MITIGATING THE PHYTOTOXIC EFFECT OF ALS INHIBITING HERBICIDES IN RICE *(Oryza sativa* L.).

By

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(2014-11-228)

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THESIS

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Kerala Agricultural University, Thrissur

Department of Plant Physiology

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DECLARATION

I hereby declare that the thesis entitled **" Mitigating the phytotoxic effect of ALS inhibiting herbicides in rice"** 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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CERTIFICATE

Certified that thesis entitled **"Mitigating the phytotoxic effect of ALS inhibiting herbicides in rice"** is a bonafide record of research work done independently by **Ms. Nithya.N (2014-11-228)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associate ship or fellowship to her.

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We, the undersigned members of the advisory committee of Ms**. Nithya. N (2014-11-228)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant physiology**, agree that the thesis entitled **"Mitigating the phytotoxic effect of ALS inhibiting herbicides in rice"** may be submitted by **Ms. Nithya. N (2014-11-228)**, in partial fulfillment of the requirement for the degree.

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Abbreviations

ALS	Acetolactate synthase
ai/ha	Active ingredient per hectare
DAS	Days after sowing
CGR	Crop growth rate
RGR	Relative growth rate
NAR	Net assimilation rate
LAI	Leaf area index
DF	Dry flowable
SC	Soluble concentration
NRase	Nitrate reductase enzyme activity
CAT	Catalase enzyme activity
NAA	Naphthalene acetic acid
IAA	Indole acetic acid
ppm	Parts per million
Kg/ha	Killogram per hectare
No/ m^2	Number per meter square
t/ha	Tonnes per hectare

Introduction

1. Introduction

Rice (*Oryza sativa*) is a major cereal crop and staple food for more than half of the world's population. About 90% of the world's rice is produced and consumed in Asia. Rice is mainly grown by transplanting seedlings into irrigated (conventional wet tillage) soil and kept saturated for most part of the growing season. The prevalent method of rice crop establishment requires a more amount of water, labour and energy, which are gradually becoming insufficient and more valuable. Dry direct-seeded rice (DSR) is considered as probable substitute to transplanted rice. The benefits of DSR include faster and easier planting, improved soil health, higher tolerance to water deficit, less methane emission, and often higher profit in areas with an assured water supply. However weed management is the major challenge in direct seeded rice. In this method, weeds and crop seedlings emerge simultaneously resulting in severe competition for resources (Chauhan, 2012), failure to control weeds result in yield losses ranging from 50 to 90% .The traditional methods of weed control in rice include hand weeding by hoe or hand pulling, but this is becoming less common because of labour scarcity at critical time of weeding and increasing labour costs. Moreover, seedlings of some grassy weeds such as *Echinochloa crusgalli* (L.) look similar to rice seedlings, making hand weeding more tedious, difficult, and less effective. In this situation, use of herbicides is becoming more popular in direct seeded rice because they are more effective, easy to apply, provide selective control, saves on labour and costs less.

Among the herbicides, post emergent herbicides play a major role in specific weed management. Acetolactate synthase (ALS) inhibitors are a major class of herbicide which is used for selective control of weeds in rice ecosystem. This group of herbicides has diverse molecules belonging to different chemistries including: imidazolinones, pyrimidinyl thiobenzoates, sulfonyl amino carbonyl triazolinones, sulfonylureas, and triazolopyrimidines. These herbicides inhibit acetolactate synthase, a key enzyme in the pathway of biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine. Plants death results from events occurring in response to inhibition of branched - chain amino acids. Application of ALS inhibiting herbicides causes growth inhibition resulting in 5-17% yield decline in rice (Ramanarayana, 2014). Reduction in photosynthetic rate, stomatal conductance, IAA content and Nitrate reductase enzyme activity has been identified as factors contributing to the growth inhibition. This necessitates management interventions.

Application of nitrogen to cereals by foliar spray leads to rapid utilization of nutrients and permits correction of observed deficiencies in less time than soil application. Some of the hormones like NAA and micronutrients also have a pivotal role in the overall development of the rice plant. Molybdenum , is a co factor of the key enzymes of assimilatory nitrogen metabolism in plants. Zinc is an essential element for tryptophan synthesis which is the precursor of IAA (Taiz and Zeiger, 2010). Application of boron is helpful for cell growth and further development in plants. The study has been proposed to develop a management technology to improve the physiological efficiency of the rice plant by additional inputs such as nutrients and hormones after the application of the post emergent ALS inhibiting herbicides.

Objective:

The study aims to mitigate the growth inhibition due to the application of post emergent ALS (Aceto Lactate Synthase) inhibitors, Bispyribac sodium and Azimsulfuron and to improve the productivity of rice.

Review of Literature

2. Review of Literature

Weeds reduce the productivity potential of crops by interfering with agricultural operations (Parthipan *et al*., 2013). In rice, uncontrolled weed growth reduces the grain yield by 33- 45% (Manhas *et al*., 2012). Farmers mostly follow the chemical method of weed control. Among the herbicides used, ALS inhibitors are very popular in rice cultivation as they are required in very less quantities and control weeds effectively. However some of these chemicals cause a suppression in growth of the rice plant leading to yield reduction. For early recovery of growth some micronutrients like Zn, Mo, B and hormones like NAA can be used. The influence of these mitigating treatments on physiological biochemical and yield attributes of the rice plant is reviewed.

2.1 Effect of herbicide on growth parameters

2.1.1Height

Kumar *et al*. (2013) reported that bispyribac-sodium 20 and 25 g/ha resulted in significantly taller plants as compared to other weed control treatments. Taller plants with higher leaf area index were recorded by two hand weedings fb application of bispyribac sodium @ 25 g/ha (Parthipan *et al*., 2013). Bhurer *et al*. (2013) observed that the highest plant height was measured in weed free plots throughout the cultivation period. Higher dose of azimsulfuron caused yellowing and stunted growth of rice cultivar ‘Rajashree’(Singh *et al*., 2010). Begum *et al*.(2008) found that the height of the plants recorded in cinosulfuron treatment was similar to weed-free, bentazon, fentrazamide+propanil, bispyribac-sodium, pretilachlor+safener and bensulfuron treatments.

2.1.2Tiller number

The herbicides bispyribac-sodium and cinosulfuron+pretilachlor produced lower number of productive and total tillers compared to other herbicide, but were very effective against the weeds (Begum *et al*., 2008). Azimsulfuron 10.0-12.5 g/ha at 15 DAT and 10.0-22.5 g/ha dose at 25 DAT provided less number of effective tillers (Yadav *et al*., 2008). Menon *et al.* (2014) reported that at 30 DAS, the highest number of tillers was in hand weeded plot. Singh *et al*. (2014) observed the highest number of tillers/m^2 in weed free conditions which was on par with the application of bispyribac-sodium @ 20-25 g/ha.

2.1.3 Leaf area index

Khaliq *et al*. (2012) observed that the highest leaf area indices were associated with weed free treatment and tank mixture of bispyribac sodium+ethoxysulfuron. Ramanarayana (2014) reported that among the growth indices LAI showed a significant reduction irrespective of the herbicide throughout the growth stages as compared to hand weeded control in rice. The hand weeded plots developed the highest LAI as compared to herbicide applied plots in rice (Saqib *et al*., 2015).

2.1.4 Crop growth rate

Khaliq *et al*. (2012) noticed that there was a reduction in CGR at the end of peak vegetative growth and phase transition to flowering stage. Application of different herbicides did not contribute to significant difference in CGR during 0-30 DAS, but at later stages (30-60DAS) maximum CGR was recorded in weed free plot, and among the herbicidal treatments, maximum CGR was recorded under pendimethalin fb penoxsulam, pendimethalin fb bispyribac-Na (Singh *et al.*, 2014). Muhammad *et al.* (2015) observed that the crop growth rate was higher in hand weeded plots (25.14 g m^{-2} day^{-1}) as compared to herbicide treated ones (24.38 g /m^2/day). Saha *et al*.(2016) conducted a field experiment at ICAR-National Rice

Research Institute, Cuttack to study the efficacy of azimsulfuron for controlling broad spectrum of weeds in transplanted rice (*Oryza sativa* L.), in this experiment they have observed that there was a reduction in the crop growth rate in weedy check plots as compared to other plots.

2.1.5 Relative Growth Rate

There was an increase in relative growth rate at 30-60 days by herbicides but not at the later growth stages (Moursi *et al*., 1980).Yu *et al*. (2010) noticed that in rice plantsthe RGR was higher in first crop season as compared to second crop season. Maximum RGR in the treatments was observed at early growth stage and there after it decreased linearly (Abbasian *et al*., 2014).

2.1.6 Net Assimilation Rate

NAR of the plants increased rapidly from emergence and decreased by the end of growing period (Nichiporovich, 1960). Singh *et al*. (1985) reported that the application of herbicides like pendimethalin and fluchloralin on green gram increased the NAR with increasing rates of herbicide during first three weeks.

2.2 Mitigation of morphological attributes of rice by different chemicals

2.2.1 Height

Soil application of nitrogen fertilizer and foliar urea application increased plant height in broccoli cultivar AG 3317 and AG 3324 (Yildirim *et al*., 2007). Alam *et al*. (2010) reported the highest plant height in rice variety BRRI dhan29 and BRRI dhan 50 when it was treated with 94 kg/ha urea as soil application and 1% urea as foliar spray as compared to control that is without urea spray. Bhuyan *et al*.(2012) observed taller plants in foliar applied nitrogen fertilizer of rice.

A pot experiment conducted with 100ppm and 200ppm NAA showed an increase in growth rate in rice at 60 DAS (Chaudhuri *et al*., 1980). Adam and Jahan (2011) noticed that application of 200ppm of NAA produced the longest plant.

Ahmad and Irshad (2011) noticed that the application of boron as borax increased plant height in rice, wheat and cotton in all the treatment compared to control. The foliar application of zinc had significant influence on height of lentil , maximum height recorded was 42.2 cm at 0.08% Zn concentration while minimum (32.8 cm) height was recorded in the control plots where no Zn was applied (Singh and Bhatt, 2013). Naeini *et al.* (2014) observd that foliar application of nutrients such as boron, copper, manganese and zinc in alkaline soils increased plant height significantly.

2.2.2 Tiller number

Parvin *et al.*(2013) reported that spraying of urea had significant effect in producing more number of tillers/hill and it was more in 5 times urea spray. Saleem *et al.* (2013) observed that foliar application of urea increased tiller number of wheat crop.

Jahan and Adam (2013) observed that the application with 25ml/l NAA and 50ml/l NAA increased tiller number in wheat.

Rashid (2006) observed that the foliar spray of boron increased all the growth parameters including tillering capacity, shoot and root height etc. Boron application at any time increased average number of tillers/hill over control in rice (Ahmad and Irshad, 2011). Increase in number of tillers/m^2 was recorded by foliar application of Zn in rice varieties ADT 49 which varied from 226 to 327 (Sudha and Stalin, 2015).

2.2.3 Leaf area index

Mondal *et al*.(2012) reported that the application of urea as foliar spray increased LAI in soyabean and the highest was recorded when urea was sprayed three times followed by two times. Kaur *et al*.(2015) observed that the maximum LAI was noted with 2% urea application which was 26% at the time of flowering and 19% at the time of pod development as compared to control in pigeon pea.

Increase in LAI in groundnut was observed when it was supplied with 80ppm of NAA (Mondal, 2003).

Gowthamy and Rao (2014) noticed that the foliar application of zinc,boron and potassium increased LAI in soyabean and significant difference was noticed at 45 to 60 DAS after that it declined upto 90 DAS. Amirani and Kasraei (2015) conducted a field trial on effect of foliar application of microelements on phenological and physiological characterestics of mung bean under drought stress. The results revealed that LAI was decreased during drought stress condition but the foliar application of microelements increased LAI in presence or absence of drought stress.

2.2.4 Crop growth rate

Kaur *et al* .(2015) observed that the application of nutrients increased the CGR, among which 2% urea spray caused the major difference which was 22% increase at flowering and 28% increase at podding stage.

Chaudhari *et al.* (1980) noted that CGR at peak tillering stage of growth was significantly high because of application of NAA in both the rice varieties, Jaya and Mahsuri. Baghel and Yadav (1992) reported that foliar application of 40 ppm NAA increased the CGR at pod filling stage in blackgram.

Foliar application of molybdenum on rice plants attributed to greater rates of carbon assimilation, which has a direct relationship with the CGR (Das Gupta and Basuchaudhuri ,1976). Khan (2013) reported that the plot cultivated with wheat crop was having maximum CGR when it was supplied with 2 kg/ha boron and minimum was obtained in control plots. Foliar application of potassium nitrate @ 2 per cent + boric acid @ 50 ppm + zinc sulphate @ 1 per cent at 30 and 60 DAS showed higher CGR (36.44 g $m^{-2}d^{-1}$) at 45-60 DAS (Gowthamy and Rao , 2014). Tariq *et al*. (2014)

conducted an experiment on influence of Zn nutrition on growth and behavior of maize hybrids and they have observed that there was 46% increase in CGR in foliar applied $ZnSO_4$ plants as compared to control.

2.2.5 Relative growth rate

Arun Kumar Shankar (1990) noted higher values of RGR in soybean, when sprayed with urea. Nicoulaud and Bloom (1996) observed that there was an increase in RGR after urea spray on tomato especially at 0.2%. Kaur *et al.* (2015) conducted an experiment to determine the effect of foliar application of mineral nutrients on growth of pigeonpea and they have observed that application of mineral nutrients enhanced the RGR at all the stage of development compared to control and it is high in 2% urea spray.

Jahan and Adam (2011) reported that 100ppm and 200ppm NAA had significant effect on RGR of rice variety BRRI dhan29 and BRRI dhan50 and it was maximum at early stages of growth and then declined in both varieties.

Gupta and Basuchaudhari (1976) reported that there was an increase in RGR of rice variety IR8 with foliar application of molybdenum. Khan (2013) conducted a field trial on effect of integrated plant nutrients on wheat and for this he used different micronutrients and among the nutrients maximum relative growth rate (80.75 & 89.59 mg $g^{-1}day^{-1}$) was obtained with the application of boron @ 2 kg ha^{-1}. Gowthamy and Rao (2014) observed that the maximum relative growth rate (0.0430g $g^{-1}d^{-1}$) was in potassium nitrate @ 2 per cent + boric acid @ 50 ppm + zinc sulphate @ 1 per cent at 30 and 60 DAS followed by potassium nitrate @ 2 per cent + boric acid @ 50 ppm (0.0417 g $g^{-1}d^{-1}$).

2.2.6 Net assimilation rate

Surendar *et al*. (2013) conducted a field experiment on physiological effects of nitrogen and growth regulators on crop growth attributes and yield of blackgram and the result of this experiment revealed that the highest NAR was observed in the treatment with N 25 kg ha ha^{-1}+ Urea 2% + Brassinosteriod 0.1 ppm at vegetative and flowering stage. Sritharan *et al.* (2014) observed that the application of 2% urea in blackgram recorded higher NAR (0.58) at 50-65 DAS than control.

Field trials conducted by Karim and Fattah (2007) in Bangladesh with the application of potassium naphthenate (KNap) and naphthalene acetic acid (NAA) as foliar spray in chick pea showed the maximum NAR in pre flowering to pod development and after that it decreased until maturity.

Gupta and Basuchaudhuri (1976) observed stimulating effect of the molybdenum on protein synthesis in vegetative organs of rice which was further confirmed by the positive correlation with NAR. Heidarian *et al.* (2011) reported that maximum NAR was exhibited by foliar application of Zn+Fe (2.7 g /plant) and minimum NAR was observed in control treatment (Zn+ Fe @ 0.4 g /plant) in soybean. Nadim *et al.* (2013) found that use of micronutrients increased the NAR, and among the various nutrients boron produced the maximum NAR (2.78 mg m^2day) followed by copper and manganese (2.41 and 2.34 mg m^2day). Gowthamy and Rao (2014) reported that foliar application of potassium nitrate @ 2 % + boric acid @ 50 ppm + zinc sulphate @ 1 % at 30 and 60 DAS showed higher NAR (0.75 mg cm^2d) as compared to control (0.63 mg cm^2d).

2.3 Effect of herbicides on physiological and biochemical parameters

2.3.1 Chlorophyll content

Channappagoudar *et al.* (2006) conducted an experiment on influence of herbicides on physiological and biochemical parameters of radish and the results revealed that all herbicides significantly increased the total chlorophyll content and among the herbicides, butachlor had higher chlorophyll content (1.045 mg g-1 frwt). Begum *et al.* (2008) conducted an experiment on effect of herbicides on controlling *Fimbristyllis miliacea* in rice field for this they used nine different post emergent herbicides such as bensulfuron, cinosulfuron, pyrazosulfuron, cinosulfuron+ pretilachlor+safener, 2,4-D, pretilachlor+safener, bentazone, fentrazamide+ propanil, and bispyribac sodium which were applied singly or as mixtures , the results showed that highest chlorophyll a,b and total chlorophyll content in cinosulfuron+ pretilachlor and bispyribac sodium applied plots. Zein *et al.* (2012) reported that application of bispyribac sodium resulted in reduction of chlorophyll a, b and total chlorophyll content in rice at 7, 14 and 21 days after treatment.

2.3.2 Total soluble protein

Peng *et al* .(2006) conducted an experiment on the effect of triazine herbicide, atrazine on the chromosome structure, protein content and compositions of rice, the result showed that the soluble protein content in rice decreased from 40.4 mg g FW(control) to 29.3 mg/g FW in treatment with 0.1 mg/L atrazine. Fentrazamide is a tetrazoline class of herbicide and the application of this herbicide above 1µM caused phytotoxicity on watergrass at 15 days after application but it had less toxicity on rice, concentration above 0.1 and 1µM caused reduction in soluble protein content in water grass and 10 and 100 µM in rice (Lim *et al*., 2007).

2.3.3 Proline accumulation

Ramanarayana (2014) reported that the application of herbicide increased the proline content in rice as compared to control but by the flowering time all the herbicide treated plants showed recovery. Bhasker (2015) observed high proline content in wheat with increase in herbicide concentration. Langaro *et al.* (2016) conducted an experiment on biochemical and physiological changes in rice after herbicide application for this they used different types of herbicides which included

Imazapyr + Imazapic, Quinclorac, Bentazon, Cyhalofop-butyl, Penoxsulan, Bispyribac-sodium, Carfentrazone-ethyl, and the result showed that the greatest proline content was observed in treatment with carfentrazone-ethyl followed by other herbicides.

2.3.4 Nitrate reductase activity

Application of 3 L ha^{-1} butachlor and 20 g ha^{-1}chlorimuron-ethyl caused reduction in nitrate reductase activity of wheat plants (Alla *et al*., 2008). Channapagoudar *et al*. (2008) conducted a field trial on the effect of herbicides on physiological and biochemical parameters in radish for this they used twelve different herbicides , the results showed that the nitrate reductase activity increased from 15 DAS to 30 DAS, and weed freecheck plots showed higher nitrate reductase activity followed by butachlor and alachlor treatments. Varshney *et al*. (2015) reported that the application of isoprotouron inhibited the nitrate reductase activity in plants.

2.3.5 IAA content

The herbicide butachlor decresed the level of endogenous IAA but it increased the abscisic acid content (Wen, 1995). Simon and Petrasek (2010) reported that in the class of auxin, IAA is the most commonly and naturally seen hormone and it has a major role in abiotic stress response. Ramanarayana (2014) reported that the application of ALS inhibiting herbicides reduced the IAA content in rice plant.

2.3.6 Catalase activity

Agostinetto *et al.* (2016) observed that the catalase enzyme activity was high for the treatment of the herbicides 2,4-D, and clodinafop compared to control in wheat plants. Nohatto *et al.* (2016) noticed lower catalase activity in rice with bentazon and atrazine as compared to control. Langaro *et al.*(2016) conducted an experiment on biochemical and physiological changes in rice plants due to the application of herbicides and the treatments consisted of imazapic + imazapyr, quinclorac, bentazon, cyhalofop-butyl, penoxsulan, bispyribac-sodium and carfentrazone-ethyl and they observed that among the treatments high catalase activity was noticed 120 hours after spraying of the herbicides and it was high for the herbicide bentazon followed by atrazine.

2.3.7 Stomatal conductance and Photosynthetic rate

Lehnoff *et al.* (2013) conducted an experiment on multiple herbicide resistant in wild oat and its impact on germination and physiology and they have observed that the herbicide susceptible plants showed low stomatal conductance as compared to others. Agostinetto *et al.* (2016) also observed reduction in stomatal conductance when wheat plants were sprayed with herbicides, metribuzin, metsulfuron and 2,4-D. Langaro *et al.* (2016) reported that the application of herbicide carfentrazone-ethyl caused reduction in stomatal conductance in rice.

2.4 Effect of mitigation treatments on biochemical parameters

2.4.1 Chlorophyll content

Nouriyani *et al.* (2012) observed that different nitrogen rates significantly increased chlorophyll a, b and total chlorophyll content in wheat plants. Malik *et al.* (2015) conducted an experiment to understand the effect of Zn, Mo, and urea on mungbean. He observed that Zn (500mg/l), Mo (3ppm) and urea (2%) significantly improved the content of total soluble protein, chlorophyll a ,b and total chlorophyll.

Senthil *et al.* (2003) tested the the effect of NAA 40ppm and IAA on physiological and biochemical parameters of soybean and the results showed that the application of IAA increased the total chlorophyll content.

Arif *et al.* (2012) noticed that there was an increase in chlorophyll a,b and total chlorophyll content in Zn and B applied rice plants. Eleiwa *et al*. (2013) observed that spraying with IAA + NAA (50 + 25; 100 + 50 and 150 + 75 ppm) or with ZnSo4 (0, 50, 100 and 150ppm) increased the chlorophyll content in barley plants. Kazemi (2013) observed that when the concentration of Zn increased there was a reduction in chlorophyll content in cucumber plants Chatterjee and Bandyopadhyay (2015) reported that the foliar application of boron increased the leaf chlorophyll content(a,b and total) in cowpea.

2.4.2 Total soluble protein

The total soluble protein content decreased at pod filling stage but it improved by foliar spray of urea in soybean (Martignone *et al*., 1987). Sritharan *et al*. (2014) reported that the total soluble protein content in blackgram increasesd when it was applied with 2% urea as foliar spray. Rao *et al*.(2015) observed that 2% urea spray under irrigated conditions resulted in higher total soluble protein content in mung bean.

Senthil *et al*. (2003) reported that the application of NAA at 40ppm increased total soluble protein content in soyabean at 35 DAS and 60 DAS.

Keles *et al*. (2004) conducted an experiment on citrus plants with high and low boron levels and the results showed that the presence of boron in plants increases the total soluble protein. Ashraf *et al*.(2014) conducted an experiment on modulation of physiological and biochemical characters in salt stressed rice by foliar application of Zn and the result revealed that there was a decrease in total soluble protein under stressed condition, which can be reversed by application of Zn. Shaimaa *et al*. (2014) observed that there was a gradual increase in total soluble protein with increase in boron concentration in tomato plants.

2.4.3 Proline accumulation

Borowski and Michalek (2010) conducted an experiment on spinach to test the effect of foliar nutrition of magnesium and urea to the crop and the result revealed that the addition of urea to the magnesium salt solution increased the proline content in plants. Jain *et al*. (2015) noticed that the application of nitrogenous compound increased the proline content in sugarcane.

Treatment of plants with plant growth regulator increases the proline content in jatropha plants. (Joshi *et al*., 2011).

Moeinian *et al*. (2011) conducted a field trial at varamin agricultural research centre to test the effect of boron foliar spray on wheat grain under drought stress and the results revealed that the application of boron increased the proline content from 0.803 ig/g to 1.01 ig/g. Dominic and Jithin (2012) observed that the higher concentration of boron increased proline content in rice plants. Vinod *et al*. (2012) observed that the application of Zn, Cu increased the proline content in wheat.

2.4.4 Nitrate reductase activity

Nitrate reductase activity in mungbean increased with urea spray (Prabakaran, 2002). Sritharan *et al*. (2014) reported that the application of 2% urea increased the nitrate reductase activity over control.

Muthulakshmi and Pandiyarajan (2013) conducted an experiment on effect of IAA on growth , physiogical and biochemical parameters in *Catharanthus roseus* and the result revealed that NAA increased the invivo nitrate reductase activity. Netam and Sharma (2014) observed that the application of a combination of GA3, NAA, 2,4-D @ 10 ppm, 20 ppm and 1 ppm respectively increased the nitrate reductase activity in brinjal plants as compared to control from 30 DAT to 90 DAT.

Seethambaram and Das (1986) found that the reduction in the Zn content reduced the nitrate reductase activity in rice and pearl millet. Shen *et al*. (1993) reported reduced nitrate reductase activity in boron deficient plants. Yu *et al*. (1999) reported that molybdenum is involved in nitrate reductase induction process. Molybdenum is a component of enzyme nitrate reductase which reduces nitrate to nitrite (Malavoltta, 2006).

2.4.5 IAA content

Alloway (2004) observed that zinc is required for the synthesis of auxin . Sun *et al.* (2009) noticed that molybdenum application in winter wheat increasesd the ABA and IAA content in leaves under low temperature stress. Soomro *et al.* (2011) reported that one of the main function of boron is IAA metabolism**.** Ahmed *et al.* (2012) conducted an experiment on the effects of zinc, tryptophan, IAA on growth, chemical compostion and yield of Valencia orange trees and he observed that IAA at the rate of 100, 200 and 300 ppm can replace foliar application of zinc.

2.4.6 Catalase activity

Habibzadeh *et al.* (2013) observed that the application urea in canola plants decreased the catalse activity. Jasim *et al.* (2014) estimated the effect of polyethylene mulch and foliar application of urea , seaweed in alleviating salt stress of broccoli and for this they used 0.5% urea spray at 4-6 leaf stage and observed that the foliar application of urea increased the catalase activity in leaves and flowers.

Barakat (2011) found that the application of IAA in most saline condition increased the catalase activity of wheat plants.

Pattanaik (1950) reported that catalase activity of roots and leaves was increased where boron was applied. Rout and Das (2002) conducted an experiment on the effect of molybdenum (0.8µM) on enzyme activity of different cultivars of rice and they observed that the highest catalse activity was for the variety subhadra (38.9) and the least value was for the variety ratna (25.2). Upadhyaya *et al.* (2012) studied the effect of Ca, K, Mn, B on different varieties of tea, and the results revealed that the catalase activity was high in boron applied plants.

Moghadham *et al.* (2013) reported that the foliar application of Zn increased the antioxidant activity in maize. Saeidnejad and Kafi (2013) conducted an experiment on the effects of Zn on physiological properties and antioxidant activity of maize plants under salinity stress and they observed that the Zn could increase the catalase activity in both saline and non saline conditions.

2.4.7 Stomatal conductance and photosynthetic rate

Jin *et al.* (2014) observed that nitrogen spraying increased the stomatal conductance and photosynthetic rate in wheat plants. Shabbir *et al.* (2015) reported that the foliar application of NPK increased the stomatal conductance and photosynthetic rate in wheat plants under water stress.

Eamus (1986) noticed that in *Pisum sativum* and *Phaseolus vulgaris,* IAA spraying increased the stomatal conductance in presence of CO_2. According to Kumar *et al.*(2006) the application of NAA (20ppm) at 90 DAS increased the photosynthetic rate and stomatal conductance in hybrid cotton.

Cohen and Bandurski (1978) reported that boron deficiency adversely decreased the photosynthetic rate. According to Lovatt and Bates,(1984) boron deficiency reduced the stomatal conductance. Ilyas *et al.* (2015) reported that the application of micronutrients Zn, Cu, B caused a significant increase in photosynthetic rate and stomatal conductance in citrus. Mir *et al.* (2015) observed that the application of Zn reduced the gas exchange properties such as photosynthetic rate and stomatal conductance in *Vigna radiata.*

2.5 Effect of herbicides on yield and yield attributes

Rawat *et al. (*2012) reported that bispyribac sodium @ 80g ha^{-1} recorded maximum grain yield in rice . According to Khwaja and Deva (2014) hand weeding at 20 and 40 DAT and also application of bispyribac sodium @ 20g ha^{-1} recorded maximum panicle weight, filled grains/panicle, test weight, grain yield and straw yield in rice. Nayak *et al.* (2014) observed that the application of herbicide pendimethalin followed by bispyribac sodium at 30 DAS recorded higher grain yield. Bispyribac sodium @ 25g ha $^{-1}$ at 15 DAT in rice recorded higher straw yield, grain yield, number of productive tillers/hill, number of filled grains per panicle, panicle weight and test weight in rice (Prashanth *et al*., 2016)..

Yadav *et al.* (2008) reported that azimsulfuron treatments could not raised the yield equivalent to weed free check in rice. Kumar *et al.* (2012) observed that maximum yield attributes such as highest panilcle/hill, panicle weight, grains/ panicle , grain yield and straw yield was recorded in weed free and handweeded plot at 30 and 50 DAT. Teja *et al.* (2015) reported that the highest number of panicles/m^2 , number of filled grains, grain yield and straw yield was recorded in hand weeding at 20 and 40 DAT which was statistically on par with bensulfuron- methyl 0.6%+ pretilachlor 6% at 60+600 g ha^{-1}, metsulfuron methyl + chlorimuron-ethyl (Almix) + azimsulfuron at 4+35 g ha^{-1}and bispyribac-Na at 25 g ha $^{-1}$.

2.6 Mitigation of yield attributes of rice variety manupriya by different chemicals

Hasanuzzaman *et al.* (2009) reported that in boro rice cv. BRRI dhan29 the highest number of panicles/ hill, grain weight and highest grain yield was observed in granular application of urea as compared to the foliar application of urea . Bhuyan *et al.* (2012) reported that foliar application of nitrogen fertilizer increased grain yield, number of panicles, grains per panicle and thousand grain weight in aman rice. Parvin *et al.* (2013) conducted an experiment on effect of foliar spray of urea and hand weeding on yield and yield components of boro rice for this they included four different weeding treatments such as , no weeding (W_0), one weeding (W_1), two weedings (W_2) , three weedings (W_3) and six methods of urea application viz. foliar spray @ 0, 60, 80,100 and120 kg ha^{-1} and soil application @ 220 kg ha^{-1} and the results revealed that highest number of grains/ panicle ,more number of filled grains and grain yield was recorded in 5 times urea spray @ 100 kg ha $^{-1}$ where as highest straw yield was in 4 times urea spray @ 120 kg ha^{-1}.

Lilani *et al.* (1991) reported that NAA can be used for enhancement of growth and yield of cereals. Application of 20 ppm of NAA showed better performance and higher straw and grain yield in wheat and rice cultivars (Alam *et al*., 2002). Adam and Jahan (2011) studied the effect of NAA at two different concentration such as 100 ppm and 200 ppm on yield attributes of rice variety BRRI dhan-29,BRRI dhan-50. The results showed that yield attributes such as number of branches per panicle, number of grains per panicle and filled grains per panicle increased in BRRI dhan-29, in both 100 and 200 ppm NAA.

Hossain *et al.* (2001) observed that the application of Zn, B and Mo increased the grain yield in aman rice BRRI Dhan 30. Chaturvedi (2006) observed that the combination of micronutrients Zn, B and Mn produced higher grain yield , filled grains and thousand grain weight in rice plants. Ali *et al.* (2009) reported that foliar application of Zn and B @ 20g l^{-1} and 30 g/l increased the number of spikes m^{-2} and grain yield in rice . Khan *et al.* (2011) conducted an experiment on residual ,cumulative and direct effect of boron application on wheat and rice under rice- wheat system, and the results showed that the application of boron @ 1kg/ha increased the grain yield both in rice and wheat. Application with Zn and B increased the yield and yield attributes in rice (Arif *et al*., 2012). Yadi *et al.* (2012) conducted an experiment on effect of Zn on grain yield and quality parameters in Iranian rice genotypes and the results revealed that Zn application @ 40 kg/ha and 20 kg/ha produced more number of filled grains, spikelets/ panicle and grain yield as compared to control. According to Singh *et al.* (2014) grain yield increased with application boron @ 3.75 kg ha $^{-1}$ followed by molybdenum @ 0.75 kg ha^{-1} in mung bean. Alam and Kumar

(2015) reported that application of zinc @ 10 kg ha^{-1} was optimum for improving the yield and yield attributes of rice crop.

Materals and Methods

3. Materials and Methods

Investigations on "Mitigating the phytotoxic effect of ALS inhibiting herbicides in rice (*Oryza sativa* L.)." was conducted in agricultural research station mannuthy. The details of materials used and methods adopted are presented in this chapter.

3.1 General details

3.1.2 Location

The experiment was conducted in Agricultural research station, mannuthy.

3.1.3 Variety used

The rice variety Manupriya, a red kernelled, long , bold, short duration variety of 105-110 days duration was used for the trial. The variety is suitable for direct seeding and transplanting for all seasons. It is tolerant to sheath blight, brown spot, blast, stem borer, gall midge 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 july 2015 to october 2015 .

3.2 Treatments

The experiment was laid out in randomized block design (RBD) with 2 herbicide treatments and 5 different nutrient sprays in bispyribac and azimsulfuron herbicide treatment with 3 replications (Table.1) . The plot size was 5m X 3m ($15m^2$). The herbicides and nutrients tried and the dosages used are given below:

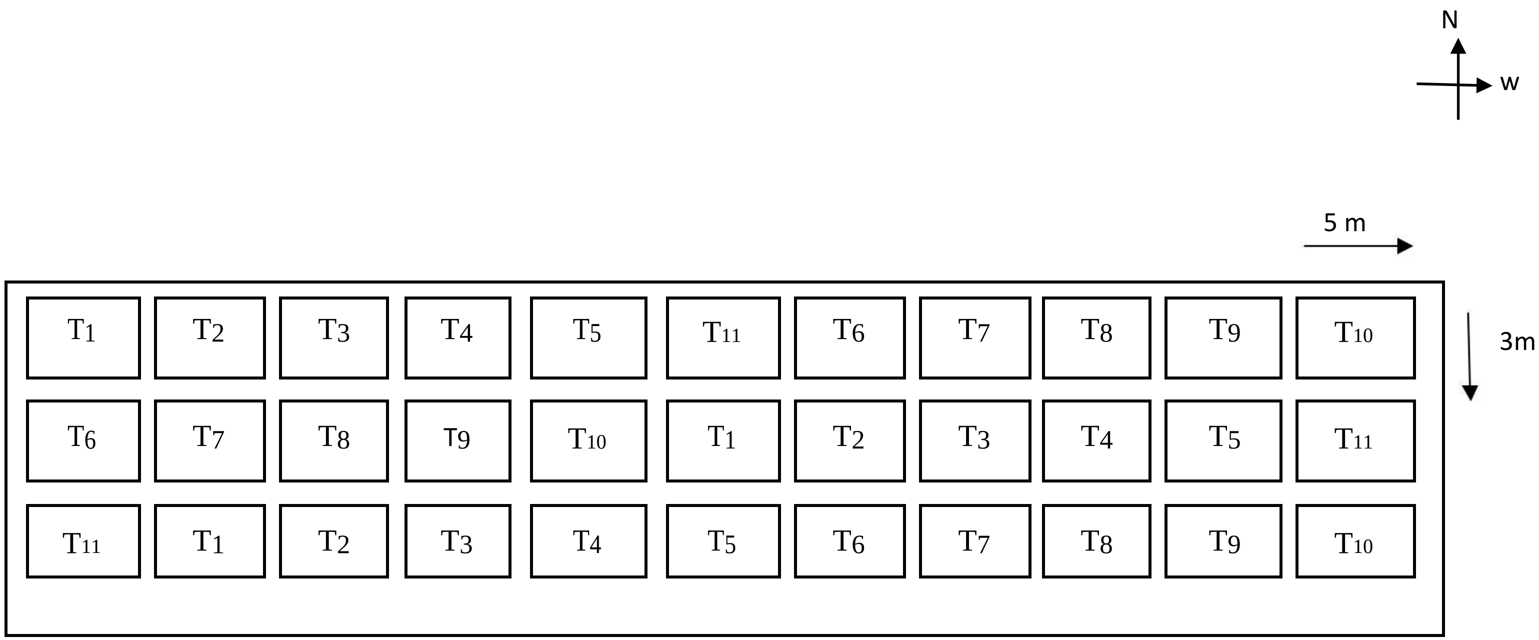

Fig. 1. Layout of the experiment

Bispyribac sodium

T_1 - Urea

T_2 - Micronutrient spray

T_3 - NAA

T_4 - Tank mix

T_5 - Water spray

Azimsulfuron

T_6 - Urea

T_7 - Micronutrient spray

T_8- NAA

T_9 – Tank mix

T_{10} – Water spray

T_{11} – Hand weeded control

Pla te 1. General view of the experimental plot

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

Sl No	Herbicides	Trade Name	Formulation	Dosage (g *a.i.*/ha)
1	Bispyribac sodium	Nominee gold	10SC	30
2	Azimsulfuron	Segment	50DF	35

DF- Dry flowable SC- Soluble concentrate

Table 2. Treatments and quantity of the nutrients used for the study

Sl No	Treatments	Concentration
1	Urea spray	1%
2	Micronutrient spray (Zinc sulphate,Borax,Molybdic acid)	1g each of Zinc sulphate and Borax, 0.01g Molybdic acid dissolved in 1000 ml water
3	NAA spray	100ppm
4	Tank mix	1% urea spray, 1g each of Zinc sulphate and Borax, 0.01g Molybdic acid and 100ppm of NAA.
5	Water spray	1000 ml

* Spray volume – 400 litre ha^{-1}.

3.3 Field operations

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

3.3.1 Land preparation, sowing and fertilizer application

Before starting the experiment soil samples were collected from the experimental site and analyzed for the basic properties like pH, EC , macro and micro nutrients . 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 20 cm width and height. After levelling, fertilizers N:P:K was applied as per POP @ 70:35:35 kg/ha . Secondary and micronutrients were applied as per soil test data except in treatments T2, T4, T7 and T9, where Zinc, Boron and Molybdenum was given as spray. Urea, Factamphos , Muriate of potash, Borax and Magnesium sulphate were used for supplying the nutrients. Full dose of P was applied basally. N , K, B, Mg were applied in two equal splits during land preparation and panicle initiation stages. After basal fertilizer application, the seeds were dibbled at a spacing of 15×10 cm at the rate of 150g/plot (90 kg ha^{-1}). In Hand weeded treatment, hand weedings were done at 20 and 40 DAS. Both the herbicides were sprayed at 15 DAS using knapsack sprayer at the recommended doses (Table 1) and all nutrients were sprayed at 35 DAS at the recommended doses (Table2). After harvesting, the soil and plant nutrients were again analyzed. The methods used for soil and plant analysis is given in table 3 and 4.

Plate 2. Mitigation treatments sparying at 35 DAS

Table 3: Methods used for soil analysis

Sl.No	Analysis	Method	Reference
1	Soil reaction(pH)	Determined in 1:2.5 soil water suspension,potentiometrically using a pH meter	Jackson,1958
2	Electrical conductivity (EC)	Estimated in the supernatant liquid of the soil water (1:2.5) used for pH estimation with the help of conductivity meter.	Jackson,1958
3	Organic carbon	Wet digestion method	Walkey and Black, 1934
4	Available Phosphorus	Available phosphorus in the soil samples extracted using Bray No.1 reagent and estimated colorimetrically by reduced molydate ascorbic acid blue colour method using spectrophoto meter	Bray and Kurtz, 1945: Watnabe and Olsen, 1965.
5.	Available Pottassium	Extracted using neutral normal ammonium acetate and its content in the extract was estimated by flame photometery	Jackson,1958
6	Available Calcium	Extracted using neutral normal ammonium extract and its content in the extract was estimated using Perkin Elmer Atomic Absorption Spectrophotometer	Jackson,1958

7	Available Magnesium	Extracted using neutral normal ammonium extract and its content in the extract was estimated using Perkin Elmer Atomic Absorption Spectrophotometer	Jackson,1958
8	Available Sulphur	Extracted using neutral normal ammonium extract and its content in the extract was estimated using Perkin Elmer Atomic Absorption Spectrophotometer	Jackson,1958
9	Iron,Copper, Manganese, Zinc	Available micronutrients in samples were extracted using 0.1 M Hcl. Four grams soil was shaken for 5 minutes with 40ml 0.1 M Hcl. It was filtered through Whatmann No.1 filter paper and the filtrate was collected and analysed for Fe, Cu, Mn, Zn using Perkin Elmer AtomicAbsorption Spectrophotometer. Extracted by DTPA method and reading taken by using Atomic Absorption Spectrophotometer	Sims and Johnson, 1991
10	Boron	Extracted with hot water and estimated Colorimetrically by Azomethine-Husing spectrophotometer	Berger and Truog, 1939)

Table 4: Methods for plant nutrient analysis

Sl.No	Nutrient	Method	Reference
1	Nitrogen	MIicrokjeldhal digestion and distillation method	Jackson,1958
2	Phosphorus	Diacid digestion of leaf sample followed by filtration. Vandadomolybdate phosphoric yellow colour in nitric acid system	Piper, 1966
3.	Pottassium	Diacid extract using Flame Photometer	Piper, 1966.
4	Zinc	Diacid using Atomic Absorption Spectrophotometer	Piper, 1966
5	Boron	By dry ashing and Azomethine- H method	Gaines and Mitchell, 1979: Bingham, 1982.
6	Molybdenum	Diacid extraction method and reading taken by using ICP (Inductively coupled plasma)	Piper,1966

3.3.2 Plant protection

Timely plant protection measures were taken up as per package of practices (POP) of KAU (KAU, 2007). Thrips and leaf folder attack was noticed during the seedling stage of the crop and “rogur” for thrips attack and “fame” for leaf folder was sprayed. In milky stage attack of rice bug was controlled by spraying malathion @ 1ml/l

3.3.3 Harvesting

The crop was harvested during last week of october when the grains were fully matured. Threshing was done manually and the produce was cleaned, dried and yield was expressed as t/ha.

3.4 Sample collection

Prior to application of treatment the field was maintained uniformly. Herbicides were applied on the 15th day and sample collection was done one week after application of herbicides to compare treatment effects. Morphological observations were taken at 15 days interval where as physiological studies were conducted one week after herbicide application and at the time of flowering.

3.5 Morphological Observations

3.5.1 Height

Five plants from each plot were selected and marked and these plants were used for taking morphological observation throughout the experiment . The plant height was measured from base to the longest leaf that is before panicle initiation and after panicle initiation, the measurement was made from base to the longest panicle and expressed in cm.

3.5.2 Tiller production

The total number of tillers in one square meter area from each experimental plot was taken at 15 days interval from 30DAS.

3.5.3 Days to flowering

When about 50% of the plants flowered, days to flowering was recorded.

3.6 Growth indices

Growth indices were worked at 15 days interval upto harvest. Sampling unit consisted of 9 plants per treatment (Three from each replication). The plant samples were uprooted and dried in hot air oven and the growth indices were computed as per the procedure given below.

3.6.1 Leaf area index (LAI)

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

3.6.2 Relative growth rate (g g^{-1} d^{-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 g g^{-1} d^{-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.

3.6.3 Crop growth rate (g m^{-2} d^{-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 m^{-2} d^{-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)

3.6.4 Net assimilation rate (mg cm^{-2} d^{-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 mg cm^{-2} d^{-1}.

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

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.

3.7 Physiological studies

The physiological and biochemical parameters were estimated one week after herbicide application and at flowering stage.

3.7.1 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 Thermo Spectonic, 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 the following formulae.

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

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

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

Where,

A = Absorption at given wavelength

V = Total volume of sample in extraction medium

W = Weight of sample

3.7.2 Net photosynthesis

Photosynthetic rate was measured by using the instrument infrared gas analyzer (Model LI-6400 of ICOR inc. Lincoln, Nebreska, USA) one week after application of herbicides and at the time of flowering and the reading was taken from
8 to 10 am and expressed as µ mol CO_2 m^{-2} s^{-1}.

3.7.3 Stomatal conductance

Stomatal conductance was measured by using the instrument infrared gas analyzer (Model LI-6400 of ICOR inc. Lincoln, Nebreska, USA) one week after application of herbicides and at the time of flowering reading were taken from 8 to 10 am and expressed as m mol H_2O $m^{-2}s^{-1}$

3.7.4 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 mg g^{-1} of unoxidised auxin g^{-1} hr^{-1}

3.8 Biochemical characters

3.8.1 Total soluble protein

Total soluble proteins was estimated by the method suggested by Lowry *et al.* (1951) and expressed as mg g^{-1}.

3.8.2 Estimation of Catalase enzyme activity

Catalase enzyme activity (CAT) was estimated by permanganate titration method of Barber (1980). The activity of the catalase enzyme was expressed in µg of H_2O_2 g^{-1} min^{-1}.

Plate 3. Gas exchange measurement using IRGA

3.8.3 Nitrate reductase activity

To estimate nitrate reductase enzyme 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 expressed in µ moles of NO_2^- formed g^{-1} fresh weight hr^{-1}.

3.8.4 Proline content

Proline content was determined by the method of Bates *et al.* (1973) and expressed as µ moles g^{-1} tissue. Absorbance was taken at 520 nm.

3.9 Yield attributes and yield

3.9.1 Number of productive tillers

The number of productive tillers square meter area of each experimental plot at harvesting stage was counted , recorded and express the value as tillers /m^2 .

3.9.2 Number of spikelets per panicle

The number of spikelets was counted from five plants and the average was calculated.

3.9.3 Number of filled grains per panicle

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

3.9.4 Chaff percentage

Samples were collected from randomly selected five plants from each plot and the percentage of chaff grains per each panicle was worked out.

Plate 4: Harvesting

3.9.5 Thousand grain weight

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

3.9.6 Grain and straw yield

The crop harvested from each replication was threshed, winnowed and weighed separately. The straw and grain weight were recorded separately and expressed in t ha^{-1}.

3.10 Data analysis

The data were subjected to stastical analysis using the stastical package AGRES and WASP. Multiple comparison among treatment means, where the F test was significant (at 5 % level) were done with Duncan's Multiple Range Test.

Results

4. Results

The present study aims to mitigate the growth inhibition due to the application of post emergent ALS (Aceto Lactate Synthase) inhibitors bispyribac sodium and Azimsulfuron and by the application of hormones, nutrients to improve the productivity of rice. The results of the study are detailed below.

4.1.1 Plant height

On the 15th day the height of the plant varied between 31.87 and 25.13. Here the variation in the plant height between the treatments was not due to any treatment effect. To negate these variations subsequent observations were taken from the same plants which were demarkated and retained in the field till the end of the experiment. The herbicides bispyribac sodium and azimsulfuron were sprayed on the 15th day. Observations taken on the 30th day showed the effect of herbicides on the rice plant. Seedlings in T_{11} (control) plot had the highest plant height (53.90) which was on par with seedlings in bispyribac applied plots , T_2,T_3,T_4 & T_5. Seedlings in T_1 alone had lower height (46.73) as compared to the other bispyribac applied plants. Seedlings in azimsulfuron applied plots showed significantly lower plant height as compared to control. The lowest height was recorded in treatments T8 (44.83) & T9 (44.83) followed by T_6,T_{10} & T_7.

Mitigation treatments were given on the 35th day and the height was recorded on the 45th day. Maximum height was observed in T_1 (75.67) which was on par with treatment T_2, T_4, T_7 & T_9 this was followed by the treatment T_3 & T_8 . Treatments T_{10} (63.4) & T_5 (62.93) recorded the lowest plant height which were on par with T_6 & T_{11}. On 60th day maximum height was recorded in T_1, T_4 and T_7 which was on par with T_2, T_6 and T_9 this was followed by the treatment T3 which were significantly superior to the treatment T_5, T_8 and T_{10}. Lowest height was recorded for the treatment T_{11} (86.20). On 75th day, maximum height was observed in treatment T_4 which was on par with T_1, T_2, T_6 and T_9. Plants in treatment T_7 were taller than that of plants in treatment T_8, T_3 and T_5. The lowest plant height was recorded for treatment T_{11} & T_{10}. On 90th day, maximum plant height was recorded in treatment T_4 (125.93) which was on par with T_1, T_6 & T_9 and significantly higher than T_2, T_7 and T_8. Lowest plant height was observed in T_5, T_{10} and T_{11} which were on par. At the time of harvest also the same height difference were retained. Maximum height was recorded for plants in treatment T_4 (127.53) which was on par with T_1 and T_9. This was followed by plants from T_6 & T_2 which was on par with T_7, T_8 and T_5. Lowest was recorded in treatments T_{10} (111.47) and T_{11} (109.80), water spray of azimsulfuron and control respectively.

Table 5: Effect of mitigating treatments on plant height (cm) .

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS
T_1	28.00 ^{cd}	46.73 ^{bcd}	75.67 ^{a}	100.40 ^{a}	120.47 ^{ab}	120.80 ^{ab}	123.40 ^{ab}
T_2	27.37 ^{cde}	49.70 ^{abc}	72.87 ^{ab}	97.80 ^{ab}	118.33 ^{abc}	119.47 ^{bc}	122.73 ^{b}
T_3	29.67 ^{abc}	49.70 ^{abc}	70.06 ^{bc}	93.53 ^{bc}	111.67 ^{de}	115.00 ^{cd}	116.67 ^{c}
T_4	25.700 ^{de}	49.37 ^{abcd}	72.60 ^{ab}	99.87 ^{a}	122.93 ^{a}	125.93 ^{a}	127.53 ^{a}
T_5	31.67 ^{ab}	50.37 ^{ab}	62.93 ^{e}	88.00 ^{cd}	108.00 ^{ef}	112.47 ^{de}	119.86 ^{bc}
T_6	25.13 ^{e}	45.16 ^{cd}	67.53 ^{cde}	98.06 ^{ab}	117.67 ^{abc}	121.13 ^{ab}	122.53 ^{b}
T_7	28.33 ^{c}	47.63 ^{bcd}	70.73 ^{abc}	99.87 ^{a}	115.87 ^{bcd}	119.00 ^{bc}	120.60 ^{bc}
T_8	27.500 ^{cde}	44.83 ^{d}	68.83 ^{bcd}	90.60 ^{cd}	114.27 ^{cd}	117.73 ^{bc}	119.53 ^{bc}
T_9	29.17 ^{c}	44.83 ^{d}	72.67 ^{ab}	97.87 ^{ab}	119.20 ^{abc}	121.93 ^{ab}	123.33 ^{ab}
T_{10}	29.40 ^{bc}	45.00 ^{cd}	63.40 ^{e}	88.33 ^{cd}	105.00 ^{f}	109.27 ^{e}	111.47 ^{d}
T_{11}	31.87 ^{a}	53.90 ^{a}	64.06 ^{de}	86.20 ^{d}	103.87 ^{f}	107.80 ^{e}	109.80 ^{d}
CD(0.05)	2.37	4.76	4.94	6.00	5.79	5.19	4.31

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.1.2 Number of tillers m^{-2}

Result regarding the effect of various treatments on tillers/m^2 of rice variety manupriya at different intervals is given in Table 6 . Herbicides were applied on the 15th day. The observation taken on the 30th day showed the effect of herbicides on tiller number. On the 30th day maximum number of tillers m^{-2} was observed in control (T_{11}) followed by bispyribac applied plots, among which T_3 showed the maximum value followed by the treatments T_4, T_5, and T_1 which were on par. Seedlings in T_2 (230.33) alone had lower number of tillers as compared to other bispyribac applied treatments. In azimsulfuron applied plots, the lowest value was recorded in T_7 (204.67) followed by T_9 & T_{10}.

Nutrient spray for mitigating the effect of herbicides were applied on 35^{th} day and observations were recorded on the 45^{th} day. The maximum number of tillers m^{-2} was observed in treatment T_2 (435) , micronutrient spary which was on par with the treatments T_1 and T_4 followed by the treatments T_3,T_9,T_7,T_8, and T_5. Treatments T_{10} (403.33) & T11 (408.33) recorded the lowest values which was on par with T_6. On 60^{th} day maximum number of tillers m^{-2} was observed in treatment T_4 (529) which was on par with the treatments T_1,T_2, T_3 followed by the treatments T_5 and T_9 which were significantly superior than the treatments T_6,T_7 & T_8. The lowest number of tillers m^{-2} was observed in treatments T_5 (516.66) & T10 which were on par. By the 75^{th} day maximum number of tillers m^{-2} was observed in treatment T_4 which was followed by the treatments T_1, T_2 and T_3. The treatments T_5 and T_9 showed lesser number of tillers compared to the treatments T4 but significantly superior to the treatments T_8 & T_7. The lowest number of tillers m^{-2} was recorded in the treatments T_6, T_{10} & T_{11} which were on par. By the 90^{th} day maximum number of tillers m^{-2} was recorded in the treatment T_4 (340.33) followed by the treatments T_1 & T_2 which was higher than that of the treatments T_3 & T_9 (330.33) and were on par. The treatments T_5 and T_6 recorded less number of tillers m^{-2} compared to other treatments which was on par with the treatments T_7, T_8, T_{10} and T_{11} that recorded the lowest number of tillers. On 105^{th} day maximum number of tillers m^{-2} was observed in tank mix applied plots (T_4) which was on par with the treatment T_2 (324.33) both of which were significantly superior to treatment T_3 & T_1. The lowest number of tillers m^{-2} was observed in treatments T_{11}, T_{10} and T_8.

Table 6: Effect of mitigating treatments on tiller m^{-2}.

Treatments	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS
T_1	234.33 ^{bc}	430.67 ^{ab}	522.67 ^{b}	421.33 ^{b}	334.00 ^{bc}	314.67 ^{cd}
T_2	230.33 ^{c}	434.00 ^{a}	522.00 ^{b}	421.67 ^{b}	336.00 ^{b}	324.33 ^{ab}
T_3	240.33 ^{b}	426.67 ^{abc}	520.67 ^{b}	420.33 ^{bc}	330.33 ^{cd}	315.67 ^{bc}
T_4	238.33 ^{bc}	433.33 ^{ab}	529.000 ^{a}	427.00 ^{a}	340.33 ^{a}	329.67 ^{a}
T_5	235.67 ^{bc}	419.67 ^{cd}	516.66 ^{c}	416.67 ^{c}	327.33 ^{de}	309.00 ^{cdef}
T_6	205.67 ^{ef}	416.33 ^{de}	505.00 ^{d}	402.67 ^{e}	324.33 ^{e}	302.33 ^{efg}
T_7	204.67 ^{f}	418.33 ^{cd}	506.33 ^{d}	404.33 ^{de}	316.00 ^{f}	306.00 ^{defg}
T_8	213.00 ^{de}	417.67 ^{cd}	506.00 ^{d}	407.33 ^{d}	315.67 ^{f}	307.00 ^{g}
T_9	216.00 ^{d}	424.33 ^{bcd}	515.33 ^{c}	416.33 ^{c}	330.33 ^{cd}	311.00 ^{cde}
T_{10}	217.67 ^{d}	403.33 ^{f}	500.66 ^{e}	402.00 ^{e}	312.33 ^{f}	298.67 ^{g}
T_{11}	249.00 ^{a}	408.33 ^{ef}	500.66 ^{e}	402.33 ^{e}	313.00 ^{f}	300.67 ^{fg}
CD(0.05)	8.13	9.29	3.67	4.08	3.67	9.27

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.1.3 Days to flowering

Data regarding the effect of various mitigation treatments on days to flowering for different intervals are given in table 7 .The different treatments showed significant difference in days to flowering. The handweeded plot took more number of days to flower (67) followed by the treatment T_{10} (66.33). The treatments T_3 ,T_4 and T_8 recorded least number of days for flowering followed by T_1, T_7 and T_9.

Table 7 : Effect of mitigating treatments on days to flowering .

Treatments	Days to flowering
T_1	64.67 c
T_2	62.33 d
T_3	60.67 e
T_4	62.67 d
T_5	65.33 bc
T_6	65.00 bc
T_7	64.33 c
T_8	62.67 d
T_9	64.67 c
T_{10}	66.33 ab
T_{11}	67.00 a
CD(0.05)	1.61

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.1.4 Leaf area index

Leaf area index was recorded on 15th, 30th, 45th, 60th, 75th and 105th day is given in table 8. On 15th day maximum leaf area index was observed in treatment T_{10} (0.69) followed by treatments T_1 and T_6. Least value was recorded for the treatment T_3 (0.39). By the 30th day hand weeded plot recorded maximum leaf area index (1.92) which was significantly superior than treatments in bispyribac applied plots treatments T_1, T_3,T_4 and T_2.The seedlings in T_5 (1.67) alone had lower leaf area index compared to other bispyribac applied plots. Azimsulfuron applied plots had lower leaf area index as compared to control and bispyribac applied plots.In the azimsulfuron applied plots T_7 (1.51) had the lowest LAI and all others were on par.

On 35th day mitigating treatments were applied and the observations were taken on the 45th day. By the 45th day the maximum leaf area index was observed in treatment T_4 (3.62) followed by the treatments T_1, T_2 and T_3 which were on par and significantly superior than the treatments T_9 and T_{11}. The treatments T_5, T_6,T_7 and T_9 showed similar trend in leaf area index and these treatments showed higher value of leaf area index compared to the treatment T_8 (3.43) which recorded the lowest leaf area index. On the 60th day the maximum leaf area index was observed in treatment T_4 (4.65) followed by the treatment T_1 this was significantly higher than that of the treatments T_2 and T_3 and T_9 which were on par. In treatments T_5, T_6, T_8 and T_7 similar values of leaf area index was recorded. The lowest value was observed in treatment $T1_0$ & T_{11} which were on par. On 75th, 90th, 105th day the LAI followed the same pattern. Maximum LAI was recorded in T_4 (1.67) and minimum was observed in treatments T_{10} & T_{11}.

Table 8: Effect of mitigating treatments on LAI .

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS
T_1	0.61 ^{ab}	1.78 ^{b}	3.57 ^{ab}	4.58 ^{ab}	5.38 ^{ab}	3.76	1.63
T_2	0.46 ^{de}	1.71 ^{bcd}	3.58 ^{ab}	4.52^{bc}	5.37 ^{ab}	3.70	1.68
T_3	0.39 ^{e}	1.76 ^{bc}	3.57 ^{ab}	4.55^{bc}	5.40 ^{ab}	3.73	1.69
T_4	0.50 ^{cd}	1.75 ^{bc}	3.62 ^{a}	4.65 ^{a}	5.407 ^{a}	3.76	1.67
T_5	0.56 ^{bc}	1.67 ^{cde}	3.47 ^{cd}	4.46 ^{cde}	5.32 ^{b}	3.63	1.60
T_6	0.51 ^{cd}	1.57 ^{ef}	3.46 ^{cd}	4.47 ^{cde}	5.14 ^{cd}	3.61	1.56
T_7	0.57 ^{bc}	1.51 ^{f}	3.48 ^{cd}	4.45 ^{de}	5.15 ^{c}	3.63	1.51
T_8	0.57 ^{bc}	1.56 ^{ef}	3.43 ^{d}	4.47 ^{cde}	5.08 ^{bcd}	3.67	1.54
T_9	0.58 ^{bc}	1.59 ^{ef}	3.52 ^{bc}	4.55 ^{bc}	5.15 ^{c}	3.68	1.52
T_{10}	0.69 ^{a}	1.62 ^{def}	3.49 ^{cd}	4.34 ^{f}	5.07 ^{de}	3.59	1.57
T_{11}	0.62 ^{ab}	1.920 ^{a}	3.54 ^{bc}	4.39 ^{ef}	5.04 ^{e}	3.60	1.62
CD(0.05)	0.090	0.107	0.077	0.150	0.076	NS	NS

4.1.5 Crop growth rate

Crop growth rate estimated for the rice variety manupriya for different intervals is indicated in table 9. Herbicides were sprayed at 15 DAS and its effect was observed in CGR value at 15-30 days interval. Maximum CGR was observed for the hand weeded control (4.89) which was on par with the treatments in bispyribac sodium T_2 and T_4 followed by the treatment T_5. Azimsulfuron applied plots showed significantly lower values compared to control and bispyribac applied plots. The lowest value was observed in treatment T_7 (3.72). The mitigating treatments were given on 35 DAS, and the effect was observed in 30-45 days CGR value. The maximum CGR was observed in the treatment T_4 (14.15) which was followed by the treatments T_1, T_3 and T_6. The treatments T_2 , T_7 and T_9 recorded significantly lower value this was followed by the treatment T_8. Control and water spray in both herbicide treatments recorded the lowest value. At 45-60 days interval, among the treatments T_4 (16.68) had the highest value followed by the treatments T_7 and T_3. Which was significantly superior than the treatments T_1 and T_6. All other treatments were inferior to these treatments. The lowest value was observed for the treatment T_{10} (11.89) and T_{11} (11.92) that is azimsulfuron fb water spray and also hand weeded control. The same trend was observed at 60-75 days interval. Maximum value was recorded for the treatment T_4 (18.32) which was followed by the treatmentsT_1, T_2, and T_9 which were superior than the treatments T_3, T_6 and T_7. All other treatments recorded lower CGR value. The lowest value was observed for the treatment T_{10} (12.26). At 75-90 days and 90-105 days there was a reduction in CGR in all the treatments and difference between the treatments was non significant.

Table 9 : Effect of mitigating treatments on crop growth rate (g m^{-2} d^{-1})

Treatments	15-30 days	30-45 days	45-60 days	60-75 days	75-90 days	90-105 days
T_1	4.27^{c}	11.89 ^{b}	13.68 ^{c}	15.87 ^{b}	9.65	3.24
T_2	4.77 ^{a}	11.54 ^{c}	13.47 ^{e}	15.86 ^{b}	9.91	2.83
T_3	4.19 ^{c}	11.84 ^{b}	13.87 ^{b}	15.62 ^{c}	10.65	3.01
T_4	4.77 ^{a}	14.15 ^{a}	16.68 ^{a}	18.32 ^{a}	9.36	4.27
T_5	4.43 ^{b}	10.14 ^{h}	11.47 ^{i}	13.63 ^{f}	5.90	3.15
T_6	3.87 ^{de}	11.86 ^{b}	13.53 ^{d}	15.43 ^{d}	9.59	5.10
T_7	3.72 ^{f}	11.36 ^{d}	13.85 ^{b}	15.46 ^{d}	8.43	4.53
T_8	3.76 ^{ef}	11.23 ^{e}	13.26 ^{g}	15.23 ^{e}	9.44	4.43
T_9	3.98 ^{d}	11.35 ^{d}	13.37 ^{f}	15.83 ^{b}	8.25	4.49
T_{10}	3.90 ^{d}	10.33 ^{g}	11.89 ^{h}	12.26 ^{h}	5.33	3.28
T_{11}	4.89 ^{a}	11.08 ^{f}	11.92 ^{h}	12.96 ^{g}	5.48	3.87
CD(0.05)	1.00	0.068	0.033	0.124	NS	NS

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.1.6 Relative growth rate

Estimation of relative growth rate on rice variety manupriya is indicated in table 10 .The herbicides were applied on the 15 days and the effect was observed in RGR value at 15-30 days interval. The maximum value was recorded for the handweeded control which was on par with the bispyribac applied treatments T_1 (0.204) and T_4 (0.204) followed by T_2 (0.202) and T_3 (0.201). All the treatments in the azimsulfuron applied plots recorded lower value as compared to handweeded control and bispyribac applied plots. The mitigating treatments were sprayed on 35 DAS and the effect was observed in 30-45 days interval. The maximum RGR value was recorded for the treatment T_4, T_2 and T_9 which were on par and followed by the treatments T_3 and T_1. The least value was observed for the treatment T_{11} (0.076) .At 45-60 days maximum RGR value was observed for the treatment T_4 (0.042) followed by the treatment T_3. The least value was observed for the treatment T_{11} (0.029). At

60-75 days interval the maximum value was observed for the treatment T_4 (0.024) followed by the treatment T_1, T_2, T_3, T_6, and T_9. The least value was observed for the treatments T_5, T_{10} and T_{11}. At 75-90 days and 90-105 days no significant difference between the treatments was noticed.

Table 10 : Effect of mitigating treatments on relative growth rate (g g^{-1} d^{-1})

Treatments	15-30 days	30-45 days	45-60 days	60-75 days	75-90 days	90-105 days
T_1	0.204[a]	0.093 [c]	0.037 [bc]	0.024 [ab]	0.013	0.004
T_2	0.202[ab]	0.097 [a]	0.035 [d]	0.023 [bc]	0.014	0.003
T_3	0.201[ab]	0.095 [b]	0.037 [b]	0.023 [bc]	0.015	0.004
T_4	0.204[a]	0.098 [a]	0.042 [a]	0.024 [a]	0.012	0.005
T_5	0.198[abc]	0.083 [f]	0.035 [cd]	0.021 [e]	0.010	0.003
T_6	0.187[cd]	0.088 [e]	0.032 [efg]	0.023 [bc]	0.014	0.004
T_7	0.189[cd]	0.089 [d]	0.034 [de]	0.022 [cd]	0.012	0.004
T_8	0.187[cd]	0.089 [d]	0.034 [def]	0.022 [cd]	0.014	0.003
T_9	0.192[bcd]	0.097 [a]	0.032 [fg]	0.023 [bc]	0.012	0.005
T_{10}	0.186[d]	0.086 [f]	0.031 [gh]	0.021 [e]	0.009	0.002
T_{11}	0.202[ab]	0.076 [h]	0.029 [h]	0.021 [de]	0.011	0.002
CD(0.05)	0.01	0.001	0.002	0.002	NS	NS

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.1.7 Net assimilation rate

Net assimilation rate was measured at 15 days interval and data regarding that is given in table 11 . At 15-30 days the maximum NAR was recorded for the treatment T_5 and T_{11} followed by the treatments T_1 and T_6 which were on par. The herbicides were applied during this interval and the effect of this was seen among the treatments. Compared to bispyribac applied plots azimsulfuron applied plots had lesser NAR value. Lowest value was recorded for the treatments T_8 and T_9. All other treatments were superior to these two treatments. At 35 DAS the mitigating treatments were applied and at the 30-45 days interval the effects was noticed. Highest value was recorded for the treatment T_4 (0.75) followed by the treatments T_3, T_6, T_7 and T_9

which were on par. Lowest value was noticed for the treatment T_{11} (0.63). Maximum NAR value during 45-60 days was noticed for the treatment T_4 (0.67) followed by the treatments T_1, T_2 and T_6. The lowest value was observed for the treatment T_{11} (0.50). At 60-75 days interval a decline in NAR value was noticed and the maximum was recorded for the treatment T_4 (0.56) followed by the treatments T_1, T_6 and T_9. All other treatments were inferior to these treatments and the lowest NAR value was noticed for the treatment T_{11} (0.41) . At 70-95 and 90-105 days interval the treatments effect was non significant.

Table 11 : Effect of mitigating treatments on net assimilation rate (mg cm^{-2} d^{-1})

Treatments	15-30 days	30-45 days	45-60 days	60-75 days	75-90 days	90-105 days
T_1	0.87^{ab}	0.71^{b}	0.63^{ab}	0.53^{a}	0.077	0.032
T_2	0.83^{bc}	0.70^{b}	0.64^{ab}	0.52^{ab}	0.076	0.033
T_3	0.79^{cd}	0.73^{ab}	0.61^{b}	0.51^{ab}	0.080	0.031
T_4	0.74^{ef}	0.75^{a}	0.67^{a}	0.56^{a}	0.080	0.032
T_5	0.88^{a}	0.63^{c}	0.55^{cd}	0.45^{c}	0.077	0.032
T_6	0.87^{ab}	0.72^{ab}	0.63^{ab}	0.53^{a}	0.077	0.035
T_7	0.79^{cd}	0.73^{ab}	0.53^{cd}	0.45^{c}	0.078	0.037
T_8	0.73^{f}	0.70^{abc}	0.55^{c}	0.46^{bc}	0.081	0.029
T_9	0.73^{f}	0.73^{a}	0.61^{b}	0.53^{a}	0.079	0.032
T_{10}	0.78^{de}	0.65^{bc}	0.52^{cd}	0.43^{c}	0.072	0.032
T_{11}	0.89^{a}	0.63^{c}	0.50^{d}	0.41^{c}	0.071	0.031
CD(0.05)	0.044	0.036	0.048	0.062	NS	NS

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.2 Biochemical parameters

4.2.1 Chlorophyll content

The chlorophyll content in rice variety manupriya is given in the table 12 &13. Herbicides were sprayed on 15 DAS and chlorophyll content was measured one week after herbicide application. There was a significant difference observed between herbicide applied plots and hand weeded control. The hand weeded control recorded maximum value of chlorophyll a (3.77), chlorophyll b (1.17) and total chlorophyll

(4.95) followed by the bispyribac applied plants. There was a significant reduction in chlorophyll content of azimsulfuron applied plots in chlorophyll a , b and total chlorophyll.

Table 12: Chlorophyll content (mg g^{-1} fresh wt) 7 DAH application.

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Bispyribac	3.72 b	1.14 b	4.86 b
Azimsulfuron	3.68 c	1.12 c	4.80 c
Handweeded	3.77 a	1.17 a	4.95 a
CD(0.05)	0.016	0.029	0.016

Mitigating treatments were sprayed on 35 DAS and the second analysis was at the time of flowering. The effect of treatment was observed in chlorophyll content of the plants. In chlorophyll a , maximum value was for the treatment T_4 (2.78). All other treatments were on par with each other. Treatment T_4 showed maximum chlorophyll b (0.97) content followed by the treatments T_8 and T_9 and it was superior than the treatments T_1, T_3, T_6, T_7, T_{10} and T_{11}. The least chlorophyll b content was observed in treatment T_5 (0.40) that is water spray in bispyribac applied plot. In case of total chlorophyll also the maximum value was observed in treatment T_4 (3.75) followed by the treatments T_3 and T_9 which were on par and significantly superior to the treatments T_1, T_2, T_6, T_7, T_8, T_{10} and T_{11}. The least value for total chlorophyll content was observed in treatment T_5 (1.41).

Table 13: Effect of mitigating treatments on chlorophyll content (mg g^{-1} fresh wt)

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T_1	1.53^{b}	0.50^{bcd}	2.03^{bc}
T_2	1.72^{b}	0.47^{cd}	2.19^{bc}
T_3	1.78^{b}	0.62^{bcd}	2.40^{b}
T_4	2.78^{a}	0.97^{a}	3.75^{a}
T_5	1.01^{b}	0.40^{d}	1.41^{c}
T_6	1.13^{b}	0.60^{bcd}	1.73^{bc}
T_7	1.32^{b}	0.59^{bcd}	1.92^{bc}
T_8	1.25^{b}	0.78^{ab}	2.03^{bc}
T_9	1.67^{b}	0.74^{abc}	2.42^{b}
T_{10}	1.18^{b}	0.55^{bcd}	1.74^{bc}
T_{11}	0.98^{b}	0.56^{bcd}	1.55^{bc}
CD(0.05)	0.84	0.29	0.97

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.2.2 Total soluble protein content

The total soluble protein content in rice variety manupriya is given in table 14 & 15 . Herbicides bispyribac and azimsulfuron were applied on 15 DAS and the analysis was done at 7 days after herbicide application. There was significant variation between hand weeded control and herbicide applied plots. The maximum total soluble protein content was observed in hand weeded control (37.6) which was on par with bispyribac (36.5) applied plots. The least total soluble protein content was for azimsulfuron (32.6) applied plots.

Table 14: Total soluble protein content (mg g^{-1}) 7 DAH application

Treatments	After application of herbicide
Bispyribac	36.5 [a]
Azimsulfuron	32.6 [b]
Handweeded	37.6 [a]
CD(0.05)	1.51

Mitigating treatments were applied on 35 DAS . The analysis was done at the time of flowering and the treatment effect was observed. More soluble protein content was seen in the treatment T_4 (24.50) followed by the treatment T_9 which was superior to the treatments T_1 and T_6 which were on par. Treatments in azimsulfuron applied plots had less soluble protein content than bispyribac applied plots. The least soluble protein content was recorded in treatment T_{11} (12.23) and T_{10} (13.83), all other treatments were superior to these treatments

Table 15 : Effect of mitigating treatments on total soluble protein content (mg g^{-1})

Treatments	At flowering
T_1	22.16 [abc]
T_2	20.66 [cd]
T_3	18.66 [de]
T_4	24.50 [a]
T_5	14.50 [e]
T_6	21.00 [bcd]
T_7	20.16 [cd]
T_8	17.16 [e]
T_9	23.26 [ab]
T_{10}	13.83 [e]
T_{11}	12.23 [e]
CD(0.05)	2.44

4.2.3 Proline accumulation

Effect of herbicides on proline content of rice is given in table 16 & 17 . There was a significant variation in herbicide applied plots and handweeded control. More proline content was observed in herbicide applied plots among which azimsulfuron showed maximum proline (2.28) accumulation as compared to bispyribac and handweeded plots

Table 16 : Proline content (μ moles g $^{-1}$ tissue) 7 DAH application

Treatments	After application of herbicide
Bispyribac	2.13^{b}
Azimsulfuron	2.28^{a}
Handweeded	1.79^{c}
CD(0.05)	0.08

After flowering proline accumulation was recorded in different treatment. Among the treatments T_4, T_8 and T_9 showed maximum proline content followed by the treatments T_3, T_6 and T_7. The least proline content was noticed in water spray treatments (T_5, T_{10}) and control (T_{11}) .

Table 17 : Effect of mitigating treatments on Proline content (µ moles g $^{-1}$ tissue)

Treatment	At flowering
T_1	1.04[cd]
T_2	1.20[bc]
T_3	1.36[ab]
T_4	1.44[a]
T_5	0.87[d]
T_6	1.40[ab]
T_7	1.29[ab]
T_8	1.44[a]
T_9	1.45[a]
T_{10}	0.89[d]
T_{11}	0.89[d]
CD(0.05)	0.19

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.2.4 Nitrate reductase enzyme acivity

The nitrate reductase activity in rice after herbicide application is given in table 18 & 19 .The maximum nitrate reductase activity was noticed in hand weeded control (438.45) followed by bispyribac sodium (405.92). The least nitrate reductase activity was observed in azimsulfuron applied plots (378.45).

Table 18 : Nitrate reductase enzyme activity (µ moles of NO_2^- formed g^{-1} fresh weight hr^{-1}) 7 DAH application

Treatments	After application of herbicide
Bispyribac	405.92[b]
Azimsulfuron	378.45[c]
Handweeded	438.45[a]
CD(0.05)	17.08

The enzyme activity in rice at the time of flowering is given in table. Nitrate reductase activity was more in treatment T_4 (375.52) that is tank mix in bispyribac applied plots followed by the treatments T_2 which is superior than the treatments T_1, T_3 and T_4. After flowering there was a reduction in nitrate reductase activity in all treatments and the least value was recorded in treatments T_{10} and T_{11} which were on par.

Table 19 : **Effect of mitigating treatments on Nitrate reductase enzyme activity (μ moles of NO_2^- formed g^{-1} fresh weight hr^{-1})**

Treatments	At flowering
T_1	360.85 c
T_2	369.92 b
T_3	364.85 c
T_4	375.52 a
T_5	363.78 c
T_6	306.98 ef
T_7	318.98 d
T_8	310.72 e
T_9	316.05 d
T_{10}	303.78 f
T_{11}	304.05 f
CD(0.05)	4.33

4.2.5 IAA content

Effect of herbicides on IAA content is showen in table 20 & 21 . Herbicide applied plots showed significantly lesser amount of IAA than handweeded plots. Maximum value was recorded for handweeded plot (1.04) followed by bispyribac applied plots (0.91) . Least value of IAA content was noticed in azimsulfuron applied plots.

Table 20: IAA content 7 DAH application (mg g $^{-1}$ of unoxidised auxin g $^{-1}$ hr $^{-1}$)

Treatments	After application of herbicide
Bispyribac	0.91 ^{ab}
Azimsulfuron	0.71 ^{b}
Handweeded	1.04 ^{a}
CD(0.05)	0.204

Mitigating treatments were sprayed on 35 DAS. The effect of these treatments was noticed at the time of flowering is given in table 21. Among the treatments T_9 (0.946) & T_4 (0.917) recorded maximum value of IAA content followed by the treatments T_3 and T_8. Effect of these treatments in the azimsulfuron applied plots was significantly lesser than bispyribac applied plots. Least value was observed in treatments T_{10} and T_{11}.

Table 21 : Effect of mitigating treatments on IAA content (mg g $^{-1}$ of unoxidised auxin g $^{-1}$ hr $^{-1}$)

Treatments	At flowering
T_1	0.54 ^{e}
T_2	0.85 ^{c}
T_3	0.88 ^{bc}
T_4	0.91 ^{ab}
T_5	0.49 ^{f}
T_6	0.53 ^{ef}
T_7	0.73 ^{d}
T_8	0.88 ^{bc}
T_9	0.94 ^{a}
T_{10}	0.44 ^{g}
T_{11}	0.44 ^{g}
CD(0.05)	0.047

4.2.6 Catalase enzyme activity

Effect of herbicides on catalase enzyme activity is given in table 22 & 23 . There was a significant variation between herbicide applied plots and handweeded control. The maximum value was recorded for azimsulfuron applied plots (5.80) which was followed by bispyribac applied plots (5.24). Minimum catalase enzyme activity was observed in hand weeded control (4.39).

Table 22 : Catalase enzyme activity 7 DAH application (µg of H_2O_2 g $^{-1}$ min $^{-1}$)

Treatments	After application of herbicide
Bispyribac	5.24 [b]
Azimsulfuron	5.80 [a]
Handweeded	4.39 [c]
CD(0.05)	0.490

There was an increase in catalase enzyme activity at the time of flowering. Significant variation between the treatments was also noticed. More catalase enzyme activity was observed in treatment T_1 (55.95) followed by the treatments T_6 and T_7. Treatment T_{10} recorded least value of catalase enzyme activity followed by treatments T_5 and T_2. All other treatments were inferior to these treatment.

Table 23: Effect of mitigating treatments on catalase enzyme activity (µg of H_2O_2 g $^{-1}$ min $^{-1}$)

Treatments	At flowering
T_1	51.00 [de]
T_2	50.15 [ef]
T_3	51.14 [de]
T_4	52.98 [c]
T_5	49.30 [fg]
T_6	55.95 [b]
T_7	56.24 [b]
T_8	56.52 [ab]
T_9	57.37 [a]
T_{10}	48.73 [g]
T_{11}	51.70 [d]
CD(0.05)	1.00

4.2.7 Net Photosynthesis

The net photosynthesis observed after herbicide application on 15 DAS is given in table 24 & 25. Observations were taken seven days after application. There was no significant variation observed in photosynthetic rate of herbicide applied plots and hand weeded control.

Table 24 : Photosynthetic rate 7 DAH application (μ mole CO_2 $m^{-2}s^{-1}$)

Treatments	After application of herbicide
Bispyribac	31.47
Azimsulfuron	30.76
Handweeded	31.56
CD(0.05)	NS

The net photosynthesis of rice plants at the time of flowering is given in table 19 . The mitigation treatment effect was noticed on the photosynthetic rate. The maximum photosynthesis was noticed for the treatment T_4 (38.00) and T_9 (38.23) followed by the treatment T_1 (35.78) and it was superior to the treatment T_6 . Least value of net photosynthesis was observed in water spray treatments T_5 (32.07) and T_{11} (32.04).

Table 25 : Effect of mitigating treatments on Photosynthetic rate (μ mole CO_2 $m^{-2}s^{-1}$)

Treatments	At flowering
T_1	35.78^{b}
T_2	34.04^{cd}
T_3	34.03^{cd}
T_4	38.00^{a}
T_5	32.07^{e}
T_6	34.87^{bc}
T_7	33.85^{cd}
T_8	33.62^{cd}
T_9	38.23^{a}
T_{10}	33.36^{de}
T_{11}	32.04^{e}
CD(0.05)	1.38

4.2.8 Stomatal conductance

Stomatal conductance of plants seven days after herbicide application was noted and data regarding this is given in table 26 & 27. There was no significant variation observed between herbicide applied plots and handweeded control

Table 26 : Stomatal conductance 7 DAH application (mol H_2O $m^{-2}s^{-1}$)

Treatments	After application of herbicide
Bispyribac	0.249
Azimsulfuron	0.239
Handweeded	0.304
CD(0.05)	NS

Stomatal conductance of plants at the time of flowering is given in table 27. The effect of treatments on stomatal conductance was more in treatment T_4 (1.07) followed by the treatments T_9 (0.92) and T_6 (0.91). All other treatments were inferior to these treatments. Least value of stomatal conductance was recorded for the treatments T_5, T_{10} and T_{11} .

Table 27 : Effect of mitigating treatments on Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)

Treatments	At flowering
T_1	0.82^{bc}
T_2	0.79^{c}
T_3	0.73^{c}
T_4	1.07^{a}
T_5	0.48^{d}
T_6	0.91^{b}
T_7	0.71^{c}
T_8	0.74^{c}
T_9	0.92^{b}
T_{10}	0.58^{d}
T_{11}	0.57^{d}
CD(0.05)	0.11

4.3 Yield attributes

4.3.1 Number of productive tillers m^{-2}

Number of productive tillers m^{-2} at the time of harvest is given in table 29. Maximum number of tillers was observed in treatment T_4 (330.67) followed by the treatment T_2 which is superior to the treatments T_1 and T_3. All other treatments recorded lesser number of productive tillers/m^2 as compared to these treatments. Minimum number of tillers was recorded in the treatments T_6 and T_{11}.

4.3.2 Spikelets/panicle.

Spikelets / panicle varied in different mitigating treatments .The maximum number of spikelets was observed in treatment T_4 (120.33) followed by the treatments T_1, T_3 and T_9 which was superior than the treatments T_5, T_7, T_8 and T_{11}. Treatment T_{10} (97.67) recorded least number of spikelets .

4.3.3 Filled grains/panicle

The number of filled grains/panicle varied between treatments and it ranged from 116 to 92. The maximum number of filled grains was noticed in treatment T_4 (116.33) followed by the treatment T_2 (109.67) and T_3. All other treatments showed less number of filled grains as compared to these treatments. Treatment T_{10} recorded least number of filled grains (90.00) followed by the treatment T_{11} (92.00) and T_8 (93.67).

4.3.4 Chaff percentage

Chaff percentage of different treatments was estimated after harvest. Highest chaff percentage was noticed in treatment T_{11} (9.14) followed by the treatments T_{10},T_6 and T_2. All other treatments were on par except the treatments T_4 (3.35) and T_7 (5.47) which had lowest chaff percentage.

4.3.5 Thousand grain weight

The highest thousand grain weight was recorded for the treatment T_4 (33.06) followed by the treatments T_3 and T_1. The treatments T_5 (29.81) and T_6 (29.67) were on par and had less weight as compared to the treatment T_4. All other treatments recorded less value as compared to these treatment. The lowest grain weight was observed in treatment T_{10} (29.47) followed by the treatment T_{11} and T_6.

Table 28 : Effect of mitigating treatments on yield attributes

Treatments	No.of Productive tillers m^{-2}	No.of Spikelets $panicle^{-1}$	No.of Filled grains $panicle^{-1}$	Chaff percent age	Thousan d grain weight
T_1	316.00^{bc}	109.67^{b}	102.33^{cd}	6.81^{bcd}	31.17^{bc}
T_2	324.33^{ab}	117.00^{a}	109.67^{b}	6.23^{cd}	30.90^{bcd}
T_3	315.67^{c}	110.67^{b}	$104.^{33\ c}$	5.71^{d}	31.51^{b}
T_4	330.67^{a}	120.33^{a}	116.33^{a}	3.35^{e}	33.06^{a}
T_5	309.67^{cde}	104.33^{cd}	97.67^{ef}	6.32^{cd}	29.81^{ef}
T_6	301.67^{ef}	103.00^{d}	95.00^{fg}	7.77^{abc}	29.67^{ef}
T_7	305.33^{def}	105.00^{cd}	99.33^{de}	5.47^{d}	30.08^{def}
T_8	305.33^{def}	101.33^{de}	93.67^{fgh}	7.56^{bc}	29.28^{fg}
T_9	311.00^{cd}	108.00^{bc}	101.33^{cde}	6.33^{cd}	30.43^{cde}
T_{10}	299.33^{f}	97.67^{e}	90.00^{h}	8.08^{ab}	28.73^{g}
T_{11}	302.00^{ef}	101.00^{de}	92.00^{gh}	9.14^{a}	29.47^{fg}
CD(0.05)	8.51	4.47	4.00	1.54	0.90

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.3.6 Grain yield

The highest grain yield was observed in treatment T_4 (4.97) followed by the treatment T_9 (4.83) which was superior to the treatments T_1, T_2, T_3, T_6, T_7 and T_8 which were on par. Lowest grain yield was obtained in treatments T_5, T_{10} and T_{11}.

4.3.7 Straw yield

The highest straw yield was obtained in treatment T_6 (6.22) followed by the treatments T_1 ,T_3 and T_8 which were superior to the treatments T_2, T_4, T_7 and T_9 which were on par. The lowest straw yield was obtained in treatments T_5, T_{10} and T_{11} which were on par.

Table 29: Effect of mitigating treatments on yield (t ha^{-1})

Treatments	Grain yield	Straw yield
T_1	4.32 ^{b}	5.88 ^{ab}
T_2	4.37 ^{b}	5.16 ^{bc}
T_3	4.35 ^{b}	5.38 ^{abc}
T_4	4.97 ^{a}	5.16 ^{bc}
T_5	4.00 ^{c}	4.94 ^{c}
T_6	4.30 ^{b}	6.22 ^{a}
T_7	4.48 ^{b}	5.22 ^{bc}
T_8	4.26 ^{b}	5.44 ^{abc}
T_9	4.83 ^{a}	5.16 ^{bc}
T_{10}	3.98 ^{c}	4.66 ^{c}
T_{11}	4.00 ^{c}	4.88 ^{c}
CD (0. 05)	0.251	0.837

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

4.4 Soil analysis

Table 30: Soil analysis before sowing

Parameters	Quantity	Remarks
pH	5.0	Very strongly acidic
Electrical Conductivity (dS/m)	0.09	Normal
Organic Carbon (%)	0.78	Medium
Available Phosphorus (Kg/ha)	88.11	High
Available Potassium (Kg/ha)	299.04	High
Available Calcium (mg/Kg)	426.50	Sufficient
Available Magnesium (mg/Kg)	50.00	Deficient
Available Sulphur (mg/Kg)	5.29	Sufficient
Micronutrients		
Copper (mg/Kg)	7.77	Sufficient
Iron (mg/Kg)	145.09	Sufficient
Zinc (mg/Kg)	2.32	Sufficient
Manganese (mg/Kg)	89.85	Sufficient
Boron (mg/Kg)	0.18	Deficient

Soil analysis was done before sowing and after harvesting. As per the soil test data fertilizers were applied. Before sowing magnesium and boron content were deficient in soil but after harvest there was an improvement in soil magnesium content though it was still deficient. However the content of boron changed from deficient to sufficient due to borax application in soil .The pH also changed from very strongly acidic to strongly acidic this change was due to the application of lime. All other nutrients were found to be sufficient. Phosphorus and potassium content was high in both cases but the quantity was reduced after harvesting.

Table 31: Soil analysis after harvesting

Parameters	Quantity	Remarks
pH	5.2	Strongly acidic
Electrical Conductivity (dS/m)	0.05	Normal
Organic Carbon (%)	0.81	Medium
Available Phosphorus (Kg ha $^{-1}$)	69.59	High
Available Potassium (Kg/ha)	197.12	High
Available Calcium (mg/Kg)	335.5	Sufficient
Available Magnesium (mg/Kg)	77.0	Deficient
Available Sulphur (mg/Kg)	5.17	Sufficient
Micronutrients		
Copper (mg/Kg)	4.86	Sufficient
Iron (mg/Kg)	31.86	Sufficient
Zinc (mg/Kg)	5.70	Sufficient
Manganese (mg/Kg)	29.67	Sufficient
Boron (mg/Kg)	0.60	Sufficient

4. 5 Effect of mitigating treatments on plant nutrient (N, P,K, B, Zn, Mo) content

Plant nutrient analysis were done after harvesting. The percentage of nitrogen, phosphorus and potassium,boron, zinc and molybdenum content in the plants after harvest is given in table 33. The results showed that higher nitrogen content was in treatment T4 (1.63) and T2 (1.57) which was stastically on par with the treatments T2, T3 and T9. Least nitrogen content was observed in the control (T11 0.99). Boron content was higher for the treatment T2 (8.77) followed by T9, T4, and T7. Least value was recorded for the treatment T5 (4.67). There was no significant difference between treatments in phosphorus, potassium and zinc. Molybdenum content in the plant samples was in non detectable level.

Table 32: Effect of mitigating treatments on plant nutrient content

Treatments	Nitrogen (%)	Phosphorus (%)	Pottasium (%)	Boron (µg g $^{-1}$)	Zinc (mg kg $^{-1}$)	Molybdenum
T_1	1.40 ^{ab}	0.39	1.41	6.87 ^{bcd}	45.66	NDL
T_2	1.57 ^{a}	0.38	1.29	8.77 ^{a}	43.50	NDL
T_3	1.40 ^{ab}	0.34	1.13	6.72 ^{cde}	42.75	NDL
T_4	1.63 ^{a}	0.40	1.18	7.89 ^{abcd}	45.08	NDL
T_5	1.16 ^{bcd}	0.33	1.13	4.67 ^{g}	48.08	NDL
T_6	1.10 ^{cd}	0.31	1.25	6.57 ^{def}	44.25	NDL
T_7	1.22 ^{bcd}	0.34	1.43	8.18 ^{abc}	49.66	NDL
T_8	1.28 ^{bc}	0.33	1.39	6.43 ^{def}	47.25	NDL
T_9	1.40 ^{ab}	0.38	1.18	8.33 ^{ab}	45.66	NDL
T_{10}	0.99 ^{d}	0.32	1.22	5.11 ^{fg}	42.83	NDL
T_{11}	1.05 ^{cd}	0.35	1.14	5.26 ^{efg}	39.33	NDL
CD (0.05)	0.280	NS	NS	1.587	NS	

T1, T6 – Urea; T2, T7 – Micronutrients; T3, T8 – NAA; T4, T9 – Tank mix;
T5, T10- Water spray T11- Control

Discussion

5. Discussion

Herbicides effectively control weed flora in the rice ecosystem. The ALS inhibiting herbicides are used for the effective control of weeds in a short period of time. But it causes yield reduction in rice plants.

The main focus of the present study was to mitigate the effect of ALS inhibiting herbicides by different chemical and hormone treatments also to identify the physiological and biochemical changes in crop after the application of herbicides and mitigating treatments. An attempt is made from the results obtained to discuss the effect of these parameters on yield attributes of rice.

5.1. Herbicides and mitigation treatments effect on morphological characters

In the present study ALS inhibiting herbicides bispyribac and azimsulfuron were applied on the 15 DAS. A comparison of the morphological attributes such as height and tiller number indicated that as compared to the hand weeded plot there was significant reduction in height when estimated on the 30th day. The reduction in height was more for azimsulfuron applied plots as compared to bispyribac applied plots. Such a reduction in plant height by the application of herbicide has also been noticed by Menon (2012) and Ramanarayana (2014). This might be due to the effects of ALS inhibitors on protein and enzyme synthesis (Koschnik *et al.*, 2007). To mitigate the effect of ALS inhibitors urea, micronutrients and NAA were applied on 35 DAS. Observations taken on the 45th day indicated that these treatments are capable of boosting the crop growth. Among the treatments, plants recovered faster with urea spray as this treatment recorded maximum height on the 45th and 60th day for both the herbicides. Improvement in plant height by urea spray has also been reported by Parvin *et al.* (2013). Later on by the 45th day tank mix application treatment T_4 and T_9 which is a mixture of urea, micronutrient and NAA gave the better result which sustained till harvest. The positive influence of urea, micronutrients and NAA on plant height has been individually reported by (Yildirim *et al*., 2007: Singh and Bhatt, 2013) respectively.

Observation of Tiller number m^{-2} was taken on the 30^{th} day .Maximum tillering was observed in T_{11} where no herbicides were applied. The bispyribac applied plots had significantly more number of tillers m^{-2} as compared to azimsulfuron applied plots. With the application of mitigating treatment on the 35^{th} day significant increase in tiller number was observed in herbicide applied plots as compared to the control. Among the treatments T_4 (Tank mix) had the maximum number of tillers (bispyribac). All other treatments in bispyribac applied plots were on par and significantly lower than T_4. Among the azimsulfuron applied plots also tank mix gave the best result (T_9). A 5 % increase in tiller number as compared to T_5 and T_{10} was observed in both the herbicide applied plots. Jahan and Adam (2011) have reported increase in number of tillers by application of 100 ppm NAA. Parvin *et al.* (2013) observed that urea spray improved tillering in rice. The positive influence of micronutrients Zn, B and Mo has been mentioned by (Sudha and Stalin, 2015: Rashid 2006) respectively. Tank mix is a combination of all these chemicals. The increase in tiller number by the application of these chemicals might be due to the increased activity of different enzymes and hormones resulting in better growth and production of productive tillers (Radhika *et al* ., 2013).

5.2. Herbicides and mitigating treatments effect on growth indices

On 30^{th} day the LAI of the hand weeded plots were significantly superior to the herbicide applied plots and among the herbicide applied plots LAI was significantly higher for bispyribac applied plots as compared to azimsulfuron applied plots. Ramanarayana (2014) has also observed that azimsulfurom applied plots had the lowest LAI on the 30^{th} day. Mitigation treatments significantly improved the LAI of the rice plant by the 45^{th} day. However improvement in LAI for azimsulfuron applied plots even with mitigation treatments was significantly lower than the bispyribac applied plots upto 75th day. After the active growth period there was a decline in LAI in all the treatments.

The relative growth rate of rice plant was affected by the herbicide azimsulfuron than bispyribac application. The RGR values for bispyribac applied plots were on par with the contol while the azimsulfuron applied plots showed significant reduction in RGR by the 30th day. Mitigation treatments given on the 35th day improved the growth of the plant. Application of combination treatment T_4 (combination of urea, micronutrients & NAA) improved the RGR value of both azimsulfuron and bispyribac sodium applied plots. Increase in leaf area might have accounted for increase in dry matter accumulation of plant resulting in high RGR (Kaur *et al.,* 2015).

The CGR of the plants on 15 to 30 days of bispyribac applied plots was on par with control and significantly superior to azimsulfuron applied plots. Mitigation treatments T_4 (combination of urea, micronutrients & NAA) had higher CGR values for both azimsulfuron and bispyribac applied plots. At 30-45 days interval to 45-60 days interval there was a 17 % increase in crop growth rate whereas from 45-60 days interval to 60-75 days interval the improvement was 10% only. The improvement in CGR was higher during active growth stage of the plant, ie 45-60 days. Improvement in CGR may be attributed to the increase in LAI & NAR of the plant as indicated in table 9 . The micronutrient spray contributes to leaf expansion and increased photosynthetic activities which might have improved the plant growth (Tahir *et al* ., 2009).

The net assimilation rate of the plants depends on LAI. NAR was significantly lower for azimsulfuron applied plots during 15-30 days. However application of mitigation treatments improved the NAR significantly. By the 45-60 days tank mix applied for bispyribac and azimsulfuron gave the highest result and was significantly superior to other treatments. The improvement in LAI would have contributed to the improvement in growth, photosynthetic efficiency and yield of the plant (Chen and Black, 1992).

5.3 Herbicides and mitigating treatments effect on physiological parameters

5.3.1 Chlorophyll content

When chlorophyll content was estimated 7 DAH application there was a significant reduction in herbicide applied plots as compared to hand weeded plots. Among the herbicide applied plots there was higher reduction in chlorophyll a, b and total chlorophyll in azimsulfuron applied plots as compared to bispyribac applied plots. Studies conducted by Ramanarayana (2014) also revealed that the reduction in chlorophyll content induced by the herbicide bispyribac was lesser as compared to azimsulfuron. The chlorophyll biosynthetic pathway is more complex as the chloroplast has their own DNA, mRNA & protein synthesis. The ALS inhibitors affect the biosynthesis of amino acids (Ray, 1984) this might be the reason for the reduction in chlorophyll content by the application of ALS inhibiting herbicides. Mitigation treatment given on the 35th day had contributed to an improvement in the chlorophyll content as indicated by the observations taken at the time of flowering (table 13). For bispyribac applied plots, among the treatments, T_4 (combined application of urea, micronutrients & NAA) has shown significant improvement in chlorophyll a, b and total chlorophyll content. While in the case of azimsulfuron applied plots tank mix application has helped to improve the chlorophyll b content which was found to be significantly superior to all other treatments. The improvement in chlorophyll content may be due to higher availability of nitrogen from urea spray and other micronutrients. Improvement in the nitrate reductase activity by treatment T_4 may be due to improved nitrogen assimilation of plants as earlier reported by Chatterjee and Bandyopadhyay, 2014. This is also validated by the improvement in the NRAse activity as given in (table 19).

5.3.2 Total soluble protein

When the total soluble protein content was estimated on 7 DAH application there was a significant reduction in herbicide applied plots as compared to hand weeded control. Among the herbicide plots bispyribac had higher soluble protein as compared to azimsulfuron applied plots. Studies conducted by Ramanarayana (2014) also showed that there was a reduction in total soluble protein, 7 DAH application and at the time of flowering. Mitigation treatments given on the 35 DAS showed an improvement in total soluble protein and best results were observed in T_4 (combined application of urea, micronutrient and NAA) in bispyribac applied plots where as in case of azimsulfuron applied plots, the micronutrient spray (T_7) recorded high total soluble protein. Barak and Helmke (1993) reported that Zn is an important component of enzymatic protein in plants. The application of Zn increases RNA content and protein content in plants (Brown et al., 1993) **.** Redondo *et al.* (2003) reported that application of boron may effect several genes associated with nitrogen metabolism which may cause increase in soluble protein content in plants. Application of urea increases nitrogen pool of plants which might have contributed to increase in soluble protein content in plants (Kaur and Jagetiya , 2005). Variation in total soluble protein content between treatments is given in figure 2.

5.3.3 Proline content

Proline is a free amino acid synthesized in the plant cell during stress condition. In the present study there was significant increase in proline content one week after herbicide application, which indicates a stress condition due to herbicide application. This result was in concurrence with the observations of Ibrahim *et al*. (2004). Among the herbicide applied plots azimsulfuron applied plots was having higher amount of proline content as compared to bispyribac applied plots. Mitigation treatments were given on the 35th day, observations taken at the time of flowering showed higher proline content in the combined application of urea, micronutrient and

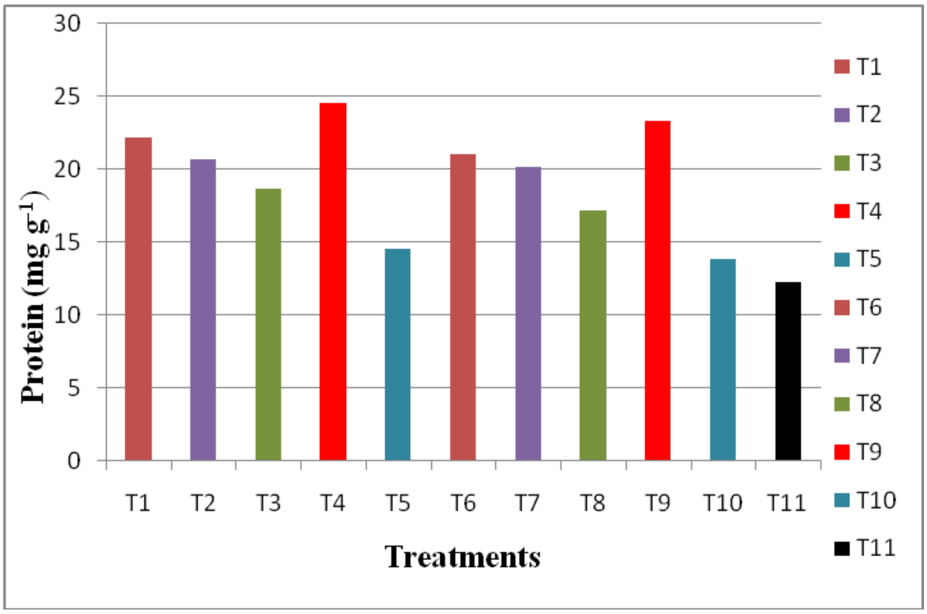

Figure 2: Variation in total soluble protein content (mg g $^{-1}$) between treatments

T1 to T5= Bispyribac sodium

T1- Urea spray

T2- Micronutrient spray

T_3- NAA

T4- Tank mix

T5- Water spray

T6 to T10 = Azimsulfuron

T6- Urea spray

T7- Micronutrient spray

T8- NAA

T9- Tank mix

T10- Water spray

T11- Control

NAA spray (T4 & T9) in both bispyribac and azimsulfuron applied plots. Rastgoo (2014) observed that Zn application lead to proline accumulation in plants. This might be due to the denovo synthesis or decline in degradation (Kavi- Kishore *et al*., 2005)of proline. It also has the unique property to act as an antioxidant and as a osmolyte in osmoprotective process (Hartzendorf and Rolletschek, 2001). It helps in preservation of enzyme structure & activity, scavenging singlet oxygen and hydroxyl radical (Matysik *et al*., 2002) and in storage of nitrogen and carbon during plant recovery. These factors might have contributed to the increase in proline content by the application of mitigation treatments.

5.3.4 Nitrate reductase enzyme activity

Reduction in NRAse activity was noticed in 7 DAH application and at the time of flowering. Among the herbicides, azimsulfuron showed maximum reduction as compared to bispyribac applied plots. Similar results was reported by Singh *et al.* (2013). Mitigation treatments given 35 DAS showed an improvement in NRAse activity and it was more in T_4 and T_9 in bispyribac and azimsulfuron applied plots respectively. Singh *et al.* (2002) reported that NRA is an indicator of nitrogen assimilation potential. Zinc increased the NRAse activity by increasing the net photosynthetic rate in plants (Seethambaram and Das, 1986) .The element molybdenum is required for normal assimilation of nitrogen in plants, as it is a component of two enzymes xanthine oxidase and nitrate reductase (Malik *et al*., 2015). Muthulakshmi and Pandiyarajan, (2013) reported that the application of IAA increases the protein content in plants .

5.3.5 IAA content

In the present study application of herbicides significantly reduced the IAA content when the observations were taken on 15-30 days. However at the time of flowering the IAA content in the case of azimsulfuron (T_{10}) and bispyribac (T_5) applied plots were on par with the control (T_{11}). Mitigation treatments improved the

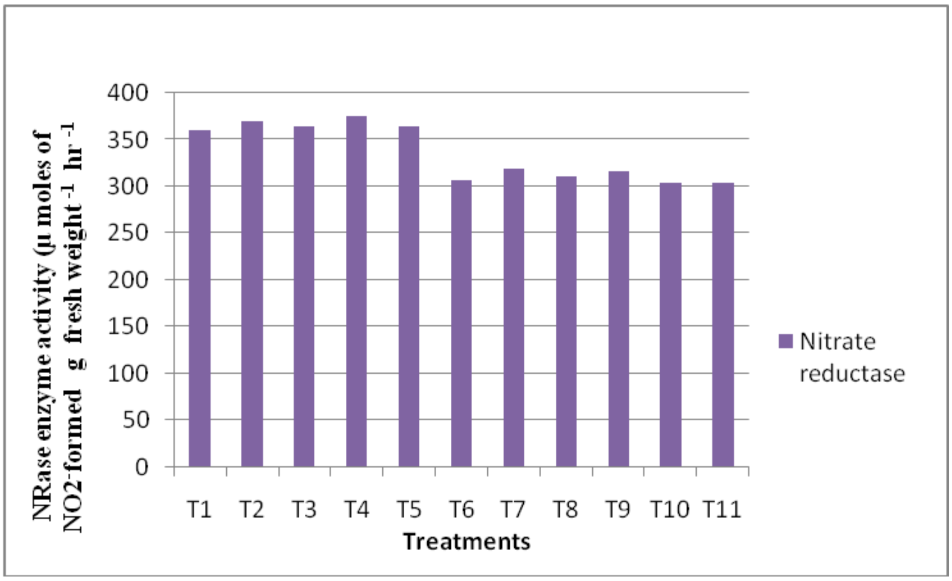

Fig. 3. Variation in NRAse enzyme activity
(µ moles of $NO2^-$ formed g fresh weight $^{-1}$ hr $^{-1}$) between treatments

T_1 to T_5= Bispyribac sodium

T1- Urea spray

T2- Micronutrient spray

T_3- NAA

T4- Tank mix

T5- Water spray

T_6 toT_{10}= Azimsulfuron

T6- Urea spray

T7- Micronutrient spray

T8- NAA

T9- Tank mix

T10- Water spray

T11- Control

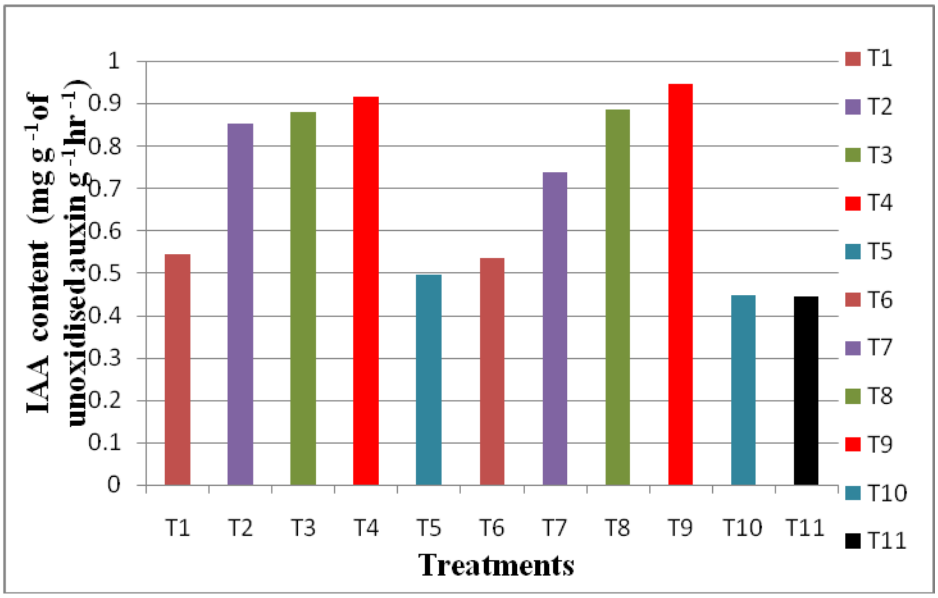

Figure 4: Variation in IAA (mg g $^{-1}$ of unoxidised auxin g $^{-1}$ hr $^{-1}$) content between treatments

T1 to T5= Bispyribac sodium

T1- Urea spray

T2- Micronutrient spray

T_3- NAA

T4- Tank mix

T5- Water spray

T6 to T10= Azimsulfuron

T6- Urea spray

T7- Micronutrient spray

T8- NAA

T9- Tank mix

T10- Water spray

T11- Control

IAA content in both the herbicide applied plots. Among the treatments urea and tank mix application gave the best result for bispyribac applied plots. IAA is structurally related to the amino acid trypotophan and to the tryptophan indole 3- glycerol phosphate both of which serve as a precursor for IAA biosynthesis (Taiz and Zeiger, 1991) . Zinc has also been identified as a component of tryptophan which enhances IAA synthesis (Kazemi, 2013). Hence combined application of these chemicals might have contributed to the better performance of these treatments. Variation in IAA content between treatments is given in figure 4.

5.3.6 Catalase enzyme activity

Catalase enzyme activity was estimated on 7 DAH application. The results revealed that the activity was more in azimsulfuron applied plots as compared to the bispyribac applied plots and hand weeded control. This result was in concurrence with Ramanarayana (2014). Mitigation treatments given on the 35 DAS, showed an increase in catalase activity in rice plants. Under rapid metabolic activity the formation of H_2O_2 may be activated (Knott, 1927). In the present study improvement in photosynthetic rate and net assimilation rate by the plants may lead to higher respiration and metabolic activity of the plant leading to activation of H_2O_2 production. Cakmak (2005) and Fageria *et al* . (2011) reported that some of the macro and micro nutrients have direct or indirect role in increasing the activity of many enzymes and others act as precursors to some hormones and enzymes thus nutrient spray might have activated the antioxidant system leading to an increase in the catalase enzyme activity. Variation in catalase enzyme activity between treatment is given in figure 5.

5.3.7 Photosynthetic rate

There was no significant reduction in photosynthetic rate by the application of herbicides. Mitigation treatments applied on the 35^{th} day improved the net photosynthetic rate of rice plants. Mixed application of IAA, urea and micronutrients

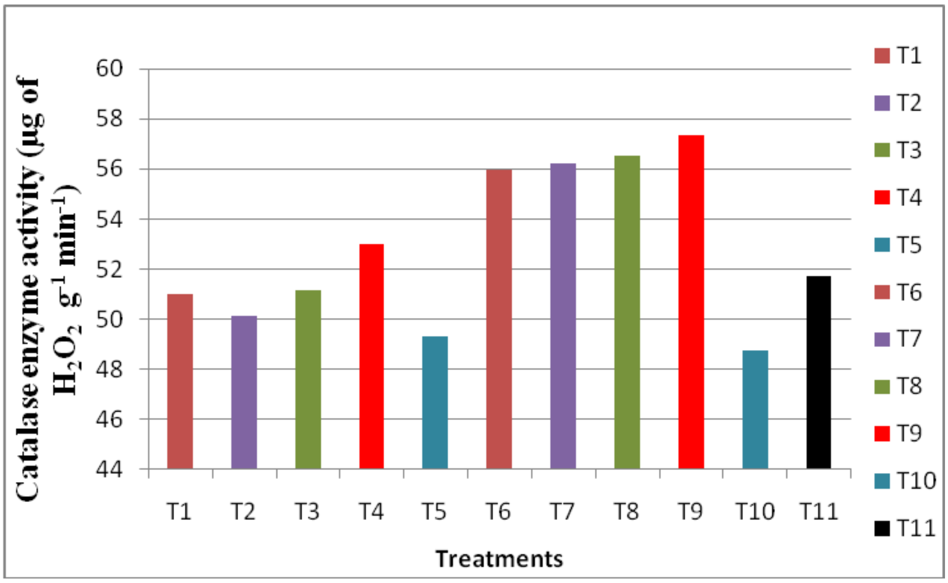

Figure 5: Variation in catalase activity (µg of H_2O_2 $g^{-1}min^{-1}$) between treatments

T1 to T5= Bispyribac sodium

T1- Urea spray

T2- Micronutrient spray

T_3- NAA

T4- Tank mix

T5- Water spray

T6 toT10= Azimsulfuron

T6- Urea spray

T7- Micronutrient spray

T8- NAA

T9- Tank mix

T10- Water spray

T11- Control

gave significantly higher photosynthetic rate in both the herbicide applied plots. The lowest photosynthetic rate was observed in treatments T_{11} and T_{10} where only water spray was given after herbicide application. Improvement in photosynthetic rate by application of urea, micronutrients and NAA has been reported by (Jin *et al.*, 2014, Ilyas *et al.,* 2015, Kumar *et al.,* 2002) respectively.

5.3.8 Stomatal conductance

The application of herbicides on 15 DAS did not show any significant reduction in stomatal conductance. Mitigation treatments given on the 35^{th} day improved the stomatal conductance of rice plants. Among the treatments combined application of urea, micronutrients and IAA showed better result in both bispyribac and azimsulfuron applied plots. Increase in photosynthetic rate and stomatal conductance by urea spray in maize has been reported by Zhang *et al*. (2012). Sharma and Ramchandra, (1990) reported that boron plays a major role in stomatal conductance as it has a role in maintaining stomatal frequency and stomatal aperture size. Application of IAA increases photosynthetic rate this increase was due to increase in stomatal aperture which facilitates more CO_2 conductance (Guinn and Brummett, 1993). These might be the factors which had contributed to increase in stomatal conductance of rice crop.

5.4 Herbicides and mitigation treatments effect on yield and yield attributes

The productivity components, such as number of productive tillers, number of spikelets, number of filled grains and thousand grain weight was significantly higher for the treatment T_4 and T_9 in the case of bispyribac sodium and azimsulfuron applied plots, indicating that among the mitigation treatments, combined application of urea, micronutrients and NAA gave the highest improvement in productivity components of the rice plants. The mixture also considerably reduced the chaff percentage.

When the grain and straw yield of water sprayed treatments were compared with the hand weeded control there was no reduction in grain yield in the bispyribac applied plots while 0.5 % reduction was noticed in the azimsulfuron applied plots. Straw yield showed 2- 4% reduction in bispyribac and azimsulfuron applied plots respectively. Ramanarayana (2014) had also reported that there was a 5-17% reduction in grain yield and 6-17% reduction in straw yield by the application of ALS inhibiting herbicides. The mitigation treatments, urea, micronutrients and NAA spray improved the yield by 20- 24% in azimsulfuron and bispyribac applied plots respectively over the control. 24 % improvement in grain yield was observed when treatment T_4 of the bispyribac applied plots was compared with T_5 where only water spray was given after bispyribac application. In the case of azimsulfuron 20 % increase in grain yield was obtained when the treatment T_9 was compared with T10 (water spray). The micronutrients applied included Zn, B and Mo. The effect of Zn application on yield components of rice has been reported by Brown *et al*. (1993), he has pointed out that Zn has higher effect on yield rather than vegetative growth. Zinc also improves the anther and pollengrain development in plants possibly by its influence on IAA and protein synthesis (Cakmack ,1989). Boron has been identified as an element that increases tillering in rice. This may be due to its positive influence on photosynthate translocation through vascular bundles of petioles (Ali *et al*., 1996). It can also act as a catalyst in the uptake and use of certain other macronutrients (Phillips, 2004). ALS inhibiting herbicides effect the protein metabolism of plants. Application of urea, Mo and IAA would have improved the protein synthesis in plants as observed by the improvement in NRAse activity and IAA content is given in figure 3 and figure 6. These might be the factors which have contributed to an increase in the productivity of rice.

5.5 Soil analysis

Soil analysis were done before sowing and after harvesting , based on the soil test data fertilizers were applied. The result showed that before sowing magnesium

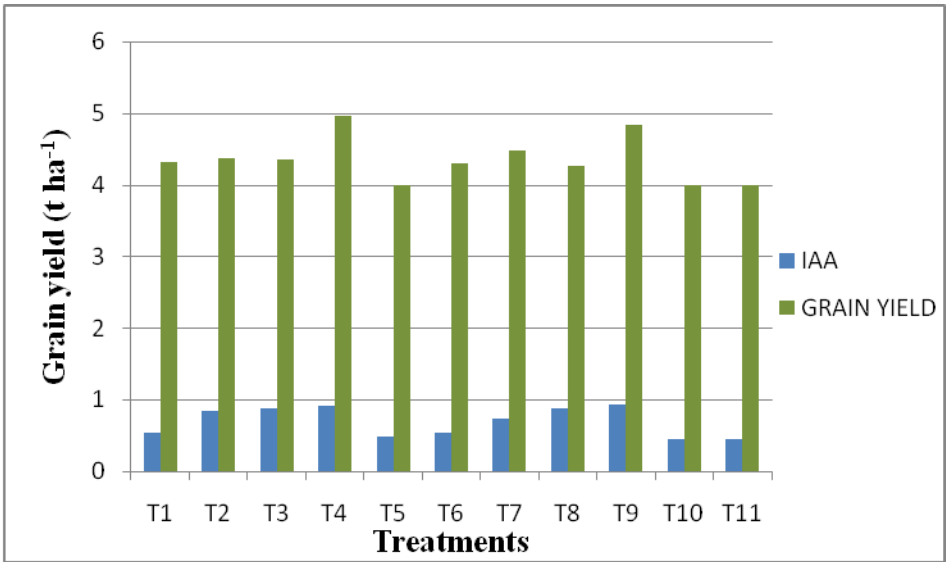

Fig. 6 . Relationship between the IAA content and grain yield

T_1 to T_5= Bispyribac sodium

T1- Urea spray

T2- Micronutrient spray

T_3- NAA

T4- Tank mix

T5- Water spray

T_6 toT_{10}= Azimsulfuron

T6- Urea spray

T7- Micronutrient spray

T8- NAA

T9- Tank mix

T10- Water spray

T11- Control

and boron was deficient in soil but after harvesting boron content became sufficient this may be due to the application of borax. All other nutrient showed a reduction, which may be due to utilization of nutrients by the plants.

5.6 **Plant nutrient analysis**

Nutrient analysis done after harvesting indicated that among the nutrients, nitrogen and boron content varied significantly between the treatments. Foliar application of urea and boric acid might have contributed to improved availability of nutrients for the growing plants.

Summary

6. Summary

Weed control is one of the most costly and labour intensive operation in rice production. Herbicides have played a major role in reducing the burden of the farmers, however a reduction in growth of rice plants after herbicide application has been noticed by farmers and researchers. The aim of the study has been to evaluate the morphological, biochemical and physiological changes in rice after application of ALS inhibiting herbicides such as bispyribac sodium and azimsulfuron and to overcome the growth and yield reduction by different chemicals and hormones.

A field trial was conducted in ARS manuuthy from July 2015 to October 2015 using the rice variety Manupriya. The soil of the land is acidic in nature with pH 5. The size of the plot was 15 m^2 (5m×3m). Treatments included 2 ALS inhibiting herbicides *viz* , bispyribac sodium and, azimsulfuron. Mitigation treatments included urea, micronutrients (Zn, B, Mo) , NAA , and a combined (Tank mix) application of these chemicals and water spray. Hand weeding was the control for comparison and it was done at 20 DAS and 40 DAS. The herbicides were sprayed at 15 DAS and mitigation treatments were given on 35 DAS using knapsnack sprayer at the recommended doses. The crop was harvested during the last week of October when the grains were fully matured. Plant height, number of productive tillers, filled grains,and chaff percentage were noted. Grain and straw yields were recorded and expressed in t ha^{-1}. The present investigations came out with the following findings.

6.1 Effect of herbicides and mitigation chemicals on morphological observations

Herbicides were applied on 15 DAS and observations taken on the 30^{th} day showed reduction in plant height as well as tiller number. More reduction was observed in azimsulfuron applied plots as compared to bispyribac applied plots. Hand weeded plots recorded highest value during this period. Mitigation treatments applied

on 35 DAS showed an improvement in morphological parameters. Among the treatments tank mix recorded higher value of plant height and tiller number up to harvest. The treatments in the bispyribac applied plots recorded higher value as compared to azimsulfuron applied plots.

6.2 Effect of herbicides and mitigation chemicals on growth analysis

Effect of herbicides and mitigation treatments on growth attributes such as RGR, CGR and NAR showed that there was no significant difference between the treatments at the final stages of the crop. Significant variation was observed at the active growth period (30-75 days).Among the treatments; highest value of RGR, CGR, and NAR was recorded for tank mix applied plots.

Maximum reduction in LAI was noticed after herbicide application. The mitigation treatments showed an improvement in LAI in all the treatments and it was more in tank mix applied plots. Treatments in azimsulfuron applied plots recorded lower value of LAI as compared to bispyribac applied plots.

6.3 Effect of herbicides and mitigation treatments on physiological parameters

Herbicides applied on 15th day caused slight reduction in physiological parameters like chlorophyll content, IAA content, stomatal conductance and photosynthetic rate compared to hand weeded plot. There was no significant variation in photosynthetic rate and stomatal conductance of crops after herbicide spray. Mitigation treatments showed an improvement in physiological parameters. More chlorophyll content, IAA content, photosynthetic rate and stomatal conductance was observed in tank mix (T4 & T9) applied plots of bispyribac and azimsulfuron

6.5 Effect of herbicides and mitigation treatments on biochemical observations

Effect of herbicides on biochemical attributes showed that there was significant variation in total soluble protein, proline, nitrate reductase enzyme activity and catalase activity between herbicide applied plots and hand weeded control. Proline content and catalase activity was more in azimsulfuron applied plots as compared to bispyribac and hand weeded control. The nitrate reductase activity and total soluble protein content was more in hand weeded control as compared to herbicide applied plots when analysed one week after herbicide spray. Mitigation treatments significantly improved all the biochemical parameters studied. However maximum improvement was seen in the tank mix application in both the herbicides applied plots.

6.6 Effects of herbicides and mitigation treatments on yield

All productive parameters like productive tillers, filled grains, and thousand grain weight was higher in tank mix applied plots of both bispyribac and azimsulfuron which showed maximum grain yield and straw yield and least chaff percentage.

Conclusion

Application of ALS inhibiting herbicides suppressed growth resulting in yield reduction. The herbicides azimsulfuron and bispyribac sodium caused slight reduction in grain yield of rice . Mitigation treatments with urea, micronutrients and NAA improved the yield attributes of the rice plant. A combination of all the three chemicals gave the best results for both herbicides. Improvement in grain yield was 20 - 24% when the herbicides azimsulfuron and bispyribac applied plots were mitigated with tank mix application and compared with unsprayed control (T11). The result of the study indicated tank mix application of micronutrients, IAA and urea can be recommended as a booster for mitigating the yield suppression resulting from herbicide application.

Future line of work

- ¾ The optimum time for application of mitigating treatments can standardized.
- ¾ Organic compounds can be tried as mitigating treatments.

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Abstract

MITIGATING THE PHYTOTOXIC EFFECT OF ALS INHIBITING HERBICIDES IN RICE (*Oryza sativa* L.).

By

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ABSTRACT OF THE THESIS

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Abstract

The study on "Mitigating the phytotoxic effect of ALS inhibiting herbicides in rice (*Oryza sativa* L.)." was conducted during 2015-2016 at Agricultural research station, Mannuthy, with the prime objective of mitigating the growth inhibition due to the application of post emergent ALS (Aceto Lactate Synthase) inhibitors, bispyribac sodium and azimsulfuron and to improve the productivity of rice.

The experiment was laid out in RBD with three replications and there were eleven treatments comprising of two Aceto Lactate Synthase inhibitors *viz.*, bispyribac sodium (30 g ai/ha) , azimsulfuron (35g ai/ha) and five mitigating treatments *viz.*, Urea spray (0.1%), Micronutrient spray (1 gm each of zinc sulphate and borax, 0.01gm of molybdic acid/litre), NAA spray (100ppm), Tank mix and water spray (1000 ml) with hand weeded as control. Herbicides were sprayed on 15 DAS whereas mitigating treatments were sprayed on 35 DAS. Observations on morphological, physiological, biochemical, yield and yield attributes of rice were recorded. Morphological characters were studied at 15 days interval and biochemical estimations were done 7 days after herbicide application and at the time of flowering.

Mitigation treatments resulted in a 10- 13 % increase in height and 4-8 % improvement in tiller number of rice plant at harvest compared to plants in the hand weeded plot. Effect of mitigating treatments on growth attributes *viz.*, relative growth rate, crop growth rate , net assimilation rate, and leaf area index indicated that at the 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 observed. Among the mitigation treatments, bispyribac sodium followed by tank mix applied plots had higher values as compared to the other treatments whereas azimsulfuron applied plots had the lowest value for these parameters.

The biochemical parameters such as nitrate reductase enzyme activity and total soluble protein content showed an initial decline when estimated one week after application of herbicide. At the time of flowering, recovery was higher in the plants where foliar spray of mitigating treatments were given as compared to water spray. Tank mix applied plots

recorded more value for the above biochemical parameters in both bispyribac sodium and azimsulfuron applied plots.

Proline content and catalase enzyme activity showed increase with herbicide application when 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.

The physiological parameters such as chlorophyll content, and IAA content showed decline but there was no significant variation in photosynthetic rate and stomatal conductance between treatments 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. Treatments in azimsufuron applied plots showed maximum reduction in these constituents.

In the present study, among the ALS inhibiting herbicides azimsulfuron contributed to higher inhibition in growth and yield of rice when compared with bispyribac sodium. Mitigating treatments contributed to 20 to 24 % increase in grain yield and 20 to 27 % increase in straw yield of rice. Tank mix applied plots recorded highest grain yield and urea applied plots recorded highest straw yield.