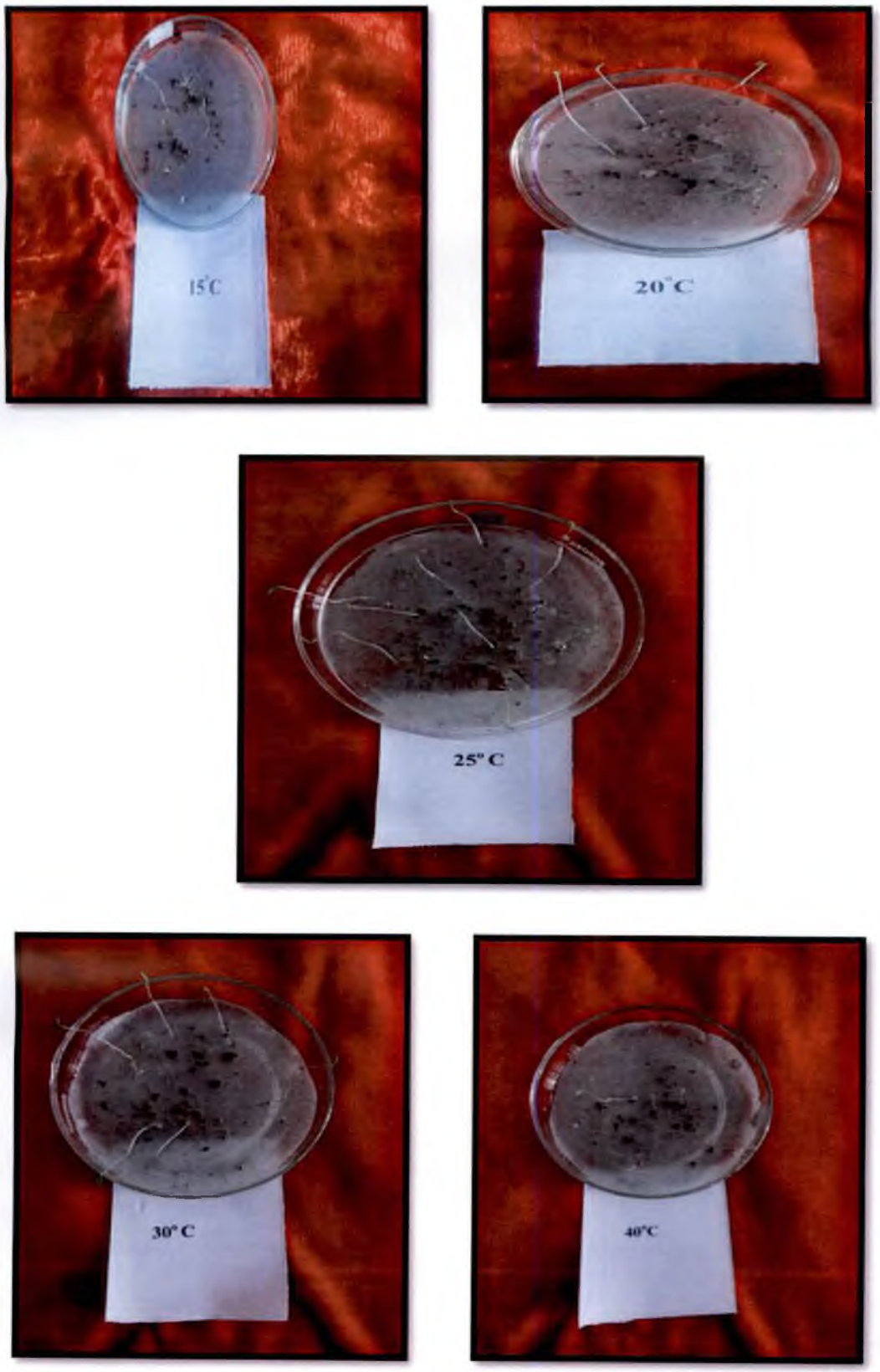


Plate 7: Effect of temperature on seed propagation of *I. miliacea*

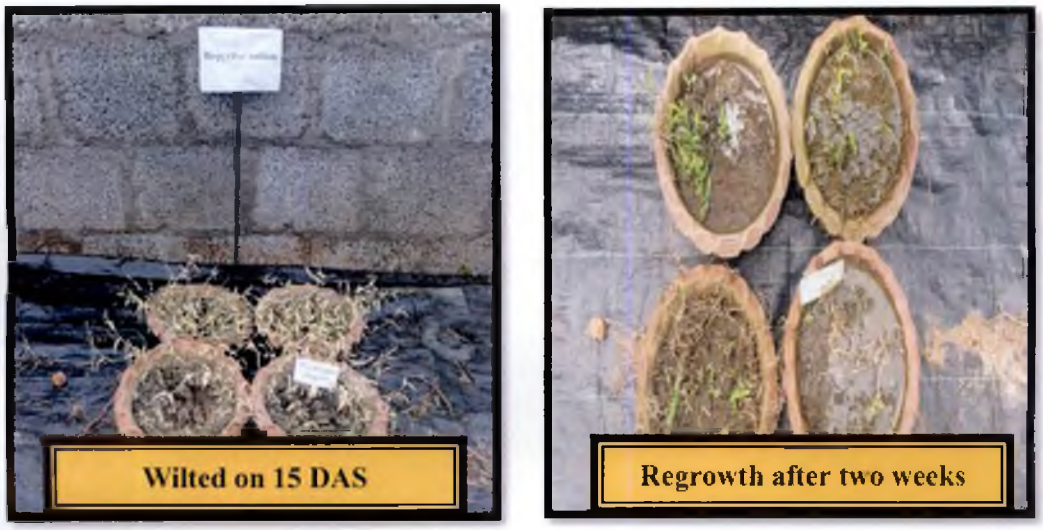


Plate 8: Response of *I. miliacea* plants to bispyribac sodium

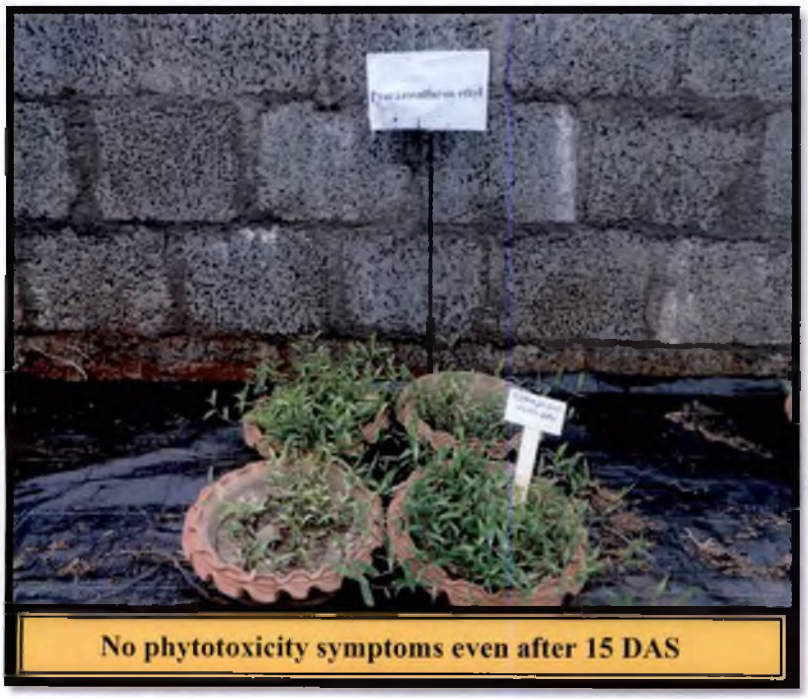


Plate 9: Response of *I. miliacea* plants to pyrazosulfuron ethyl

Plate 10: Response of *I. miliacea* plants to penoxsulam

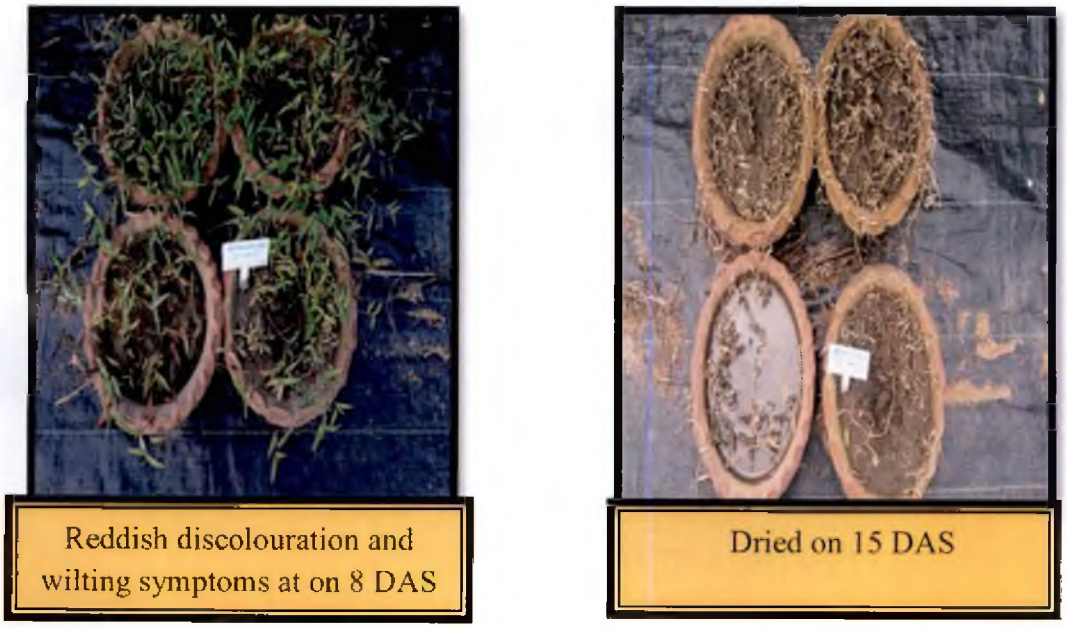
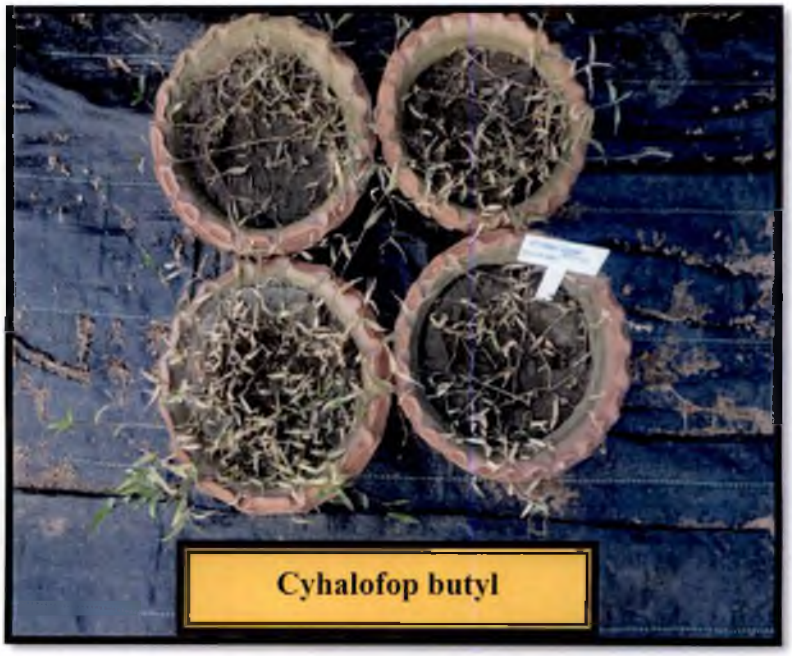


Plate 11: Response of *I. miliacea* plants to azimsulfuron



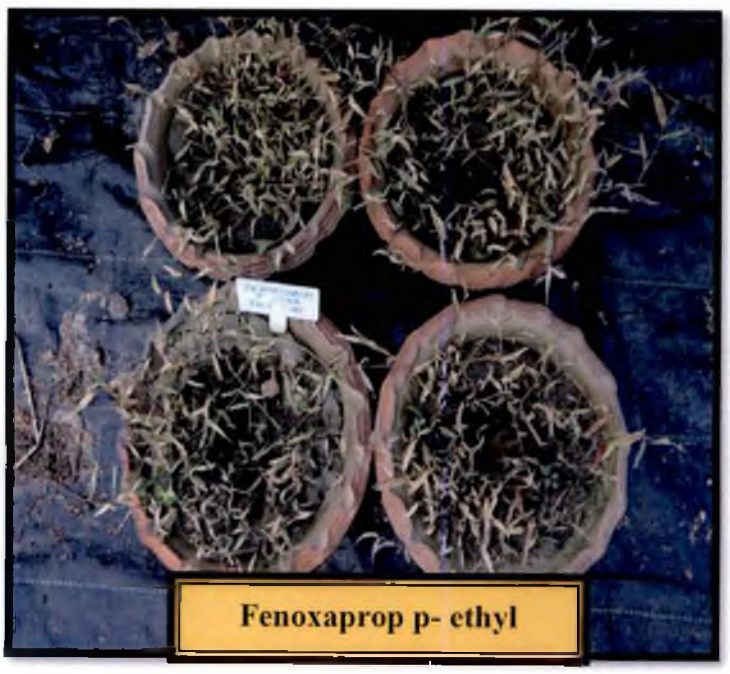


Plate 12: Response of *I. miliacea* plants towards cyhalofop butyl and fenoxaprop p-ethyl

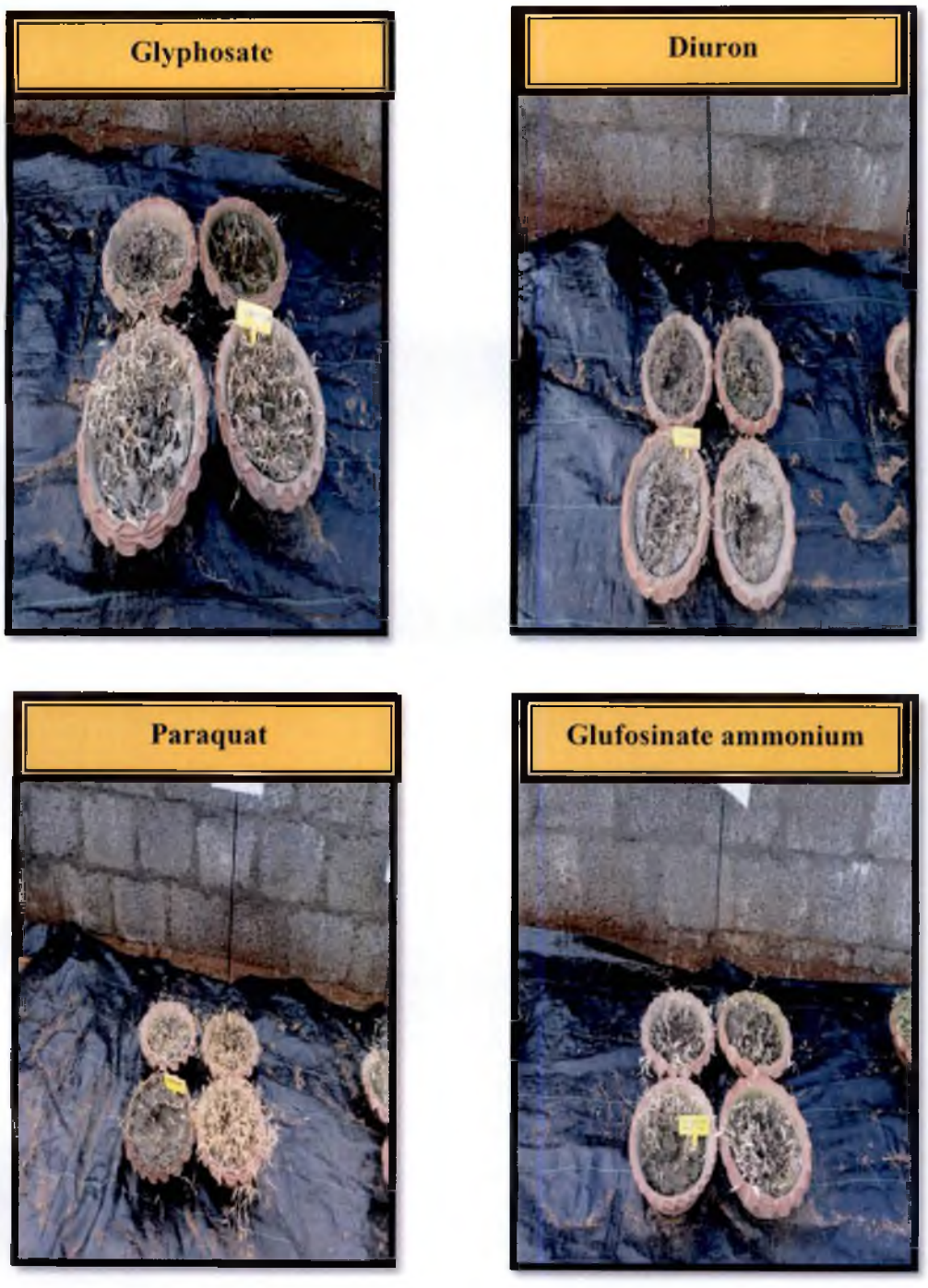


Plate 13: Response of *I. miliacea* plants to non- traditional rice herbicides

Discussion

5. DISCUSSION

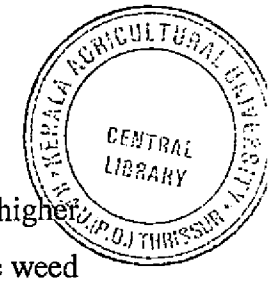
The present investigations were carried out to study the phenology, morphology and propagation of *I. miliacea* and to test the sensitivity of the weed to the herbicides commonly used in the state. The results obtained are discussed in this chapter.

5.1 Phenology and morphology of*Isachne miliacea*

Phenological studies conducted with *I. miliaceae* in different rice growing soils of Kerala revealed that the days required to attain different phenophases varied with soil type. The growth of the weed was faster and the lifespan of the weed was longer in Kuttanad soil (Table 4). The phenophases of the weed were almost similar in Kole and Onattukara soil while growth and development was delayed in Palakkad soils indicating that soil type had a profound influence on the phenology of the plant.

The variation of *I. miliacea* plants in different soil types can be attributed to its phenotypic plasticity. According to Pigliucci (2003) phenotypic plasticity is the property of a given genotype to produce different physiological or morphological phenotypes in response to different environmental conditions. Delucia *et al.* (1989) have reported that soil type can also influence phenotypic plasticity in plants.

The morphological characters such as shoot length, leaf number, internodal length, panicle number, seed number and biomass of the weed also varied significantly with soil type. The variation in shoot length (Table 5.) was almost double (22.50 cm to 43.00cm). Sajith *et al.* (2013) has reported that the shoot length of *I. miliaceae* varied from 23.40 cm to 25.60 cm in the wet lands of Vellayani, Kerala. They also found that shoot length was significantly influenced by nutrient management and plant density in the rice fields. In the current study plants were grown in the soil without any competition to estimate the growth potential of the plant. This might have contributed to higher shoot length in Kole land and Onattukara soils.



Leaf number, panicle number and seeds per panicle were significantly higher for plants grown in Kuttanad soil and the lowest values were recorded for the weed grown in Palakkad soil. This indicates that the seed production potential and growth rate is higher in Kuttanad soil followed by soils of Onattukara and Kole lands which were on par in most of the characters. The reason might be due to the higher nutrient status and organic carbon content of Kutannad soils. Lechowicz *et al.* (1988) found that different edaphic factors were correlated with performance for two vegetatively similar annual species of *Impatiens*. In *I. capensis,* growth and reproductive characters were highest in areas where potassium, phosphorus and organic content were in the highest level. In the present study the growth and reproductive characters of *I. miliacea* were high in Kuttanad soil, where the potassium, phosphorus and organic content was higher compared to other soils. As the nutrient status of Onattukara and Kole land soils were similar (potassium, phosphorus, magnesium, zinc, manganese and sulphur), the performance of the weed in those two soil types were also on par for many of the morphological characters (Table 5, 7, 8,9,10 and 11, Fig. 1 and 3).

I. miliacea grown in Kole land soil had the highest chlorophyll content throughout the growing period (Table 12). This may be due to higher magnesium (Mg) and iron (Fe) content of the Kole land soil. Huner and Hopkins (1998) have reported that Mg and Fe play an essential role in chlorophyll formation and photosynthesis.

The organic carbon content of the soil varied from 3.50 % in Kuttanad soil to 0.60 % in Palakkad soil (Table 13). The organic carbon content of the soil had a direct influence on the growth and phenology of *I. miliacea*. Soil organic carbon is the basis of soil fertility, as it releases nutrients for plant growth, biological and physical health of soil and is a buffer against harmful substances (Salazar *et al.*, 2011). The response of the weed to organic carbon content is evident from the study. The biomass production and fecundity of the weed was highest in Kuttanad soil (fig.4 and 5), which may be attributed to the high organic carbon content of the soil. Palakkad soil had the lowest organic carbon content and low growth and

establishment of the weed. The influence of soil organic content on the enhancement of weed growth has been reported by Emmanuel (2015) in the case of *Tithonia diversifolia.*

The dehydrogenase enzyme activity of the soil is an indicator of soil health (Burns, 1978). It is related with the soil organic carbon content. As per Yuan and Yue (2012), the higher organic carbon content in the soil will provide a substrate to support higher microbial biomass which is indicated by higher dehydrogenase activity. The dehydrogenase activity of soil has a direct relationship with plant reproductive growth (Moeskops, 2010). In the present investigation the reproductive growth of *I. miliacea* in different soil types seems to have a direct relationship with dehydrogenase enzyme activity (Table 14 and fig.6).

5.2 Propagation of *Isachne miliacea*

The weed *I. miliacea* can be propagated both by seeds and stem cuttings. The seeds show dormany and maximum germination is obtained during the month of May (Varghese, 1996). In the present study the influence of different abiotic factors on the propagation of seeds and stem cuttings was analysed.

Experiment conducted to study effect of depth of seed burial on the germination of the weed revealed that when the depth of seed burial increased from 0 to 6 cm the germination per cent declined from100 to 15 % .The establishment frequency of the stem cuttings was reduced to 7.76% for the same depth (Table 14 and 15). Similar results were reported by Budd *et al.* (1997) for *Commelina benghalensis,* where the seed germination per cent decreased from 19.5 % to 0.5 % from surface to eight cm depth. The present result was further confirmed with the observation of Budd *et al.* (1999) and Reissig *et al.* (1999) in stem cuttings of *Commelina benghalensis* and *Paspalum distichum* respectively. Smith and Fox (1993) and Vamadevan *et al.* (1994) also reported that the biomass and number of weeds decreased with increase in depth of burial.

Fig.1 Total shoot length of *I. miliacea* in different soil types (cm)

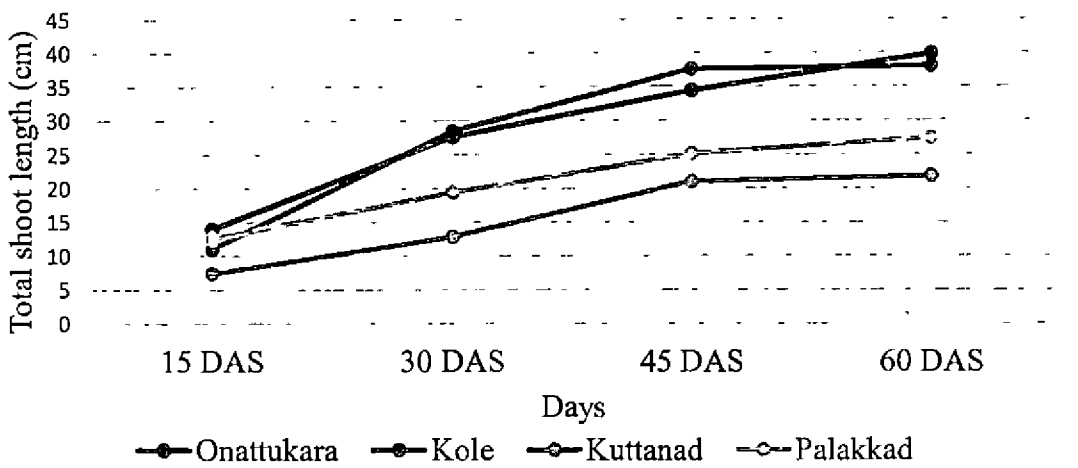


Fig. 2 Leaf number of *I. miliacea* in different soil types

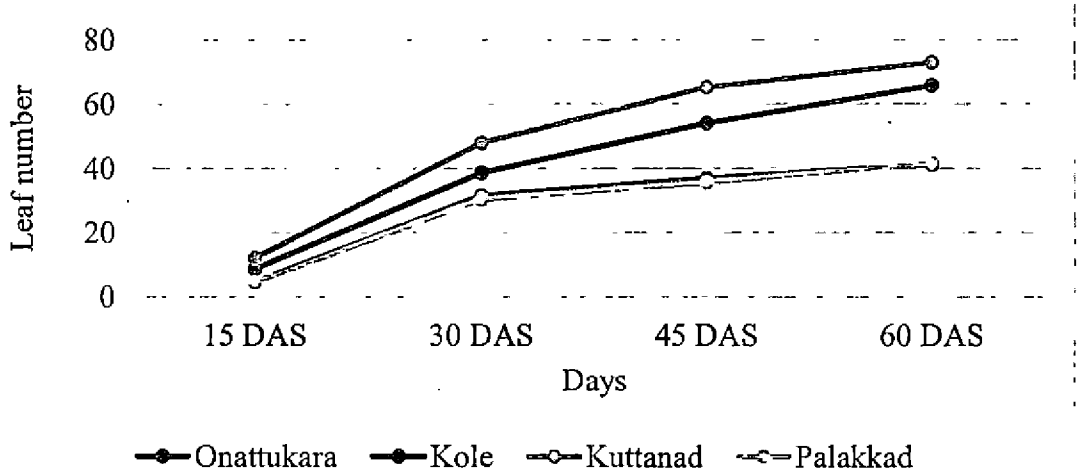


Fig.3 Internodal length of *I. miliacea* in different soil types (cm)

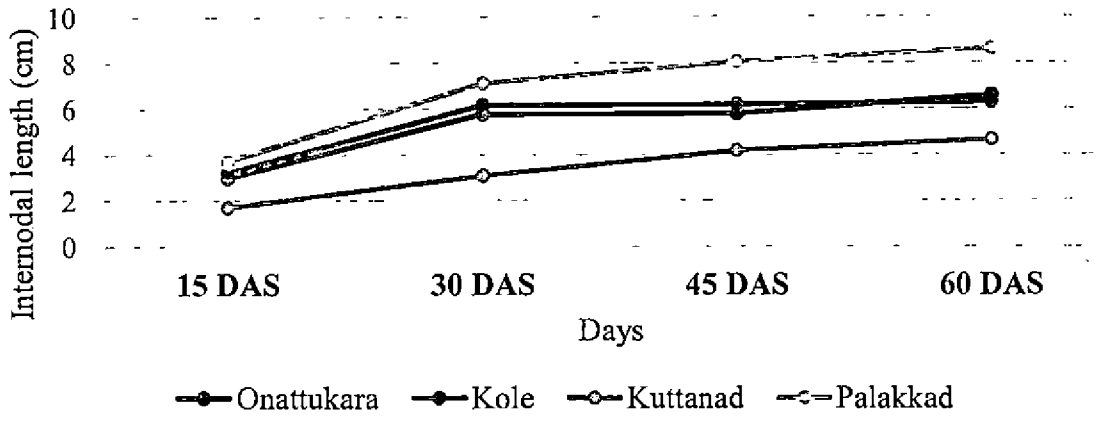


Fig. 4 Panicle number per plant of *I. miliacea* in different soil types

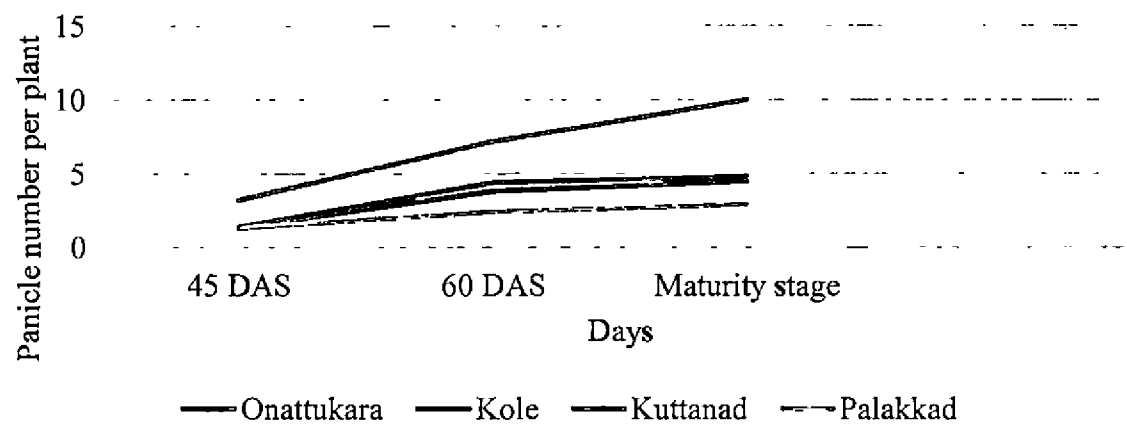
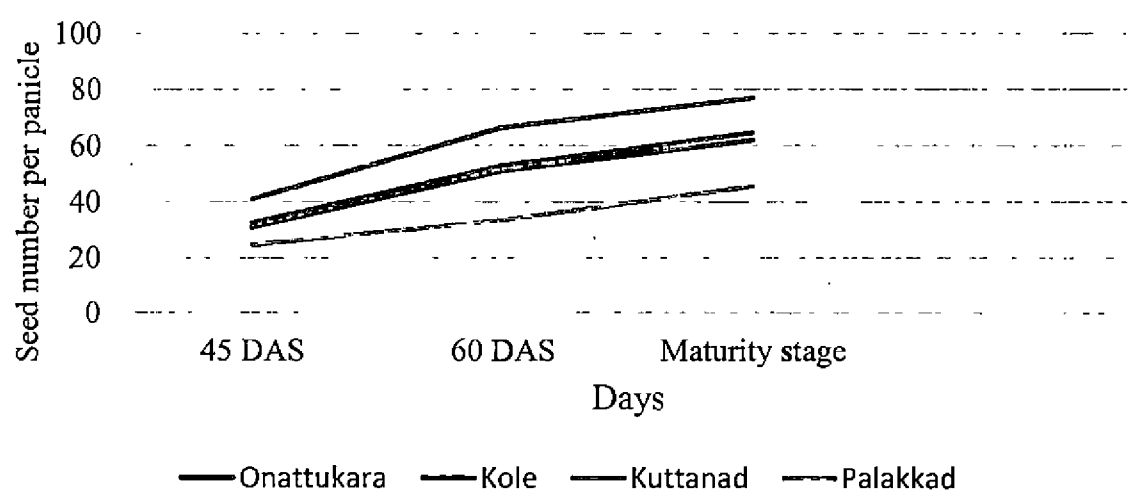


Fig. 5 Seed number per panicle of *I. miliacea* in different soil types



Chauhan and Johnson (2012) have reported that smaller sized seeds germinate better from the soil surface and the ability of seedlings to emerge from deeper depths depends on the energy reserves in the seeds; larger weed seeds with greater reserves can support seedling emergence from deeper depths better than small weed seeds. *I. miliacea* has very small sized seeds and this might be the reason for the lower germination percentage when the seed is buried deeper.

The effect of light on the propagation of both seeds and stem cutting in *I. miliacea* varied significantly (Table 16 and 17, Fig. 7 and 8). In open condition there was higher germination per cent, establishment frequency and biomass production. Under 50 % shade, seed germination declined by 68% and was much lower (14 %) in 75 % shade. In the case of stem cuttings the weed biomass was lower in plants grown under shade than plants receiving direct sunlight. This result is in agreement with the results of earlier studies conducted by Hendrix (1995) in the grass weed *Ischaemum rugosum*. The study indicates that *I. miliacea* requires maximum sunlight for its germination and growth and cannot be categorised as a shade loving plant.

Soil moisture level influenced the propagation of *I. miliacea*. (Table 18 and 19). Water at field capacity was the ideal condition for the germination of the weed seeds. The highest germination percentage and biomass were observed at field capacity, while in the dry condition and flooded condition the seeds did not germinate, both lack of water and excess of water inhibited the germination of *I. miliacea*. However in the case of stem cuttings the establishment frequency was highest in flooded condition followed by field capacity. Previous studies conducted by Gill *et al.* (2005) in *Echinochloa crusgalli* have also shown that weed emergence was maximum at field capacity, and that five to six centimetre standing water for four weeks significantly reduced seedling emergence of the weed.

Temperature also had significant influence on germination of *I .miliacea* seeds (Table 20, Fig. 9). Highest germination was observed at 25°C followed by 20°C and 30°C. The lowest germination per cent were observed at 40°C and 15°C

Fig. 6 Dehydrogenase enzyme activity of different soil types

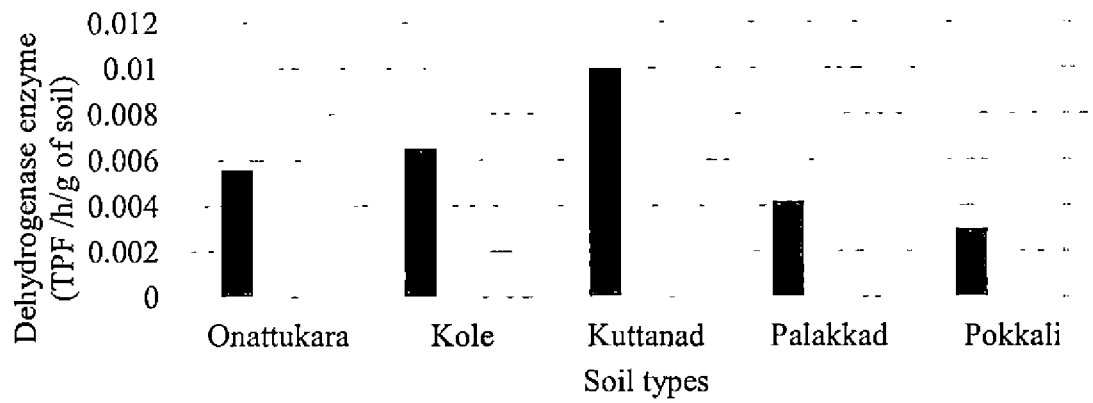


Fig. 7 Effect of light on seed propagation of *I. miliacea*

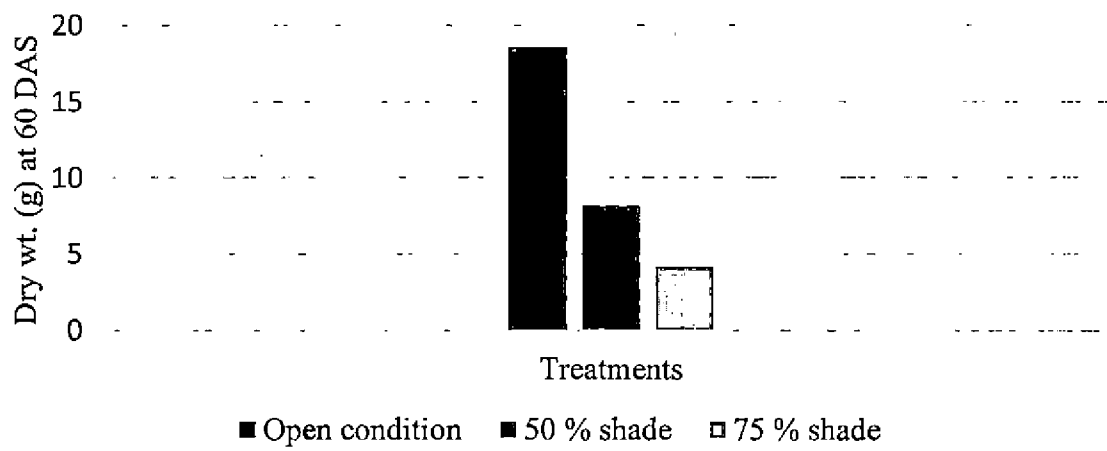


Fig. 8 Effect of light on stem cutting of *I. miliacea*

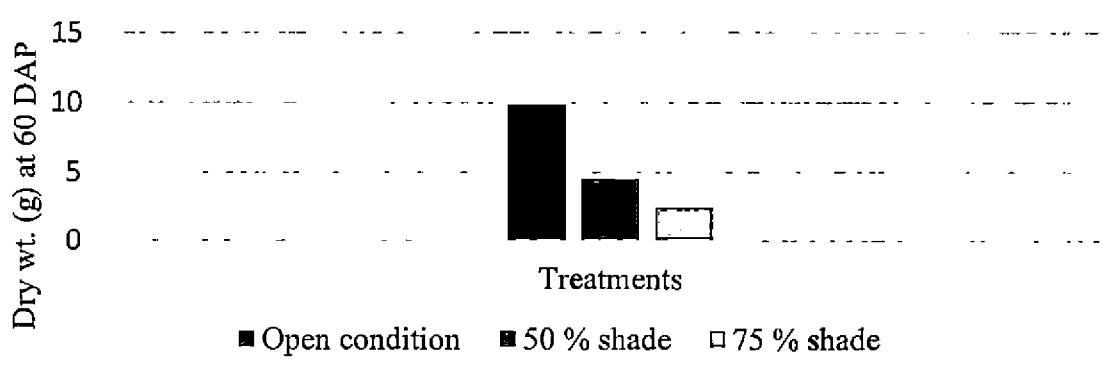


Fig. 9 Effect of temperature on seed propagation of *I. miliacea*

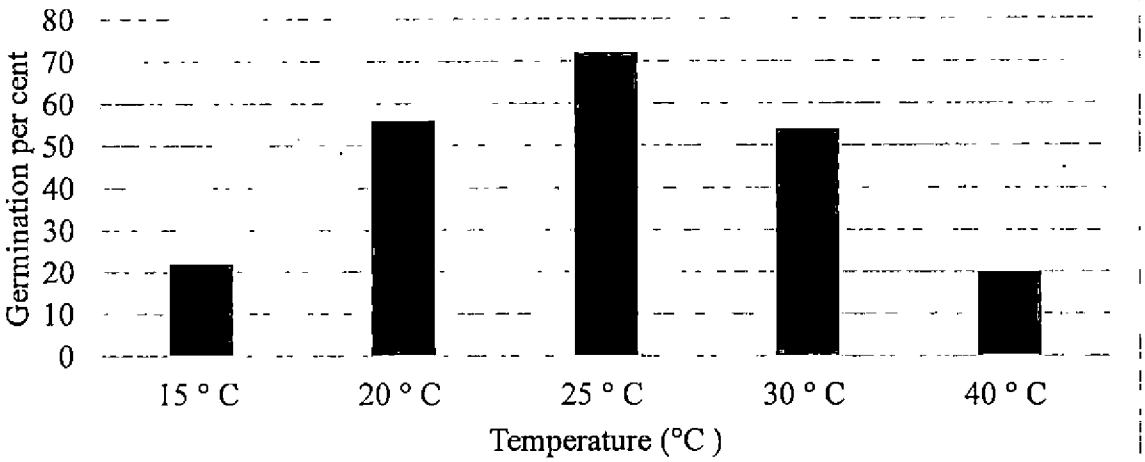
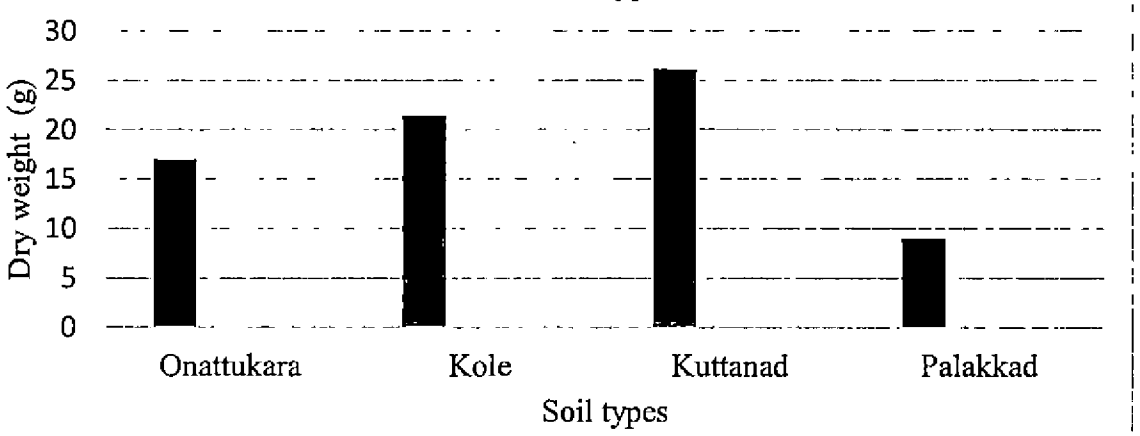


Fig. 10 Dry weight per plant of *I. miliacea* at 75 DAS from different soil types



respectively. According to Bewley and Black (1994) seeds have the capacity to germinate over a defined range which is characteristic for each species; hence, there are clear minimum and maximum temperatures for germination and between them, a broad range over which germination of all seeds can be attained.

5.3 Herbicides sensitivity on weeds using bioassay techniques

5.3.1 Seed bioassay using pre emergence herbicides

Evaluating *I. miliacea* to pre emergence herbicides using bioassay techniques showed that among the pre emergence herbicides butachlor and oxyfluorfen were most effective herbicides for the control of the weed (Table 21). Here zero percentage germination was observed when the herbicides soaked paper was placed on the top of the seed placed in petriplates, indicating that the herbicides acts on the germinating plumule. Chang *et al.* (1985) also reported that butachlor is a selective, systemic herbicide, absorbed primarily through germinating shoots and secondarily by roots.

The next best herbicides for the pre emergence control of *I. miliacea* was pretilachlor, which gave only 5 % germination. Among the pre emergence herbicides pendimethalin was the least effective where 32.5 % of the seeds germinated. Pendimethalin was the only herbicide which gave better control in TP method as compared to BP method I, this may be because pendimethalin acts a root inhibitor as it affects cell division and it is not translocated by xylem (Ross, 1994). Hence we can give preference may be given to butachlor and oxyfluorfen as pre emergence herbicides in the rice fields for the management of *Isachne miliacea.* These two herbicides have already been included in the package of practices (KAU, 2011) as pre emergence herbicides for weed control in paddy field.

5.3.2 Whole plant bioassay using post emergence herbicides

In whole plant bioassay technique, the best results were obtained for azimsulfuron (ALS inhibiting herbicide), cyhalofop butyl, and fenoxaprop-p- ethyl (ACCase inhibiting herbicides) (Table 22). Bura (2003) reported that azimsulfuron

is taken up mainly by leaves, shoots and to a lesser extent by roots, it is translocated via both xylem and phloem and inhibition of ALS leads to the cessation of cell division and subsequent growth processes in plants. Singh *et al.* (2004) and Kim *et al.* (2003) have earlier reported the effectiveness of fenoxaprop p-ethyl against *Leptochloa.* Saini (2003) noticed reduction of annual grasses by the application of cyhalofop-butyl in wet seeded rice.

Bipyribac sodium and Penoxsulam sprayed *I. miliacea* showed wilting symptoms at 8 DAS, but regrowth was seen after two weeks. Similar observations were reported in *Echinochloa colona* by Nady *et al.* (2012). They have suggested that the resistance of *Echinochloa colona* to bispyribac-sodium may be due to the faster metabolism of bispyribac-soduim below the physiologically active concentration or the insensitivity of its target enzyme (Acetolactate synthase). Yasuor *et al.* (2009) attributed that the resistance to penoxsulam in *Echinochloa phyllopogon* to the increased herbicide metabolism by Cytochrome P450 monooxygenase (CYP).

Pyrazosulfuron ethyl showed least effectiveness towards *I. miliacea* and there was no wilting symptom. *Cyperus difformis* resistance to pyrazosulfuron-ethyl was reported as the insensitivity of the ALS enzyme to the herbicide (Margo *et al.*, 2010). The same may be the reason for the resistance of *I. miliacea.*

The study clearly shows the effectiveness of azimsulfuron, cyhalofop butyl, and fenoxaprop-p- ethyl against *I. miliacea.* Any of these herbicides can be recommended in rice fields prone to infestation of this weed.

5.3.3 Whole plant bioassay using non-traditional rice herbicides

Non-traditional rice herbicides were the most effective against *I. miliacea.* Glyphosate is an aromatic aminoacid inhibitor. Symptoms include yellowing of new growth and death of treated plants in days to weeks (Ross, 1994).

Diuron, a broad spectrum herbicide, with residual activity in soil, primarily functions by inhibiting the Hill reaction in photosynthesis, limiting the production

of high-energy compounds such as adenosine triphosphate (ATP) used for various metabolic processes. Lipids and proteins are attacked and oxidized, resulting in loss of chlorophyll and carotenoids, and in leaky membranes which cause cells and cell organelles to dry and disintegrate rapidly (Hess and Warren, 2002).

Paraquat belongs to contact herbicide group and it causes rapid destruction of cell membranes, prevents translocation to other regions of the plant. Severe injury is evident hours after application, first as water-soaked areas which later turn yellow or brown. Maximum kill is attained in a week or less (Childs *et al.*, 1985).

Glufosinate-ammonium acts by inhibiting the enzyme glutamine synthetase in plants, leading to a complete breakdown of ammonia metabolism and consequent inhibition of photosynthesis (Kidd and James, 1991). Leaf chlorosis and necrosis follows and the plant dies in 1-2 weeks (KEMI, 2002).

Among these herbicides paraquat is banned in Kerala and glufosinate-ammonium is not readily available. Diuron is a selective herbicides used in pineapple and banana however, because of its long residual action it cannot be recommended in rice fields for pre plant application. Thus the choice for the farmers at present is only glyphosate. It can be recommended for control of existing *I. miliacea* in paddy fields before land preparation. The herbicide can be applied about a week before tillage operation, this will kill all the existing *I. miliacea.* Infestation through the seeds from the weed seed bank can be checked by spraying pre emergence herbicides like butachlor or oxyfluorfen to keep the field free of *I. miliacea* for a reasonable period of the crop.

Summary

6. SUMMARY

Isachne miliacea Roth. is one of the dominant weeds in the low land rice ecosystems of Kerala. Presently, the weed is reported to be spreading to other rice growing regions of the state. Currently weed shifts have become a common phenomenon in the rice ecosystems and this has been attributed to opportunistic germination, habit, fecundity and competitive ability of the weeds together with the natural resistance of some species to newer herbicides which are more specific in action. The prostrate nature of *I. miliacea* and its ability to germinate from both seed and stem cuttings might be factors that contribute to the fast spread of the weed in the rice ecosystems. The weed escapes attention in a mature rice field due to its prostrate nature. Information on the sensitivity of the weed to new herbicides is also meagre. Hence a study was conducted to understand the phenology and morphology of the weed in different rice soils of Kerala and also to find the factors affecting its propagation. The sensitivity of the weed to pre and post emergence herbicides was evaluated using seed and whole plant bioassay techniques respectively. The present investigation came out with the following findings:

- *I. miliacea* had high phenotypic plasticity which was influenced by soil charecteristics.
- The plants from Kuttanad soil showed earlier flowering and fruit set as compared to Palakkad soil.
- Morphological attributes of *I. miliacea* such as total shoot length, leaf number and internodal length also depended on the soil type. Plants grown in Palakkad soil showed slower growth but higher shoot length and internodal length as compared to Kuttanad soil. The seed production and duration of the weed was higher in Kuttanad soil. The variation in organic carbon content and nutrient status of the soil may be the reason for the variation in the growth attributes of the weed. This may be the major reason for the fast spread of the weed in this area.

- Propogation of *I. miliacea* was affected by factors such as light, temperature, moisture and depth of burial. Growth of the weed was highest in open condition compared to shaded condition, under 50 % shade the growth declined by 70 %.Higher germination per cent of seed was obtained under moist condition, while stem cuttings performed well in both moist and submerged conditions. 25^0 C was found to be optimum for the germination of *I. miliacea* seeds. The germination per cent of *I. miliacea* reduced with depth of burial.
- Herbicide sensitivity studies using bioassay techniques revealed that among the pre-emergence herbicides tested, higher weed suppression was observed in butachlor and oxyfluorfen. Among the post emergence herbicides, best control was obtained for azimsulfuron followed by cyhalofop butyl and fenoxaprop p- ethyl. All the four non- traditional rice herbicides tested *viz.* glyphosate, diuron, paraquat and glufosinate ammonium gave excellent control of *I. miliacea* and no regrowth was observed after two weeks.

Future line of work

The current study was proposed to understand the phenological and morphological attributes of *I. miliacea* in different soil types and also the factors affecting its propagation. The sensitivity of the weed to pre and post emergence herbicides was also evaluated. This will help to develop better management practices and to keep the rice field free of *I. miliacea* for a reasonable period of the crop.

- Interaction between growth stages of *I. miliacea* and herbicide action has to be studied further
- Effect of herbicide application and water management on regrowth of the weed has to be further evaluated for devising effective control measures.

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GROWTH AND PHYSIOLOGY OF *Isachne miliacea* ROTH. IN DIFFERENT SOIL TYPES AND ITS SENSITIVITY TO COMMON HERBICIDES

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ABSTRACT OF THE THESIS

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ABSTRACT

Isachne miliacea Roth. is one of the predominant weed in the low land rice fields of Kerala. The weed belongs to the family poaceae. Locally it is known as 'Chovverippullu, Naringa, Njammal, Changalipullu.' Previous studies conducted in Kerala Agricultural University showed that *I. miliacea* alone can contribute to 61 per cent reduction in the production of rice in Onattukara region. Presently, the weed is reported to be spreading to other rice growing regions of Kerala. It is found both during the *Rabi* and *Kharif* seasons. Eradication of the weed is very difficult, as it is propagated through seeds and stem cuttings. Hence a study was proposed to understand the growth and propagation of *I. mliacea* in different rice growing soils and also to understand the response of the weed to common herbicides popular in the state.

The effect of different soil types on growth and phenology of *I. miliacea* was studied in pot culture with soil collected from five different rice growing regions *viz.* Onattukara, Kole, Kuttanad, Pokkali and Palakkad. Ten kg soil each was filled in plastic boxes of size 50 x 25 cm and five replications was maintained for each soil type. Ten seeds were sown in each box and the germination was noted. At two leaf stage when the seedlings were identifiable, a single seedling was retained in each box and the excess seedlings were removed, irrigated regularly and the different phenophases and the morphological attributes were observed at 15 days interval.

The study showed that soil type had a profound influence on the phenology and growth pattern of the weed. Due to high acidity (pH -3.6) and salinity (EC – 4.46 dS/m) seeds sown in Pokkali soils did not germinate. Plants grown in Kuttanad soil showed earlier germination, tillering, flowering, seed formation and seed maturation, while those from Palakkad soil required more number of days to reach these phenophases. The variation in morphological attributes such as total shoot length, leaf number, internodal length, number of panicles and seeds produced per plant were significant. Plants grown in Palakkad soil had higher total shoot length

and internodal length as compared to Kuttanad soil, but the leaf number, number of panicles per plant and number of seeds produced per panicle were higher in Kuttanad soil, where the potassium, phosphorus and organic content was higher compared to other soils. As the nutrient status of Onattukara and Kole land soils were similar (potassium, phosphorus, magnesium, zinc, manganese and sulphur), the performance of the weed in those two soil types were also on par for many of the morphological characters.

Propagation of *I. miliacea* was affected by ambient temperature, shade, depth of burial and moisture level of soil. The seeds germinated when the temperature range was between 15 - 40^{0}C, the highest germination was obtained at 25^{0}C. Growth of the weed *was* highest in open condition compared to shaded condition. Under 50 per cent shade, seed germination declined by 70 per cent. Higher germination per cent of weed was obtained under moist condition, while stem cuttings performed well in both moist and submerged condition. The germination per cent of the weed, reduced with depth of burial.

Sensitivity of the weed to pre emergence herbicides was tested using seed bioassay technique. In the first set, herbicide soaked filter paper was placed at the bottom of petri plates and seeds were placed on it (TP method). In the second set, the herbicide soaked filter paper was placed on top of the seeds and another water soaked filter paper was placed at the bottom (BP method I). In the third set, seeds were placed in between two filter paper soaked with herbicides (BP method II). BP method II gave the best result for all the pre emergence herbicides. However, oxyfluorfen and butachlor showed higher weed suppression in all the methods tried.

Sensitivity of the weed to post emergence herbicides was tested using whole plant bioassay technique. Here, the weed was grown in mud pots and the herbicides were sprayed when the weed was in the vegetative phase. Among post emergence herbicides tested, best control was obtained for azimsulfuron followed by cyhalofop butyl and fenoxaprop p- ethyl. The weed was susceptible to these chemicals. In the case of bispyribac sodium and penoxsulam though initial control was observed,

regrowth was seen after two weeks and so the chemical was classified as moderately resistant to the weed. The weed was resistant to pyrazosulfuron, where no drying symptoms were observed. All the four non-traditional rice herbicides tested *viz.*, glyphosate, diuron, paraquat and glufosinate ammonium gave excellent control of *I. miliacea* and no regrowth was observed after two weeks.