MORPHO-PHYSIOLOGICAL CHANGES IN RICE DUE TO APPLICATION OF SELECTED POST EMERGENT HERBICIDES

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Morpho-physiological changes in rice due to application of selected post emergent 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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C. V. Ramanarayana

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Abbreviations

ACCase	Acetyl CoA carboxylase
ALS	Acetolactate synthase
ai/ha	Active ingredient per hectare
DAT	Days after transplanting
DAS	Days after sowing
CGR	Crop growth rate
RGR	Relative growth rate
NAR	Net assimilation rate
LAI	Leaf area index
DMP	Dry matter Production
WGR	Weed growth rate
PE	Pendimithalin
HW	Hand weeding
EC	Emulsifiable concentrate
DF	Dry flowable
WP	Wettable powder
SC	Soluble concentration
NRase	Nitrate reductase enzyme activity
GS	Glutamine synthetase
MCPA	2-Methyl-4-Chlorophenoxyacetic Acid
MCPP	Mecoprop
POD	Peroxidase
PQ	Paraquat
SOD	Superoxide dismutase
CAT	Catalase
AOPP	Aryloxy phenoxy propionate
ppm	Parts per million
Kg/ha	Kilogram per hectare
No/m^2	Number per meter square
t/ha	Tonnes per hectare

Introduction

1. Introduction

Rice is the staple food for more than 60 percent of worlds' population. It is grown in about 42.5 Mha with a production of 95 Mt contributing 45 percent to total food grain production of India. Among the various systems of cultivation of rice, direct seeding of sprouted seeds in puddle offers a good alternative to transplanting system. But weed problems are more critical in direct seeding (Moorthy and Saha, 2002) contributing to a yield loss of 40 to 100 percent (Choubey *et al.*, 2001).

Manual weeding is considered to be the best, the undependable labour availability and escalating wages in many cases has given impetus to the development. Chemical control is the most commonly used and reliable method for controlling weeds in rice. Some of the weeds are not controlled by the traditional herbicides and mostly regenerate after hand weeding. With changing scenario of weed management farmers need herbicides having high efficacy, low phytotoxicity and cost effective as well as no residual effect on succeeding crops. Introduction of new post-emergence herbicides may help to have a wide spectrum of application time as well as according to weed specificity and may be an effective tool for specific weed management.

Acetyl Co A Carboxylase (ACC ase) inhibitors and Acetolactate Synthase (ALS) inhibitors are two major class of herbicides successfully used in the selective control of weeds in the rice ecosystem. They are used for the control of many grass and broad leaved weeds such as *Echinochloa spp.,Cynotis axillaris, Sacciolepis interrupta, Leptochloa chinensis, Marselia quadrifolia* etc.

Acetyl Co A carboxylase is a multifunctional protein located in the stroma of plastids. It catalyses the carboxylation of Acetyl Co A to Malonyl Co A which is the precursor in the fatty acid synthesis in plants. This inhibition of fatty acid synthesis blocks production of phospholipids used in building new cell membranes required for cell growth. Herbicide Cyhalofop butyl, Clodinafop, Diclofop, Fenoxaprop and Cyclohexanediones are ACCase inhibitors and these herbicides affect the fatty acid synthetic pathway of plants and contribute to their destruction and control. The tolerance to these groups of herbicides is attributed to the alteration in the target site, presence of detoxification mechanism and overproduction of ACCase (Gronwald, 1992).

ALS is the first enzyme common in the biosynthesis of the branched chain amino acid leucine, isoleucine and valine (Stidham,1991). The selective herbicides belonging to the chemical class sulfonyl ureas, imidazolinones, triazolopyrimidines and pyrimidinyl benzoate are the major ALS inhibitors used in weed control. These herbicides will bind with ALS and make the enzyme inactive there by affecting the protein synthetic pathway of plants leading to their death. Resistant plants are able to detoxify these herbicides. According to Vidal (2002) these products control a broad spectrum of weed flora, low toxicity to humans and animals and high capacity of translocation in plants.

The new herbicides currently popular in Kerala belong to the above mentioned categories. In many cases, the application of these herbicides does not show any visible symptoms of toxicity to the rice plant. However, morphological changes such as reduction in height have been observed in the field. Hence the current study has been proposed to identify the physiological and biochemical factors that contribute to such morphological changes in the plant. This will help to develop measures to improve the efficiency of the plant and nullify the negative effects of herbicide application.

Objective

The study aims to identify

1. The physiological changes due to application of Acetyl CoA Carboxylase (ACC) and Acetolactate Synthase (ALS) inhibiting herbicides.

2. Morphological changes due to application of Acetyl CoA Carboxylase (ACC) and Acetolactate Synthase (ALS) inhibiting herbicides and their effect on the growth and yield of rice.

Review of Literature

2. Review of Literature

In India weed problem is one of the most important biotic constraints that limits rice productivity (Saha and Rao, 2010). Weeds that emerge simultaneously with crop deplete considerable amount of costly fertilizer and native plant nutrients, which result in lower yield. Effective control of weeds is, therefore, vitally important. The yield loss due to weeds was as high as 40-100 per cent in direct seeded rice (Choubey *et al.*, 2001).

Among the popular weed control options chemical weed control is widely used by farmers. Among the herbicides, the ALS and ACCase inhibitors are very popular in rice cultivation as they are required only in small quantities and can effectively control most of the weeds. The influence of these chemicals on the physiological and biochemical parameters of the rice plant is reviewed.

2.1 Weed spectrum in rice

In rice more than 80 species of poaceae family have been reported as weeds. Species in Cyperaceae family rank next with more than 50 species. Other families with 10 or more weed species include Alismataceae, Asteraceae, Lythraceae and Scrophulariaceae (Hosmani, 1995). Choubey *et al.* (2001) observed *Echinochloa colona, Commelina benghalensis, Cyperus iria* and *Cynotis axillaris* as major weeds in dry sown rice.

Moorthy and Manna (1982) reported that the weed flora in puddled rice composed of *Fimbristylis miliacea, Cyperus difformis* and *Scirpus supines.* At IRRI, the major weed species observed in wet seeded rice in the order of importance include *Paspalum distichum, Monochoria vaginalis, Sphenoclea zeylanica, Echinochloa glabrescens* and *Cyperus difformis* (Mabbayad and Moody, 1984).

In direct wet sown fields of Pattambi (Kerala), Nair *et al.* (1974) reported the presence of *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.

Jayakumar *et al.* (1994) reported that the major weeds at Coimbatore condition were *E. crus-galli* (22.0 %), *E. colona* (23.5 %) in grasses, *Cyperus difformis* (21.3 %) and *Cyperus iria*

(21.0 %) in sedges, *Marselia quadrifoliata* (5.8 %) and other broad leaved weeds (6.4 %) in *rabi* season.

The pre-dominant 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 *Echinocloa colonum, E. crus-galli* 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.*

Roy *et al.* (2009) reported that weed vegetation in boro rice, Assam were *Monochoria vagnialis, Panichum repens, Echinochloa colonum, Echinochloa crus-gali, Fimbristylis miliacea, Cyperus micheliamus, Cyperus esculenta, Sciepus juncoides* and *Cynodon dactylon.*

2.2 Chemical control of weeds

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 labor (Singh *et al.,* 2006; Anwar *et al.,* 2012a).

Among the post emergence herbicides, Ethoxysulfuron, Cyhalofop-butyl, Pretilachlor, Chlorimuron, Metsulfuron, Bispyribac sodium, Azimsulfuron, Fenoxaprop and Penoxsulam effectively controlled weeds in direct seeded rice (Mann *et al.*, 2007; Singh *et al.,* 2008; Mahajan *et al.,* 2009; Juraimi *et al.,* 2010).

2.3 Herbicide toxicity to rice

Cyhalofop butyl applied to rice plants did not show any visual phytotoxicity symptoms (Abeysekhara, 1999). There was no phytotoxicity for Fenoxaprop -p-ethyl from 45-90 g/ha at different stages of application (Singh *et al.,* 2003). Katiyar and Kolhe (2006) observed that Fenoxaprop -p-ethyl+ Ethoxysulfuron and Metamifop had no phytotoxic effect on rice. Application of Metamifop 10 EC at 100 g a.i/ha at 2-3 leaf stage was found to be better in controlling grass weeds in direct seeded rice (Nithya *et al.*, 2011). Yadav *et al.* (2009) noted no phytotoxicity for Bispyribac sodium on rice and no residual toxicity for succeeding crop of wheat. Rao *et al.* (2009) also reported that Bispyribac sodium is a safe herbicide to rice and to rice fallow crops. There was no phytotoxicity for Azimsulfuron on rice at 15 and 30 days after spray and there was no residual toxicity on succeeding crop of wheat (Yadav *et al.*, 2008). Yadav *et al.* (2010) also observed there was no phytotoxicity for Azimsulfuron on rice and no residual toxicity on succeeding wheat. Almix @ 25g/ha showed moderate to severe toxicity in rice which persisted up to 30 DAT (Yun *et al.*, 2005).

2.4 Effect of herbicides on growth paremeters

Singh *et al.* (2010) reported phytotoxicity symptoms like yellowing and stunted growth of rice cultivar 'Rajshree' at higher doses of Azimsulfuron (27.5 and 30 g/ha). Owing severe phytotoxicity on variety 'Malwa 36', application of Almix led to lower plant height, fresh and dry weight of plant (Mukherjee and Singh, 2006). Sreedevi *et al.* (2009) observed that unweeded control recorded lower plant height as compared to herbicide applied plots. Petter *et al.* (2013) reported that application of imazethapyr + imazapic and nicosulfuron affected the plant height in rice.

Kumar and Gill (1982) registered a reduction of 63 percent and 11 percent in effective tillers and test grain weight respectively in unweeded plot over herbicide treated plots. Jain *et al.* (1996) reported that CGR was positively correlated with seed yield and crop biomass. Tiwari *et al.* (2010) conducted a field experiment during 2001 kharif season (June to October) to determine the effect of herbicides on phytotoxicity and yield of direct sown rice cv. IR-36 in Chattisgarh, India. During 0-25 DAS, post emergence application of Chlorimuron + Metsulfuron, irrespective of rates, showed maximum crop growth rate (CGR), which was significantly higher than CGR under hand weeding and the unweeded control. During 25-50 DAS, all herbicide treatments as well as hand weeding twice were equally effective in producing higher CGR and were significantly superior to the control. During 70-90 DAS, hand weeding twice showed maximum CGR of 0.109 g $day^{-1}plant^{-1}$, which was significantly superior than all the lower rates of herbicide treatments and the control, except preemergence application of 1.0 kg Pendimethalin/ha. Manjunath and Panchal (1989) reported that herbicides at optimum doses reduced weed growth effectively, thus increasing crop growth rate and leading to higher DM production, yield and yield components. Rana and Angiras (1999) found that Pretilachlor at 0.80 kg/ha was statistically at par with hand weeding twice, and increased crop growth rate (CGR), paddy grain yield and decreased growth rate of weeds (WGR). Aishy *et al.* (1976) found that Linuron, alone or mixed with Treflan, and hoeing produced the highest DM yield, CGR and the highest seed yield. Veeramani *et al.* (2006) revealed that the plant growth indices such as LAI, CGR, RGR and Plant DMP values were increased appreciably by Paraquat application in 14 days after first irrigation compared to unweeded control in cotton

Satao and Nalamwar (1991) reported that pre-emergence application of 1 kg Atrazine + 2 weedings 45 and 60 days after germination gave the highest grain and fodder yields which were 44 and 75% respectively and greater than the untreated control. NAR and RGR were also highest with this treatment. Singh *et al.* (1985) carried out an experiment on the influence of various rates of Nitrofen, Fluchloralin and Pendimethalin (0.5, 1, 1.5 and 2 kg/ha) on the growth parameters of green gram and they concluded that RGR values increased with increasing rates of herbicides during weeks 3-6 and these values were lower than those recorded with hand weeded plants. Later during weeks 6-9 no significant variations were noted in RGR values due to herbicide treatments. Moursi *et al.* (1980) reported that RGR at 30-60 days were significantly increased by herbicides but not at later growth stages. Field trials conducted by Bhargavi and Reddy (1993) in Andhra Pradesh with pre-emergent herbicides *viz.,* Butachlor, Thiobencarb and Pendimethalin and post-emergent herbicides, 2,4-D ethyl ester at 0.9 kg (7 DAS) and 2,4-D sodium salt at 1 kg, (30 DAS) with hand weeding twice at 20 and 40 DAS showed that in all the treatments the RGR of rice increased, and that of the weeds decreased, as compared with the untreated control. Mehta *et al.* (2010) conducted a field experiment consisting of six weed control methods (weedy check, weed-free, hand weeding at 20 and 40 DAS, HW at 20+interculturing at 40 DAS, application of Pendimethalin @ 0.75 kg ha^{-1} (PE) and Pendimethalin @ 0.75 kg ha^{-1} (PE)+Interculturing at 40 DAS) and their results revealed that RGR and yield attributes were higher for pre-emergence application of Pendimethalin @ 0.75 kg per ha+IC at 40 DAS followed by hand weeding (HW) at 20 and 40 DAS this was statistically at par with weed free treatment and significantly superior over rest of the treatments.

Nichiporovich (1960) was of the opinion that NAR increased rapidly from emergence to a stable optimum level with a decline at the end of the growing period. Williams (1946) and

Thorne (1960) however, found that NAR of all the species based on leaf area, fell linearly with time. Murti and Khan (2005) conducted a field study at Kumarganj, Faizabad, Uttar Pradesh to evaluate the effect of two Dinitroaniline herbicides on growth parameters and yield. The results revealed that application of Pendimethalin and Fluchloralin at 0.75, 1.0 and 1.25 kg/ha did not produce significant effect on net assimilation rate (NAR) and yield. Singh *et al.* (1985) carried out experiments on the influence of various rates of Nitrofen, Fluchloralin and Pendimethalin (0.5, 1, 1.5 and 2 kg/ha) on growth parameters of green gram and they concluded that NAR values increased with increasing rates of herbicides during 1-3 weeks.

Channappagoudar *et al.* (2008) carried out a field experiment at University of Agricultural Sciences, Dharwad during kharif season for two years to study the influence of herbicide on physiological parameters in potato. The experiment consisted of 13 treatments comprising five herbicides viz. Alachlor (1.0 and 1.5 kg ai/ha), Fluchloralin (1.0 and 1.5 kg ai/ha), Pendimethalin (1.0 and 1.5 kg ai/ha), Metribuzin (0.75 and 1.0 kg ai/ha) and Diuron (1.0 and 1.5 kg ai/ha) compared with farmers practice, weed free check and unweeded control results revealed that the influence of all the herbicides on the physiological parameters of potato was similar. The herbicides substantially increased LAI and photosynthetic rate than unweeded control. Pre emergence application of Metribuzin @ 0.75 kg ai/ha recorded higher LAI (2.82) and photosynthetic rate (28.0 u mol/m^2/s). The lower LAI and photosynthetic rate (2.088, 22.7 μmol/m^2/s and 66.1%, respectively) was recorded in unweeded control.

2.5 Effect of herbicides on physiological and biochemical parameters

2.5.1 Chlorophyll content

Singh *et al.* (1987) conducted an experiment on field evaluation of Pendimethalin, Butachlor, Thiobencarb and 2,4-D and the results revealed that the herbicides applied after sowing or transplanting affected the chlorophyll content of the leaves of rice. Pre emergent application of Pendimethalin or Oxadiazon at 0.75 kg/ha and Butachlor or Thiobencarb at 1.5 kg/ha in rice followed by wheat showed non-significant differences in the chlorophyll and carotenoid content of the leaves of wheat. However leaf chlorophyll *a* was highest in Thiobencarb-treated rice crop followed by wheat and lowest in Butachlor-treated rice. Chlorophyll *b* was highest in Pendimethalin-treated rice and lowest in Butachlor-treated rice.

Total chlorophyll content in wheat following herbicide-treated rice was in the order Thiobencarb> control > Pendimethalin > Oxadiazon > Butachlor (Randhawa and Gill, 1989)

Efficacy of herbicides *viz.*, Butachlor (1.0 kg/ha), 2,4-D (0.72 kg/ha), Oxyfluorfen (0.2 kg/ha), Anilofos (0.4 kg/ha) and Thiobencarb (1.0 kg/ha) on rice cv. Mahsuri showed the mean chlorophyll content in the rice cv. Mahsuri was maximum in plants treated with Butachlor (2.35 mg/g at 20 DAF) and minimum in unweeded control (1.94 mg/g). The chlorophyll content of the leaves reduced in the order Butachlor > 2,4-D > Anilofos > Oxyfluorfen > Thiobencarb > unweeded control (Deka *et al.,* 1996).

Field experiment in Karnataka conducted by Nagaraju *et al.* (1995) in rice cv. IR-20 with 2,4-D + Anilofos, Butachlor, 2,4-D, Anilofos, Pretilachlor and Oxyfluorfen revealed that the application of 0.30 kg Anilofos + 0.60 kg 2,4-D/ha 4 days after transplanting increased chlorophyll content. Application of Saturn (thiobencarb) on rice field stimulated the chlorophyll 'a' content in rice leaves (Dawah *et al.*, 2005).

Sahoo *et al.* (1993) conducted a field experiment and evaluated the herbicides 2,4-D and 2,4,5-T, both applied at 10-1000 ppm, on plant pigments (chlorophylls '*a*' and '*b*' and total chlorophyll) and reported that Chlorophyll *a* and *b* and total chlorophyll were generally reduced by application of both 2,4-D and 2,4,5-T. Peng *et al.* (2006) studied the effects of Triazine herbicide Atrazine on rice seedlings and reported that chlorophyll contents decreased from 1.07±0.013 mg/g FW (control) to 0.97±0.013 mg/g FW.

2.5.2 Total soluble protein

Perumal *et al.* (2005) estimated the effect of application of the herbicides sodium salt of 2,4-D at 500 g/acre and Butachlor 50% EC at 400 ml/ha on the weed *Eclipta alba* and rice. They reported that the decrease in soluble protein content was more in *Eclipta alba* than rice and Butachlor showed more effect than 2,4-D in reducing the soluble protein content of leaves. Application of Fentrazamide at and above 1 μM on the early growth stage of water grass and rice showed phytotoxicity symptoms and 95 percent reduction in dry weight due to degradation of protein (Jin *et al.*, 2007). Peng *et al.* (2006) reported that soluble protein content decreased from

40.4 mg/g FW (control) to 29.3 mg/g FW when rice seedlings were treated with 0.1 mg/litre Atrazine for 7 days.

Ting (2009) studied the effects of cadmium (Cd), Acetochlor (AC) and Bensulfuron-methyl (BSM) on nitrogen metabolism and plant growth in rice seedlings with aquatic culture experiments and the results demonstrated that in all exposure groups there were marked decrease in the fresh weight of rice seedlings and the activities of nitrate reductase (NRase) and glutamine synthetase (GS) in the roots and shoots, consequently leading to an increase in free amino acids content and decrease in soluble protein content and nitrate content in the roots and shoots.

2.5.3 Nitrate reductase activity

The nitrate reductase activity (NRase) which is the key enzyme in nitrogen metabolism is known to be regulated by various environmental factors and nitrite availability. It is also believed that the reduction of nitrate by NRase is the rate limiting process for the utilization of nitrogen in the form of nitrate (Bertero *et al.*, 2003).

Deka *et al.* (1996) conducted pot culture experiments to evaluate the herbicides Butachlor at 1 kg/ha, 2,4-D at 0.72 kg/ha, Oxyfluorfen at 0.2 kg/ha, Anilofos at 0.4 kg/ha and Thiobencarb at 1 kg/ha applied at 3 days after sowing or transplanting to direct-sown and transplanted rice and concluded that irrespective of herbicide application, *in vivo* nitrate reductase activity (NRase) was greatest at the early tillering stage, after which nitrate reductase activity gradually declined. The greatest levels of nitrate reductase activity were recorded with Butachlor-treated rice at the tillering stage, and the lowest nitrate reductase activity was observed in the unweeded control at 20 DAF. The effect of Butachlor on nitrate reductase activity in the leaves of rice cv. RP-2421 at different stages of growth in Palampur, showed a positive correlation with grain protein and leaf protein (Neelam and Ruchi, 2005).

Antony (1995) reported that nitrate reductase activity was correlated with TDM at early stage but did not have any positive correlation with any of the yield and yield components in soybean. Prakash *et al.* (1989) reported that application of Thiobencarb did not affect nitrate reductase activity in rice plants where as in *E. crus-galli*, the treatment strongly lowered nitrate reductase activity.

2.5.4 Proline content

Chalko (1970) reported that application of 4-chloro-2-methylphenoxyacetic acid (MCPA) increases the proline content in barley compared to control from 17 to 28%.

Lakimova *et al.* (2002) reported application of herbicides Fluazifop at 375 and 500 g/ha and Quizalofop at 150 and 200 g/ha increased proteases and proline content in lily. Ibrahim *et al.*, (2004) reported that combined applications of Fenoxaprop-p and Fluroxypyr increased the proline content in maize.

Application_of 0.3 kg/ha Simazine, 0.3 kg/ha Simazine + 0.3 kg/ha 2,4-D, and 1.5 kg/ha MCPP [mecoprop], reduced the proline content in winter rye (Panamarioviene,1996).

2.5.5 Total amino acids

Davies and Humphrey (1978) reported that the plant changed its enzyme complement by rapidly synthesizing new proteins from amino acid formed by degradation of old proteins. Application Bensulfuron-methyl had very little influence on the free amino acid content of *O. sativa* and *P. dichotomiflorum* belonging to the poaceace family (Chang *et al.*, 2008).

Zhong *et al.* (2001) conducted an experiment on the influences of herbicides on the physiological and biochemical parameters of potted rice plants and they reported an increase in free amino acids in rice leaf sheath but the increase was not significant compared with the control. Young and Chu (1975) conducted an experiment in pot culture with rice, Simazine or Simetryne applied to leaves at 1, 5 or 20 ppm and soil at 2.8 or 5.6 g at flowering stage of rice resulted in an increase in the content of total amino acid.

2.5.6 Catalase enzyme activity

Catalase is a universal enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen and protects the cell from oxidative damage (Chelikani *et al.*,2004). Lin *et al.* (2010) evaluated the effect of fluroxypyr at 0-0.8 mg on selected metabolic and stress related parameters in *Oryza sativa* plants after 6 days of exposure and concluded that Fluroxypyr-induced significant changes in activities of superoxide dismutase, catalase, ascorbate peroxidase and peroxidase (POD).

Sood *et al.* (2011) conducted an experiment on the effects of different Paraquat (PQ) dosages on growth, lipid peroxidation, and activity of antioxidant enzymes of *Azolla microphylla* and the results revealed that supplementation of 10 µM Paraquat increased the activities of superoxide dismutase (SOD), catalase (CAT) as compared with control. Mei *et al.* (2009) reported that catalase has a much lesser role in the defense against Quinclorac or Bensulfuron-methyl induced oxidative stress. Prokopenko and Musina (1983) reported that catalase activity during early growth and at flowering and pod formation stages of soyabeans was not adversely affected by applying 1-3 kg Dacthal [chlorthal-dimethyl] but decreased by further increasing the herbicides rates.

Kun *et al.* (2013) studied the effects of Atrazine on superoxide dismutase (SOD), and catalase (CAT) activity in leaves of *Pennisetum hydridum* and revealed that low level of Atrazine at (5, 10 $mg.L^{-1}$) elevated O_2^-, production rate and CAT activity in leaves leading to a decreased H_2O_2 content.

Studies by Singh *et al.* (2013) in wheat crop at Faizabad revealed that the catalase and peroxidase activities in leaves were significantly increased due to herbicides over untreated check. Minimum increase was observed with Sulfosulfuron, while Isoproturon showed maximum increase.

2.5.7 IAA content

Indole-3-acetic acid (IAA) is the most common, naturally-occurring, plant hormone of the auxin class Simon and Petrasek (2010). Kimura *et al.* (1971) reported that Benthiocarb competitively inhibited the elongation of laminar joints of excised rice leaves induced by IAA. Inhibition of protein synthesis through competition at the auxin-acting site appears to be one of the modes of action of Benthiocarb. Wen (1995) reported that Butachlor greatly decreased levels of endogenous IAA, but increased endogenous abscisic aci in rice seedlings.

2.5.8 Stomatal conductance

Stomatal conductance is the measure of the rate of passage of carbon dioxide (CO_2) entering, or water vapor exiting through the stomata of a leaf. It is under direct biological control of the leaf through guard cells, which surround the stomatal pore (Taiz and Zeiger, 1991).

Oosterhuis *et al.* (1990) conducted an experiment to elucidate the morphological and physiological responses of rice to post-emergent application of Fenoxaprop. The results revealed that net photosynthesis was reduced by 35% in Fenoxaprop-treated plants after 11 days, although stomatal conductance was not affected. Carvalho *et al.* (2012) reported that glyphosate doses in a range of 180-360 g ai ha^{-1}increased photosynthesis, transpiration and stomatal conductance. Ivanova *et al.* (1999) reported that Atrazine inhibited the growth of the trifoliate leaves, and decreased photosynthesis, transpiration rates and stomatal conductance in bean leaves.

2.5.9 Photosynthetic rate

Sousa *et al.* (2014) reported that the application of chemical herbicides of the imidazolinone group on rice plants contributed to changes in the photosynthetic metabolism of plants. Sharma *et al.* (1989) conducted experiment on 15 day old rice plants with 1, 3, 5 or 7 ppm Oxyfluorfen. The results revealed that decreased leaf chlorophyll contents inhibited photosynthesis in rice plants. Argenta and Lopes (1992) reported that there was increase in inhibition of photosynthesis with Chlomazone doses above 0.75 kg in rice.

Basuchaudhuri (1989) reported that application of Simazine at 12 ppm caused 50 percent inhibition of photosynthesis in leaf discs of rice and application of Butachlor caused 90 percent inhibition in rice.

2.5.10 Phenol

Klepacka *et al.* (2011) reported that organisms that synthesize phenolic compounds do so in response to ecological pressures such as pathogen and insect attack, UV radiation and wounding. Duke et al. (1985) found those herbicides, especially Glyphosate, Chlorsulfuron and Acifluorfen influence the synthesis of various phenolic compounds that instances where auxin and gibberellic acid are partially or completely inactivated the inhibitory activity of phenolic compounds and ABA is increased, under such conditions phenolics exert a direct influence on growth.

2.6 Effect of herbicides on yield and yield attributes

Cyhalofop butyl available as Clincher . 10 EC, is an aryloxy phenoxy propionate herbicide developed for post emergence control of grass weeds in dry as well as wet seeded rice. It is phloem mobile, systemic herbicide that inhibits Acetyl Co-A carboxylase enzyme activity (Sharma *et al.*, 2004). Cyhalofop butyl @ 90g/ha resulted in more number of panicles and higher grain yield of rice (4.5 t/ha) (Angiras and Attri, 2002). Saini (2003) reported application of

Cyhalofop butyl at 10, 15 and 20 DAS resulted in higher paddy yield. Cyhalofop butyl applied to rice plants did not show any visual phytotoxicity symptoms (Abeysekhara and Wickrama, 2004). Saini (2005) reported Cyhalofop butyl at 120g/ha (15 DAS) followed by 2,4-D at 1kg/ha (20 DAS) and 2,4-D at 15 DAS followed by Cyhalofop butyl at 20 DAS being on par with each other and recorded higher number of panicles/m^2, panicle length, grains/panicle and 1000 grain weight. Sangeetha *et al.* (2009) reported application of Cyhalofop butyl (15 DAS) + 1 HW (45 DAS) produced more panicles/m^2, filled grains/panicle and yield which was on par with HW twice.

Bahar and Singh (2004) reported that **Fenoxaprop p-ethyl** available as Rice star, is a new aryl oxy phenoxy propionate (AOPP) post emergence herbicide inhibiting Acetyl Co-A carboxylase enzyme and is effective against grass weeds in rice. Saini and Angiras (2002) recorded higher grain yield (30.3 t/ha) as well as yield attributing factors with application of Fenoxaprop p-ethyl @ 90g/ha (20 DAS). Fenoxaprop p-ethyl at 56.25 g/ha applied at 10 days stage produced grain yield of 6798 kg/ha which was at par with weed free treatment (Singh *et al.*, 2004). Dixit and Varshney (2008) observed higher number of panicles/m^2 (236) and grains/panicle (71) under post emergence application of Fenoxaprop p-ethyl. Sreedevi *et al.* (2009) recorded higher mean plant height with application of Fenoxaprop p-ethyl. Fenoxaprop p-ethyl (0.06 kg/ha) mixed with ethoxysulfuron (0.015 kg/ha) produced higher grain yield (Tiwari *et al.*, 2010).

Metamifop is a new aryl oxy phenoxy propionate (AOPP) post emergence herbicide which shows an exclusive whole plant safety to rice with a high control efficacy to annual grass weeds, especially *Echinochloa crus-galli*. Selectivity of Metamifop between rice and barnyard grass could be due to both differential foliar absorption rate and differential Acetyl Co-A carboxylase sensitivity (Kim *et al.*, 2003).

Bispyribac sodium, available as Nomineegold is a pyrimidinyl carboxy herbicide, effective to control many annual and perennial grasses, sedges and broad leaved weeds in rice fields (Yun *et al.*, 2005). Walia *et al.* (2008) recorded highest grain yield of 4684 kg/ha with post emergent application of Bispyribac sodium (30g/ha) at 30 DAS. Bispyribac sodium 10%SC @ 30g/ha gave higher grain yield (Rao *et al.*, 2009). Yadav *et al.* (2009)

eported 41 percent increase in grain yield with application of Bispyribac sodium @ 25g/ha at 15-25 DAT. Application of Bispyribac sodium @ 25g/ha on 20 DAT registered higher grain yield (ARWR, 2011). Walia *et al.* (2008) obtained more tillers/m^2 (310), grain weight/panicle (21.7 g), and a yield of 5016 kg/ha with pre emergence application of Pendimethalin followed by Bispyribac sodium @ 20 g/ha.

Azimsulfuron is a post emergence sulfonyl urea herbicide useful for controlling weeds in rice fields (Valle *et al.*, 2006). Yadav *et al.* (2008) noticed a yield of 6242 kg/ha with application of 30g/ha Azimsulfuron along with 2g/ha Metsulfuron at 25 DAT. Weight of grains/panicle was higher with post emergence application of Azimsulfuron @ 25 and 30g/ha (Walia *et al.*, 2008). Singh *et al.* (2010) reported that Azimsulfuron @ 30g/ha + Metsulfuron @ 2g/ha produced higher grain (5.54q/ha) and straw yield (8.5q/ha). They also observed some yellowing of rice leaf when Azimsulfuron was applied but plants recovered in 15-20 days. Singh *et al.* (2010) obtained the highest grain and straw yield with application of Azimsulfuron @ 25 g/ha alone or tank mixed with Almix. Application of Azimsulfuron 50 DF @ 30 g/ha resulted in increased yield components and yield of rice (Sakthivel and Balasubramanian, 2010).

Almix is a ready mix formulation of Metsulfuron methyl and Chlorimuron ethyl belonging to sulfonyl urea group, inhibiting ALS activity and is effective against broad leaved weeds and sedges in rice (Yun *et al.*, 2005). Tank mix of Almix + Butachlor (4+1250g/ha) and ready mix of Almix + Anilofos (Alkombo) @ 280.5g/ha produced higher grain yield without any phytotoxicity on rice (Singh *et al.*, 2003). Grain and straw yield of 5837 kg and 7132 kg/ha respectively was obtained with tank mix application of Almix and 2,4-D which was on par with HW twice (Mukherjee and Singh, 2004). Almix + 2,4-D (15+500g/ha) at 8 DAT was found most effective in maximizing rice grain yield (58.3q/ha) (Mukherjee and Singh, 2006). A higher grain yield were obtained with application of Almix and Butachlor 3 DAT (Patra *et al.*, 2006). Metsulfuron-methyl @ 8g/ha applied 10 DAT increased yield by 72 percent (Saha and Rao, 2010).

Materials and Methods

3. Materials and Methods

Investigations on the "Morpho-physiological changes in rice (*Oryza sativa* L.) due to application of selected post emergent herbicides" was conducted in the *Kole* lands of Thrissur district. The details of materials used and methods adopted are presented in this chapter.

3.1 General details

3.1.1 Location

The experiment was conducted in the field of a farmer M. Kesavaraj in the Alappad *Kole* located at $10^0 31'$ latitude and $76^0 11'$ longitude and 1m below MSL.

3.1.2 Climate and weather conditions

The experimental site enjoys typical humid tropical climate. The mean monthly averages of important meteorological parameters observed during the experimental period are presented in Appendix I.

3.1.3 Variety used

The rice variety Jyothi (PTB 39), a red kernelled, short duration variety of 110-115 days duration was used for the trial. The variety is suitable for direct seeding and transplanting during both first (Virippu) and second crop (Mundakan) seasons. It is tolerant to BPH and rice blast disease, moderately susceptible to sheath blight and capable of yielding over 8 t/ha under favorable situations and gives moderate yields even under adverse conditions.

3.1.4 Season

The crop period was from November 2013 to February 2014 (Mundakan).

3.1.5 Cropping history of the experimental site

Alappad *Kole* is a single cropped land, where rice is grown during September-October to February-March. The land remains submerged during the rest of the year.

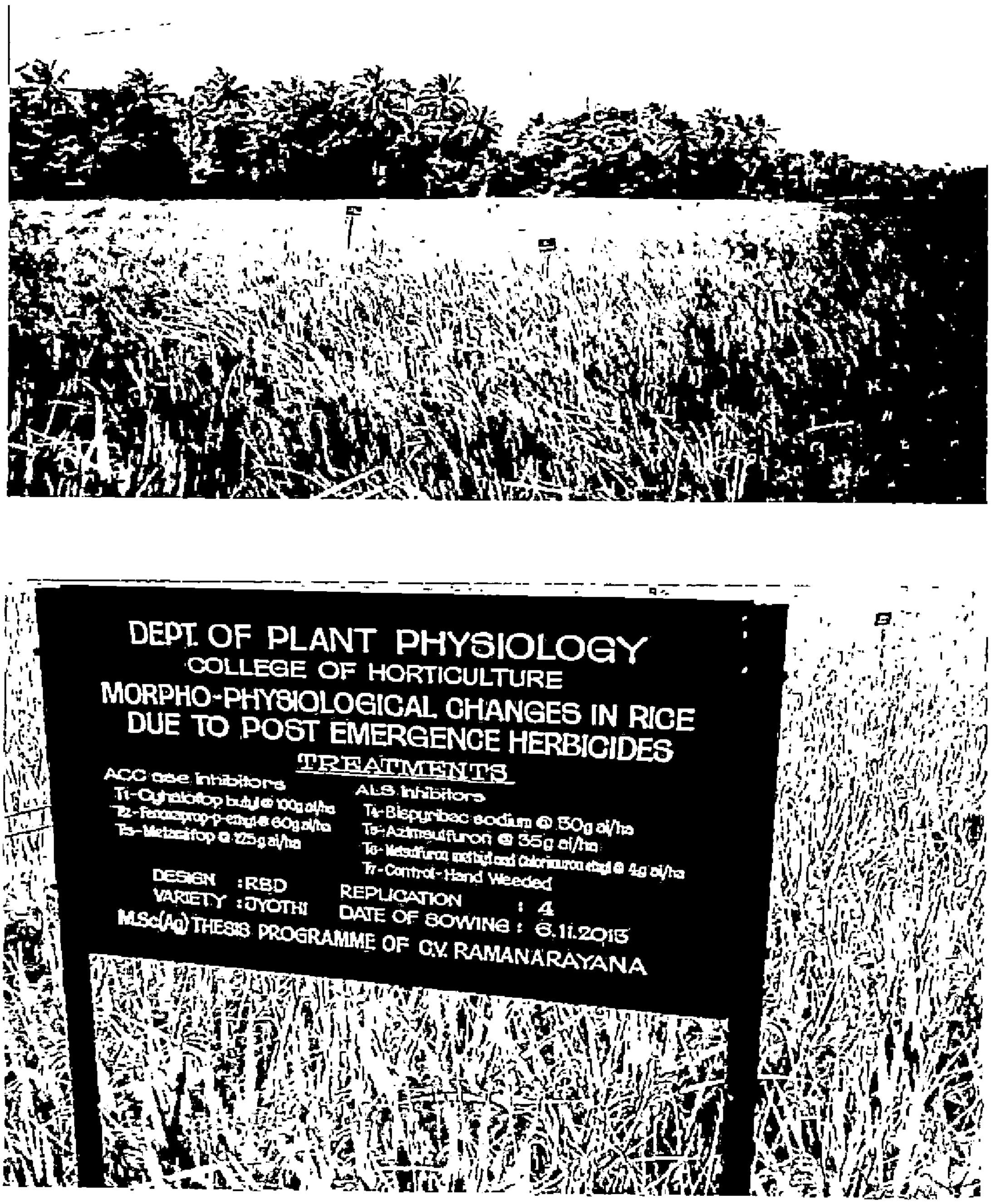

Plate 1. General view of the experimental plot

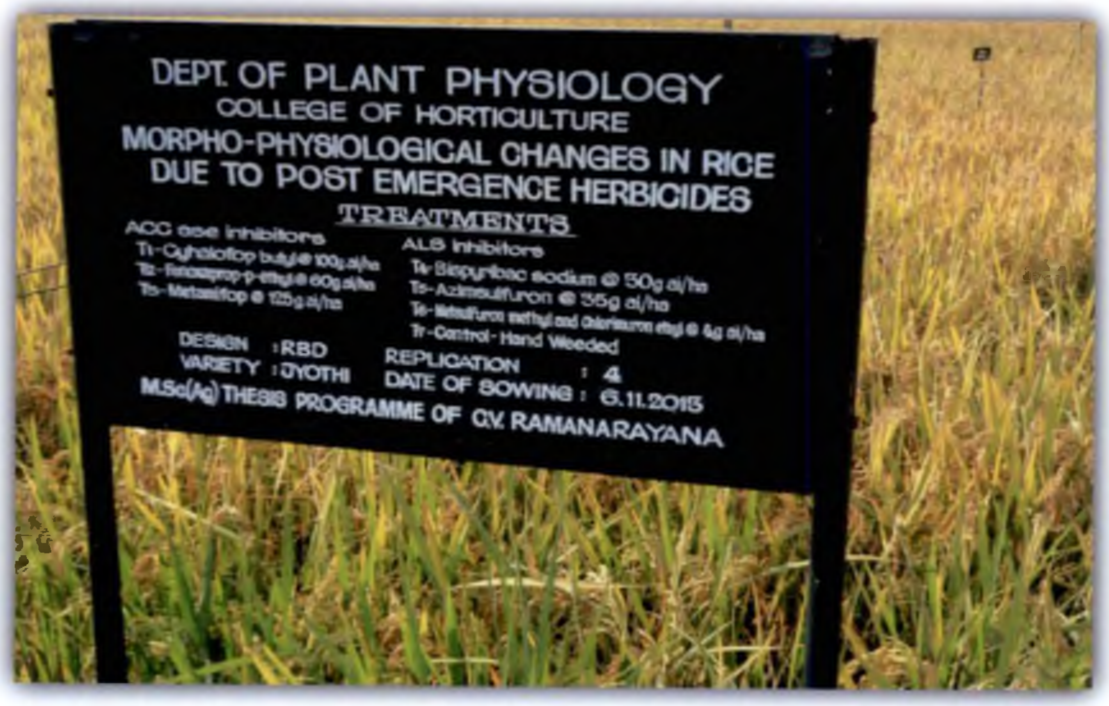

Plate 1. General view of the experimental plot

3.2 Treatments

The experiment was laid out in randomized block design (RBD) with 7 treatments and 4 replications (Table.1) and the plot size was 5m X 3m (15m^2). The herbicides tried and the dosages used are given below:

Table 1. Chemical name, trade name, formulation and dosage of the herbicides used for the study

Group	Herbicides	Trade Name	Formulation	Dosage (g *a.i.*/ha)
ACCase enzyme inhibitors	1. Cyhalofop butyl	Clincher	10EC	100
	2. Fenoxaprop - p - ethyl	Rice star	6.7% w/w	60
	3. Metamifop	Metamifop	10EC	125
ALS enzyme inhibitors	4. Bispyribac sodium	Nomineegold	10SC	30
	5. Azimsulfuron	Segment	50DF	35
	6. Metsulfuron methyl and chlorimuron ethyl	*Almix	6.7% w/w	4
	Control- Hand Weeded			

*Almix – A combination of chlorimuron ethyl 10% + metsulfuron methyl 10%

EC- Emulsifiable concentrate

DF- Dry flowable WP- Wettable powder SC- Soluble concentrate

3.3 Field operations

The details of various field operations from land preparation to threshing are given below.

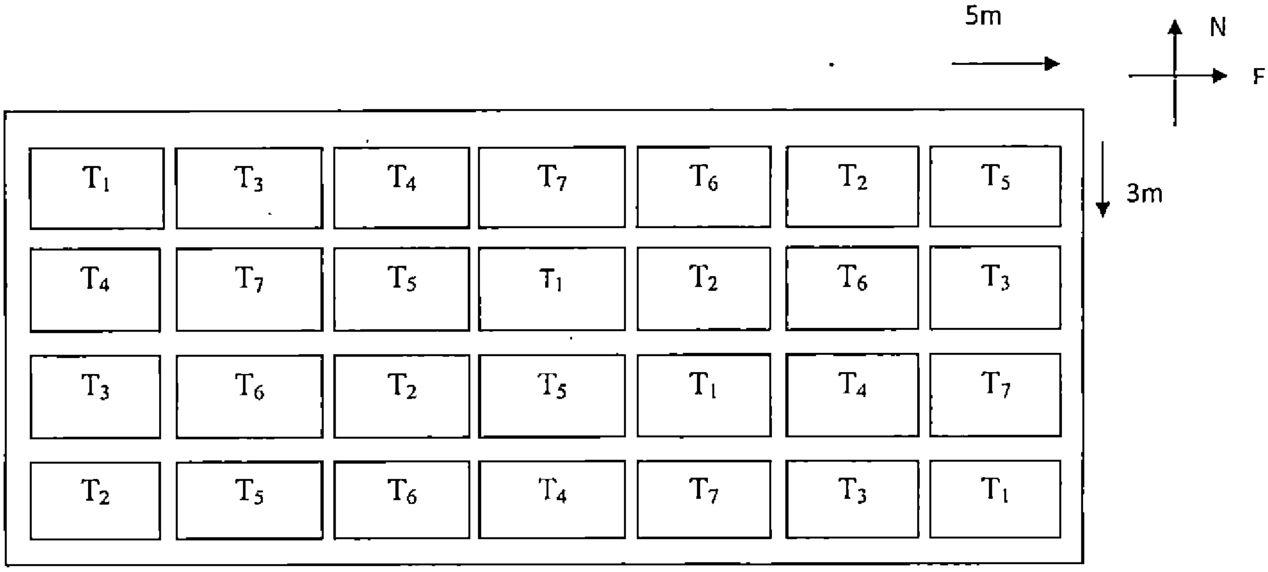

Fig. 1. Layout of the experiment

ACCase enzyme inhibitors

T_1 - Cyhalofop butyl

T_2 - Fenoxaprop - p - ethyl

T_3 - Metamifop

ALS enzyme inhibitors

T_4 - Bispyribac sodium

T_5 - Azimsulfuron

T_6 - Almix

T_7 - Hand weeded

3.3.1 Land preparation, sowing and fertilizer application

The area was ploughed, puddled and levelled. The plot size adopted was $15m^2$ (5m x 3m). Plots of 5m x 3m were made by taking bunds of 25 cm width and height. After levelling, fertilizers to supply NPK @ 90:35:45 kg/ha were applied. Urea, Factamphos and Muriate of potash were used for supplying the nutrients. Full dose of P was applied basally. N and K were applied in three equal splits at land preparation, maximum tillering and panicle initiation stages. After basal fertilizer application, the seeds were broadcasted at the rate of 150g/plot (100 kg/ha). In Hand weeded treatment, hand weedings were done at 20 and 40 DAS. All herbicides were sprayed at 15 DAS using knapsack sprayer at the recommended doses (Table 1).The population of rice seedlings was maintained at 66 plant / m^2 by thinning and gap filling.

3.3.2 Plant protection

Timely plant protection measures were taken up as per package of practices (POP) of KAU (KAU, 2007). Leaf folder attack was noticed during the seedling stage of the crop and acephate (Acetaf) @ 2g/L was sprayed against it.

3.3.3 Harvesting

The crop was harvested during second week of February where the grains fully matured. Threshing was done manually and the produce was cleaned, dried and yield was expressed as kg/ha.

3.4 Sample collection

Prior to application of treatment the field was maintained uniformly. Herbicides were applied on the 14^{th} day and sample collection was done one week after application of herbicides to compare treatment effects.

On the 15th day before application of herbicide 15 plant samples were randomly collected from different plots and the biochemical and physiological parameters were analyzed and the mean values have been reported.

Plate 2. Herbicide spraying at 15 days after sowing

3.5 Visual phytotoxicity symptoms

Visual phytotoxicity rating of crop was done on third and seventh day after spraying. Symptoms of injury like leaf scorching, Leaf curling, Tip burn, Yellowing and Recovery time were obtained and graded from 0 – 5 using the toxicity scale given (Thomas and Abraham, 2007).

Table 2. Scale for rating herbicide phytotoxicity in crop and weeds

Rating	Effect on weeds	Effect on crop
0	None	No injury
1	Slight control	Slight injury
2	Moderate control	Moderate injury
3	Good control	Severe injury
4	Very good control	Very severe injury
5	Complete control	Complete destruction

3.6 Morphological observations

3.6.1 Height

Three plants from each plot were selected and the height was measured from base of the plant to the longest leaf before panicle initiation and from base to the longest panicle after panicle initiation were recorded and expressed as cm.

3.6.2 Tiller production

The number of tillers in one square meter area of each experimental plot at 15 days interval from 30DAS was counted and recorded using a 0.25 m^2 quadrat.

3.6.3 Days to flowering

Time of flowering (days) was recorded when about 50% of the plants flowered.

3.7 Growth indices

The growth indices were worked at 15 days interval for 7 stages. The sampling unit consisted of 12 plants per treatment (Three from each replication). The plant samples were uprooted and dried and the growth indices were computed as per the procedure given below.

3.7.1 Leaf area index (LAI)

Leaf area index as estimated measuring the length and average width of leaf and multiplying by a factor of 0.75 as suggested by Yoshida (1981).

3.7.2 Relative growth rate (g g^{-1} day^{-1})

Relative growth rate (RGR) is the rate of increase in the dry weight per unit time. It was calculated by using the formula of Blackman (1919) and expressed as mg g^{-1} day^{-1}.

$$RGR = \frac{\log W_2 - \log W_1}{T_2 - T_1}$$

Where,

W_1 and W_2 = Dry weight of plant at time intervals T_1 and T_2 respectively.

3.7.3 Crop growth rate (mg cm^{-2} day^{-1})

Crop growth rate (CGR) is the rate of dry matter production per unit ground area per unit time. CGR was calculated by adapting the formula suggested by Watson (1952) and expressed as g cm^{-2} day^{-1}.

$$CGR = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{A}$$

Where,

W_1 and W_2 = Dry weight of plant at time intervals T_1 and T_2 respectively.

A = Unit land area occupied by the plant (cm^2)

3.7.4 Net assimilation rate (g cm^{-2} day^{-1})

Net assimilation rate (NAR) is the rate of dry weight increase per unit leaf area per unit time, which was calculated by the formula given by Gregory (1926) and expressed as g cm^{-2} day^{-1}.

$$NAR = \frac{(W_2 - W_1)}{(T_2 - T_1)} \quad X \quad \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)}$$

Where,

L_1 and W_1 = Leaf area (cm^2) and dry weight of the plant (g) at time T_1

L_2 and W_2 = Leaf area (cm^2) and dry weight of the plant (g) at time T_2.

3.8 Physiological studies

The physiological and biochemical parameters were estimated before herbicide application (14^{th} day), one week after application and at flowering stage.

3.8.1 Chlorophyll content

The chlorophyll a, Chlorophyll b and total chlorophyll were estimated by the method suggested by Hiscox and Israelstam (1979). The chlorophyll content was estimated in spectrophotometer ((Model-4001/4 ThermoSpectonic, Thermo Electron Corporation, USA) at two wavelength 663 nm and 645 nm and expressed as milligram g^{-1} fresh weight of plant tissue. The calculation was done by using 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

3.8.2 Net photosynthesis

Photosynthetic rate was measured by using the instrument infrared gas analyzer (Model LI-6400 of ICOR inc. Lincoln, Nebreska, USA) at morning 08-10AM. It was estimated in 14 DAS and one week after application of herbicides and expressed as μ mol CO_2 m^{-2} s^{-1}.

3.8.3 Stomatal conductance

Stomatal conductance was measured by using the instrument infrared gas analyzer (Model LI-6400 of ICOR inc. Lincoln, Nebreska, USA) at morning from 08-10AM. It was estimated in 14 DAS one week after application of herbicides and expressed as m mol H_2O m^{-2} s^{-1}.

3.8.4 IAA content

IAA (Indol acetic acid) was estimated by the method suggested by Parthasarathy *et al.* (1970) with little modification using Garden Weber reagent. The IAA was expressed in μg of unoxidised Auxin g^{-1} fresh weight.

3.9 Biochemical characters

3.9.1 Total soluble protein

Total soluble proteins was estimated by Lowry *et al.* (1951) and expressed as mg/g of sample.

3.9.2 Total free amino acids

Amino acid concentration in leaves of the sample plants was estimated by using ninhydrin method described by Moore and Stein (1948) and expressed as mg g^{-1} plant sample.

3.9.3 Estimation of Catalase in leaf

Catalase (CAT) activity was estimated by permanganate titration method of Barber (1980). The activity of the catalase was expressed in enzyme units. One unit

Plate 3.Observations taken by using IRGA

of catalase was defined as that amount of enzyme, which breaks down 1 μ mol. of H_2O_2 / min.

3.9.4 Nitrate reductase activity

To estimate nitrate reductase activity in the leaf, the method suggested by Hageman and Flesher (1960) was followed. The nitrite formed was estimated by the method described by Nicholas *et al.* (1976), by measuring the absorbance of the pink colour at 540 nm using spectrophotometer and it was expressed in μmoles of NO_2^- formed g^{-1} fresh weight hr^{-1}.

3.9.5 Total phenols

Phenol content was estimated by the method suggested by Malick and Singh (1980). The phenol content was expressed in mg g^{-1} of plant sample. Standard solution was prepared with catechol and absorbance taken at 650 nm.

3.9.6 Proline content

Proline content was determined by the method of Bates *et al.* (1973) and expressed as mg/g plant tissue. Absorbance was taken at 520 nm.

3.10 Yield attributes and yield

3.10.1 Number of productive tillers

The number of productive tillers in 0.25 square meter area of each experimental plot at harvesting stage was counted and recorded, using a 50m X 50m quadrat and multiplied by 4 to express the value as tillers/m^2.

3.10.2 Number of spikelets per panicle

The number of spikelets was counted from five plants collected randomly from each plot and and the average worked out.

3.10.3 Number of filled grains per panicle

The number of filled grains was counted from five plants collected randomly from each plot and the average worked out.

3.10.4 Chaff percentage

Randomly selected five plants from each plot were collected and the chaff grains per each panicle were worked out.

3.10.5 Thousand grain weight

One thousand grains were counted from the produce of each plot and their weights were recorded in grams.

3.10.6 Grain and straw yield

The crop harvested from each replication was threshed, winnowed and weighed seperately. The straw and grain weight were recorded and expressed in kg/ha.

3.11 Observation on weeds

3.11.1 Weed count

Species wise weed count was taken using a 50cm x 50cm (0.25 m^2) quadrat. The quadrat was placed at random and samples were taken from each plot at 60 days and were reported as number/m^2.

Plate 4. Field observations

3.11.2 Biomass of weeds

The weeds uprooted from the quadrat at 60 days were cleaned, air dried and then oven dried at 80 ± 5^0C. The dry weight was recorded in kg/ha

3.12 Data analysis

The data were subjected to analysis of variance using the statistical package 'MSTAT-C' (Freed, 1986). Data on weed biomass, which showed wide variation, were subjected to square root transformation $\sqrt{(x+0.5)}$ to make the analysis of variance valid (Gomez and Gomez, 1984). Multiple comparisons among treatment means, where the F test was significant (at 5% level) were done with Duncan's Multiple Range Test (DMRT).

Results

4. Results

The present investigation aims to study the effects of ALS inhibitors and ACCase inhibiting herbicides on the physiological, biochemical and morphological parameters of rice. The results of the investigation are given below.

Table 3. Physiological and biochemical parameters of rice seedlings before application of herbicides (14 days)

Parameter	Mean	Standard error
LAI	0.17	0.01
Chlorophyll'a' (mg/g)	1.710	0.08
Chlorophyll'b' (mg/g)	0.845	0.11
Total chlorophyll (mg/g)	3.14	0.06
Total soluble protein (mg/g)	27	3.44
Proline content (mg/g)	0.05	0.007
Nitrate reductase enzyme activity (µmoles of NO_2^- formed g^{-1} fresh weight hr^{-1})	.340	10.66
IAA content (mg of unoxidised auxin/g)	0.729	0.01
Total phenol content (mg/g)	1.74	0.01
Net photosynthesis (µ mole CO_2 $m^{-2}s^{-1}$)	35	2.66
Stomatal conductance (mol $H_2Om^{-2}s^{-1}$)	1.13	0.05
Catalase enzyme activity (units/gm)	9.6	0.59
Total amino acid content (mg/g)	10	1.63

Biochemical and physiological parameters of the rice seedlings were taken on the 14th day before application of herbicides. 15 seedlings were collected randomly from different plots and the results of the estimation are given above.

LAI was observed in the range 0.16 - 0.18. Chlorophyll 'a' was observed in the range of 1.702 - 1.718. Chlorophyll 'b' was 0.834 - 0.856 and total chlorophyll was 3.08 - 3.20 mg/g. On the 14th day the range of total soluble protein was observed as 23.56 - 30.44 mg/g and the range of proline content was 0.043 - 0.057 mg/g and the range of IAA content was recorded as 0.728 - 0.730mg of unoxidised auxin/g. The range of phenol content was observed as1.73 -1.75 mg/g and the range of net photosynthesis was observed as 32.34 - 37.66 μ mole CO_2 / $m^{-2}s^{-1}$, stomatal conductance was 1.08 - 1.18 mol H_2O $m^{-2}s^{-1}$, catalase enzyme activity 9.01 - 10.19 units/g and the range of total amino acid content was recorded as 8.37 - 11.63mg/g.

4.1.1 Plant height

The herbicides were applied on the 15th day and plant height was recorded on 30th, 45th, 60th, 75th, 90th and105th day. The results revealed that 15 days after the herbicide application there was significant variation in plant height between the hand weeded and herbicide applied plots. The highest plant height was recorded in the Hand weeded plot (53.35 cm). This was on par with the plants in the Bispyribac sodium applied (53.20 cm) plot. There was significant reduction in plant height in all other herbicide treatments. Maximum reduction was observed in Fenoxaprop (49.40 cm) applied plot. By the 45th day there was a slight improvement in plant height and it was on par with other herbicide treatments excluding Bispyribac sodium (63.45) which was significantly superior. By the 60th day the height of plants in the plots applied with Fenoxaprop, Metamifop, Azimsulfuron and Almix showed significant reduction as compared to Hand weeded, Bispyribac and Cyhalofop applied plots. By the 75th day there was improvement in the Eenoxaprop and Metamifop applied plots but the height of the plants in the Azimsulfuron and Almix plot showed significant reduction. This trend continued till at harvest time. Plant height in all the herbicide applied plots were significantly inferior to Hand weeded plot. However, among the herbicide treated plots the height of the plants in the Azimsulfuron, Almix and Fenoxaprop plots were significantly inferior to other herbicides (Table 4).

The summary analysis of variance (Appendix II) of height indicated that there was no significant difference between the ACCase and ALS herbicide groups. Among the ACCase herbicides Cyhalofop showed maximum values compared to Metamifop and Fenoxaprop. Among the ALS herbicides Bispyribac showed less reduction in height than Azimsulfuron and Almix.

Table 4. Effect of herbicides on plant height (cm) at different intervals

Treatment	30 days	45 days	60 days	75 days	90 days	105 days
Cyhalofop	50.7^{bc}	62.12^{b}	81.10^{bc}	96.07^{b}	102.90^{c}	108.50^{c}
Fenoxaprop	49.40^{c}	61.40^{b}	80.45^{c}	95.45^{bc}	101.70^{d}	106.0^{e}
Metamifop	50.02^{bc}	62.00^{b}	80.65^{c}	95.20^{bc}	102.40^{cd}	107.4^{d}
Bispyribac	53.20^{a}	63.45^{a}	82.07^{b}	97.20^{a}	104.50^{b}	109.6^{b}
Azimsulfuron	50.97^{b}	61.87^{b}	80.12^{c}	94.70^{c}	101.20^{e}	105.3^{e}
Almix	50.52^{bc}	61.05^{b}	80.05^{c}	94.80^{c}	101.90^{d}	105.6^{e}
Handweeded	53.35^{a}	64.0^{a}	84.05^{a}	97.55^{a}	105.30^{a}	112.1^{a}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.1.2 Number of tillers

Data regarding the effect of various treatments on tiller number / m^2 of rice plants at different intervals are given in Table 5. On the 30th day maximum tiller number / m^2 was observed in Hand weeded control (256) followed by Bispyribac (242) applied plot. The minimum was recorded in Fenoxaprop applied plot (229). By the 45^{th} day , maximum tiller / m^2 was noticed in Hand weeded (441) which was on par with Bispyribac sodium (438).

Minimum tiller number / m^2 was observed in Azimsulfuron (419). By the 60^{th} day rice plants in hand weeded plot (536) showed more tiller number / m^2 followed by Bispyribac sodium (534). Almix application had the least number of tillers (514). By the 75^{th} day, the tiller decline was observed in all the treatments. Maximum tillers / m^2 was seen in the Hand weeded plot (435) followed by Bispybac (430). Minimum was observed in Azimsulfuron (414) and Almix (414) applied plots. By the 90^{th} day, Hand weeded treatment showed more number of tillers (369) followed by Bispyribac sodium.(362). Azimsulfuron showed less tillers / m^2 (346). By the 105^{th} day, Hand weeded (351) showed more number of tillers which was on par with Bispyribac (346) and Cyhalofop (338). Azimsulfuron had the least number of tillers / m^2 (329) at the time of harvest.

The summary analysis of variance (Appendix II) of tiller number indicated that there was no significant difference between the ACCase and ALS herbicide groups. Among the ACCase herbicides Cyhalofop showed maximum values compared to Metamifop and Fenoxaprop. Among the ALS herbicides Bispyribac showed less reduction in tiller number than Azimsulfuron and Almix.

Table 5. Effect of herbicides on tiller number/m^2 at different intervals

Treatment	30 days	45 days	60 days	75 days	90 days	105 days
Cyhalofop	239^{bc}	433^{ab}	526^{bc}	422^{bc}	358^{b}	338^{a}
Fenoxaprop	229^{de}	424^{bc}	517^{d}	416^{c}	349^{cd}	333^{bc}
Metamifop	234^{cd}	428^{bc}	520^{cd}	420^{c}	355^{bc}	336^{bc}
Bispyribac	242^{b}	438^{a}	534^{ab}	430^{ab}	362^{ab}	346^{a}
Azimsulfuron	227^{e}	419^{c}	515^{d}	414^{c}	346^{d}	329^{c}
Almix	232^{c}	423^{c}	514^{d}	414^{c}	348^{cd}	331^{bc}
Handweeded	256^{a}	441^{a}	536^{a}	435^{a}	369^{a}	351^{a}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.1.3 Days to flowering

Data regarding the effect of various treatments on days to flowering are given in Table 6. Herbicide application resulted in a slight delay in days to flowering. Flowering was observed by the 64.75 days in Hand weeded plot but it was delayed by 2 days in Azimsulfuron applied plot (66.75 days) followed by Almix (66.25).

Table 6. Effect of herbicides on days to flowering (Days)

Treatment	Days to flowering
Cyhalofop	65.50^{bc}
Fenoxaprop	65.75^{b}
Metamifop	66.00^{ab}
Bispyribac	65.50^{bc}
Azimsulfuron	66.75^{a}
`Almix	66.25^{ab}
Handweeded	64.75^{c}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.1.4 Leaf area index

Herbicides were applied on the 15th day and leaf area index was recorded on 15th, 30th, 45th, 60th, 75th, 90th and 105th day. By the 30th day, Hand weeded control showed more leaf area index (2.06) which was significantly superior to Bispyribac sodium (1.89) applied plot. Azimsulfuron (1.66) and Fenoxaprop (1.66) showed least leaf area index. On the 45th, 60th and 75th day the same trend was seen. By the 90th day leaf area index showed uniform decline but the treatment effect continued. Application of herbicides resulted in a significant reduction in LAI as compared to the Hand weeded plots. However, LAI in herbicide treated plots was on par with each other (Table7).

Table 7. Effect of herbicides on LAI at different intervals

Treatment	30 days	45 days	60 days	75 days	90 days	105 days
Cyhalofop	1.81^{bc}	3.44^{c}	4.33^{c}	5.27^{c}	2.98^{b}	1.77^{b}
Fenoxaprop	1.66^{d}	3.37^{cd}	4.20^{de}	5.15^{de}	2.85^{cd}	1.72^{b}
Metamifop	1.76^{cd}	3.41^{c}	4.26^{cd}	5.21^{cd}	2.89^{c}	1.74^{b}
Bispyribac	1.89^{b}	3.63^{b}	4.52^{b}	5.45^{b}	3.06^{b}	1.86^{b}
Azimsulfuron	1.66^{d}	3.30^{d}	4.13^{e}	5.03^{f}	2.79^{cd}	1.75^{b}
Almix	1.73^{cd}	3.36^{cd}	4.19^{de}	5.10^{ef}	2.81^{d}	1.76^{b}
Handweeded	2.06^{a}	3.75^{a}	4.67^{a}	5.77^{a}	3.15^{a}	1.90^{a}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.1.5 Crop growth rate

The data pertaining to crop growth rate for different intervals are indicated in (Table 8). There was not much variation between the treatments for 15-30 and 30-45 days. However, there was significant variation in the crop growth rate at 45-60 days as well as 60-75 days. Minimum CGR was recorded in the case of Azimsulfuron (3.058) followed by Almix (3.157) applied plot which were significantly inferior to all other treatments. The Hand weeded plot showed the highest CGR values (3.783). However, during 60-75 days the CGR value for the Hand weeded plot (4.861) was significantly superior to all other herbicide treated plots. Among the herbicide treated plot the CGR in the Bispyribac (4.262) applied plot followed by the Cyhalofop (3.757) and Metamifop (3.602) applied plots were significantly higher than the CGR for the Almix (3.371) applied plot.

Table 8. Effect of herbicides on crop growth rate (mg cm^{-2} d^{-1})

Treatment	15-30 days	30-45 days	45-60 days	60-75 days	75-90 days	90-105 days
Cyhalofop	1.230	2.322	3.577^{abc}	3.757^{c}	0.800	0.500
Fenoxaprop	1.191	2.271	3.452^{bc}	3.488^{cd}	0.630	0.460
Metamifop	1.217	2.283	3.589^{abc}	3.602^{c}	0.580	0.300
Bispyribac	1.308	2.229	3.653^{ab}	4.262^{b}	0.760	0.410
Azimsulfuron	1.089	2.103	3.058^{d}	3.571^{cd}	0.400	0.430
Almix	1.132	2.181	3.157^{c}	3.371^{d}	0.580	0.320
Handweeded	1.473	2.390	3.783^{a}	4.861^{a}	1.140	0.400

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.1.6 Relative growth rate

Estimation of relative growth rate of the rice plant (Table 9) indicated that there was no variation due to the herbicide application for RGR during 15-30, 30-45 and 45-60 days. However there was significant variation in the relative growth rate at 60-75 days. The maximum relative growth rate was observed in the hand weeded (33.621) plot. Among the herbicide treatments Almix (25.539) showed maximum reduction in RGR and this was significantly lower than all other herbicide treatments.

Table 9. Effect of herbicides on relative growth rate (mg g^{-1} d^{-1})

Treatment	15-30 days	30-45 days	45-60 days	60-75 days	75-90 days	90-105 days
Cyhalofop	195.797	68.383	46.515	29.868[b]	4.652	2.778
Fenoxaprop	192.376	70.116	45.466	27.752[bc]	3.903	2.706
Metamifop	195.182	68.041	46.409	27.158[bc]	3.510	1.742
Bispyribac	203.923	61.813	46.892	30.016[b]	4.192	2.147
Azimsulfuron	188.133	69.068	44.100	27.018[bc]	2.656	2.732
Almix	190.561	69.062	46.483	25.539[c]	3.779	1.999
Handweeded	205.898	62.036	43.987	33.621[a]	5.919	1.958

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.1.7 Net assimilation rate

Estimation of net assimilation rate indicated that in 15-30 days and 30-45 days there was no significant difference between the treatments. However, there was significant difference in the net assimilation rate at 45-60 days as well as 60-75 days. Hand weeded plot showed significantly higher net assimilation rate (0.455) at 45-60 days which was on par with the Bispyribac (0.435) and Cyhalofop (0.450) applied plots. Azimsulfuron (0.398) showed least net assimilation rate. By 60-75 days handweeded control showed more net assimilation rate (0.451) followed by Bispyribac applied plot (0.414). Almix showed least net assimilation rate (0.330) (Table 10).

Table 10. Effect of herbicides on net assimilation rate (mg cm^{-2} d^{-1})

Treatment	15-30 days	30-45 days	45-60 days	60-75 days	75-90 days	90-105 days
Cyhalofop	0.854	0.441	0.450^{a}	0.379^{bc}	0.094	0.045
Fenoxaprop	0.852	0.453	0.441^{ab}	0.361^{cd}	0.073	0.055
Metamifop	0.838	0.441	0.437^{ab}	0.368^{cd}	0.067	0.050
Bispyribac	0.928	0.403	0.453^{a}	0.414^{ab}	0.060	0.068
Azimsulfuron	0.781	0.424	0.398^{c}	0.377^{bc}	0.060	0.045
Almix	0.790	0.428	0.412^{bc}	0.330^{d}	0.066	0.050
Handweeded	0.857	0.409	0.455^{a}	0.451^{a}	0.142	0.068

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.2 Phytotoxicity screening

Table 11. Visual phytotoxicity screening of rice plant

Treatment	Grade (0-10 scale)
Cyhalofop	0
Fenoxaprop	0
Metamifop	0
Bispyribac	0
Azimsulfuron	0
Almix	0
Handweeded	0

Phytotoxicity screening was done on three days after application and no visible symptoms were observed in any of the herbicide applied plots (Table 11).

4.3 Biochemical parameters

4.3.1 Chlorophyll content

Data on chlorophyll content in rice is given in the Table 12 and 13. One week after herbicide application there was significant difference in the chlorophyll content of leaves in the herbicide treated and Hand weeded plots. The maximum reduction in chlorophyll 'a' was observed in Azimsulfuron (2.43) followed by Almix (2.48). Cyhalofop (2.67) and Bispyribac (2.71) application also reduced the chlorophyll 'a' content of rice which was significantly lower than Hand weeded plot (2.80) but superior to other treatments. After flowering Handweeded applied plot (1.66) had more chorophyll 'a' which was on par with Bispyribac applied plot (1.62). Azimsulfuron showed least chlorophyll 'a' content (1.49).

One week after application the chlorophyll 'b' content in the Bispyribac applied plot (1.06) was on par with the Hand weeded plot (1.08). Cyhalofop (0.87), Fenoxaprop (0.83) and Metamifop (0.84) application showed similar results and they were all on par and significantly inferior to Hand weeded and Bispyribac application but significantly superior to Azimsulfuron and Almix applied plot. After flowering Hand weeded plot (0.77) showed more chorophyll 'b' which was followed by Bispyribac sodium applied plot (0.70). Azimsulfuron (0.52) and Almix (0.52) showed least chlorophyll 'b' content.

One week after application Hand weeded plot showed more total chlorophyll (3.88) which was followed by Bispyribac sodium applied plot (3.77). Azimsulfuron showed least total chlorophyll content (3.20). After flowering same trend was seen in total chlorophyll content.

The summary analysis of variance (Applendix III) of chlorophyll 'a', 'b' and toatal chlorophyll indicated that there was no significant difference between the ACCase and ALS herbicide groups. Among the ACCase herbicides Cyhalofop showed maximum values compared to Metamifop and Fenoxaprop. Among the ALS herbicides Bispyribac showed less reduction than Azimsulfuron and Almix. However the reduction in the in the case of chlorophyll ' b' was almost uniform in the case of ACCase inhibiting enzyme group.

Table 12. Chlorophyll content of rice (mg/g fresh wt) 7 DAH application

Treatment	Chlorophyll'a'	Chlorophyll'b'	Total Chlorophyll
Cyhalofop	2.67^{b}	0.87^{b}	3.55^{c}
Fenoxaprop	2.55^{cd}	$0.83b^{cd}$	3.39^{d}
Metamifop	2.65^{bc}	0.84^{bc}	3.48^{cd}
Bispyribac	2.71^{b}	1.06^{a}	3.77^{b}
Azimsulfuron	2.43^{e}	0.77^{d}	3.20^{e}
Almix	$2.48d^{e}$	0.79^{cd}	3.27^{e}
Handweeded	2.80^{a}	1.08^{a}	3.88^{a}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

Table 13. Chlorophyll content of rice (mg/g fresh wt) at the time of flowering

Treatment	Chlorophyll'a'	Chlorophyll'b'	Total Chlorophyll
Cyhalofop	1.57[b]	0.60[c]	2.18[c]
Fenoxaprop	1.49[c]	0.55[cd]	2.05[de]
Metamifop	1.52[c]	0.59[cd]	2.12[cd]
Bispyribac	1.62[a]	0.70[b]	2.33[b]
Azimsulfuron	1.49[c]	0.52[d]	2.03[e]
Almix	1.53[bc]	0.52[d]	2.06[de]
Handweeded	1.66[a]	0.77[a]	2.44[a]

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.2 Total soluble protein

Data regarding the effect of various treatments on total soluble protein in rice plants at different intervals are given in Table 14. Hand weeded control showed more total soluble protein content (40.80) which was on par with Bispyribac sodium applied plot (40.65). Fenoxaprop (32.90), Azimsulfuron (33.15) and Almix (34.75) showed less total soluble protein which was significantly lower than the other herbicide treatments. After flowering, Hand weeded treatment had more total soluble protein content (28.8) which was followed Bispyribac treatment (27.10). The plants in the Azimsulfuron applied plots had the least protein content (24.45) which was significantly lesser than all other treatments.

The summary analysis of variance (Applendix III) of soluble protein indicated that there was no significant difference between the ACCase and ALS herbicide groups. Among the ACCase herbicides Cyhalofop showed maximum values. Among the ALS herbicides Bispyribac showed less reduction than Azimsulfuron and Almix.

Table 14. Effect of herbicides on total soluble protein content (mg/g) in rice plant

Treatment	7 DAH	At flowering
Cyhalofop	35.60[b]	26.70[b]
Fenoxaprop	32.90[c]	25.35[c]
Metamifop	34.90[bc]	25.85[c]
Bispyribac	40.65[a]	27.10[b]
Azimsulfuron	33.15[c]	24.45[d]
Almix	34.75[bc]	25.52[c]
Handweeded	40.80[a]	28.8[a]

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.3 Proline accumulation

Data regarding the effect of various treatments on proline accumilation of rice plants at different intervals are given in Table 15. The proline content reduced uniformly with the stage of the crop. One week after application of herbicides the proline content of the herbicide applied plots was significantly higher than the Hand weeded plot (0.080). Among the herbicide applied plots the proline content of Almix (0.125), Azimsulfuron (0.131) and Fenoxaprop (0.125) applied plots was significantly superior to others. Bispyribac sodium (0.084) followed by Cyhalofop butyl (0.098) showed lower values of proline. After flowering also the same trend was seen. Azimsulfuron had significantly superior values (0.091) than all other treatments.

The summary analysis of variance (Applendix III) of proline content indicated that there was no significant difference between the ACCase and ALS herbicide groups. Among the ACCase herbicides and ALS herbicides there was no significant difference in the proline content.

Table 15. Effect of herbicides on proline content (mg/g) in rice plant

Treatment	7 DAH	At flowering
Cyhalofop	0.098[c]	0.075[b]
Fenoxaprop	0.125[a]	0.078[b]
Metamifop	0.109[b]	0.076[b]
Bispyribac	0.084[d]	0.062[c]
Azimsulfuron	0.131[a]	0.091[a]
Almix	0.125[a]	0.079[b]
Handweeded	0.080[d]	0.055[c]

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.4 Nitrate reductase enzyme activity

Data given in (Table. 16) shows that one week after herbicide application nitrate reductase enzyme activity had gradually increased. Hand weeded plot (493.7) showed more nitrate reductase enzyme activity followed by Bispyribac sodium (450.0) applied plot. Azimsulfuron showed the least enzyme activity (365.0). After flowering the nitrate reductase enzyme activity declined uniformly. However the variation among treatments followed a similar trend and Hand weeded plot (373.2) showed more enzyme activity which was followed by Bispyribac sodium plot (355.2). Azimsulfuron applied plot (303.7) had the least nitrate reductase enzyme activity.

Summary analysis of variance of nitrate reductase enzyme (Appendix IV) activity indicated that there was no significant difference between the groups. Among the ACCase ase group Cyhalofop showed more nitrate reductase enzyme activity. Among the ALS herbicide group Bispyribac showed more nitrate reductase enzyme activity.

Table 16. Effect of nitrate reductase enzyme activity in rice plant (μ moles of NO_2^- formed g^{-1} fresh weight hr^{-1})

Treatment	7 DAH	At flowering
Cyhalofop	407.5^{c}	335.0^{c}
Fenoxaprop	390.0^{cd}	310.5^{de}
Metamifop	397.5^{cd}	325.0^{cd}
Bispyribac	450.0^{b}	355.2^{b}
Azimsulfuron	365.0^{d}	303.7^{e}
Almix	372.5^{cd}	308.5^{e}
Handweeded	493.7^{a}	373.2^{a}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.5 IAA content

Herbicide application significantly affected the IAA content of the plant and they were significantly lower than the Hand weeded plot (0.884) (Table 17). However the Bispyribac applied plot showed maximum IAA content (0.847) which was followed by the Cyhalofop (0.822), Metamifop (0.809) and Fenoxaprop applied plots (0.806). After flowering Bispyribac plot IAA content (1.109) improved and it was on par with the Hand weeded control (1.122). Lowest value was seen for Azimsulfuron (1.003) and Almix (1.006) applied plots.

Summary analysis of variance of IAA (Appendix III) activity indicated that there was no significant difference between the groups. Among the ACCase group Cyhalofop showed more IAA activity. Among the ALS herbicide group Bispyribac showed more IAA activity.

Table 17. Effect of herbicides on IAA content in rice plant (mg of unoxidised Auxin g^{-1} fresh weight)

Treatment	7 DAH	At flowering
Cyhalofop	0.822[bc]	1.075[b]
Fenoxaprop	0.806[bc]	1.025[cd]
Metamifop	0.809[bc]	1.050[bc]
Bispyribac	0.847[ab]	1.109[a]
Azimsulfuron	0.788[c]	1.003[d]
Almix	0.803b[c]	1.006[d]
Handweeded	0.884[a]	1.122[a]

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.6 Total phenols

Estimation of phenol content in the rice plants one week after application revealed that the total phenol content of plants in the Hand weeded plot (2.761) was significantly higher than plants from the herbicide applied plots (Table 18). Among the herbicide treatments Bispyribac had higher phenol content (2.244) which was on par with Cyhalofop (2.009), Fenoxaprop (1.995) and Metamifop (1.856). The least values was observed in the Azimsulfuron (2.890) and Almix (2.969) applied plots. After flowering a similar trend was observed however the phenol content of the Bispyribac applied plot (3.316) was on par with the Hand weeded plot (3.372) and phenol content of the plants in the Azimsulfuron applied plot (2.890) was significantly lower than all other treatments.

Summary analysis of variance of Total phenals (Appendix IV) indicated that there was no significant difference between the groups. Among the ACCase group Cyhalofop showed more phenol activity. Among the ALS herbicide group Bispyribac showed more phenol activity.

Table 18. Effect of herbicides on total phenol content (mg/g) in rice plant

Treatment	7 DAH	At flowering
Cyhalofop	2.009^{bc}	3.152^{b}
Fenoxaprop	1.995^{bc}	3.000^{bcd}
Metamifop	1.856^{bc}	3.095^{bc}
Bispyribac	2.244^{b}	3.316^{a}
Azimsulfuron	1.764^{c}	2.890^{d}
Almix	1.794^{c}	2.969^{cd}
Handweeded	2.761^{a}	3.372^{a}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.7 Photosynthetic rate

The photosynthetic rate showed variation among treatments (Table 19). One week after application of herbicides the Hand weeded (44.3) and Bispyribac (42.79) applied treatments were on par and significantly higher than other treatments. Cyhalofop (41) and Metamifop (40.15) were on par. However Fenoxaprop (39.96), Almix (38.4) and Azimsulfuron (39.4) application significantly reduced the photosynthetic rate of the plants.

Summary analysis of variance of (Appendix III) photosynthetic rate indicated that there was no significant difference between the groups. Among the ACCase group Fenoxaprop showed less photosynthetic rate. Among the ALS herbicide group Almix showed less photosynthetic rate.

Table 19. Effect of herbicides on photosynthetic rate in rice plants (μ mole CO_2 $m^{-2}s^{-1}$)

Treatment	7 DAH
Cyhalofop	41^{bc}
Fenoxaprop	39.96^{c}
Metamifop	40.15^{bc}
Bispyribac	42.79^{ab}
Azimsulfuron	39.4^{c}
Almix	38.4^{c}
Handweeded	44.3^{a}

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.8 Stomatal conductance

Stomatal conductance was found to be influenced by herbicide application (Table 20). Among the herbicides Bispyribac (1.29), Cyhalofop (1.28) and Metamifop (1.20) application were on par with Hand weeding plot (1.41). Fenoxaprop (1.04) and Metamifop (1.20) application were on par but they were significantly higher than Azimsulfuron (0.92) and Almix (0.92) application which showed the least value of stomatal conductance.

Summary analysis of variance of (Appendix III) stomatal conductance indicated that there was no significant difference between the groups. Among the ACCase group Fenoxaprop showed less stomatal conductance. Among the ALS herbicide group Almix showed less stomatal conductance.

Table 20. Effect of herbicides on stomatal conductance in rice plants (mol $H_2Om^{-2}s^{-1}$)

Treatment	7 DAH
Cyhalofop	1.28[a]
Fenoxaprop	1.04[bc]
Metamifop	1.20[ab]
Bispyribac	1.29[a]
Azimsulfuron	0.92[c]
Almix	0.92[c]
Hand weeded	1.41[a]

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.9 Catalase enzyme activity

One week after application of herbicides Azimsulfuron applied plot showed more catalase activity (17.73) this was on par with Almix (17.20) applied plot which was significantly higher than other herbicide treatments (Table 21). Hand weeded (10.21) plot showed least catalase enzyme activity. After flowering Azimsulfuron (66.11) applied plot showed more catalase activity which was followed by Almix (63.96) applied plot. Hand weeded (53.21) plot showed least catalase enzyme activity.

Summary analysis of variance of (Appendix IV) catalase activity indicated that there was no significant difference between the groups. Among the ACCase group Fenoxaprop showed more catalase activity. Among the ALS herbicide group Azimsulfuron showed more catalase activity.

Table 21. Effect of herbicides on catalase enzyme activity in rice plant (Catalase enzyme units/g)

Treatments	7 DAH	At flowering
Cyhalofop	11.83[bcd]	61.28[bc]
Fenoxaprop	13.98[b]	62.89[b]
Metamifop	12.90[bc]	59.66[c]
Bispyribac	11.28[cd]	54.29[d]
Azimsulfuron	17.73[a]	66.11[a]
Almix	17.20[a]	63.96[ab]
Hand weeded	10.21[d]	53.21[d]

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.3.10 Total amino acid content

Application of herbicide contributed to a significant reduction in the total amino acid content of the rice plant (Table 22). Among the herbicides Bispyribac (8.910), Almix (8.780) and Azimsulfuron (8.790) showed maximum reduction in the total amino acids and they were all on par and significantly lower than Hand weeded (12.280), Cyhalofop (11.895), Metamifop (10.505) and Fenoxaprop (10.425). Fenoxaprop treatment showed significant reduction in amino acid as compared to the other two herbicides. Observations taken after flowering indicated the Hand weeded plot (9.415) showed maximum total amino acid content which was on par with Metamifop (8.935), Fenoxaprop (8.915) and Cyhalofop (8.890) applied plots. Azimsulfuron (8.730) and Almix (8.560) and Bispyribac (8.420) applied plots showed lower total amino acid content.

Summary analysis of variance of (Appendix IV) total amino acid indicated that there was no significant difference between the groups. Among the ACCase group Cyhalofop showed more amino acid acontent. Among the ALS herbicide group there were no significant difference was observed.

Table 22. Effect of herbicides on total amino acid content (mg/g) in rice plant

Treatment	7 DAH	At flowering
Cyhalofop	11.895[a]	8.890[ab]
Fenoxaprop	10.425[b]	8.915[ab]
Metamifop	10.505[b]	8.935[ab]
Bispyribac	8.910[c]	8.420[b]
Azimsulfuron	8.790[c]	8.730[b]
Almix	8.780[c]	8.560[b]
Hand weeded	12.280[a]	9.415[a]

In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

4.4 Yield parameters

4.4.1 Number of productive tillers

The maximum number of productive tillers / m^2 was recorded in Hand weeded treatment (351) which was on par with Bispyribac sodium (346) and Cyhalofop (338) applied plot. Plants in the Metamifop (336), Fenoxaprop (333) and Azimsulfuron (329) applied plots showed significant reduction in tiller number and they were all on par (Table 23).

Summary analysis of variance (Appendix V) of productive tillers showed that there was no significant difference between the herbicide groups. Among the ACCase group Cyhalofop showed maximum productive tillers than Metamifop and Fenoxaprop. Among the ALS group Bispyribac showed maximum productive tillers than Azimsulfuron and Almix.

4.4.2 Number of spikelets

The number of spikelets / panicle ranged from 108.6 to 111. The maximum spikelets /panicle (111.0) was recorded in handweeded treatment which was on par with Bispyribac sodium (110.4), Fenoxaprop (110.1), Metamifop (109.8), Cyhalofop (109.7) and Azimsulfuron (109.3). Azimsulfuron showed least spikelets / panicle (108.6).

Summary analysis of variance (Appendix V) of spikelets showed that there was no significant difference between the herbicide groups. Among the ACCase group Cyhalofop showed maximum values than Metamifop and Fenoxaprop. Among the ALS group Bispyribac showed maximum values than Azimsulfuron and Almix.

4.4.3 Number of filled grains

The number of filled grains/ panicle ranged from 100.8 to 105.6. The maximum filled grains / panicle was recorded in Hand weeded treatment (100.8) followed by Bispyribac sodium applied plot (103.7). Almix showed least number of filled grains / panicles (100.8).

Summary analysis of variance (Appendix V) of filled grains showed that there was no significant difference between the herbicide groups. Among the ACCase group Fenoxaprop showed the least values and among the ALS group Almix showed the least filled grains per panicle.

4.4.4 Chaff percentage

Chaff percentage in the Azimsulfuron applied plot was the highest (9.435). Plants in the Hand weeded plot had the lowest chaff percentage (6.313) which was on par with Metamifop (6.412) applied plot. Chaff percentages with all other herbicide treatments were on par.

Summary analysis of variance (Appendix V) of chaff percentage showed that there was no significant difference between the herbicide groups. Among the ACCase group Fenoxaprop showed the maximum values and among the ALS group Azimsulfiron showed the maximum chaff percentage.

Table 23. Effect of herbicides on yield attributes

Treatment	Productive tillers/m^2	Spikelets/panicle	Filled grains/panicle	Chaff percentage	1000 grain wt (g)
Cyhalofop	338^a	110.1^{ab}	103.4^b	7.063^b	29.35^{bc}
Fenoxaprop	333^{bc}	109.7^{ab}	102.4^{bc}	7.710^b	29.52^{ab}
Metamifop	336^{bc}	109.8^{ab}	102.6^{bc}	6.412^c	29.49^{abc}
Bispyribac	346^a	110.4^{ab}	103.7^b	7.600^b	29.53^a
Azimsulfuron	331^{bc}	108.6^b	101.4^{cd}	9.435^a	29.34^c
Almix	329^c	109.3^{ab}	100.8^d	7.613^b	29.46^{abc}
Hand weeded	351^a	111.0^a	105.6^a	6.313^c	29.58^a

4.4.5 Grain yield

The highest grain yield of 6.07 t / ha was recorded in Hand weeded plot followed by Bispyribac sodium applied plot (5.733) Azimsulfuron plot (4.950) recorded the lowest grain yield (Table 24).

Summary analysis of variance (Appendix V) of grain yield showed that there was no significant difference between the herbicide groups. Among the ACCase group Cyhalofop showed the maximum values than Metamifop and Fenoxaprop. Among the ALS group Bispyribac sodium showed the maximum grain yield compared to Azimsulfuron and Almix.

4.4.6 Straw yield

Maximum straw yield was obtained from the Handweeded plot 7.913 t / ha followed by Bispyribac sodium applied plot (6.727). Lowest straw yield was obtained from the Azimsulfuron plot (5.975).

Summary analysis of variance (Appendix V) of straw yield showed that there was no significant difference between the herbicide groups. Among the ACCase group Cyhalofop showed the maximum values than Metamifop and Fenoxaprop. Among the ALS group Bispyribac sodium showed the maximum straw yield compared to Azimsulfuron and Almix.

Table 24. Effect of herbicides on yield (t/ha)

Treatment	Grain yield	Straw yield
Cyhalofop	5.575[c]	6.552[b]
Fenoxaprop	5.137[e]	6.137[cd]
Metamifop	5.325[d]	6.278[c]
Bispyribac	5.733[b]	6.727[b]
Azimsulfuron	4.950[f]	5.975[d]
Almix	4.988[f]	6.168[c]
Handweeded	6.037[a]	7.193[a]

4.5 Weed count

4.5.1 Studies on weeds

Major weed species found in experimental plot were grasses, sedges and broad leaved weeds. The grasses are *Echinochloa colona, Echinochloa crusgalli, Echinochloa stagnina* and *Leptochloa chinensis.*

Ludwigia perennis, Lindernia crustacea, Monochoria vaginalis, Sphaeranthes indicus and *Alternanthera* sp. were the broad leaved weeds.

Fimbristylis mileacea, Cyperus iria and *Cyperus difformis* were the sedges present. Species wise weed count was taken at 60 DAS and at harvest which is given in Table 25.

The observations related to weed spectrum, that is, weed population and biomass of weeds from the experiment is furnished below.

Table 25. Effect of herbicides on weed count (No/m^2)

Treatment	Grasses	Sedges	Broad leaved weeds
Cyhalofop	1.9^{b} (3.2)	2.5^{c} (6.0)	2.9^{a} (8.5)
Fenoxaprop	2.1^{ab} (4.2)	2.7^{abc} (7.2)	3.1^{a} (9.7)
Metamifop	2.2^{a} (4.7)	3.1^{a} (9.2)	2.7^{a} (7.0)
Bispyribac	1.0^{c} (0.7)	1.4^{d} (2.0)	1.4^{b} (2.2)
Azimsulfuron	2.1^{ab} (4.2)	1.8^{ab} (4.0)	1.9^{ab} (3.0)
Almix	2.0^{ab} (3.7)	1.4^{d} (2.0)	1.0^{b} (0.7)
Hand weeded	0.7^{d} (0.0)	0.7^{e} (0.0)	1.0^{b} (0.7)

G- Grasses, S- sedges, B- broad leaved weeds. *$\sqrt{x+0.5}$ transformed values, Original values in parentheses. In a column, means followed by common letters do not differ significantly at 5% level in DMRT.

By 60 DAS, the lowest grass count was noticed in hand weeded plot. Among the treatments Bispyribac sodium applied plots showed least number of grass count and was free of sedges also. The highest population of sedges (14/m^2) was registered in Metamifop applied plot as well as in Azimsulfuron applied plot. In Hand weeded plot, broad leaved weed mainly *Lindernia crustacea* was present (8/m^2). Among herbicide treatment Fenoxaprop and Cyhalofop showed more broad leaved weeds.

4.5.2 Biomass of weeds removed

By 60 DAS, the lowest accumulation of dry weight of 38 kg/ha was noticed in Hand weeded plots followed by Bispyribac sodium with 125 kg/ha (Table. 26).

Table 26. Effect of herbicides on weed dry weight (kg/ha)

Treatment	Weed dry weight
Cyhalofop	18.6[b] (346)
Fenoxaprop	19.3[a] (350)
Metamifop	18.2[b] (340)
Bispyribac	11.2[c] (125)
Azimsulfuron	12.5[c] (152)
Almix	18.4[b] (342)
Hand weeded	6.1[d] (38)

$\sqrt{x+0.5}$ transformed values, Original values in parentheses. In a column, means followed by common letters do not differ significantly at 5% level by DMRT.

Discussion

5. Discussion

Chemical weed control is the easiest and economical option for controlling weeds in a cropped ecosystem. The new generation weedicides such as ALS and ACCase inhibitors are reported to be ecofriendly and target specific.

The main focus of the present investigation was to evaluate the morphological, physiological and biochemical changes on the rice plant by the application of ACCase and ALS inhibiting herbicides. An attempt is made from the results obtained to discuss the effect of these parameters on the yield and yield attributes of rice.

Phytotoxicity screening of the rice plant conducted 3 days after herbicide application revealed that these groups of herbicides caused no visual symptoms of toxicity to the rice plant. This has been validated by the findings of Menon (2012).

5.1 Effect of herbicides on morphological characters

In the present study herbicide application resulted in a 2-4 percent reduction in height (Table. 4) and 1 to 6 percent reduction in tiller number (Table. 5) of rice plant at harvest as compared to plants in the Hand weeded plot. The Summary analysis (Appendix II) revealed that both ALS and ACCase inhibitors had similar effect on the rice plant. However the extent of suppression in morphological attributes depended on the chemical formulations. Menon (2012) and Mukherjee and Singh (2006) have also observed reduction in the height and tiller number of rice applied with the herbicides, Fenoxaprop (ACC) and Almix (ALS) as compared to Hand weeded control. The negative effect of ALS inhibitors on the plant height and tiller number may be due to the inhibiting effect of the chemical on amino acid production resulting in a decrease protein and enzyme synthesis leading to cessation of growth (Koschnick *et al.*, 2007). According Gonsolus and Curran (1996) in ACCase inhibitors, the active ingredients are absorbed by the leaves and translocated to the meristematic tissues through phloem. The process halts the growth of young developing leaves in susceptible plants by damaging the membrane structure of the cells in the meristematic region inhibiting cell division and extension of the growing point.

5.2 Effect of herbicides on growth indices

Effect of herbicides on growth attributes *viz.,* RGR, CGR and NAR indicated that at the initial and final stages of growth there was no significant difference between the treatments. However during the critical stages of growth *i.e.,* at 45-75 DAS (Yoshida, 1981) significant variation was seen among the treatments for growth indices except RGR and they were all significantly lower than Hand weeded crop. For RGR significant variation was observed during 60-75 days. Total dry matter is influenced by CGR, RGR, LAI and NAR (Hunt, 1982). The crop growth rate is an important growth factor that reflects the assimilation capacity of the plant canopy which in turn depends on LAI and NAR (Aishy *et al,* 1976).

LAI was significantly lower in all the growth stages studied. The maximum reduction in LAI was observed on the 30^{th} day (Table. 7). Among the herbicides, maximum reduction was observed in the Fenoxaprop and Azimsulfuron applied plots. This is as per the findings of Singh *et al.* (2013). They have reported that application of post emergent herbicides Phenoxaprop - p - ethyl and Sulfosulfuron has resulted in reduction in LAI of wheat as compared to untreated check. The drastic reduction in LAI would have affected the photosynthesis and dry matter accumulation in the plant. Chen and Black (1992) have reported that LAI is an important growth attribute that influences the photosynthetic efficiency and yield of the plant and can be used as reference tool for crop growth. The improvement in LAI during the later stages would have stabilized the dry matter accumulation process in the plant and hence subsequent variation in the CGR, NAR, and RGR was not significant. Among the ALS group, Bispyribac showed minimum reduction in both CGR and NAR. Among ACC group, Cyhalofop showed the minimum reduction in both CGR and NAR. This can be substantiated by the lower reduction in LAI by application of these chemicals. Despite sharing common modes of action, herbicides are known to have different effects on plant species (Koschnick *et al.*, 2007).

5.3 Effect of herbicides on physiological parameters

Physiological parameters like chlorophyll content, photosynthetic rate, stomatal conductance and IAA content showed no significant difference between the ACCase and ALS herbicide groups as per the summary analysis (Appendix III). Among the ACCase herbicides, Cyhalofop showed higher values compared to Metamifop and Fenoxaprop. Among the ALS herbicides, Bispyribac showed less reduction in physiological parameters than Azimsulfuron and Almix.

5.3.1 Chlorophyll content

Chlorophyll content is the most common indicator for various stresses and abnormal conditions to plant due to its simplicity and rapidity (Kim *et al.*, 2008). In the present study herbicide application reduced the chlorophyll a, b and total chlorophyll content (Table. 12&13) of the rice plant. Significant reduction was seen one week after application as compared to the flowering stage. The results were in concurrence with the findings of Huang *et al.* (2006). They found that application of Bensulfuronmethyl (ALS) affect the biomass and total chlorophyll content in the shoots of rice. The ACCase enzyme affects the plastid of the chloroplast leading to a reduction in the chlorophyll content (Burton *et al.*, 1987). The ALS enzyme affects the biosynthesis of amino acid and there by affecting the protein synthesis in plants (Ray, 1984). Chloroplasts have their own DNA, mRNA and protein synthesizing machinery. The chlorophyll biosynthetic pathway is very complex and consists of more than a dozen steps (Taiz and Zeiger, 1991). This might be the reason for the reduction in the chlorophyll content of the rice plant by the application of these herbicides.

5.3.2 Photosynthetic rate

Photosynthetic rate increases with stomatal conductance (Farquhar and Sharkey, 1982). The photosynthetic rate is also associated with the chlorophyll per leaf (Sestak, 1966.). Application of ALS and ACCase inhibiting herbicides has resulted in significant reduction in the photosynthetic rate and stomatal conductance of the rice plant. In grapes 34.5 percent decrease in photosynthetic rate due to application of post emergent herbicides has been reported by Tan *et al.* (2012). The effect of the herbicides on the chlorophyll a, b and total chlorophyll content coupled with a decrease in stomatal conductance might have contributed to a reduction in photosynthetic rate of rice plants. Among the herbicides, Azimsulfuron and Almix applied plots had lower chlorophyll content in the leaves (Table no 12&13) and lower stomatal conductance (Table. 20) and this might be the reason for the lower photosynthetic rate. Photosynthetic rate is positively correlated to the yield (Fig. 2).

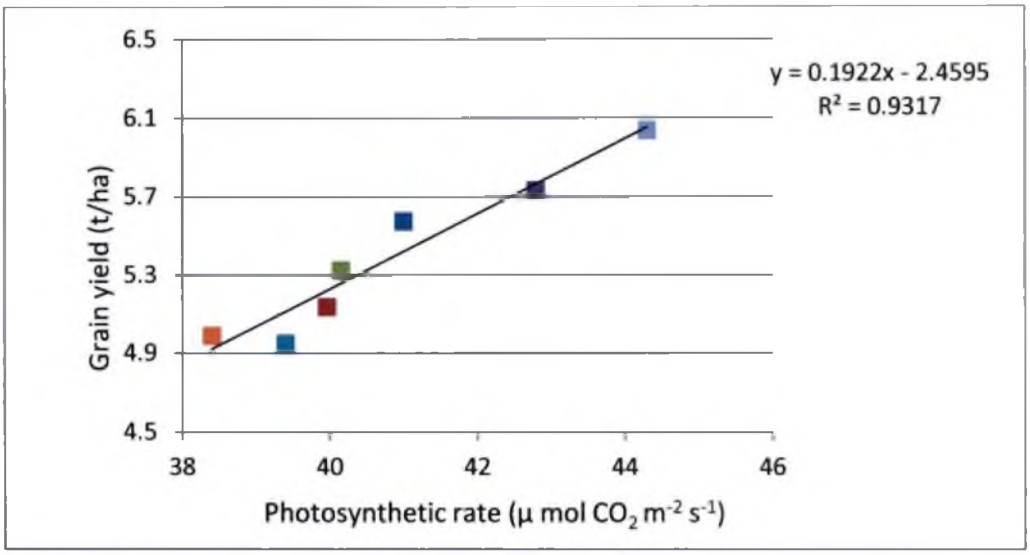

Fig. 2. Relationship between the Photosynthetic rate and grain yield

Treatments

- Hand weeded
- Bispyribac sodium
- Cyhalofop butyl
- Metamifop
- Fenoxaprop –p- ethylAzimsulfuron
- Almix
- Azimsulfuron

5.3.3 Stomatal conductance

Influence of stomatal conductance on grain yield is given in (Fig. 3). Stomatal conductance has a positive relationship with yield. Stomatal conductance regulates the entry of CO_2 into the plant and there by the accumulation of dry matter in the plant (Taiz ad Zeiger, 1991). The stomatal conductance of the rice plant was reduced significantly one week after application of herbicides. This result was in accordance with the observation of Oosterhuis *et al.* (1990). They found that application of Fenoxaprop reduced 35 percent stomatal conductance in rice plant. Among the herbicide applied plots maximum reduction was noticed in Almix and Azimsulfuron while minimum reduction was observed in the case of Bispyribac followed by Cyhalofop. Fayez (2000) reported that higher stomatal conductance results in high yield under good irrigation system.

5.3.4 IAA content

The hormone Indole acetic acid is mainly produced at the shoot tip and moves basipetally to the root tip and induces cell division and cell elongation of the roots. It also serves as signaling molecule necessary for development of plant organs and coordination of growth (Taiz and Zeiger, 1991). IAA induced cell division and cell elongation subsequently affects plant growth and development (Sugawara *et al.,* 2009). The ACCase enzyme inhibiting herbicides are reported to inhibit cell division and cell elongation of the growing tip (Snipes *et al.,* 1984). The inhibition of IAA in the herbicide applied plots might have contributed to the reduction in the growth of the plants. IAA content of the rice plant was significantly reduced one week after herbicide application and at flowering stage uniformly for all the herbicides (Fig. 4). The IAA content in the Almix and Azimsulfuron applied plots was significantly lesser. However by the time of flowering the IAA content of Bispyribac applied plot were on par with Hand weeded plot followed by Cyhalofop. This might be the reason for better performance of plants applied with Bispyribac and Cyhalofop.

5.4 Effect of herbicides on biochemical parameters

The results of the summary analysis of variance between the groups of herbicides and Hand weeded control (Appendix IV) indicated that the biochemical parameters like soluble protein, phenols, nitrate reductase enzyme activity and amino acid content were significantly

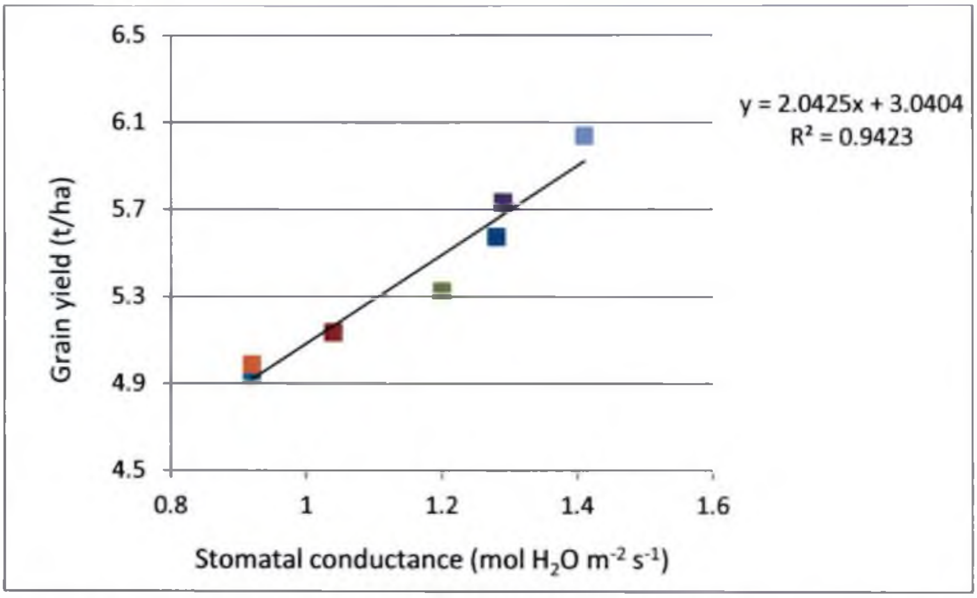

Fig. 3. Relationship between the stomatal conductance and grain yield

Treatments

- Hand weeded
- Bispyribac sodium
- Cyhalofop butyl
- Metamifop
- Fenoxaprop –p- ethylAzimsulfuron
- Almix
- Azimsulfuron

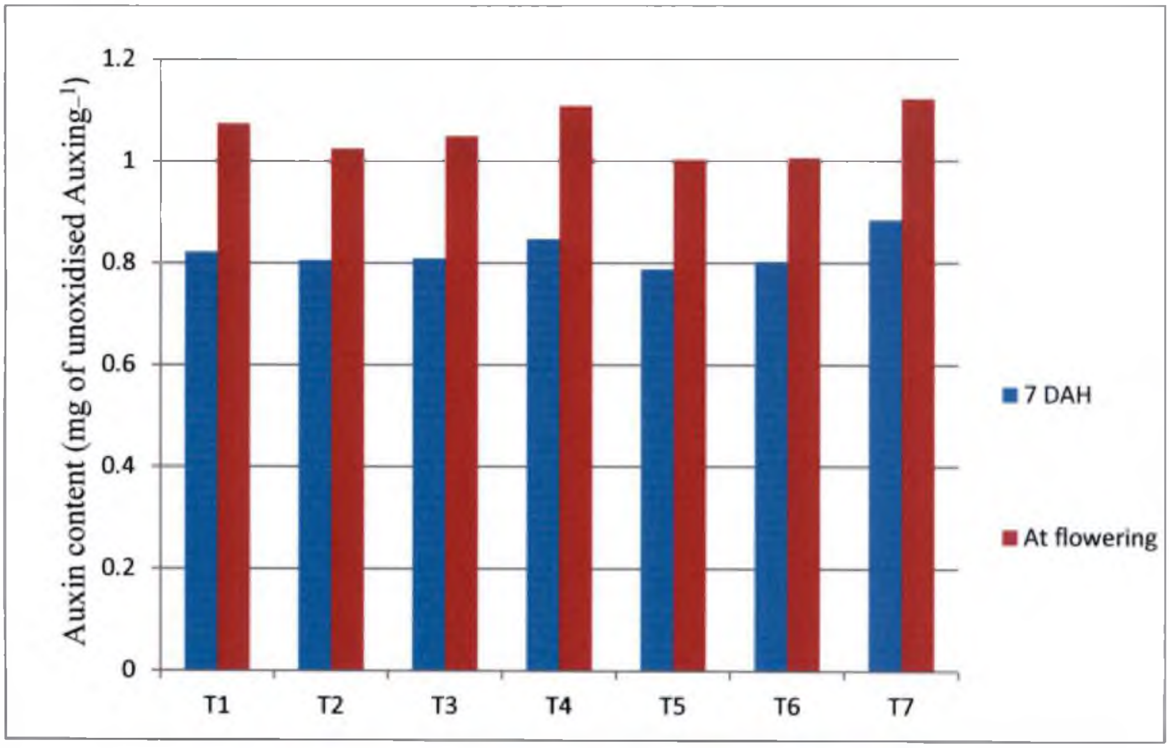

Fig. 4. Auxin content at 7 DAH and at the time of flowering

ACCase enzyme inhibitors

T1 - Cyhalofop butyl

T2 - Fenoxaprop - p – ethyl

T3 - Metamifop

ALS enzyme inhibitors

T4 - Bispyribac sodium

T5 - Azimsulfuron

T6 - Almix

T7 - Hand weeded

7 DAH – 7 days after herbicide application

lower for the herbicide applied plots as compared to Hand weeded plot. Among the ACCase inhibitors Cyhalofop showed higher values compared to Metamifop and Fenoxaprop in all the above parameters. Among the ALS enzyme inhibitors Bispyribac showed less reduction for all the above mentioned biochemical parameters. Proline content and catalase enzyme activity increased uniformly with herbicide application. However, these parameters were higher for those herbicides which showed maximum suppression in growth. The maximum value of these parameters was seen in Azimsulfuron and Almix applied plots.

5.4.1 Soluble protein

There was significant reduction in the soluble protein content one week after application of herbicides and at flowering stage. This result was in concurrence with the observation of Fedtke (1973). He observed that application of Metabenzthiazuron herbicide inhibited the protein content in rice as well as weeds. Kim *et al.* (2008) reported that leaf soluble protein is highly related to the content of Rubisco, a key enzyme of photosynthesis. The lowered leaf protein content reflects the decreased physiological activities in leaves including photosynthesis.

5.4.2 Proline content

There was significant increase in the proline content one week after application of herbicide and at flowering. This result is in concurrence with the observation of Ibrahim *et al.* (2004). They reported that combined applications of Fenoxaprop (ACCase) and Fluroxypyr increases the proline content in Maize. Panamarioviene *et al.* (1996) found that the application of Mecoprop increased the proline content in rye by 10-18 percent. Fayez and Kristen (1996) found that application of Chlorosulfuron (ALS), Norflurazon, and Triallate resulted in a reduction of root growth of *Pisum sativum, Phaseolus vulgaris and Vicia faba* and attributed this to the increase in proline content in the plants. Accumulation of the amino acid proline in plants occurs when the plant is experiencing stress (Salisbury and Ross, 2001). In the present study plants sprayed with herbicides Almix, Azimsulfuron (ALS group) and Fenoxaprop, Metamifop (ACCase group) seem to accumulate more proline indicating (Fig. 5) that these chemicals induced greater stress to the plant resulting in yield reduction.

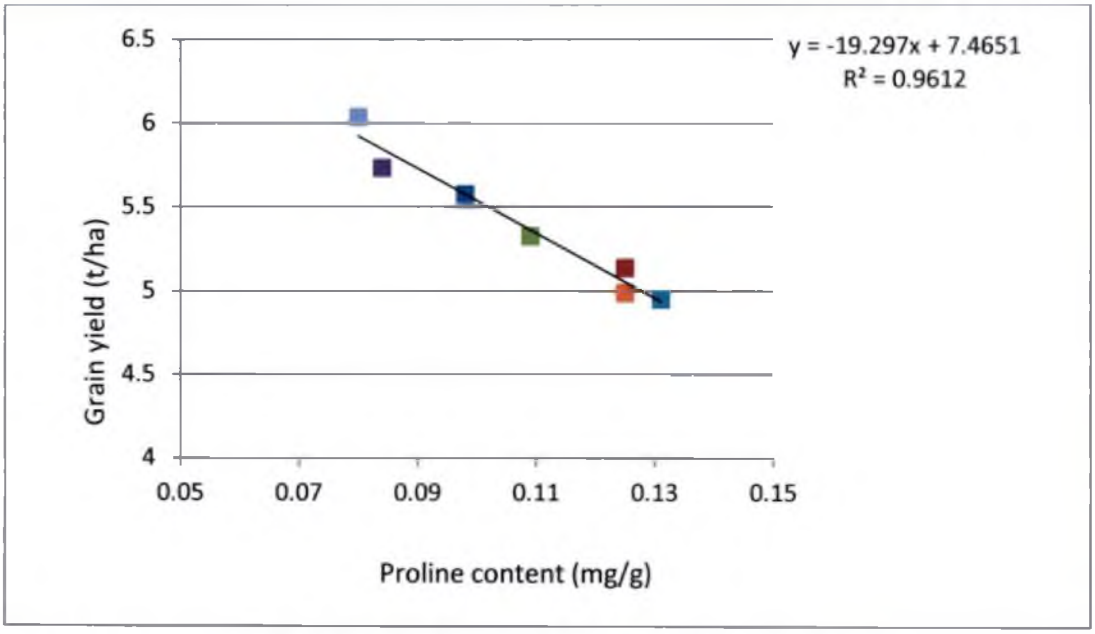

Fig. 5. Relationship between the proline content and grain yield

Treatments

- Hand weeded
- Bispyribac sodium
- Cyhalofop butyl
- Metamifop
- Fenoxaprop –p- ethylAzimsulfuron
- Almix
- Azimsulfuron

5.4.3 Nitrate reductase enzyme activity

Garg (2013) reported that nitrate reductase is one of the most important enzymes in the assimilation of exogenous nitrate, the most available nitrogen to green plants growing in soil. Activity of the enzyme in plants gives a best estimate of the nitrogen status of the plant and is very often correlated with growth and yield. Among the biochemical parameters nitrate reductase enzyme activity was found to be a major factor that influenced the grain yield (Fig. 6). In the present study the maximum reduction in NRase was observed in the case of Almix and Azimsulfuron applied plots. Both these herbicides belong to the sulfonyl urea group. ALS inhibiting herbicide affects the protein metabolism in plants. Hence reduction of the enzyme to have a direct relationship with yield of rice. Photosynthesis is dependent on nitrogen assimilation as this process provides the building blocks for the synthesis of the proteins and pigments that comprise the photosynthetic machinery. Decrease in CO_2 assimilation is accompanied by decrease in nitrogen assimilation which is due to the inhibition of the nitrate reductase enzyme (Nicholas *et al.,* 1976). The nitrate reductase enzyme activity of the rice plant was reduced significantly one week after herbicide application and at flowering stage. The results were in concurrence with the observation of Singh *et al.* (2013). They found that the application of Phenoxyprop and Sulfosulfuron reduced the nitrate reductase activity in wheat.

5.4.4 Catalase enzyme activity

Catalase is a scavenging enzyme which removes the active oxygen species (Taiz and Zeiger, 1991) accumulating in the plant due to stress. Herbicide application has resulted in an increase in the activity of catalase indicating that the plant has experienced stress which in turn has contributed to the production of active oxygen species in the rice plant. Those plants which had higher catalase activity with herbicide application had lower yield (Fig. 7) indicating that higher catalase activity had a negative influence on yield attributes of rice. There was significant increase in the activity of catalase enzyme in rice one week after application of herbicides and at flowering stage compared to Hand weeded plots. This result was in concurrence with the observation of Singh *et al.* (2013). They observed that the application of Phenoxyprop and sulfosulfuron increased the catalase enzyme activity in wheat. Lin *et al.* (2011) reported that Fluroxypyr induced oxidative stress in plants triggered the activities of scavenging enzymes like SOD, catalase and peroxidase. Hence increase in catalase activity indicated that application of

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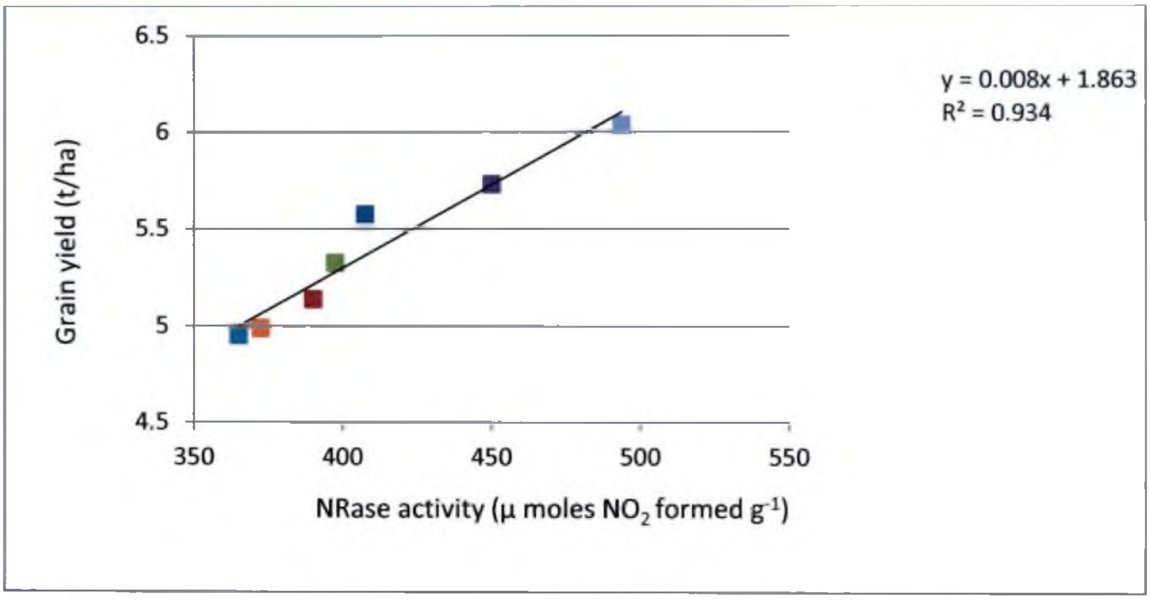

Fig.6. Relationship between NRase enzyme activity and grain yield

Treatments

- Hand weeded
- Bispyribac sodium
- Cyhalofop butyl
- Metamifop
- Fenoxaprop –p- ethylAzimsulfuron
- Almix
- Azimsulfuron

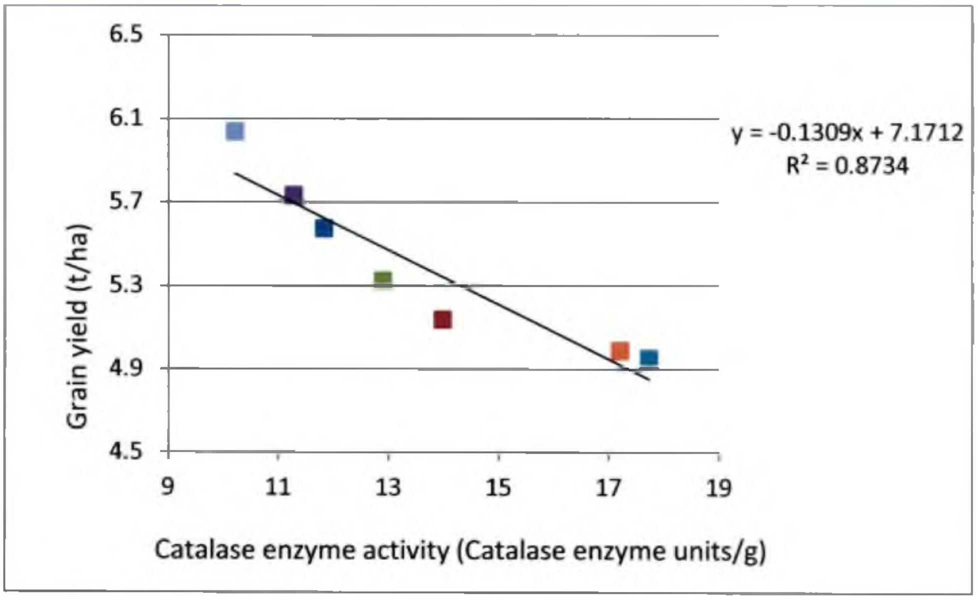

Fig. 7. Relationship between the catalase enzyme activity and grain yield

Treatments

- Hand weeded
- Bispyribac sodium
- Cyhalofop butyl
- Metamifop
- Fenoxaprop –p- ethylAzimsulfuron
- Almix
- Azimsulfuron

herbicides may be contributing to oxidative stress in the plants. In the study Azimsulfuron contributed to maximum oxidative stress for the plants followed by Almix and Fenoxaprop.

5.4.5 Phenol content

In the rice seedlings the phenol content is reported to increase with the growth of the plant. Valentine *et al.* (2003) has reported that as plants grow, phenolics accumulates in the cell vacuoles, or polymerize to lignin, which strengthens the secondary cell walls. In the present study a significant decrease in the phenol content one week after application was observed for all the herbicides. However there was variation between the herbicide treatments. In Bispyribac applied plots the phenol content improved by flowering and was on par with Hand weeded control. This might have contributed to the better performance of Bispyribac as compared to other herbicides. Ibrahim *et al.* (2004) reported that combined application of Fenoxaprop and Fluroxypyr increased the phenol content in maize compared to unweeded control.

5.4.6 Amino acid content

There was significant decrease in the total amino acid content one week after application of herbicides and at flowering stage. This was in concurrence with the observation of Kim *et al.* (2008). They found that application of Bensulfuron-methyl slightly decreased amino acid content in rice. ALS inhibiting herbicides expressed significant reduction in amino acid content when compared to ACCase inhibitors. ALS inhibitors contribute to reduction in free amino acid content which may lead to altered levels of chlorophyll, protein and sugar content in the rice plant (Moreland *et al.,* 1969).

5.5 Effect of herbicides on yield and yield parameters

The yield parameters of rice such as number of productive tillers, number of spikelets, and number of filled grains were significantly reduced in the herbicide applied plots. Among the yield attributes number of spikelets decides the grain number. The grain number is a major determinant of yield and is dependent on the partitioning efficiency of the plant (Sharma *et al.*, 2004). In the current study the spikelet number and filled grain were found to be significantly higher for the Hand weeded plot as compared to the herbicide applied plots. This was in concurrence with the observation of Menon (2012). She reported higher number of panicles/m^2, filled grains per panicle which resulted in higher grain and straw yield in the hand weeded plot as compared to herbicide treated plots. In the present study, 5 to 17 percent reduction in grain yield and 6 to 17 percent reduction in straw yield of rice was observed by herbicide application. The lowest reduction in grain and straw yield was seen in the case of Bispyribac sodium (5 percent) followed by Cyhalofop bütyl (7 percent). Petter *et al.* (2013) had reported that application of ALS inhibitors such as Penoxulam, Pyrazosulfuron and Bispyribac sodium had reduced the number of grains per panicle at the rate of 27 to 37 percent. The effect of ALS inhibiting enzyme on the biosynthesis of amino acid and photosynthesis (Ray, 1984) might be the major reason for the reduction in the yield attributes of rice. The ACCase enzyme affects carboxylation of acetyl CoA to form melonyl CoA which is the first step in the synthesis of fatty acid (Gronwald, 1992). Meloñyl CoA is an intermediate of a number of metabolic pathways. Hence inhibition of the ACCase enzyme will contribute to a disruption in the metabolic activities of the plant there by affecting the yield. The results of the summary analysis of variance between the groups of herbicides and Hand weeded control (Appendix V) indicated that the yield and yield parameters were significantly lower for the herbicide applied plots as compared to the Hand weeded control. There was no significant difference between the ACCase and ALS inhibitors. Among the ACCase group, Cyhalofop showed maximum yield and yield attributes compared to the other two herbicides. Among ALS inhibitors Bispyribac showed maximum yield. This might because of the faster recovery of the plants from the stress imposed by the application of these chemicals. The estimation of proline and catalase content one week after application and at the time of flowering substantiate the findings (Table. 15 & 21). Several authors like Suganthi *et al.* (2005) and Subhalakshmi and Venkataramana (2009) reported an increase in yield and yield attributes due to hand weeding.

The biochemical parameters which contribute to yield such as nitrate reductase enzyme activity, phenol content, total soluble protein content and total amino acid content showed an initial decline when estimated one week after application of herbicide but by the time of flowering the Bispyribac treated plants showed drastic improvement and they recovered completely. The stress induced biochemical constituent's proline content and catalase enzyme activity which showed an initial increase with herbicide application showed reduction by flowering time and were on par with the Hand weeded control. Cyhalofop was the next best chemical which showed better recovery.

5.6 Studies on weeds

5.6.1 Population and dry matter production of weeds

All herbicides were post emergent in action and were sprayed at 15 DAS. The weed count taken on 60 DAS revealed that among the herbicide applied plots, the weed population was lowest in Bispyribac sodium. Yadav *et al.* (2009) reported that this was due to the broad spectrum action of Bispyribac sodium. Azimsulfuron also a broad spectrum herbicides, was found less effective in the present study compared to Bispyribac sodium. Weed dry weight recorded on 60 DAS, showed minimum values for Hand weeded treatment followed by Bispyribac sodium applied plots. This corroborates with the findings of Rekha *et al.* (2002) who reported a lower weed dry matter production in Hand weeded control. Among the ACC ase group Cyhalofop was the next best performer giving good control of weeds. This might be because of the higher population of *Echinochloa* species in the *kole* lands. Joy *et al.* (1993) has also observed higher proportion of grasses as compared to sedges and broad leaved weeds in the *kole* lands. Sindhu (2008) compared the grasses, sedges and broad leaved weeds in rice in *Kole* lands and reported that among the grasses, *Echinochloa* spp. was the predominant one.

Summary

6. Summary

Herbicidal weed control is widely adapted in rice cultivation and recently many new herbicides are being introduced in the market. These herbicides are effective for the control of weeds and have helped to improve the profitability of rice cultivation. However, farmers have observed an inhibition in the growth of the rice plant after the application of the herbicides. The focus of the study has been to evaluate the morphological, physiological and biochemical changes in the rice plant by the application of different formulations of ACCase and ALS inhibiting herbicides on the rice plant and to identify the stage at which the inhibition is observed.

A field experiment was conducted in Alappad *Kole* from November 2013 to February 2014, using the rice variety, Jyothi. The soil of the *Kole* lands is clayey in texture with pH 5.5 and belongs to the soil order Inceptisol. The plot size was $15m^2$ (5mx3m). The treatments included 2 groups of hebicides *ie.,* ACCase and ALS enzyme inhibitors. ACCase enzyme inhibitors were Cyhalofop butyl, Fenoxaprop-p-ethyl and Metamifop. ALS enzyme inhibitors were Bispyribac sodium, Azimsulfuron and Almix. Hand weeding was the control for effective comparison. Hand weeding was done at 20 and 40 DAS. All herbicides in the study were post emergence in action and were sprayed at 15 DAS using knapsack sprayer at the recommended doses. Visual phytotoxicity rating of the crop was done on the third and seventh day after spraying. The crop was harvested during the fourth week of February after the grains were fully matured. After manual threshing, cleaning and drying, yield was expressed in t/ha.

Species wise weed count and weed dry matter production at 60 DAS were recorded. Plant height, numbers of tillers of rice, yield attributes like number of panicles, filled grains per panicle, test weight of grains, chaff percentage were taken. Grain as well as straw yields were recorded and expressed in t/ha. The present investigation came out with the following findings.

6.1 Effect of herbicides on physiological parameters

There was slight reduction in the physiological parameters like chlorophyll content, photosynthetic rate, stomatal conductance and IAA content compared to Hand weeded control plots.

The maximum chlorophyll content was noticed in the Hand weeded control plot compared to herbicide applied plots. Among the herbicide applied plots, Bispyribac applied plot showed maximum chlorophyll content compared to other herbicide treated plots.

Hand weeded plots showed the least reduction in photosynthetic rate, stomatal conductance and IAA content compared to all other treatments. Among the herbicide applied plots, Bispyribac showed the least reduction compared to all other herbicide applied plots. Azimsulfuron applied plot showed the maximum reduction in physiological parameters compared to other treatments.

6.2 Effect of herbicides on growth analysis

Effect of herbicides on growth attributes *viz.*, RGR, CGR and NAR indicated that at the initial and final stages of growth there was no significant difference between the treatments. However during the critical stages of growth 45-75 DAS significant variation was seen among the treatments except RGR and they were all significantly lower than Hand weeded crop. For RGR 60-75 DAS showed significant variation and Hand weeded showed maximum RGR.

The herbicides were applied on the 15th day and maximum reduction in LAI was noticed on the 30th day in all the herbicide treated plots compared to Hand weeded plot. Among the herbicides maximum reduction was observed in the Fenoxaprop and Azimsulfuron applied plots.

6.3 Effect of herbicides on morphological observations

Hand weeded plots showed the least reduction in plant height and tiller number compared to herbicide applied plots. Among the groups, on the 60th day a significant difference was observed between the different herbicide formulations. In ALS enzyme inhibitors, plants in the Bispyribac applied plots showed least reduction in plant height (2 percent) as compared to Azimsulfuron and Almix (4 percent) applied plots. The reduction in tiller number followed the same trend. In the case of ACCase inhibitors, the reduction in plant height and tiller number was significantly lower for Cyhalofop treated plants as compared to Fenoxaprop and Metamifop treated plants.

6.4 Effect of herbicides on biochemical observations

The results of the summary analysis of variance between the groups of herbicides and Hand weeded control (Appendix IV) indicated that the biochemical parameters like soluble protein, proline, phenols, catalase, nitrate reductase enzyme activity and amino acid content were significantly different between the herbicide applied and Hand weeded control. However ACCase and ALS inhibitors were on par. Among the ACCase enzyme inhibitors, Cyhalofop showed maximum values compared to Metamifop and Fenoxaprop in all the parameters except in catalase enzyme activity and proline content. Among the ALS enzyme inhibitors Bispyribac showed maximum values for all biochemical parameters except catalase enzyme activity and proline content. Azimsulfuron showed maximum catalase enzyme activity and proline content compared to other herbicides. Proline and catalase enzyme accumulate under stress. The results indicate that herbicide application induced stress in the plants.

6.5 Effect of herbicides on yield

Hand weeded plot showed maximum grain yield and straw yield compared to herbicide applied plots. Among the ALS enzyme inhibitors, Bispyribac applied plot showed the maximum grain and straw yield. Azimsulfuron and Almix showed the least grain and straw yield. Among the ACCase enzyme inhibitors, Cyhalofop showed maximum grain yield and straw yield. Fenoxaprop and Metamifop showed least grain and straw yield.

6.6 Effect of herbicides on weeds

Cyhalofop, Fenoxaprop and Metamifop applied plots showed the maximum number of sedges and broad leaved weeds as compared to grasses. Almix controlled the sedges and broad leaved weeds. Bispyribac sodium controlled all types of weeds in the rice ecosystem. Azimsulfuron also a broad spectrum herbicide, was found less effective in the present study compared to Bispyribac sodium.

Conclusion

Herbicide application caused stress to the rice plant which in turn resulted in growth suppression during the critical period of growth 45-75 DAS. This resulted in a decrease in the biochemical constituents such as chlorophyll content, IAA, phenols, nitrate reductase enzyme activity, free amino acid content and soluble proteins and affected the physiological efficiency of the rice plant as seen from a reduction in the photosynthetic rate and stomatal conductance of the plant. This in turn resulted in a decrease in the yield attributing characters such as panicle number, grain number, 1000 grain weight, height and tiller number of rice. Among the herbicides, Bispyribac applied plot showed the least reduction in the above mentioned characters while Azimsulfuron applied plot showed the maximum reduction. Among the ACCase enzyme inhibitors, Cyhalofop butyl showed the least reduction.

Future line of work

The current study has been proposed to identify the effect of ACCase and ALS inhibiting herbicides on the physiological and biochemical factors that contribute to yield reduction in the plant. The results may be revalidated for confirmation and better management practices may be developed to improve the efficiency of the plant and nullify the negative effects of herbicide.

- Such studies can be undertaken for other groups of herbicides.
- Methods to mitigate the metabolic inhibition due to herbicide application may be developed.

References

References

Abeysekhara, A.S.K. 1999. Current status of weed control in rice in Sri Lanka. In: *Proceedings of the 17th Asian Pacific Weed Science Society Conference*, Bangkok, Thailand, 22-27 November, pp.174-179.

Abeysekaera, A.S.K. and Wickrama, U.B. 2004. Control of *Leptochloa chinensis* (L.) Nees. in wet-seeded rice fields in Sri Lanka. In: *Proceedings of the World Rice Research Conference*, Japan, 4-7 November, pp. 215-217.

Adeyemi, A.A. 1999. Weed infestation in cocoa (*Theobroma càcao* L): physiological basis of the effects of weeds on the establishment and growth of cocoa seedlings after transplanting to the field; *Nigerian J. Tree Crop Res.* 3. pp 20-34.

Aishy, S.M., Zahran, M., and El-Ashry, M. 1976. Effect of weed control treatments on both weeds and soybean plants. *Indian J. Weed Sci.* 143(1): 18-26.

Angiras, N.N. and Attri, S.P. 2002. Efficacy of herbicide mixture to control mixed weed flora in direct seeded puddled rice. *Indian J. Weed Sci.* 34: 42-44.

Antony, E. 1995. Nitrogen utilization studies in groundnut (*Arachis hypogaea* L.) genotypes. *MSc. (Ag) Thesis*, University of Agricultural Sciences, Dharwad, 69p.

Anwar, M.P., Juraimi, A.S., Puteh, A., Man, A., and Rahman, M.M. 2012a. Efficacy, phytotoxicity. and economics of different herbicides in aerobic rice. *Acta Agric. Scand.* 62: 604-615.

Argenta, L.Z. and Lopes, N.F. 1992. Pigments accumulation, photosynthetic, respiratory and growth rates in soybean, rice and water-grass as effected by chlomazone dosages. *Revista Brasileira de Fisiologia Vegetal.* 4(2): pp 81-86.

ARWR [Annual Research Workshop on Rice]. 2011. 30th Annual Research Workshop on Rice – 2011. Crop management. Tamilnadu Rice Research Institute. Aduthurai, TNAU, Coimbatore. p. 15, 45 &50

Bahar, F.A. and Singh, G. 2004. Effect of herbicides on dry seeded rice and associated weeds. *Indian J. Weed Sci.* 36: 269-270.

Barber J.M. 1980. Catalase and peroxidase in primary leaves during development and senescence. *Z. Pflan. Physiol.* 97: 135–144.

Basuchaudhuri, P. 1989. Inhibition of photosynthesis in leaf discs by herbicides. *Curr. Sci.* 58(14): 807-808.

Bates, L., Waldren, R.P., and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil.* 39: 205-207.

Bertero, M.G., Rothery, R.A., Palak, M., Hou, C., Lim, D., Blasco, F., Weiner, J.H., and Strynadka, N.C. 2003. "Insights into the respiratory electron transfer pathway from the structure of nitrate reductase A". *Nat. Struct. Biol.* 10 (9): 681–712.

Bhargavi, K. and Reddy, T.Y. 1993. Growth pattern of weeds and semi-dry rice (*Oryza sativa* L.) under weed-management practices. *Indian J. Agron.* 38: 295-298.

Blackman, V.N. 1919. The compound interest law and plant growth. *Ann. Bot.* 33: 353-360.

Burton, J.D., Gronwald, J.W., Somers, D.A., Connelly, J.A., Gengenbach, B.G., and Wyse, D.L. 1987. Inhibition of plant acetylcoenzyme A carboxylase by the herbicides sethoxydim and haloxyfop. *Biochem. Biophysical Res. Commun.* 148: 1039–1044.

Carvalho, L.B., Alves, P.L.C.A., Bianco, S. and Prado, R. 2012. Physiological dose-response of coffee (*Coffea arabica* L.) plants to glyphosate depends on growth stage. *Chilean J. Agric. Res.* 72(2): 182-187.

Chandrashekhara Reddy, P. 1980, Studies on photosynthetic productivity of sorghum (*Sorghum bicolor* L.) genotypes under rainfed condition. *MSc(Ag) Thesis*, University of Agricultural Sciences, Bangalore, 72p.

Channappagoudar, B.B., Biradar, N.R., Bharamagoudar, T.D., and Koti, R.V. 2008. Influence of herbicides on physiological and biophysical parameters in potato. *Karnataka J. Agric. Sci.* 21: 4-7.

Chalko, A. 1970. Influence of some herbicides on the chemical composition and on the enzymic activity of barley and malt. *Rocznik Technologii Chemii zywnosci.* 18: 77- 90.

Chang, K.H., Sakamoto, M., Jin, Y.H., Murakami, T., Miyabara, Y., Nakano, S., Imai, H., Doi, H., and Hanazato, T. 2008. Comparative Study of Pesticide Effects (Herbicide and Fungicide) on Zooplankton Community. *Interdisciplinary Studies on Environmental Chemistry—Biological Responses to Chemical Pollutants.* pp. 361–366.

Chen, J.M. and Black, T.A. 1992. Defining leaf area index for non-flat leaves. *Plant: Cell Environ.* 15: 421-429.

Chelikani, P., Fita, I., and Loewen, P.C. 2004. "Diversity of structures and properties among catalases". *Cell. Mol. Life Sci.* 61 (2): 192–208.

Choubey, N.K., Kolhe, S.S., and Tripathi, R.S. 2001. Relative performance of cyhalofop-butyl for weed control in direct seeded rice. *Indian J. Weed Sci.* 33: 132-135.

Davies, D.D. and Humphrey, T.J. 1978. Amino Acid recycling in relation to protein turnover. *Plant Physiol.* 61(1): 54–58.

Dawah, A.M.A., Elmageed, S.A., and Farag, M. 2005. Effects of thiobencarb herbicide on some chemical parameters and plankton communities of rice fields. *Egyptian J. Agric. Res.* 83(4): 1783-1796.

Deka, S.C., Gogoi, A.K., and Pathak, D. 1996: Effect of herbicides on chlorophyll content and nitrate reductase activity in relation to grain protein yield and grain yield of rice (*Oryza sativa* L.) *Ann. Agric. Bio Res.* 1(1/2): 107-111.

Dixit, A. and Varshney, J.G. 2008. Assesment of post emergence herbicides in direct seeded rice. *Indian J. Weed Sci.* 40: 144-147.

Duke, S.O., Vaughn, K.C., and Wauchope, R.D. 1985. Effects of glyphosate on uptake, translocation, and intracellular localization of metal cations in soybeans (*Glycine max* L.) seedlings. *Pest. Biochem. Physiol.* 24:384–394.

Farquhar, G.D. and Sharkey, T.D. 1982. Stomatal conductance and photosynthesis. *Ann. Rev. Plant Physiol.* 33: 317–345.

Fayez, K.A. 2000. Action of photosynthetic diuron herbicide on cell organelles and biochemical constituents of the leaves of two soybean cultivars. *Pest. Biochem. Physiol.* 66, 105–115.

Fayez. K.A. and Kristen, U. 1996. The influence of herbicides on the growth and proline content of primary roots and on the ultrastructure of root caps, *Environ. Exp. Bot.* 36: 71.

Fedtke, C. 1973. Effects of the herbicide methabenzthiazuron on the physiology of wheat plants. *Pesticide Sci.* 4(5): 653-664.

Freed, R. 1986. MSTAT version 4.0. Department of Crop and Soil Sciences, Michigan State University.

Garg, S.K. 2013. Role and Hormonal Regulation of Nitrate Reductase Activity in Higher Plants: *A Rev. Plant Sci.* 3(1): 13-20.

Gomez, A.K. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research* (2nd edition). John Wiley and Sons, New York, 657p.

Gonsolus, J.L. and Curran, W.S. 1996. Herbicide mode of action and injury symptoms. *North Central Extension Publication.* 377p.

Gregory, F.G. 1926. The effect of climatic conditions on the growth of barley. *Ann. Bot.* 40: 1-26.

Gronwald, J.W. 1992. Lipid biosynthesis inhibitors. *Weed Sci.* 39: 435-449.

Hageman, R.H. and Flesher, D. 1960. Nitrate Reductase Activity in Corn Seedlings as Affected by Light and Nitrate Content of Nutrient Media. *Plant Physiol.* 35(5): 700–708.

Hiscox, J.D. and Israelstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian j. Bot.* 57: 1332-1334.

Hosmani, M.M. 1995. Cotton. Integrated weed management in field crops. *J. weed sci.* pp.176-186.

Huang, H. Xiong, Z.T., Li, M.J., Li, S.L. Huang, Y., Gao, J.Q., Qiu, H.J., and Mba, F.O. 2006. Effect of cadmium and herbicides on the growth, chlorophyll and soluble sugar content in rice seedlings. *J. Nat. Sci.* 3 742–748.

Hunt, R. 1982. *Plant growth curves: the functional approach to plant growth analysis.* London: Edward Arnold.

Ibrahim, I.S., Sanaa, A.M.Z., El-Shahawy, H.S., and Mahmoud, M.M. 2004. Physiological responses to fluroxypyr, fenoxaprop-p-ethyl and their combinations in shoot tip tissues of soybean and maize. *Ann. Agric. Sci.* 49(2): 449-466.

Ivanova, A., Stefanov, K. and Yordanov, I. 1999. Effect of the herbicide atrazine on the bean leaf lipids. *Biologia Plantarum.* 42(3): 417-422.

Jain, H.C., Tiwari, J.P., and Jain, K.K. 1996. Effect of spacing, seed rate and weed control measures on physiological parameters of soybean (*Glycine max* (L.) Merrill). *World Weeds* 3(3/4): 157-163.

Jayakumar, A., Chirala, S.S., Chinault, A.C., Baldini, A., Abu-Elheiga, L., and Wakil, S.J. 1994. Isolation and chromosomal mapping of genomic clones encoding the human fatty acid synthase gene. *Genomics.* 23: 420-424.

Jin, S., Sunohara, Y., and Matsumoto, H. 2007. Action of fentrazamide on protein metabolism and cell division in plants. *J. Pesticide Sci.* 32(3): 249-254.

Joy, P.P., Syriac, E.K., Ittyaverah, P.J., and Joseph, C.A. 1993. Herbicidal technology for weed control in low land rice of Kerala. In: *Proceedings of the 5th Kerala Science Congress,* January 1993, Kottayam. Kerala State Council for Science, Technology and Environment, pp.135-137

Juraimi, A.S., Begum, M., Yusuf, M.N.M., and Man, A. 2010. Efficacy of herbicides on the control weeds and productivity of direct seeded rice under minimal water conditions. *Plant Protec. Quat.* 25(1):19-25.

Katiyar, P. and Kolhe, S.S. 2006. Weed control in drilled rice. *J. Maharashtra Agric. Univ.* 31: 284-287.

Kim, T.J., Chang, H.S., Ryu, J.W., Ko, Y. K., Park, C. H., Kwon, O.Y., and Chung, B.J. 2003. Metamifop - A new post-emergence grass killing herbicide for use in rice. In: *Proceedings of International Congress,* SECC, Glasgow, Scotland, UK, pp.81-86

Kim, S.T., Cho, K.S., Jang, Y.S., and Kang, K.Y. 2008. Two-dimensional electrophoretic analysis of rice proteins by polyethylene glycol fractionation for protein arrays. *Electrophoresis.* 22: 2103–2109.

Kimura, I., Ichizen, N., and Matsunaka, S. 1971. Mode of action of a herbicide, benthiocarb. *Weed Res.* 12: 54-59.

Klepacka, J., Gujska, E., and Michalak, J. 2011. Phenolic compounds as cultivar- and variety-distinguishing factors in some plant products. *Plant Foods for Human Nutrition.* 66: 64-69.

Koschnick, T.J., Netherland, M.D. and Haller, W.T. 2007. Effects of three ALS-inhibitors on five emergent native plant species in Florida. *J. Aquat. Plant Manage.* 45: 47-51.

Kumar, P. and Gill, H.S. 1982. Weed control in direct seeded rice under puddled condition. *Oryza* 19: 162-166.

Kun, L., Yuan, Z., and Jun, C.Z. 2013. Response characteristics of reactive oxygen metabolism in *Pennisetum hydridum* to atrazine stress. Science Press, Beijing, China, *Acta Botanica Boreali-Occidentalia Sinica.* 33(12): 2479-2485.

Lakimova, E.T., Kapchina, V.M., Bistrichanov, S.M., Denkova, S.T., and Ivanova, I.I. 2002. Physiological response of lily (*Lilium* sp.) plants to stress. *Bulgarian J. Agric. Sci.* 8(5/6): 499-506.

Lin, C.C., Jih, P.J., Lin, H.H., Lin, J.S., Chang, L.L., Shen, Y.H.,and Jeng, S.T. 2011. Nitric oxide activates superoxide dismutase and ascorbate peroxidase to repress the cell death induced by wounding. *Plant Mol. Biol.* 77: 235–249.

Lin, C.J., Ling, T., and Hong, H. 2010. Fluroxypyr triggers oxidative damage by producing superoxide and hydrogen peroxide in rice (*Oryza sativa* L.). *Ecotoxicology.* 19(1): 124-132.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* 193: 265-275.

Mabbayad, M.O. and Moody, K. 1984. Improving butachlor selectivity and weed control in wet-seeded rice. *J. Plant Prot. Trop.* 2: 117-124.

Mahajan, G., Chauhan, B.S., and Johnson, D.E. 2009. Weed management in aerobic rice in northwestern indo-gangetic plains. *J. Crop Improv.* 23: 366-382.

Malick, C.P. and Singh, M.B. 1980. *Extraction and estimation of amino acids and keto acids.* In: Plant Enzymology and Histo-Enzymology. (Eds.): CP. Malik, MB. Singh, Kalyani Publishers. pp286.

Manjunath, S. and Panchal, Y.C. 1989. Growth and yield components of cotton as influenced by herbicides.; *J. Maharashtra Agric. Univ.* 14(2): 181-183.

Mann, R.A., Ahmad, S., Hassan, G., and Baloch, M.S. 2007. Weed management in direct seeded rice crop. *Pakist. J. Weed Sci. Res.* 13(3-4): 219-226.

Mehta, R.S., Meena, S.S., and Lal, G. 2010. Effect of irrigation levels and weed control methods on dry matter accumulation, growth dynamics and yield of fenugreek. *Indian J. Hort.* 67(2): 219-224.

Mei, S., Liya, S., Zimu, L., and Hang, M. 2009. Catalase and superoxide dismutase activities in a *Stenotrophomonas maltophilia* WZ2 resistant to herbicide pollution. *Ecotoxicology and Environmental Safety.* 72(1): 136-143.

Menon,S.S. 2012. Efficacy of new post emergence herbicides for rice. MSc(Ag.) thesis,Kerala Agricultural University, Thrissur,77p.

Moore, S. and Stein, W.H.J. 1948. *Biol. Chem.* 176: 367 – 388.

Moorthy, B.T.S. and Saha, S. 2002. Bio-efficacy of certain new herbicide formulations in puddle-seeded rice. *Indian J. Weed Sci.* 34: 46-49.

Moorthy, B.T.S. and Manna, G.B. 1982. Herbicides for weed control in puddle seeded rice. In: *Ann. Conf. on Indian Soc. of Weed Sci.* Hyderabad, Abstract: 13.

Moreland, D.E., Malhotra, R.D., and Gruenhagen, E.H. 1969. Effect of herbicides on RNA and protein synthesis. *Weed sci.* 17: 556.

Moursi, M.A., Rizk, T.Y., Fayed, M.T., and Roshdy, A. 1980. Comparative evaluation for the efficiency of some herbicides on growth of soybean (*Glycine max* L.) plants and associated weeds. Research Bulletin, Faculty of Agriculture, Ain Shams University, 16p.

Mukherjee, D. and Singh, R.P. 2004. Efficacy of certain low doses herbicides in medium land transplanted rice. *Indian J. Weed Sci.* 36: 47-49.

Mukherjee, D. and Singh, R.P. 2006. Effect of low doses of herbicides on weeds, nutrient uptake and yield of transplanted rice. *Indian J. Agron.* 50: 194-196.

Murti, K. and Khan, A.H. 2005. Gaurav Effect of dinitroaniline herbicides on growth parameters, yield and protein contents in grain of urdbean (*Vigna mungo* L.) crop. *Society of Agricultural Research Information Centre, Hisar.* 6(1): 131-133.

Nagaraju, A.P., Munegowda, M.K., and Nanjundappa. G. 1995. Effect of mixed herbicides on the chlorophyll content of leaf tissues of transplanted rice. Current Research - University of Agricultural Sciences, Bangalore 24(8): 146-147.

Nair, R.R., Vidyadharan, K.K., Pisharody, P.N. and Gopalakrishnan, R. 1974. Comparative efficiency of new herbicides for direct seeded rice fields. Agric. Res. J. Kerala. 12(1): 24-27.

Neelam, S., and Ruchi, S., 2005. Nitrogen assimilation potential, periodic nitrate reductase activity and its relationship to grain protein in field grown rice in presence of butachlor. *Indian J. Plant Physiol.* 10(2): 196-198.

Nichiporovich, A.A. 1960. Photosynthesis and theory of obtaining crop yield. *Indian J. Plant Physiol.* 13(5): 169-175.

Nicholas, J.C., Harper, J., and Hageman, R.H. 1976. Nitrate reductase Activity in Soybeans (*Glycine max* L.) *Plant Physiol.* 58: 736.

Nithya, C., Chinnusamy, C., and Muthukrishnan, P. 2011. Evaluation of grass herbicide - metamifop on weed control and productivity of direct seeded rice in Tamil nadu. In: *Proceedings of 23rd Asian-Pacific Weed Science Society Conference* The Sebel Cairns, pp.108-115.

Oosterhuis, D., Wullschleger, S.D., Hampton, R.E., and Ball, R.A. 1990. Physiological response of rice (*Oryza sativa* L.) to fenoxaprop. *Weed Sci.* 38(6): 459-462.

Panamarioviene, A. 1996. The influence of herbicides on the amino acids in winter rye, barley and maize. *Lietuvos Zemdirbystes Instituto Mokslo Darbai Zemdirbyste.* 55: 68-81.

Pandey, S. and Velasco, L., 2005. *Trends in crop establishment methods in Asia and research issues. In: Rice is Life: Scientific Perspectives for the 21st Century, Proceedings of the World Rice Research Conference.* Tsukuba, Japan, pp. 178–181.

Parthasarathy, K., Balu, D.R.C., and Rao, P.S. 1970. Studies on sandal spur. VII. Polyphenol oxidase activity and metabolism of sandal (*Santalam album* L.) in healthy and diseased. *Proc. Indian. Acad. Sci.* 72: 277-284.

Patra, A.K., Haidar, J., and Tripathy, S.K. 2006. Chemical weed control in transplanted rice in Hirakud command area. *Ann. Agric. Res.* 27: 385-388.

Peng, Z.L., Ying, W.Z., Ying, J.H., and Yong, F.Z. 2006. The effect of triazine herbicide atrazine on the chromosome structure, protein content and compositions in *Oryza sativa* L. *Acta Agronomica Sinica.* 32(4): 497-502.

Perumal, R., Vijayarengan, P., and Vijayaragavan, M. 2005. Effect of herbicides on biochemical changes in *Oryza sativa* L. *Adv. Plant Sci.* 18(2): 717-720.

Petter, F.A., Zuffo, A.M., and Pacheco, L.P. 2013. Effect of Acetolactate Synthase Inhibitor Herbicides on Upland Rice (*Oryza Sativa* Linn.) Cultivars. *J. Agric. Sci.* 5(10): 99-107.

Prakash, T.R., Murthy, R.S., and Swamy, P.M. 1989. Influence of thiobencarb on nitrate reductase, nitrite reductase and DCPIP photoreduction in rice and *Echinochloa crus-galli* (L.) (barnyardgrass). *Weed Res.* 29(6): 427-432.

Prokopenko, O.I. and Musina, G.V. 1983. Effect of some herbicides on catalase activity and oil content in soyabean plants. *Sibirskii Vestnik Selskokhozyaistvennoi Nauki.* 6: 15- 17.

Rana, S. and Angiras, N. 1999. Influence of integrated weed management on physiological performance of broadcast sown puddled rice (*Oryza sativa* L.). *J. of Agric. Res.* 25(1/2): 1-9.

Randhawa, S.K. and Gill, H.S. 1989. Influence of Herbicides Applied in Transplanted Rice on Chlorophyll and Carotenoids in Wheat Leaves. *J. Res. Punjab Agric. Univ.* 26: 29-30.

Rao, R., Murthy, K.M., and Venkata Reddy, C. 2009. Efficacy of bispyribac sodium 10% SC on early post emergence herbicide on rice. *Proceedings of the National symposium on Weed Threat to Environment, Biodiversity and Agriculture Productivity,* TNAU,Coimbatore, India, pp: 117-119.

Rao, A.N., Johnson, D.E., Sivaprasad, B., Ladha, J.K., and Mortimer, A.M. 2007. Weed management in direct-seeded rice. *Adv. Agron.* 93: 153–255.

Ray, T.B. 1984. Site of action of chlorsulfuron. *Plant Physiol.* 75: 827–831.

Rekha, B.K., Raju, M.S. and Reddy, M.D. 2002. Effect of herbicides in transplanted rice. *Indian J. Weed Sci.* 34: 123-125.

Roy, H.P., Salam, M.A., Islam, M.R., Ahammed, K.U., Akhter, B., and Khalequzzaman, K.M. 2009. Weed Infestation and Yield Performance of Boro Rice in Direct Seeding Method as Influenced by Green Growth Regulator and Herbicides. *Int. J. Sustain. Crop Prod.* 4(1): 83-90.

Saha, S. and Rao, K.S. 2010. Efficacy of metsulfuron methyl for controlling broadleaf weeds in transplanted rice under rainfed shallow lowland. *Indian J. Agric. Sci.* 80: 522-526.

Sahoo, B.C., Koley, N., and Das, A.K. 1993. Dissipation and effect of chlorophenoxy herbicides on plant pigments and carbohydrates of rice plant (*Oryza sativa* L.). *Pesticide Res. J.* 5(2): 186-191.

Saini, J.P. and Angiras, N.N. 2002. Evaluation of fenoxaprop-p-ethyl for weed control in direct seeded puddle rice. *Indian J. Weed Sci.* 34: 131-133.

Saini, J.P. 2003. Efficacy of cyhalofop butyl against weeds in direct seeded puddled rice under mid hill conditions of Himachal Pradesh. *Indian J. Weed Sci.* 35: 205-207.

Saini, J.P. 2005. Efficacy of cyhalofop butyl alone and in combination with 2,4-D against mixed weed flora in direct seeded upland rice. *Indian J. Agron.* 50: 38-40.

Sakthivel, S. and Balasubramanian, R. 2010. Evaluation of the efficacy of Azimsulfuron 50DF against transplanted rice weeds as post emergent application. In: *National Conference on Challenges in Weed Management in Agro-ecosystems, Present Status and Future Strategies*, TNAU, Coimbatore, p. 153

Salisbury, F.B., and Ross, 2001. *Plant physiology.* 4th edition. 682p.

Sangeetha, S.P., Balakrishnan, A., Sathyapriya, R., and Maheswari, J. 2009. Influence of seeding methods and weed management practices on direct seeded rice. *Indian J. Weed Sci.* 41: 210-212.

Satao, R.N. and Nalamwar, R.W. 1991. Effect of herbicides and weeding on RGR, NAR and yield of sorghum. *Ann. Plant Physiol.* 5 (1): 135-138.

Sestak, Z. 1966. Limitations for finding a linear relationship between chlorophyll content and photosynthetic activity. *Biol. Plant* 8: 336-346.

Sharma, S.D., Punia, S.S., Malik, R.K., and Narwal, S. 2004. Efficacy of cyhalofop butyl against weeds in rice nursery. *Indian J. Weed Sci.* 36: 181-183.

Sharma, D., Bhardwaj, R., and Maheshwari, V. 1989. Inhibition of photosynthesis by oxyfluorfen. *Curr. Sci.* 58(23): 1334-1336.

Simon, S. and Petrasek, J. 2010. Why plants need more than one type of auxin. *Plant Sci.* 180: 454–460.

Sindhu, P.V. 2008. Eco-friendly management of weeds in rice. Ph.D thesis, Kerala Agricultural University, Thrissur, 274p.

Singh, V.P., Singh, G., and Singh, M. 2004. Effect of fenoxaprop-p-ethyl on transplanted rice and associated weeds. *Indian J. Weed Sci.* 36: 190-192.

Singh, B.P. and Ghosh, D.S. 1990. Effect of cultural practices on growth and yield of rice. *Indian Agricst.* 34(2): 83-87.

Singh, S.K., Sharam, R., Singh, J.N. 1985. Effect of nitrofen, fluchloralin and pendimethalin herbicides on the growth parameters of green gram (*Vigna radiata* L.). *Ann. Conf. Indian Soc. Weed Sci.* pp 52

Singh, V.K., Singh, A., and Singh, S.P. 1987. Effect of interaction of herbicides on chlorophyll and mineral content of rice leaves. *Narendra Deva J. of Agric. Res.* 2(2): 175-177.

Singh, V.P., Singh, S.P., Tripathi, N., Kumar, A., and Singh, M.K. 2008. Bioefficacy of Penoxsulam on transplanted rice weeds. *Indian J. Weed Sci.* 41: 28-32.

Singh, S., Bhushan, L., Ladha, J.K., Gupta, R.K., Rao, A.N., and Sivaprasad, B. 2006. Weed management in dry seeded rice cultivated on furrow irrigated raised bed planting system. *Crop Prot.* 25: 487-495.

Singh, S., Singh, H., Narwal, S., and Malik, R.K. 2003. Performance of herbicides in transplanted rice. *Indian J. Weed Sci.* 35: 114-116.

Singh, A.P., Bhambri, M.C., Dwivedi, S.K., and Shrivastava, A. 2010. Efficacy of post emergence herbicides on direct seeded rice (*Oryza sativa*) in Chattisgarh plains of India. *J. Curr. Adv. in Agrl. Sci.* 2: 47-48.

Singh, S.P. Pandey, P., Kumar, M., Singh, S., Pandey, N., and Srivastva, D. 2013. Growth and biochemical responses of wheat (*Triticum aestivum* L.) to different herbicides. *African J. Agric. Res.* 14(8): 1265-1269.

Snipes, C.E., Walker, R.H., Whitwell, T., Buchanan, G.A.J., McGuire, A., and Martin, N.R. 1984. Efficacy and economics of weed control in cotton (*Gossypium hirsutum* L.). *Weed Sci.* 32: 95-100.

Sood, A., Pabbi, S., and Uniyal, P. L. 2011. Effects of paraquat on lipid peroxidation and antioxidant enzymes in aquatic fern *Azolla microphylla*. *Russian J. Plant Physiol.* 58(4). 667-673.

Sousa, C.P., Pinto, J.J.O., Martinazzo, E.G., Perboni, A.T., Farias, M.E., and Bacarin, M.A. 2014. Chlorophyll *a* fluorescence in rice plants exposed of herbicides of group imidazolinone. *Sociedade Brasileira da Ciencia das Plantas Daninhas.* 32(1): 141-150.

Stidham, M.A. 1991. Herbicides that inhibit acetohydroxyacid synthase. *Weed Sci.* 39: 425-434.

Sreedevi, P. and Thomas, C.G. 1993. Efficiency of anilofos on the control of weeds in direct sown puddle rice. In: *Proc. of the Int. Symp. of Indian Soc. of Weed Sci.* Hisar, pp. 16-18.

Sreedevi, B., Murthy, P.K. and Singh, S.P. 2009. Nitrogen uptake and energy consumption of weeds in wet seeded rice. In: *National Symposium on Weed Threat to Environment, Biodiversity and Agriculture Productivity*, TNAU, Coimbatore, p.49

Subhalakshmi, C. and Venkataramana, M. 2009. Growth and nutrient uptake of transplanted rabi rice, weeds as influenced by different weed management practices. In: *National Symposium on Weed Threat to Environment, Biodiversity and Agriculture Productivity*, TNAU, Coimbatore, p.63

Subramanyam, D., Sumathi, V., Rao, D.S.K. and Reddy, D.S. 2009. Effect of planting pattern and weed management on nutrient uptake and economics of rabi sunflower and its associated weeds. *Ind. J. Weed Sci.* 41: 65-70.

Subramanian, E., James Martin, G., and Balasubramanian, R. 2006. Effect of integrated weed management practices on growth and yield of wet seeded rice (*Oryza sativa* L.) and their residual effect on succeeding pulse crop. *Indian J. Agron.* 51(2): 93-96.

Suganthi, M., Kandasamy. O.S., Subbian, P., and Jayakumar, R. 2005. Relative efficacy of pretilachlor 50EC for weed control in lowland transplanted rice-rice cropping system. *Indian J. Weed Sci.* 37: 101-102.

Sugawara, S., Hishiyama, S., Jikumaru, Y., Hanada, A., Nishimura, T., Koshiba, T., Zhao, Y., Kamiya, Y., and Kasahara, H. 2009. Biochemical analyses of indole-3-acetaldoxime-dependent auxin biosynthesis in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 106: 5430–5435.

Taiz, L., and Zeiger, E. 1991. *Plant Physiology*. Redwood City, CA: The Benjamin/Cummings Publishing Company, Inc. pp. 92–95.

Tan, W., Li, Q.L., and Zhai, H. 2012. Photosynthesis and growth responses of grapevine to acetochlor and fluoroglycofen. *Pesticide Biochem. Physiol.* 103: 210-218.

Thomas, C.G. and Abraham, C.T. 2007. *Methods in Weed Science*. AICRP on weed control, College of Horticulture, Vellanikkara. 108p.

Thorne, G.N. 1960. Variation with age in net assimilation rate and other growth attributes of sugar beet, potato and barley in a controlled environment. *Ann. Bot.* 24: 356-371.

Ting, H. 2009. Toxic effects of cadmium, acetochlor and bensulfuron-methyl on nitrogen metabolism and plant growth in rice seedlings. Elsevier, Amsterdam, Netherlands. *Pesticide Biochem. Physiol.* 94 (2/3): pp 64-67.

Tiwari, R.B., Pandey, T.D. and Nandeha, K.L. 2010. Weed management studies in direct seeded rice. In: *Biennial Conference of Indian Society of Weed Science on Recent Advances in Weed Science Research.* 25-26 February 2010. Indira Gandhi Krishi Vishwavidyalaya, Raipur, p.30

Valentine, I.K., Maria, V.K., and Bruno, B. 2003. Phenolic cycle in plants and environment. *J. Mol. Cell Biol.* 2: 13-18.

Valle, A., Boschin, G., Negri. M., Abbruscato, P., Sorlini, C., Agpstona, A.D., and Zanardini, E. 2006. The microbial degradation of Azimsulfuron and its effect on the soil bacterial community. *J. Appl. Microbiol.* 101: 443-452.

Vidal, R. 2002. *Acaó dos herbicidas.* Porto Alegre: Edicao do autor. Copyrights

Veeramani, A., Prema, P., and Guru, G. 2006. Effect of pre and post-sowing weed management on weeds and summer irrigated cotton. ANSInet, Asian Network for Scientific Information, Faisalabad, Pakistan, Asian Journal of Plant Sciences. 5(2): 174-178.

Walia, U.S., Bhullar, M.S., Nayyar, S., and Walia, S.S. 2008. Control of complex weed flora of dry seeded rice with pre and post emergence herbicides. *Indian J. Weed Sci.* 40: 161-164

Watson, D. J., 1952, The physiological basis of variation in yield. *Adv. Agron.* 4 :101-145.

Wen, F. 1995. Physiological responses of rice seedlings to butachlor.; Korean Journal of Weed Science. 15(4): 247-253.

Williams, R. F. 1946. The physiology of plant growth with special reference to the concept of net assimilation rate. *Ann. Bot.* 10: 41-47.

Yadav, D.B., Yadav, A., Punia, S.S., and Balyan, R.S. 2008. Evaluation of azimsulfuron for control of complex weed flora in transplanted rice. *Indian J. Weed Sci.* 40 (3&4):132-136.

Yadav, D.B., Punia, S.S., and Yadav, A. 2010. Efficacy of Bispyribac sodium, Azimsulfuron and Penoxsulam for post emergence weed control in transplanted rice. In: *Bienniel Conference of ISWS on Recent Advances in Weed Science Research* 25-26 February 2010, Indira Gandhi Krishi Vishwavidyalay, Raipur, p.75

Yadav, D.B., Yadav, A., and Punia, S.S. 2009. Evaluation of bispyribac-sodium for weed control in transplanted rice. *Indian J. Weed Sci.* 41(1&2): 23-27.

Yoshida, S. 1981. *Fundamentals of rice crop science.* International Rice Research Institute, Manila, Philippines.

Young, C.U. and Chu, C. 1975. Effects of s-triazines on the protein and amino acid contents of rice. *J. Agric. Ass. China.* 91: 7-21.

Yun, M.S., Yogo, Y., Miura, R., Yamasue, Y., and Fischer, A.J. 2005. Cytochrome p-450 monooxygenase activity in herbicide resistant and susceptible late watergrasss (*Echinochloa phyllopogen* L.). *Pesticide Biochem. Physiol.* 83:107-114.

Zhong, Y.S., Jincai, W., Jianxiang, X., and GuoSheng, L. 2001. Influences of herbicides on physiology and biochemistry of rice. *Acta Phytophylacica Sinica.* 28(3): 274-278.

Appendices

APPENDIX-1

Monthly weather data during the cropping period at COH, Vellanikkara from Nov 2013 to Feb 2014

(Latitude 10^{0}31'N, Longitude 76^{0}13' and Altitude 40.29MSL)

Month	Temperature (^{0}C)		Relative Humidity (%)		Rainfall (mm)	Mean evaporation (mm)	Rainy days	Sunshine hours (hrs/day)	Mean wind speed (Km/hr)
	Maximum	Minimum	Morning	Evening					
November	32.6	23.9	87	60	82.0	2.7	5	187.2	3.0
December	31.9	22.3	77	45	0.5	2.4	0	254.7	5.5
January	32.9	23.0	66	36	0.0	4.6	0	277.6	6.9
February	34.7	22.9	75	37	0.0	9.1	0	240.8	4.5

APPENDIX-2

Summary ANOVA for comparing ACC ase and ALS enzyme inhibitors for morphological parameters

Source	df	Mean sum of squares	
		Height	Tiller number
Bet ACC	2	22.33*	1225**
Bet ALS	2	137.3**	7600**
ACC/ALS	1	1.125	28.1
Treatments/control	1	349.6**	10214.7**

APPENDIX-3

Summary ANOVA for comparing ACC ase and ALS enzyme inhibitors for physiological parameters

Source	df	Mean sum of squares					
		Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Photosynthetic rate	Stomatal conductance	Auxin
Bet ACC	2	0.014*	0.002	0.026*	143.25*	0.058*	0.0005*
Bet ALS	2	0.09**	0.105**	0.388**	21.2**	0.181**	0.027**
ACC/ALS	1	0.8	0.00045	0.0018	0.036	0.107	0.00005
Treatment/control	1	0.12**	0.159**	0.325**	170.69**	0.308**	0.0019**

APPENDIX-4

Summary ANOVA for comparing ACC ase and ALS enzyme inhibitors for biochemical parameters

Source	df	Mean sum of squares					
		Soluble protein	Proline content	Nitrate reductase	Catalase enzyme activity	Total phenols	Total amino acids
Bet ACC	2	7.0*	0.001	3358.3*	4.62*	0.0075*	2.733*
Bet ALS	2	29.18**	0.0025	14558.3**	51.23**	0.0665**	0.021
ACC/ALS	1	0.28	0.00005	42.6	3.12	0.00245	21.12**
Treatment/control	1	92.06**	0.00995	46810.1**	87.93**	0.225**	30.9**

APPENDIX-5

Summary ANOVA for comparing ACC ase and ALS enzyme inhibitors for yield and yield attributes

Source	df	Mean sum of squares						
		Productive tillers/m^2	Spikelets/ panicle	Filled grains/panicle	Chaff	Test wt	Grain yield	Straw yield
Bet ACC	2	28*	0.236*	1.238*	1.6**	0.032	0.192**	0.178**
Bet ALS	2	91.58**	3.327*	9.556**	4.4**	0.040	0.779**	0.611**
ACC/ALS	1	0.18	0.125	0.32	0.6	0.00005	0.0072	0.00045
Treatments/control	1	291.22**	7.72*	38.95**	13.3**	0.115	2.033**	2.68**

Abstract

MORPHO-PHYSIOLOGICAL CHANGES IN RICE DUE TO APPLICATION OF SELECTED POST EMERGENT HERBICIDES

By

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ABSTRACT OF THE THESIS

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Abstract

The study on "Morpho-physiological changes in rice due to application of selected post emergent herbicides" was conducted during 2013-2014 at Alappad *kole* lands in farmer's field. The objective of the experiment was to study the morphological, physiological and biochemical changes in rice due to application of Acetyl CoA carboxylase and Acetolactate synthase enzyme inhibitors and their effect on growth and yield of rice.

The experiment was laid out in RBD with four replications and there were seven treatments comprising of three Acetyl CoA carboxylase enzyme inhibitors *viz.,* Cyhalofop butyl, Fenoxaprop -p- ethyl and Metamifop and three Acetolactate synthase enzyme inhibitors *viz.,* Bispyribac sodium, Azimsulfuron and Almix with Hand weeded as control. Herbicides were sprayed on 15 DAS. Observations on morphological, physiological, biochemical, yield attributes and yield of rice were recorded. Biochemical estimations were done on 7 days after herbicide application and at the time of flowering. Morphological characters were studied at 15 days interval.

Herbicide application resulted in a two to four percent reduction in height and one to six percent reduction in tiller number of rice plant at harvest as compared to plants in the Hand weeded plot. Effect of herbicides on growth attributes *viz.,* RGR, CGR and NAR indicated that at the initial and final stages of growth there was no significant difference between the treatments. However during the critical stages of growth *i.e.,* 45-75 DAS significant variation was seen. Among the herbicide treatments, Bispyribac sodium followed by Cyhalofob butyl applied plots had higher values as compared to the other herbicides. Azimsulfuron applied plots had the lowest value for these parameters. Among the growth indices LAI showed significant reduction irrespective of the herbicide throughout the growth stages as compared to Hand weeded control.

The biochemical parameters such as nitrate reductase enzyme activity, phenol content, total soluble protein content and total amino acid content showed an initial decline when estimated one week after application of herbicide but by the time of flowering all the herbicide treated plants showed recovery. However the recovery was higher for Bispyribac sodium in the ALS inhibiting group and Cyhalofop in the ACCase inhibiting group.

Proline content and catalase enzyme activity showed increase with herbicide application as compared to Hand weeded control indicating that application of these chemicals may be imparting stress to the plant, however by flowering time in most of the treatments there was recovery. Among the treatments, plants in the Almix and Azimsulfuron applied plots showed less recovery.

The physiological parameters such as chlorophyll content, photosynthetic rate, stomatal conductance and IAA content showed decline when estimated one week after herbicide application. . In the case of chlorophyll content the recovery of the plant was comparatively less as compared to all other chemical constituents. Azimsufuron and Almix application contributed to maximum reduction in these constituents.

Weed count and weed dry weight was taken on 60 DAS. The lowest values were recorded in Bispyribac sodium treatment. Azimsulfuron also a broad spectrum herbicide, was found less effective in the present study compared to Bispyribac sodium.

In the present study, 5 to 17 percent reduction in grain yield and 6 to 17 percent reduction in straw yield of rice was observed by herbicide application. Among the ALS enzyme inhibitors, the lowest reduction in grain and straw yield was observed in Bispyribac sodium treatment and maximum reduction was in Azimsulfuron treatment. Among the ACCase enzyme inhibitors, Cyhalofop butyl showed least reduction in grain and straw yield of rice while fenoxaprop showed maximum reduction.