PHYSIOLOGICAL EVALUATION OF HERBICIDAL EFFECTS ON RICE, BROADLEAVED WEEDS AND SEDGES

By

LINU C

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "Physiological evaluation of herbicidal effects on rice, broadleaved weeds and sedges" 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.

Vellanikkara

Date: 27-09-2019

Linu C

(2017-11-081)

CERTIFICATE

Certified that this thesis entitled "Physiological evaluation of herbicidal effects on rice, broadleaved weeds and sedges" is a bonafide record of research work done independently by **Linu C (2017-11-081)** 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.

Vellanikkara

27-09-2019

Dr. T. Girija

Major Advisor

Professor and Head

Department of Plant Physiology

Kerala Agricultural University

Thrissur, Kerala

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. **Linu C (2017-11-081)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Physiology**, agree that the thesis entitled "Physiological evaluation of herbicidal effects on rice, broadleaved weeds and sedges" may be submitted by **Ms. Linu C (2017-11-081)**, in partial fulfillment of the requirement for the degree.

Dr. T. Girija
Professor and Head
Department of Plant Physiology
College of Horticulture
Vellanikkara

Dr. P. S. Abida
Professor and Head
Division of Crop Improvement
RARS,
Pattambi

Dr. Meera V. Menon
Professor
Department of Agronomy
AICRP on Weed Management
College of Horticullture
Vellanikkara

Dr. K. M. Durga Devi
Professor
Department of SS & AC
College of Horticulture
Vellanikkara

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Abbreviations

ALS	Acetolactate synthase
ACCase	Acetyl CoA carboxylase
ai/ha	Active ingredient per hectare
DAS	Days after sowing
DAT	Days after transplanting
CGR	Crop growth rate
RGR	Relative growth rate
NAR	Net assimilation rate
LAI	Leaf area index
WPI	Weed persistence index
WP	Wettable powder
SOD	Superoxide dismutase
CAT	Catalase
ppm	Parts per million
Kg ha^{-1}	Kilogram per hectare
No m^{-2}	Number per meter square
t ha^{-1}	Tonnes per hectare

Introduction

1. INTRODUCTION

Rice (*Oryza sativa*) is the third most cultivated cereal in the world and it is the staple food for more than half of the world's population. It acts as an energy and protein source for human beings. Rice is grown in an area of 161 million ha in 114 countries, ranging from the flood plains of Bangladesh to the Himalayan foothills of Nepal, and from the rain forests of Indonesia to the desert plains of Australia. India is the second largest producer of rice, next to China with an area of 42 million ha and with a production of 90 million tonnes. It contribute to 45% of food grain production in the country. Due to population growth, the production has to be increased to meet demand (Matloob *et al.*, 2015). Weeds are the major biological constraint to the rice ecosystem and it accounts for about 43% crop loss (Choubey *et al.*, 2001). *Ludwigia parviflora, Monochoria vaginalis, Eclipta alba, Marsilea quadrifoliata* and *Limnophila heterophylla* are the major broad leaved weeds, and *Cyperus difformis, Cyperus iria* and *Fimbristylis miliacea* are the common sedges found in rice ecosystems of Kerala. To increase rice production, it is important to reduce crop loss caused by weed competition. Weeds not only reduce rice production but also reduce the grain quality.

Among the weed management practices, hand weeding is the most effective but it is laborious, costly and time consuming. So, the only option left for the farmers is to use chemical weed control methods. According to reports more than 80% reduction in weed density and 74-87% reduction in weed dry weight could be attained by herbicide application (Begum *et al.*, 2008). Post-emergence herbicides are used to control already emerged weeds which compete with the developing crop. 2,4-D, penoxsulam and ready mix formulation of metsulfuron-methyl and chlorimuron ethyl (Almix®) are the most popular post-emergence herbicides currently used in Kerala.

Auxinic herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid) is a post-emergence herbicide of phenoxyacetic acid family that is used for the control of broad leaved weeds without affecting monocots.

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Penoxsulam is a systemic herbicide which inhibit plants from the synthesis of essential enzyme acetolactate synthase (ALS). It is a member of the triazolopyrimidine sulfonamide chemical family.

Almix® is a third generation herbicide, effective against broad leaved weeds and sedges. The chemical composition is 10% metsulfuron methyl+10% chlorimuron ethyl. It belongs to the sulfonyl urea group of herbicides, which inhibit plant cell division. It enters the plant through contact *via* leaves and prevent the synthesis of acetolactate synthase (ALS) enzyme.

However none of these herbicides are reported to control all the dicot weeds and sedges of the rice ecosystem. This may be associated with the mode of action of the herbicide or the development of herbicide resistance. Herbicide resistance is a phenomenon seen in the field where herbicides are used continuously (Delye *et al.*, 2013). Identification and validation of this phenomenon is difficult in the field. Hence farmers have a tendency to use excess herbicides to control such weeds. In this context the present study has been proposed with the following objectives

- To evaluate the effect of Almix®, penoxsulam and 2,4-D on the growth, physiology and yield of rice.
- To identify broad leaved weeds and sedges that are selectively controlled by these herbicides.

Review of literature

2. REVIEW OF LITERATURE

The control of obnoxious, undesirable, unwanted plants is the main problem encountered by farmers. Weeds increase the labour and cost of production by interfering with agricultural operations, and reduce crop yields (Sharma *et al.*, 2017). The emergence of weeds along with the crop depletes the native plant nutrients, contributing to yield loss. In direct seeded rice, yield loss due to weeds was estimated to be as high as 40-100 per cent (Choubey *et al.*, 2001).

Chemical weed control is the most cost effective method used by farmers. Among the herbicides, 2,4-D, penoxsulam and Almix® are widely used herbicides in rice fields of Kerala for the control of broadleaved weeds and sedges. The effect of these chemicals on broad leaved weeds is proven, however their effect on rice plant is less understood. In this context the review covers the physiological and biochemical effect of these chemicals on rice plant.

2.1 Weed spectrum in rice

More than 80 species of Poaceae family and 50 species of Cyperaceae family have been reported as weeds in rice field. Other families with 10 or more species includes Asteraceae, Lythraceae, Alismataceae and Scrophulariaceae (Hosmani, 1995).

Prakash *et al.* (2013) reported that the major weed flora in transplanted rice field of Rajastan were grasses like *Echinochloa colonum, Echinochloa crusgalli* and sedges like *Cyperus rotundus, Cyperus difformis* and *Cyperus iria* and broad leaved weeds like *Eclipta alba* and *Ammenia baccifera.*

Yadav *et al.* (2009) stated that major weeds in transplanted rice field of Haryana were *Echinochloa colona* (L.), *Echinochloa glabrescence* among grasses, *Euphorbia* sp, *Ammannia baccifera* L., among broad leaved weeds and *Fimbristylis miliacea* (L.) Vahl, *Cyperus iria* L., *C. rotundus* L., *C. difformis* L. among sedges.

In a field experiment conducted in South Kerala using transplanted scented rice ('Pusa Basmathi1'), the major weed flora observed were *Echinochloa colona,* *Echinochloa crus-galli, Leersia hexandra* (grasses); *Cyperus iria, Cyperus difformis and Fimbristylis miliacea* (sedges); and *Ludwigia parviflora, Monochoria vaginalis* (broad-leaf weeds) (Menon *et al*., 2014). In rabi season, the major weeds in coimbatore conditions were grasses such as *E. crus-galli* (22.0%), *E. colona* (23.5%); sedges such as *Cyperus difformis* (21.3%), and *Cyperus iria* (21.0%); broad leaved weeds such as *Marselia quadrifoliata* (5.8%) and other minor weeds (Jayakumar *et al*., 1994).

Subramanian *et al*. (2006) reported that weed flora mainly found in rice field were grasses like *Echinochloa colonum, E. crus-galli* and *Cynodon dactylon* and sedges like *Cyperus rotundus, C. iria, C. difformis* and broad leaved weeds like *Eclipta alba, Ammania baccifera, Phyllanthus niruri and Ludwigia parviflora.* In a trial conducted in the rice field for two years during November-February 2006 and during December-April 2007, major weeds observed were *Cyperus iria* and *Fimbristylis miliacea*, *Ludwigia parviflora, Monochoria vaginalis* and *Lindernia* sp. (Abraham *et al*., 2012).

Raj *et al.* (2013) observed sedges such as *Fimbristylis miliacea, Cyperus difformis, Cyperus iria, Schenoplectus pungens* and broad leaved weeds such as *Monochoria vaginalis, Ludwigia perennis* and *Sphenoclea zeylanica* in a field trial conducted in Alappuzha during rabi and kharif seasons.

Benerjee *et al*. (2005) reported that *Echinochloa crus-galli, Cyperus iria, C. difformis, Fimbristylis milacea, Monochoria vaginalis, Saggittaria sagittifolia, Marsilea quadrifoliata, Ludwigia parviflora, Leersia hexandra,* and *Alternenthera sessilis* observed in transplanted rice field in boro season.

Patra and Saha (2005) observed *Echinochloa crus-galli, Cyperus difformis, Fimbristylis miliaceae, Scirpus articulatus, Eclipta alba, Monochoria vaginalis, Ammania baccifera, Sphenoclea zeylanica, Ludwigia parviflora, Leersia hexandra* and *Panicum repens* in transplanted rice field.

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2.2 Chemical control of weeds

Due to scarcity and high wages of labour, herbicide based management is the most viable and cost effective method (Singh *et al.*, 2006; Anwar *et al.*, 2012a).

Application of penoxsulam herbicide to the transplanted rice is the best chemical to control all type of weed growth (Prakash *et al.*, 2013). 2,4-D @ 500 g a.i. ha^{-1} effective in controlling most of the broadleaved weeds and sedges and also increase the grain yield. 2,4-D @ 18 g a.i. ha^{-1} was found to be least expensive and also control most of the broadleaved weeds (Mann *et al.*, 2007).

Benerjee *et al.* (2005) reported that treatment of Almix® 20 WP @ 4 g a.i./ha + Butachlor 50 EC @ 1250 g a.i./ha application to rice field control all categories of important weeds. Ramanarayana (2014) stated that ALS inhibiting herbicides control most of the broadleaved weeds and sedges.

2.3 Herbicide resistance by weeds

Schulz and Segobye (2016) reported that 28 different species of weed species had shown resistance against 2,4-D. Goggin *et al.* (2016) revealed that through transport inhibition mechanism some weeds attained resistance against 2,4-D. Studies conducted by Pericas *et al.* (2015) suggested that biotype of *Cyperus difformis* showed resistance against herbicides. Andres and Theisen (2013) claimed that *Fimbristylis miliacea* had attained resistance against 2,4-D. Baltazar (2017) observed that due to high 2,4-D use (one or two applications per cropping system) *Sphenoclea zeylanica* had evolved resistant biotypes.

2.4 Effect of herbicides on weeds

Singh *et al.* (2017) claimed that uncontrolled growth of weeds reduced the grain and straw yield by 42% and 46% respectively. Herbicide application at 25, 50, 75, or 100% full dose showed 30% less weed species compared unweeded plot. Higher weed control efficiency was noted with higher dosage of post emergent herbicides (Bostrom and Fogelfors, 2002). Noldin (1977) reported that ALS

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inhibiting herbicides showed a control for weeds such as *Ludwigia* Spp., *Fimbristyllis milicea*.

Lower weed density than weedy check was observed by the application of penoxsulam (25 g/ha) @ 12 DAT. This caused 90% reduction in mean dry weight of weeds than weedy check. When compared to application of butachlor @ 1500 g/ha, penoxsulam showed 64% reduction in dry matter of weeds. The superiority of penoxsulam over butachlor was mainly due to the effective control of sedges and broadleaved weeds. Increase in the dosage of penoxsulam increase the percentage of weed control efficiency and maximum WCE was recorded at a dosage of 25 g/ha.(Mahajan and Chauhan, 2008).

Application of penoxsulam (0.025 kg/ha) at 0-5 days after transplanting resulted in lower weed index, weed persistence index, dry weight of weeds and higher herbicidal efficiency index (Prakash *et al.*, 2013). Raj *et al.* (2015) stated that higher doses of penoxsulam + cyhalofop butyl (130 and 135 g a.i./ha) showed a reduction in dry weight and weed control efficiency (97.34% and 98.31%).

Application of 2,4-D @ 800 g/ha showed 96.7 % weed control efficiency and weed index of 10.5 (Raj *et al.*, 2013). Singh and Nam (2003) stated that application of pendimethalin 1.0 kg/ha or anilophos 0.40 kg/ha each with 2,4-D (Na-salt) 0.60 kg/ha showed 50-51% weed control efficiency and this was found to be a better substitute for hand weeding. Katara *et al.* (2012) revealed that combined application of clodinonafop and 2,4-D had a higher weed persistence index.

Patra and Saha (2005) reported that the application of butachlor @ 3 DAT followed Almix® @ 25 DAT showed better weed control efficiency which was closer to hand weeding treatment. It also effectively reduced the weed dry weight and weed density compared to other herbicide treatments. Biswas *et al.* (2018) reported that 2,4-D application caused the death of weed *Cyperus iria*.

Almix® alone @ 4 g ha^{-1} was effective in reducing density of grasses and sedges (Singh *et al.*, 2004). Singh *et al.* (2017) stated that application of metsulfuron-methyl + chlorimuron-ethyl @ 4g/ha had highest weed control efficacy against *Echinochloa glabrescens*, *Cyperus* spp. and *Ammania* spp. Benerjee *et al.* (2005) reported that application of Almix® 20 WP @ 4 g a.i./ha + butachlor 50 EC @ 1250 g a.i./ha gave a satisfactory weed control efficiency at all growth stages of crop, this may be due to the reduction in weed biomass or dry weight on herbicide application.

2.5 Effect of herbicides on growth parameters of rice

2.5.1 Height

Field experiment conducted by Langaro *et al.* (2016) reported that application of post-emergence herbicides showed a reduction in plant height compared to hand weeded control, among the herbicides penoxsulam showed the highest value. The application of 2,4-D @ 1.0-2.0 kg, cinosulfuron + piperophos @ 1.5 kg and basagran + propanil @ 1.0-3.0 kg consistently increased the plant height and number of tillers over the untreated control (Dadari and Mani, 2005). Mukherjee and Singh (2016) noted that Almix® application reduce the plant height due to the severe phytotoxicity on rice variety 'Malwa 36'. Sreedevi *et al.* (2009) reported that unweeded control recorded lowest plant height compared to the herbicide treated plot. Chakraborti *et al.* (2017) conducted a field experiment with six numbers of herbicides *viz.*, pendimethalin, 2,4-D, fenoxaprop, bispyribac sodium, metsulfuron methyl+ chlorimuron ethyl (Almix®), pyrazosulfuron ethyl and among the herbicides Almix® @ 4 g/ha showed poor plant height and number of branches per plant. In a study conducted by Shendage *et al.* 2016 revealed that the combination of bispyribac-Na and chlorimuron + metsulfuron showed the least plant height, which was on par with weedy check. Bhurer *et al.* (2013) reported that weed free plot showed more plant height than herbicide applied plots.

2.5.2 Tillers/m^2

Kumar and Gill (1982) revealed that unweeded plot showed 11% reduction in effective tillers over herbicide applied plot. The application of Almix® @ 4 g/ha on direct seeded upland rice showed a reduction in number of branches per plant (Chakraborti *et al.*, 2017). Shendage *et al.* (2016) reported that combination of bispyribac-Na and chlorimuron+metsulfuron showed the least number of tillers. Menon *et al.* (2014) reported that higher number of tillers was observed in weed free plot @ 30 DAS.

2.5.3 Leaf area index

Saqib *et al.* (2015) revealed that hand weeded plot of rice recorded higher LAI compared with the herbicide applied plots. Studies conducted in rice by Ramanarayana (2014) and Nithya (2016) reported that hand weeded plot had highest LAI compared with plots applied with ALS inhibiting herbicides. Among the ALS inhibiting herbicides azimsulfuron and fenoxaprop applied plots had the lowest LAI.

2.5.4 Days to flowering

Herbicide application reported to delay flowering in rice. Studies by Ramanarayana (2014) and Nithya (2016) showed that the flowering date was extended by 2 days in herbicide applied plots compared to hand weeded plot.

2.5.5 Relative growth rate

Moursi *et al.* (1980) stated that relative growth rate increased at early stages of the crop (30-60 days) growth and later showed a decline. Bhargavi and Reddy (1993) conducted a field trial using pre-emergence herbicides like butachlor, thiobencarb and pendimethalin and post-emergence herbicides like 2,4-D ethyl ester @ 0.9 kg (7 DAS) and 2,4 -D sodium salt @ 1 kg (30 DAS), they observed an increase in RGR for all the herbicide application due to the reduction in weed density. RGR noted higher in first season crop than second season crop in case of rice plant (Yu *et al.*, 2010). Abbasian *et al.* (2014) noted that RGR was higher in early stages of the rice crop and then reduced linearly.

2.5.6 Crop growth rate

Application of different herbicides did not show any difference in CGR up to 0-30 DAS. After 30 DAS, maximum CGR was recorded in weed free plot. Among the different herbicides, pendimethalin along with penoxsulam showed maximum CGR (Singh *et al*., 2014). Jain *et al*. (2010) disclosed a positive correlation of CGR on biomass and seed yield. Tiwari *et al*. (2010) conducted a field experiment during kharif season, they observed that application of chlorimuron + metsulfuron had maximum crop growth rate during 0-25 DAS which was higher than hand weeded condition. At 25-50 DAS, hand weeded plot and chlorimuron + metsulfuron applied field showed equal crop growth rate, which was significantly higher than unweeded control. During 70-90 DAS, hand weeded plot showed maximum crop growth rate which was superior to herbicide applied plot. Manjunath and Pañchal (1989) observed that optimum dosage of herbicides increased CGR. Khaliq *et al*. (2012) observed a reduction in CGR during the transition of crop from vegetative stage to reproductive stage. Muhammed *et al*. (2015) observed that hand weeded plot showed higher crop growth rate than herbicide applied plot.

2.5.7 Net assimilation rate

Nichiporovich (1960) stated that NAR showed an increase up to an optimal level and declined at the end of growing stage in rice crop. Williams (1946) and Thorne (1960) disclosed a positive correlation of net assimilation rate with leaf area. Ramanarayana (2014) suggested that hand weeded plot showed highest net assimilation rate and Almix® applied plot showed the least. Nithya (2016) stated that hand weeded plot showed more NAR than herbicide applied plots.

2.6 Herbicide toxicity to rice

Field experiment conducted by Benerjee *et al.* (2005) showed that Almix® application had no phytotoxicity to the rice plant. Ramanarayana (2014) stated that Almix® shows no visual phytotoxicity symptoms on rice crop even after 3 days of herbicide application. Moderate to severe toxicity was reported in rice when it was applied with Almix® @ 25g/ha which persisted up to 30 DAT (Yun *et al*., 2005).

Begum *et al.* (2008) reported that after the application of herbicides, most of the plant showed some phytotoxic symptoms like reduction in plant height, chlorosis and stunding. 2,4-D treated rice plant showed leaf spreading and appearance of adventitious roots above the soil surface. Okfor (1986) stated that 2,4-D amine applied rice crop showed only a slight tip burn and yellowing symptom in leaves. However, rice plants recovered from these symptoms. Thapa (2012) reported that 2,4- D and penoxsulam showed phytotoxicity symptoms at early stages of rice crop and later the crop recovered.

There was no phytotoxicity effect on rice due to the application of penoxsulam at any dose applied @ 3 or 12 DAT (Mahajan and Chauhan, 2008). Yadav *et al.* (2008) reported that penoxsulam showed no residual toxicity on succeeding crop of wheat.

2.7 Effect of herbicide on physiological and biochemical parameters of rice and weeds

2.7.1 Chlorophyll content

Thappa (2012) reported that 2,4-D and butachlor showed a significant reduction in chlorophyll content at initial stage of the rice crop which recovered later. Langaro *et al.* (2016) conducted an experiment using the post-emergence herbicides such as imazapic + imazapyr, quinclorac, bentazon, cyhalofop-butyl, penoxsulam, bispyribac-sodium and carfentrazone-ethyl and revealed that carfentrazole-ethyl (200 g ai. ha^{-1}) showed a reduction in chlorophyll a, chlorophyll b and total chlorophyll content of the rice crop compared to control at 24 hrs after spraying. In the case of penoxsulam (60 g ai. ha^{-1}) and bispyribac sodium (50 g ai. ha^{-1}), there was no significant difference compared to control. Ramanarayana (2014) reported that one week after herbicide application in rice plant, azimsulfuron and Almix® applied plots showed a reduction in chlorophyll a and chlorophyll b content compared to handweeded plot. Same trend was seen after flowering of the rice crop. Channappagoudar *et al.* (2008) reported that herbicides significantly increased the total chlorophyll content in radish.

Sahoo *et al.* (1993) reported that 2,4-D and 2,4,5-T herbicide application contributed to a reduction in chlorophyll a, chlorophyll b and total chlorophyll content of the rice plant. The extent of reduction increased with increase in dosage level. In a field experiment conducted by Deka *et al.* (1996) in rice cv Mahsuri with butachlor (1.0 kg/ha), 2,4-D (0.72 kg/ha), oxyfluorfen (0.2 kg/ha), anilofos (0.4 kg/ha) and thiobencarb (1.0 kg/ha), it was seen that mean chlorophyll content was maximum in butachlor treated plot followed by 2,4-D and minimum in unweeded control. ALS inhibiting herbicides application in rice showed a decline in total chlorophyll content (Nithya, 2016).

Nagaraju *et al.* (1995) stated that the application of 0.30 kg anilofos + 0.60 kg 2,4-D/ha at 4 days after transplanting showed an increase in chlorophyll content in rice crop. The study conducted by Singh *et al.* (1987) in rice using the herbicides pendimethalin, butachlor, thiobencarb and 2,4-D revealed that herbicide application reduced the chlorophyll content. Chlorophyll a was highest in thiobencarb treated plant and lowest recorded in butachlor treated plant.

2.7.2 Total soluble protein

Protein content of rice plant treated with the post-emergence herbicides such as imazapic + imazapyr, quinclorac, bentazon, cyhalofop-butyl, penoxsulam, bispyribac-sodium and carfentrazone-ethyl. Among these carfentrazone-ethyl showed maximum reduction compared with other treatments. Control had maximum protein content followed by penoxsulam (Langaro *et al.*, 2016).

Ramanarayana (2014) reported that herbicide applied plot showed a decrease in soluble protein content of rice plant. Among the herbicides, fenoxaprop (32.9), azimsulfuron (33.15) and Almix® (34.75) showed the least soluble protein content compared with other herbicides. When wheat and barley plants were treated with the butoxyethyl ester of 2,4-D @ 2,4 and 8 kg/ha, a rapid reduction in protein content was observed (Pellet and Saghir, 1971). Nithya (2016) reported that ALS inhibiting herbicides contributed to an initial decline of protein content of the rice plant.

Bovey and Meyer (1981) found that 2,4-D and 2,4,5-T affected the protein content of wheat. Manivasagaperumal *et al.* (2005) estimated the effect of application of herbicides 2,4-D, Isoproturon and 2,4-D + Isoproturon in wheat and found that 2,4-D showed maximum reduction in protein content. Perumal *et al.* (2005) conducted a study using sodium salt of 2,4-D @ 500 g /acre and butachlor 50% EC @ 400 ml/ha on the weed *Eclipta alba* and rice. They found that butachlor showed more reduction in protein content than 2,4-D. The study conducted with 2,4-D @ 0.03M and 0.04M in wheat revealed that application of 0.03M 2,4-D increased the protein content and 0.04M 2,4-D decreased the protein content (Ashraf and Murtaza, 2017).

The investigation carried out by Sharma *et al.* (2013) in wheat using 2,4- D at different concentrations 0.5 kg/ha, 1.0 kg/ha and 2.0 kg/ha revealed that increase in protein content occured 30 days after spraying irrespective of the treatment and thereafter declined up to 120 days after spray. Highest protein content was recorded in 2,4-D @ 2.0 kg/ha and it was positively correlated with nitrate reductase enzyme activity. Martin *et al.* (1990) reported that proline content of wheat crop was not influenced by 2,4-D herbicide at any stage of application.

2.7.3 Proline content

Pellet and Saghir (1971) found that application of 2,4- D in barley and wheat caused an increase in proline content. Langaro *et al.* (2016) conducted an experiment in rice plant using imazapic + imazapyr, quinclorac, bentazon, cyhalofop-butyl, penoxsulam, bispyribac-sodium, carfentrazone-ethyl, control and reported that maximum proline content observed in carfentrazone-ethyl followed by bentazon and minimum in control condition during 24 and 120 hrs after the application of herbicide. Ramanarayana (2014) revealed that proline content reduced uniformly with stages of the crop. But herbicide applied plots showed more proline content than hand weeded plot. Among the herbicides fenoxaprop, azimsulfuron and Almix® showed the minimum increase in proline content.

Nithya (2016) reported that ALS inhibiting herbicides increased the proline content in rice crop. The study conducted by Panamarioviene (1996) stated that the application of 0.3 kg/ha simazine, 0.3 kg/ha simazine + 0.3 kg/ha 2,4- D and 1.5 kg/ha MCPP [mecoprop] reduced proline content in winter rye. Bhaskar (2015) reported that increase in herbicide dosage caused an increase in proline content in rice crop.

2.7.4 Nitrate reductase enzyme activity

Nitrate reductase (NRase) is the enzyme involved in the nitrogen metabolism which is regulated by various environmental factors. The reduction of nitrate by NRase is the rate limiting process for the utilization of nitrogen in the form of nitrate (Bertero *et al.*, 2003).

The experiment conducted by Ramanarayana (2014) stated that one week after herbicide application on rice crop, nitrate reductase activity gradually increased and hand weeded plot showed the maximum. Bispyribac-sodium showed the minimum enzyme activity. During flowering stage enzyme activity gradually reduced. Nithya (2016) showed that ALS inhibiting herbicides application caused a reduction in nitrate reduction enzyme activity. Herbicides 2,4-D @ 0.5 kg/ha, 1.0 kg/ha and 2.0 kg/ha was applied to wheat at 30 days after sowing and found that significant increase in nitrate reductase enzyme activity was observed on application of 2,4-D @ 2.0 kg/ha at all the stages of development (Sharma *et al.*, 2013).

Deka *et al.* (1996) reported that Nitrate reductase enzyme activity showed a positive correlation with soluble protein content. Alla *et al.* (2008) reported that reduction in nitrate reductase enzyme activity was observed in rice due to the application of 3L per ha butachlor and 20g per ha chlorimuron-ethyl.

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2.7.5 IAA content

Indole-3-acetic acid (IAA) is the naturally occurring auxin class plant hormone (Simon and Petrasek, 2010). Herbicide application reduced the IAA content of the rice plant. Lowest value of IAA was observed in Almix® and azimsulfuron applied plots and highest was noted in handweeded condition (Ramanarayana, 2014). The experiment conducted by Nithya (2016) showed that ALS inhibiting herbicides application caused a reduction in IAA content in rice.

2.7.6 Photosynthesis rate

Application of carfentrazone-ethyl caused a reduction in photosynthetic rate in rice. Penoxsulam applied plot showed a medium level of photosynthetic rate (Langaro *et al.*, 2016). Ramanarayana (2014) revealed that among ALS inhibiting herbicides, Almix® showed the lowest photosynthetic rate and hand weeded plot showed the maximum. Nithya (2016) reported that there was no significant variation in photosynthetic rate even after the application of ALS inhibiting herbicides.

2.7.7 Stomatal conductance

Stomatal conductance is the measure of rate of CO_2 entering and water vapour exiting through the stomata of the leaf. It is controlled by guard cells in the leaf, which surrounds the stomatal pore (Taiz and Zeiger, 1991). Langaro *et al.* (2016) reported that stomatal conductance was maximum in imazapic + imazapyr, quinclorac and bentazon treated rice plots and lowest in carfentrazone-ethyl applied plot. Penoxsulam showed a medium level of stomatal conductance. Ramanarayana (2014) conducted an experiment using the ALS inhibiting and ACCase herbicides and found that Almix® applied plot showed lowest stomatal conductance compared with other herbicides. ALS inhibiting herbicides had no significant effect on stomatal conductance of rice crop (Nithya, 2016). Lehnoff *et al.* (2013) reported that herbicide susceptible wheat plant showed low stomatal conductance compared to resistant plant. Agostinetto *et al.* (2016) reported that application of metribuzin, metsulfuron and 2,4-D showed a reduction stomatal conductance in wheat plants.

2.7.8 Catalase enzyme activity

Decomposition of hydrogen peroxide to water and oxygen is catalyzed by universal enzyme called catalase which protect the plant cell from oxidative damage (Chelikani *et al.*, 2004). Rice plant applied with imazapic + imazapyr, quinclorac, bentazon, cyhalofop-butyl, penoxsulam, bispyribac-sodium, carfentrazone-ethyl herbicides had highest catalase enzyme activity at 120 hours after spraying due to fast accumulation of H_2O_2. Penoxsulam showed less catalase activity compared with other herbicides (Langaro *et al.*, 2016).

Ashraf and Murtaza (2017) reported that application of higher concentration of 2.4-D caused an increase in catalase activity compared to lower concentration in wheat. Among ALS inhibiting herbicides Almix® and azimsulfuron contributed to significantly higher amount of catalase enzyme activity in rice plant compared with other herbicides (Ramanarayana, 2014).

Nithya (2016) reported that application of ALS inhibiting herbicides at one week after sowing of rice caused an increase in catalase enzyme activity compared to hand weeded control indicating herbicide application imparts stress to the rice plant. The catalase activity was higher in initial period and most of herbicide applied plants showed recovery from stress during flowering stage. Herbicides 2,4-D and clodinafop showed higher catalase enzyme activity compared to control in wheat (Agostinetto *et al.*, 2016).

Malencic *et al.* (2008) reported that the weeds *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Convolvulus arvensis* L. and *Sinapis arvensis* L. weeds showed higher catalase content on herbicide application.

2.7.9 Total aminoacid content

Davies and Hamphrey (1978) stated that plant synthesize new protein from aminoacid which was formed by the degradation of old proteins to change its enzyme compliment. Ramanarayana (2014) reported that total aminoacid content of rice reduced due to herbicide application. Among the herbicides, Almix®, bispyribac and azimsulfuron showed the lowest aminoacid content and handweeded plot showed the maximum. Pellet and Saghir (1971) stated that higher doses of 2,4-D had a minor effect on total aminoacid content in wheat and barley.

2.7.10 Carbohydrate content

Sahoo *et al.* (1993) revealed that carbohydrate concentration decreased initially in all the rice plant parts when it was treated with 2,4-D and 2,4,5-T. However, from the 10^{th} day after treatment an increase in concentration of carbohydrate occured. Pellet and Saghir (1971) stated that the application of higher doses of 2,4-D to wheat and barley showed a rapid reduction in carbohydrate content.

Manivasagaperumal *et al.* (2005) conducted a study using 2,4-D, Isoproturon and 2,4-D + Isoproturon in wheat and found that Isoproturon showed maximum reduction in carbohydrate content followed by a combination of 2,4-D and Isoproturon. Ashraf and Murtaza (2017) reported that application of 2,4-D at lower concentration (0.03M) showed higher content of sugar compared to the higher concentration (0.04M).

2.7.11 Chlorophyll stability index

Nasrabadi and Dhumal (2014) reported that high concentration of pesticide imparts stress which adversely affect chlorophyll stability index. Paul *et al.* (2017) revealed that physiological parameters like chlorophyll a/b and chlorophyll stability index indicate the response of plants against abiotic stresses.

2.7.12 SOD enzyme activity

Langaro *et al.* (2016) reported that post-emergence herbicides application in rice showed an increase in SOD content compared to hand weeded plot. Kim *et al.* (1994) suggested that weeds like *Chenopodium album, Polygonum aviculare, Pinellia ternata* showed resistance against paraquat herbicide due to activity of superoxide dismutase. Superoxide dismutase was known to detoxify paraquat. Malencic *et al.* (2008) reported that weeds like *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Convolvulus arvensis* L. and *Sinapis arvensis* L. showed higher SOD content on herbicide application

2.7.13 Wax content of leaf

Prakash and Swamy (1987) reported that herbicide application significantly inhibited the synthesis of epicuticular wax in banyard grass (*Echinochloa crus-galli*). Wilkinson (1980) conducted a study in six ecotype of saltcedar (*Tamarix pentandra*) and found that post-emergence herbicide resistance shown by some of the ecotypes may be due to the change in quality and quantity of epicuticular wax.

2.8 Effect of herbicides on yield and yield attributes

Application of penoxsulam (25g/ha) at 12 DAT caused 13% increase in grain yield than butachlor and weedy check. Grain yield of rice was negatively correlated with dry matter of weed. Penoxsulam application increased the panicle number and panicle weight (Mahajan and Chauhan, 2008). Singh *et al.* (2014) reported that application of pendimethalin along with penoxsulam showed maximum grain yield and grain filling percentage and test weight. The highest and lowest grain filling percentage was noted in weed free and weedy check plot respectively.

The study conducted by Kogan *et al.* (2011) in the rice field of Chile disclosed that application of penoxsulam at 12 days after sowing resulted in 30 to 56% increase in rice yield than the untreated controls. Yadav *et al.* (2008) reported that application of post emergence herbicide penoxsulam @ 20-25 g/ha resulted in grain yield similar to hand weeded plot. Penoxsulam @ 0.025 kg/ha, 0-5 days after transplanting had higher grain and straw yield (Prakash *et al.*, 2013). Raj *et al.* (2015) stated that application of penoxsulam+cyhalofop butyl at its higher dose (135 g a.i./ha) contributed the highest grain yield.

Application of herbicide containing 2,4-D+methyl metsulfuron showed higher filled grain and rice grain yield, which was similar to handweeded plot (Antralina *et al.,* 2015). Application of 2,4- D Na salt (1 kg a.i. ha^{-1}) @ 20 days after transplanting increased the grain yield (Jacob and Syriac, 2005). Saini (2005) reported that application of cyhalofop butyl @ 120 g/ha (15 DAS) followed by 2,4-D @ 1 kg/ha (20 DAS) gave higher number of panicles/m^2, panicle length, grains per panicle and 1000 grain weight. Thappa (2012) reported that the phytotoxicity effect of 2,4-D and butachlor had no harmful effect on grain yield. Singh and Nam (2003) stated that application of pendimethalin 1.0 kg/ha or anilophos 0.40 kg/ha each with 2,4-D (Na-salt) 0.60 kg/ha gave an additional 9.91 q/ha grain yield.

Haffaker *et al.* (1967) conducted a study with isopropyl ester, isooctyl ester, and dimethylamine salt of 2,4-D and applied to barley, wheat and beans in the field. There was no significant effect on the yield by the application of isooctyl ester, and dimethylamine salt. Highest cereal yield was recorded in isopropyl ester. Application of bromoxynil, methabenzthiazuron, MCPA, bromoxynil + MCPA. linuron, prometryne, diuron, 2,4-D ester and 2,4-D amine herbicides resulted in yield reduction of wheat and barley (Elliott *et al.*, 1975). Katara *et al.* (2012) revealed that combined application of pinoxaden with metsulfuron-methyl, carfentrazone and 2,4-D showed higher tillers, grains/spike, 1000-seed weight and also grain yield, straw yield, which were on par with weed free condition.

Dakshayani *et al.* (2017) conducted a study to check the effect of post emergence herbicides on yield and yield attributes of transplanted rice in southern zone Karnataka and revealed that application of metsulfuron-methyl+chlorimuron-ethyl 20 WP (400 g a.i. ha^{-1}) at 15 DAS showed higher filled grain per panicle, thousand grain weight, grain yield and straw yield and lower chaff percentage which was on par with hand weeded condition. The lowest yield and yield attributes were recorded in the unweeded plot. Application of butachlor @ 3 DAT followed by Almix® at 25 DAT gave higher panicle/m^2, filled gains/panicle and also grain yield, it was closest to the handweeded plot (Patra and Saha, 2005). Almix® @ 4 g ha^{-1} recorded higher grain yield than the hand weeded plot (Singh *et al.*, 2004).

Singh *et al.* (2017) stated chlorimuron-methyl + azimsulfuron-ethyl @ 4 g/ha recorded maximum grain yield (3.97 t/ha), number of effective tillers (209.3), filled gains/panicle (83.7), 1000 grain weight (27.3), which was on par with hand weeded control. Benerjee *et al*. (2005) stated that Almix® 20 WP @ 4 g a.i./ha + butachlor 50 EC @ 1250 g a.i./ha showed higher grain yield (4.95 t/ha) and straw yield (6.08 t/ha). Singh *et al*. (2007) stated that Almix® @ 8 g/ha significantly enhanced the grain yield by 89.7% over the control. Heisnam *et al*. (2015) reported that Almix® applied field recorded higher number of effective tillers per hill, number of spikelets, filled grains, grain yield and straw yield. Almix® application resulted in higher grain yield (68.98 q/ha), which was 20-29% higher than control. Seventy two per cent increase in yield was observed by the application of metsulfuron-methyl @ 8 g/ha compared to control in rice crop (Saha and Rao, 2010).

Application of a mixture of Almix® @ 15 g/ha and 2,4-D @ 1259 g/ha showed maximum grain yield (58.3 q/ha) (Mukherjee and Singh, 2006). Tank mixture of Almix® and 2,4-D gave a similar grain yield (5837 kg/ha) and straw yield (7132 kg/ha) that of handweeded condition (Mukherjee and Singh, 2004). Chakraborti *et al*. (2017) conducted a field experiment on direct seeded rice with six herbicides *viz*., pendimethalin, 2,4-D, fenoxaprop, bispyribac sodium, metsulfuron methyl+ chlorimuron ethyl (Almix®), pyrazosulfuron ethyl and found that Almix® had lower seed yield and test weight. Combination of bispyribac-Na and chlorimuron + metsulfuron showed the least grain yield, straw yield, number and length of panicle (Shendage *et al*., 2016).

Materials and methods

3. MATERIALS AND METHODS

Investigation on "Physiological evaluation of herbicidal effects on rice, broadleaved weeds and sedges" was conducted at Agricultural Research Station, Mannuthy. Details of materials used and methods adopted are presented in this chapter.

3.1 General details

3.1.1 Location

The experiment was conducted at Agricultural Research Station, Mannuthy.

3.1.2 Variety used

Jyothi (PTB 39) a red kernelled, long, bold, short duration (110-115 days) rice variety was used for the trial.

3.1.3 Season

Crop period was from July 2018 to November 2018.

3.2 Treatments

Experiment was laid out in randomized block design (RBD) with 8 treatments and 3 replications (Table.1). Plot size was 5 × 3 m (15 m^2). Herbicides tried and dosages used are given below:

Table 1. Details of herbicides used for the study

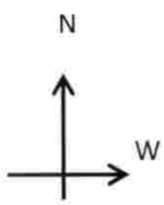
Sl.No.	Herbicides	Trade Name	Formulation	Dosage (g ai/ha)	Time of application (DAS)
1	2,4-D sodium salt	Agan	80% WP	800	
2	2,4-D sodium salt	Agan	80% WP	1600	

3	Ready mix formulation of metsulfuron methyl and chlorimuron ethyl	Almix®	10% w/w	4	20
4	Ready mix formulation of metsulfuron methyl and chlorimuron ethyl	Almix®	10% w/w	8	
5	Penoxsulam	Granite	21.7% w/w	25	
6	Penoxsulam	Granite	21.7% w/w	50	
7	Control- Hand weeded	-	-	-	
8	Control- Unweeded	-	-	-	-

Almix® - metsulfuron methyl 10% + chlorimuron ethyl 10%

3.3 Field operations

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



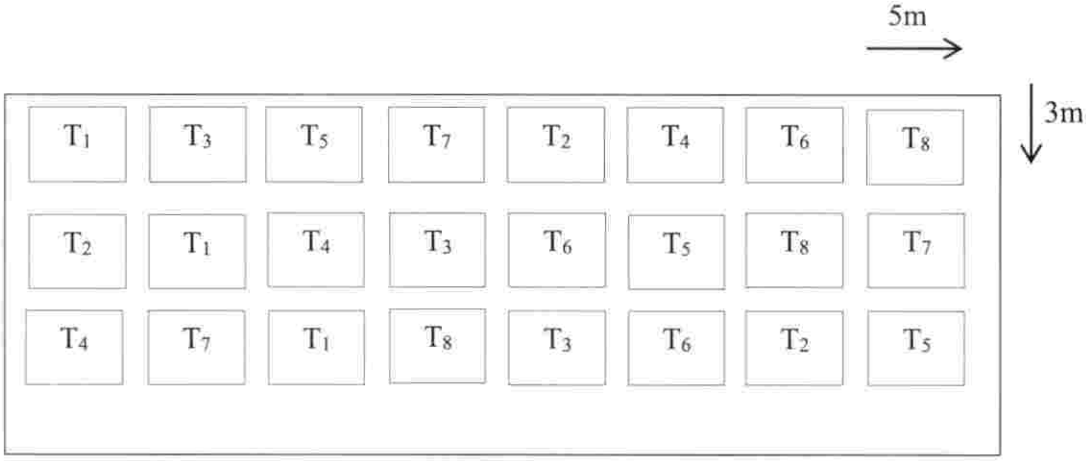


Fig. 1. Layout of the experiment

T_1- 2,4-D @ 0.8 kg ai/ha

T_2- 2,4-D @ 1.6 kg ai/ha

T_3- Almix® @ 0.004 kg ai/ha

T_4- Almix® @ 0.008 kg ai/ha

T_5- Penoxsulam @ 0.025 kg ai/ha

T_6- Penoxsulam @ 0.05 kg ai/ha

T_7- Control - Hand weeded

T_8- Control - Unweeded

3.3.1 Land preparation, sowing and fertilizer application

Field was ploughed, puddled and leveled. Plots of 5m × 3m were prepared by taking bunds of 25cm width and height. After leveling, N:P:K fertilizers were applied as per POP @ 70:35:35 kg/ha. Urea, Factomphos, Muriate of potash were used. Full dose of phosphorus was applied basally, but N and K were applied in two equal splits at land preparation and panicle initiation stages. After basal application of fertilizers, rice seeds were broadcasted at the rate of 150g/plot (100kg/ha). At 20 DAS, all herbicides were sprayed using knapsack sprayer at the recommended doses (Table 1.). In case of hand weeded plot, weeding was done at 20 DAS. Grass weeds were removed by hand at weekly intervals from 20 DAS onwards.

3.3.2 Plant protection

Timely plant protection measures were taken as per the package of practices (POP) of KAU (KAU, 2007). Leaf folder attack was noticed during the seedling stage of the crop and Fame® (50ml/ha) was applied. After the floods in Kerala, outbreak of bacterial leaf blight occurred at tillering stage and streptocyclin was applied (1.5g/3L). Rice bug attack was also observed during milky stage and was controlled by application of malathion @1ml/L.

3.3.3 Harvesting

The crop was harvested during the second week of November 2018 when the grains were completely matured. Threshing was done manually and the produce was cleaned and dried .Yield was expressed as kg/ha.

3.4 Sample collection

Prior to application of herbicides plots were maintained uniformly. Twenty four plant samples were randomly collected from all plots and biochemical and physiological parameters were estimated. Herbicides were sprayed on the 20th day. Samples were collected one week and at the time of flowering for biochemical analysis.

Plate. 1. General view of experimental plot

Plate. 2. Herbicide application at 20 days after sowing

3.5 Morphological observations

Morphological observations were recorded at 20 days intervals.

3.5.1 Height

From each plot five plants were tagged. Throughout the experiment, morphological parameters of these plants were recorded. Before panicle initiation, plant height was measured from base to the longest leaf and after panicle initiation, measurement was taken from base to the longest panicle. It was expressed in cm.

3.5.2 Tiller production

The total number of tillers in one square meter area from each experimental plot was recorded at 20 days intervals.

3.5.3 Days to flowering

Days to flowering was recorded when about 50% of plants flowered.

3.6 Growth indices

Growth indices were computed at 20 days interval up to harvest stage. The sampling unit contained 9 plants per treatment (three from each replication). The plant samples were uprooted and dried and the growth indices were calculated as per the procedures given below.

3.6.1 Leaf area index (LAI)

Leaf area index was determined by measuring average length and width of the leaf and multiplied with a factor 0.75 recommended by Yoshida (1981).

3.6.2 Relative growth rate

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

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

Where,

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

3.6.3 Crop growth rate

Crop growth rate (CGR) is the rate of dry matter production per unit ground area per unit time. It was calculated using the formula of Watson (1952) and expressed as mg cm^{-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 plants 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

Net assimilation rate (NAR) is the rate of dry weight increase per unit leaf area per unit time. NAR was computed using the formula suggested 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 Visual phytotoxicity scoring

On the third and seventh day after spraying, visual phytotoxicity rating of crop was done. Symptoms of injury like tip burn, leaf scorching, leaf curling, yellowing and recovery time were observed and graded from 0-5 with the help of toxicity scale given by Thomas and Abraham, (2007).

Table 2. Scale for rating herbicide phytotoxicity in rice crop

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

3.8 Physiological studies

The physiological and biochemical parameters were estimated before herbicide application, one week after herbicide application and at the time of flowering.

3.8.1 Chlorophyll content

Chlorophyll a, chlorophyll b and total chlorophyll were measured by the method adopted by Hiscox and Israelstam (1979). The chlorophyll content was estimated in spectrophotometer (Model- 4001/4 Thermo spectonic, Thermo Electron Corporation, USA) at three wavelengths 663 nm, 645nm and 652 nm. It was expressed as milligram per gram fresh weight of plant tissue. The calculation was done by using the following formulae:

$$\text{Chlorophyll a} = [(12.7 \times A663) - (2.69 \times A645)] \times V/1000 \times W$$

$$\text{Chlorophyll b} = [(22.9 \times A645) - (4.68 \times A663)] \times V/1000 \times W$$

$$\text{Total chlorophyll} = [(20.2 \times A663) + (8.02 \times A645)] \times V/1000 \times W$$

Where,

A = Absorption at given wavelength

V = Total volume of sample in extraction medium

W = Weight of sample

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3.8.2 Net photosynthesis

Photosynthetic rate was calculated by using the instrument infrared gas analyzer (Model LI- 6400 of ICOR Inc. Licoln, Nebraska, USA) before herbicide application, one week and at the time of flowering. The reading was taken from 8 to 10 am and expressed as μ mol CO_2 m^{-2} s^{-1}. Due to the unexpected rains, the reading could be taken only at maturity stage.

3.8.3 Stomatal conductance

Stomatal conductance was measured using infrared gas analyzer (Model LI-6400 of ICOR Inc. Lincoln, Nebraska, USA) and it was taken at 8-10 am. The observations were taken before herbicide application, one week after herbicide application and at the time of flowering. It was expressed as mol H_2O m^{-2} s^{-1}.

3.8.4 IAA Content

IAA (indole acetic acid) was estimated by the method proposed by Parthasarathy *et al.* (1970) with little modification using Garden weber reagent. The IAA content was expressed as mg of unoxidised auxin g^{-1} fresh weight.

3.9 Biochemical characters

3.9.1 Total soluble protein

The total soluble protein was estimated using the method suggested by Lowry *et al.* (1951) and expressed as mg g^{-1} of fresh weight.

3.9.2 Total aminoacid content

Total aminoacid content was estimated using ninhydrin method proposed by Moore and Stein (1948) and expressed as mg g^{-1}.

3.9.3 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. Measurement of gas exchange parameters using IRGA

3.9.4 Proline content

Proline content was estimated by the method of Bates *et al.* (1973) and expressed as mg g^{-1}. Absorbance was taken at 520 nm, using the instrument spectrophotometer.

3.9.5 Nitrate reductase activity

Nitrate reductase enzyme activity in the leaf was estimated by the method given by Hageman and Flesher (1960). 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 mol of NO_2^- formed per g fresh weight per h.

3.9.6 Total carbohydrate

Total carbohydrate was estimated (Hedge and Hofreiter, 1963) using anthrone reagent. Absorbance was taken at 630 nm, using the instrument spectrophotometer.

3.10 Yield attributes and yield

3.10.1 Productive tillers/m^2

At harvesting stage, number of productive tillers in one square meter area of each experimental plot was counted, recorded and expressed as tillers /m^2.

3.10.2 Spikelets/ panicle

The number of spikelets was computed from five plants from each treatment and the average was calculated.

3.10.3 Filled grains/ panicle

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

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3.10.4 Chaff percentage

Samples were collected from five randomly selected plants from each plot and the percentage of chaffy grains per each panicle was calculated.

3.10.5 Thousand grain weight

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

3.10.6 Grain and straw yield

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

3.11 Observations on weeds

3.11.1 Weed count

Species wise weed count was taken using a 50 cm X 50 cm (0.25 m^2) quadrat. It was multiplied by four. So as to express the weed count in a square meter area quadrat. The quadrat was randomly placed and from each plot weed count were taken before and one week after herbicide application. Species wise count was taken after 60 days of herbicide application.

3.11.2 Dry weight

Surviving weeds after herbicide application were uprooted and cleaned and air dried and also oven dried at 80 ± 5^0C. At 60 DAS again species wise weeds were uprooted and cleaned, air and oven dried. The dry weight was recorded in kg ha^{-1}.

3.11.3 Weed persistence index

This index indicates the resistance in weeds against the tested treatments and confirms the effectiveness of the selected herbicide. It was calculated by the formula given by Khaliq *et al.*, (2011).

$$\text{WPI} = \frac{\text{Weed weight in treated plot}}{\text{Weed weight in control plot}} \times \frac{\text{Weed count in control plot}}{\text{Weed count in treated plot}}$$

3.11.4 Weed control efficiency

It indicates the percentage reduction in weed dry matter by any weed control treatment in comparison to weedy check plot. It was used to compare different weed control treatments. It was calculated using the formula suggested by Mani *et al.* (1973).

$$\text{WCE (\%)} = \frac{\text{Dry matter of weeds in unweeded plot - Dry matter of weeds in treated plot}}{\text{Dry matter of weeds in unweeded plot}} \times 100$$

3.12 Net house study

A net house study was undertaken with selected weeds. Seeds of some common weeds of rice ecosystem, *viz. Marsilea quadrifoliata, Fimbristylis miliacea, Cyperus iria* and *Ludwigia parviflora* were collected from Thrissur and Palakkad districts and plants were sown in pots with three replications. Herbicides

Plate. 4. Herbicide application at 20 DAS in pots

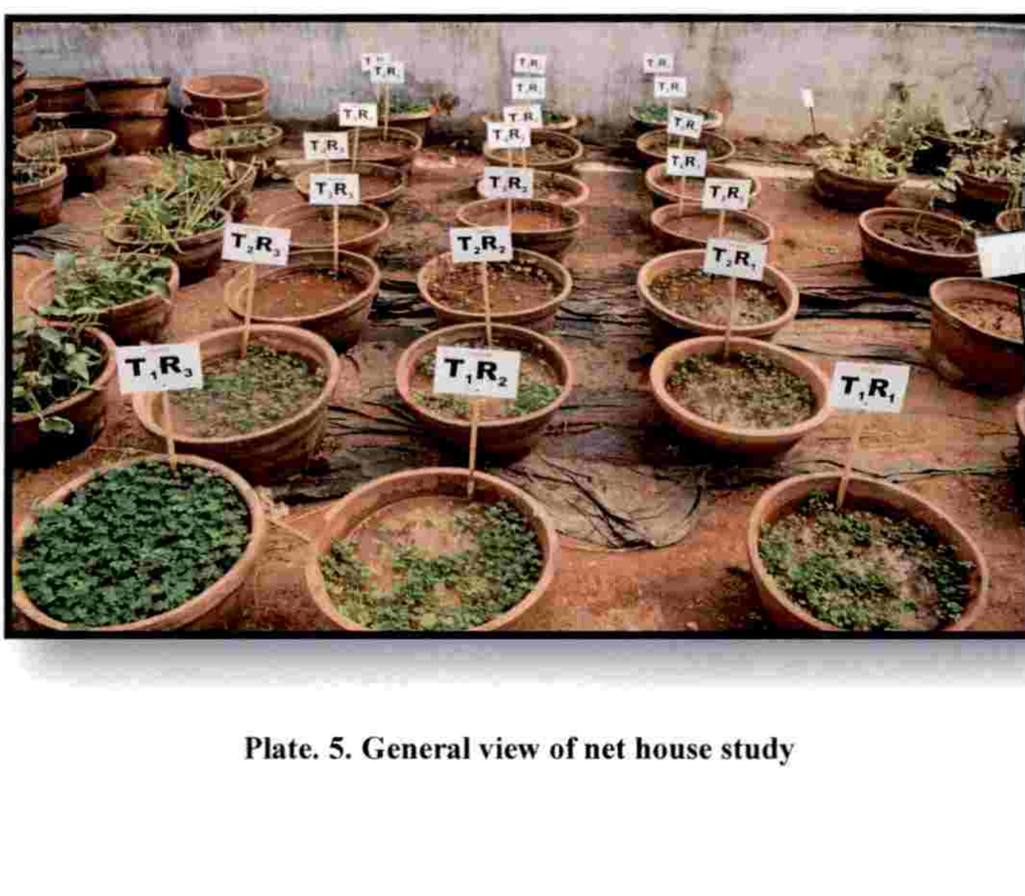

Plate. 5. General view of net house study

2,4-D, Almix®, penoxsulam were sprayed in 2 different concentrations (normal and twice the normal dose). Observations were taken in 2 days interval and graded from 0-5 with the help of toxicity scale given by Thomas and Abraham, (2007).

3.12.1 Chlorophyll stability index

Total chlorophyll content was measured by the method adopted by Hiscox and Israelstam (1979). Chlorophyll content was estimated in spectrophotometer (Model- 4001/4 Thermo spectonic, Thermo Electron Corporation, USA) at three wavelength 663 nm, 645nm and 652 nm. It was expressed as milligram g^{-1} fresh weight of plant tissue. Calculation was done by using the following formulae:

$$CSI = \frac{\text{Total chlorophyll content of the treatment}}{\text{Total chlorophyll content of the control}} \times 100$$

3.12.2 Catalase enzyme activity

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

3.12.3 SOD enzyme activity

Superoxide dismutase was determined using a slightly modified procedure (Madamanchi *et al.*, 1994) originally explained by Beauchamp and Fridovich (1971) with Nitroblue tetrazolium (NBT) salt as reagent. It was expressed in enzyme units mg^{-1} fresh weight.

3.12.4 Wax content of leaf

Wax content of weed leaf sample of weeds was estimated using the chemical chloroform. Leaf discs were soaked in chloroform for 10-15 sec and the wax content was estimated using the formula suggested by Ebercon *et al.* (1977) and expressed as mg.

Wax content = $W_b - W_a$

W_b= Weight of beaker after evaporation of chloroform

W_a= Initial weight of the beaker

Result

4. RESULT

The present investigation was carried out to evaluate the herbicidal effects on physiological, morphological and biochemical parameters on rice, broadleaved weeds and sedges.

Before herbicidal application (19^{th} day), observations on biochemical and physiological parameters of rice seedlings were taken. Ten rice seedlings were randomly collected from different plots, observations were taken and the results are presented here.

At this stage the LAI of rice seedlings ranged from 0.15-0.21. The chlorophyll content of the seedling ranged from 1.14-2.30 mg g^{-1}, 0.573-1.01 mg g^{-1} and 2.15-2.88 mg g^{-1} for chlorophyll a, b and total chlorophyll respectively. Total soluble protein ranged from 21.5-24 mg g^{-1}. The proline content of the seedling was between 0.01-0.13 mg g^{-1}. On 19^{th} day, the nitrate reductase enzyme activity ranged from 360-400 μ moles of NO_2^- formed g^{-1} FW h^{-1}). The IAA content was observed to be between 0.60-0.75 mg of unoxidised auxin g^{-1} FW. The stomatal conductance ranged from 1.01-1.18 mol CO_2 $m^{-2}s^{-1}$ and net photosynthesis ranged from 32-36 mol H_2O_2 m^{-2} s^{-1}. Catalase enzyme activity was observed between 4.99-11.56 catalase enzyme units g^{-1} while the total amino acid content varied from 9.50-11.00 mg g^{-1}.

Table. 3 Physiological and biochemical parameters of rice seedlings before application of herbicides (19 days)

Parameter	Mean	Standard error
LAI	0.179	0.006
Chlorophyll a (mg g^{-1})	1.59	0.359
Chlorophyll b (mg g^{-1})	0.830	0.132
Total chlorophyll (mg g^{-1})	2.42	0.227
Total soluble protein (mg g^{-1})	23.00	0.764
Proline content (mg g^{-1})	0.067	0.017

Nitrate reductase enzyme activity (μ moles of NO_2^- formed g^{-1} FW h^{-1})	380.00	11.55
IAA content (mg of unoxidised auxin g^{-1} FW)	0.683	0.044
Net photosynthesis (μ mol CO_2 m^{-2} s^{-1})	34.20	0.800
Stomatal conductance (mol H_2O m^{-2} s^{-1})	1.11	0.028
Catalase enzyme activity (enzyme units g^{-1})	7.33	2.12
Total amino acid content (mg g^{-1})	9.5	0.244

4.1 Morphological parameters

4.1.1 Plant height

Herbicides were applied on the 20th day after sowing and plant height of rice was recorded on 40th, 60th, 80th, 100th, 115th DAS and are given in Table 4. Treatment T_7 (73.51cm) showed an increase in height compared to herbicides applied plots on 40th DAS, while T_1 (72.13 cm) was on par with T_7 (73.51 cm). Height of T_8 (68.90 cm) was on par with T_2 (67.59 cm) treated plot. Application of twice the normal dose of herbicides showed reduction in plant height and maximum reduction was seen in T_4 (64.91 cm) which was on par with T_6 (65.79 cm). On the 60th DAS also similar trend was observed. On 80th DAS, T_5 (91.55 cm) applied plot showed a reduction in plant height. T_6 (83.58 cm) showed maximum reduction in plant height compared to other herbicide applications followed by T_4 (84.25 cm) while T_7 (94.86 cm) showed the maximum height followed by T_1 (93.13 cm). In case of 100th and 115th DAS, there was significant reduction in plant height in all the herbicides applied plots as compared to the control, however the reduction in plant height was more prominent for twice the normal doses applied plots.

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

Treatment	40 DAS	60 DAS	80 DAS	100 DAS	115 DAS
T_1 (2,4-D, X)	72.13^{ab}	83.41^{ab}	93.13^{b}	101.13^{b}	111.00^{b}
T_2 (2,4-D, 2X)	67.59^{d}	75.81^{cd}	85.25^{e}	92.80^{f}	102.87^{ef}
T_3 (Almix®, X)	69.54^{c}	82.65^{b}	92.49^{b}	99.20^{c}	109.33^{c}
T_4 (Almix®, 2X)	64.91^{e}	74.65^{de}	84.25^{f}	89.73^{g}	101.93^{fg}
T_5 (Penox., X)	71.57^{b}	81.73^{b}	91.55^{c}	98.07^{d}	107.67^{d}
T_6 (Penox. 2X)	65.79^{e}	73.62^{e}	83.58^{f}	88.80^{h}	100.80^{g}
T_7 (HWC)	73.51^{a}	84.87^{a}	94.86^{a}	103.33^{a}	114.07^{a}
T_8 (UWC)	68.90^{cd}	76.85^{c}	86.94^{d}	95.33^{e}	103.73^{e}
CD(0.05)	1.605	2.171	0.883	0.712	1.402

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.1.2 Number of tillers

Data on effect of herbicides on tiller/m^2 of rice crop were taken on the 40th DAS onwards and are given in Table 5. On 40th DAS, T_7 (434.00) showed higher tillers/m^2 followed by T_1 (430.83). T_8 (424.33) was on par with T_2 (421.67). Lowest tiller/m^2 was seen in T_4 (418.33) which was on par with T_6 (420.33). On the 60th DAS, tillers/ m^2 of T_1 (532.33) increased and became on par with T_7 (535.00). Tillers/m^2 of normal dose of T_3 (529.83) and T_5 (527.83) increased considerably. On the 80th DAS, T_7 (432.00) had maximum tillers/m^2 compared to herbicide treatments and T_6 (415.17) showed the least. Same trend was followed up to 115 DAS and on the 115th DAS, T_7 (294.33) showed higher tillers/m^2 which was on par with T_1 (293.67) while T_6 (279.50) had the least number of tillers.

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

Treatment	40 DAS	60 DAS	80 DAS	100 DAS	115 DAS
T_1 (2,4-D, X)	430.83^b	532.33^{ab}	429.50^b	355.50^b	293.67^a
T_2 (2,4-D, 2X)	421.67^e	521.17^e	421.00^e	347.33^f	282.83^{de}
T_3 (Almix®, X)	425.83^d	529.83^{bc}	427.17^c	353.00^c	289.50^b
T_4 (Almix®, 2X)	418.33^f	518.33^f	417.67^f	345.33^g	281.33^e
T_5 (Penox., X)	428.33^c	527.83^c	424.83^d	351.83^d	288.33^{bc}
T_6 (Penox., 2X)	420.33^{ef}	517.17^f	415.17^g	343.00^h	279.50^f
T_7 (HWC)	434.00^a	535.00^a	432.00^a	357.50^a	294.33^a
T_8 (UWC)	424.33^d	524.00^d	422.83^{de}	349.50^e	285.00^{cd}
CD(0.05)	2.178	2.668	2.077	0.785	3.558

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.1.3 Days to flowering

Data regarding the effect of different treatments on days to flowering are given in Table 6. The result revealed that the herbicide application delayed flowering in rice variety Jyothi. Plants in T_7 flowered by 63 days while the unweeded control T_8 took 64 days and T_6 took 66 days to flower. The maximum delay was 3 days.

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

Treatment	Days to flowering
T_1 (2,4-D, X)	63.22^g
T_2 (2,4-D, 2X)	64.67^c
T_3 (Almix®, X)	63.56^f
T_4 (Almix®, 2X)	65.00^b
T_5 (Penox., X)	63.89^e

T_6 (Penox., 2X)	65.67^{a}
T_7 (HWC)	62.89^{h}
T_8 (UWC)	64.33^{d}
CD(0.05)	0.285

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.1.4 Leaf area index

Data regarding the effect of herbicide application on LAI are given in Table 7. By the 40th DAS, T_7 (1.77) showed higher leaf area index which was on par with T_1 (1.66). T_2 (1.23), T_4 (1.13), T_6 (1.10) treatments showed a significant reduction in leaf area index. On the 60th and 80th DAS, leaf area index of T_1 (3.93, 5.07) was on par with T_7 (4.03, 5.21). Twice the normal dose of herbicides showed less leaf area index and T_6 (3.11, 4.19) showed the least. On the 100th and 115th DAS, T_7 (3.23, 1.79) had the highest leaf area index followed by T_1 (3.14, 1.70). Twice the normal dose of herbicides showed lower leaf area index and among these T_6 (2.52, 1.49) showed the least value.

Table. 7 Effect of herbicides on LAI at different intervals

Treatment	40 DAS	60 DAS	80 DAS	100 DAS	115 DAS
T_1 (2,4-D, X)	1.66^{ab}	3.93^{a}	5.07^{ab}	3.14^{b}	1.70^{b}
T_2 (2,4-D, 2X)	1.23^{e}	3.34^{cd}	4.41^{cde}	2.68^{e}	1.58^{de}
T_3 (Almix®, X)	1.49^{bc}	3.81^{ab}	4.77^{bc}	3.05^{bc}	1.69^{b}
T_4 (Almix®, 2X)	1.13^{e}	3.34^{cd}	4.24^{de}	2.63^{e}	1.54^{ef}
T_5 (Penox., X)	1.56^{cd}	3.76^{ab}	4.61^{cd}	2.97^{c}	1.66^{bc}
T_6 (Penox., 2X)	1.10^{e}	3.11^{d}	4.19^{e}	2.52^{f}	1.49^{f}
T_7 (HWC)	1.77^{a}	4.03^{a}	5.21^{a}	3.23^{a}	1.79^{a}
T_8 (UWC)	1.40^{d}	3.52^{bc}	4.60^{cd}	2.77^{d}	1.62^{cd}

CD(0.05)	0.150	0.395	0.386	0.090	0.062

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.1.5 Relative crop growth

Data on herbicidal effect on relative growth rate of rice plant are given in Table 8. T_7 (204.85) showed higher relative growth rate which was on par with normal dose of herbicides *viz*, T_1 (202.05), T_3 (198.88), T_5 (200.54)) and T_8 (196.97). T_4 (192.25) showed minimum level of relative growth between 20-40 days. In case of 40- 60 days, same trend was observed but relative growth rate of T_6 (63.79) reduced and became the lowest. By 60-80 days, T_7 (44.58) showed highest relative growth rate followed by T_1 (39.92) and least was observed in T_6 (30.70). In 80-100 days, an increase in relative growth rate of T_1 (7.48), T_3 (6.69), T_5 (6.14) and T_8 (5.70) was recorded. By 100-115 days, handweeded plot (T_7, 1.99) recorded highest relative growth rate which was on par with T_1 (2.08) while T_2 (1.65), T_4 (1.59) and T_6 (1.56) plots showed the least value.

Table. 8 Effect of herbicides on relative growth rate (mg g^{-1} d^{-1})

Treatment	20-40 DAS	40-60 DAS	60-80 DAS	80-100 DAS	100-115 DAS
T_1 (2,4-D, X)	202.05^{ab}	68.89^{ab}	39.92^{b}	7.48^{ab}	2.08^{a}
T_2 (2,4-D, 2X)	194.75^{cde}	66.11^{de}	33.92^{ef}	4.78^{cd}	1.65^{e}
T_3 (Almix®, X)	198.88^{abcd}	68.16^{bc}	37.75^{c}	6.69^{ab}	1.92^{bc}
T_4 (Almix®, 2X)	192.25^{e}	64.87^{ef}	32.10^{fg}	4.32^{cd}	1.59^{e}
T_5 (Penox., X)	200.54^{abc}	67.58^{bc}	36.25^{cd}	6.14^{bc}	1.85^{cd}
T_6 (Penox., 2X)	192.85^{de}	63.79^{f}	30.70^{g}	3.80^{d}	1.56^{e}
T_7 (HWC)	204.85^{a}	69.85^{a}	44.58^{a}	8.25^{a}	1.99^{ab}
T_8 (UWC)	196.97^{bcde}	66.86^{cd}	35.32^{de}	5.70^{bc}	1.78^{d}
CD(0.05)	6.427	1.449	1.906	1.880	0.101

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.1.6 Crop growth rate

Data on effect of herbicides on crop growth rate of rice plant are given in Table 9. The result revealed that there was no significant change in crop growth rate from 20-60 DAS. In 60-80 days, T_7 (4.39) showed higher crop growth rate followed by T_1 (4.07) and least was observed in T_6 (3.34). In 80-100 DAS, T_8 (0.667), T_2 (0.631), T_4 (0.586) and T_6 (0.548) showed lower crop growth rate compared to hand weeded and plots applied with normal herbicide dosage. In the final phase, that is 100-115 DAS, treatment T_1 (0.500) showed an increment in crop growth rate and became on par with handweeded control (T_7, 0.478). Twice the normal dose of herbicides applied plots showed lower crop growth rate compared to normal dose of herbicides applied plots.

Table. 9 Effect of herbicides on crop growth rate (mg cm^{-2} d^{-1})

Treatment	20-40 DAS	40-60 DAS	60-80 DAS	80-100 DAS	100-115 DAS
T_1 (2,4-D, X)	1.99	3.67	4.07^{b}	0.880^{b}	0.500^{a}
T_2 (2,4-D, 2X)	1.47	3.20	3.56^{f}	0.631^{def}	0.345^{de}
T_3 (Almix®, X)	1.81	3.52	3.92^{c}	0.802^{bc}	0.429^{b}
T_4 (Almix®, 2X)	1.33	3.15	3.44^{g}	0.586^{ef}	0.317^{ef}
T_5 (Penox., X)	1.88	3.40	3.78^{d}	0.715^{cd}	0.406^{bc}
T_6 (Penox., 2X)	1.36	3.43	3.34^{h}	0.548^{f}	0.305^{f}
T_7 (HWC)	2.23	3.84	4.39^{a}	1.11^{a}	0.478^{a}
T_8 (UWC)	1.68	3.27	3.65^{e}	0.667^{de}	0.381^{cd}
CD(0.05)	NS	NS	0.090	0.111	0.040

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

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4.1.7 Net assimilation rate

Data on effect of different herbicides on net assimilation rate of rice var. Jyothi are given in Table 10. The result revealed that there was no significant difference in net assimilation rate at 20-40 and 40-60 DAS. By 60-80 DAS, T_7 (0.433) showed higher net assimilation rate followed by T_1 (0.394). T_6 (0.304) showed the least NAR which was on par with T_4 (0.319). In 80-100 DAS, the value of T_8 (0.079) reduced and became on par with T_2 (0.073), T_4 (0.069) and T_6 (0.066). In case of 100-115 DAS, net assimilation rate of T_1 (0.062) increased and became on par with T_7 (0.063). T6 (0.048) showed the lowest net assimilation rate.

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

Treatment	20-40 DAS	40-60 DAS	60-80 DAS	80-100 DAS	100-115 DAS
T_1 (2,4-D, X)	0.85	0.477	0.394^{b}	0.114^{b}	0.062^{ab}
T_2 (2,4-D, 2X)	0.75	0.434	0.333^{ef}	0.073^{cd}	0.053^{e}
T_3 (Almix®, X)	0.791	0.494	0.373^{bc}	0.094^{bc}	0.06^{bc}
T_4 (Almix®, 2X)	0.718	0.451	0.319^{fg}	0.069^{d}	0.051^{f}
T_5 (Penox., X)	0.816	0.466	0.361^{cd}	0.084^{cd}	0.058^{c}
T_6 (Penox., 2X)	0.731	0.386	0.304^{g}	0.066^{d}	0.048^{g}
T_7 (HWC)	0.866	0.523	0.433^{a}	0.143^{a}	0.063^{a}
T_8 (UWC)	0.774	0.464	0.345^{de}	0.079^{cd}	0.056^{d}
CD(0.05)	NS	NS	0.024	0.022	0.002

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.2 Phytotoxicity screening

Visual symptoms of phytotoxicity was noted on the third and fifth day after herbicide application and are given in Table 11. On the 3rd DAH application, T_1 showed slight injury like tip burn and aerial root development and T_2 showed moderate injury while other herbicides had shown no phytotoxicity symptoms. 7th day after herbicide application T_1 completely recovered from phytotoxicity symptoms while the symptoms declined in the case of T_2 also.

Table. 11 Visual phytotoxicity screening of rice plant

Treatment	3 DAH application	5 DAH application
	Grade(0-5)	
T_1 (2,4-D, X)	1	0
T_2 (2,4-D, 2X)	2	1
T_3 (Almix®, X)	0	0
T_4 (Almix®, 2X)	0	0
T_5 (Penox., X)	0	0
T_6 (Penox., 2X)	0	0
T_7 (HWC)	0	0
T_8 (UWC)	0	0

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3 Biochemical parameters

4.3.1 Chlorophyll content

Data on effect of herbicides on chlorophyll content of rice plant are given in Tables 12&13. Seven days after herbicide treatment, T_7 (3.72) showed higher chlorophyll 'a' content followed by T_1 (3.55) and lowest content was observed in T_4 (2.39). In case of chlorophyll 'b' content, T_7 (1.12) showed the highest value which was on par with T_1 (1.05), T_5 (0.970), T_3 (0.951) and lowest by T_4 (0.740).

Hand weeded plot (4.84) showed significant difference in total chlorophyll content compared to unweeded control (4.02) and other herbicides applied plots. However, T_1 (4.60) was on par with hand weeded plot and the least shown by T_4 (3.13).

At flowering stage, T_7 (2.33) applied plot showed higher chlorophyll a content which was on par with T_1 (2.25). Treatments T_2 (1.88), T_4 (1.55) and T_6 (1.13) showed significant reduction in chlorophyll content which was lower than unweeded control (T_8, 1.99). Chlorophyll b content was highest in T_7 (1.90) and lowest in T_6 (0.522). In case of total chlorophyll content, highest was noted in T_7 (4.22) followed by T_1 (3.38), T_3 (3.34) and T_5 (3.27). Lowest was noted in T_6 (1.65).

Table. 12 Effect of herbicides on chlorophyll content of rice at 7 days after herbicide application (mg g^{-1})

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll
T_1 (2,4-D, X)	3.55^{b}	1.05^{ab}	4.60^{ab}
T_2 (2,4-D, 2X)	3.07^{d}	0.814^{cd}	3.88^{e}
T_3 (Almix®, X)	3.23^{c}	0.951^{abcd}	4.18^{cd}
T_4 (Almix®, 2X)	2.39^{f}	0.740^{d}	3.13^{g}
T_5 (Penox., X)	3.42^{b}	0.970^{abc}	4.39^{bc}
T_6 (Penox., 2X)	2.81^{e}	0.799^{cd}	3.60^{f}
T_7 (HWC)	3.72^{a}	1.12^{a}	4.84^{a}
T_8 (UWC)	3.15^{cd}	0.873^{bcd}	4.02^{de}
CD (0.05)	0.148	0.215	0.237

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

Table. 13 Effect of herbicides on chlorophyll content of rice at flowering (mg g^{-1})

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll
T_1 (2,4-D, X)	2.25^{ab}	1.12^{b}	3.38^{b}
T_2 (2,4-D, 2X)	1.88^{c}	0.75^{cd}	2.63^{d}
T_3 (Almix®, X)	2.25^{ab}	1.10^{b}	3.34^{b}
T_4 (Almix®, 2X)	1.55^{d}	0.70^{d}	2.25^{e}
T_5 (Penox., X)	2.21^{ab}	1.06^{b}	3.27^{b}
T_6 (Penox., 2X)	1.13^{e}	0.522^{e}	1.65^{f}
T_7 (HWC)	2.33^{a}	1.90^{a}	4.22^{a}
T_8 (UWC)	1.99^{bc}	0.903^{c}	2.89^{c}
CD (0.05)	0.287	0.157	0.191

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.2 Total soluble protein

Data on effect of herbicides on total soluble protein content of rice plant are given in Table 14. One week after herbicide application, hand weeded plot (T_7, 21.17) showed maximum total soluble protein content compared to herbicides applied plots and unweeded control. Among the herbicides, T_1 (19.83) was superior in soluble protein content compared to other herbicides. T_4 (16.25) showed the minimum value which was on par with T_6 (16.91). Same trend was maintained even at flowering stage indicating lack of recovery.

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Table. 14 Effect of herbicides on total soluble protein content of rice plant (mg g^{-1})

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	19.83^{b}	11.42^{b}
T_2 (2,4-D, 2X)	18.00^{e}	10.08^{e}
T_3 (Almix®, X)	19.50^{c}	10.83^{c}
T_4 (Almix®, 2X)	16.25^{f}	09.00^{f}
T_5 (Penox., X)	19.50^{d}	10.50^{d}
T_6 (Penox., 2X)	16.91^{f}	09.00^{f}
T_7 (HWC)	21.17^{a}	13.92^{a}
T_8 (UWC)	19.25^{d}	10.50^{d}
CD (0.05)	0.400	0.232

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.3 Proline content

Data on effect of herbicides on proline content of rice plant are given in Table 15. Seven days after herbicides application, proline content of herbicides applied plots and unweeded plots were higher than hand weeded control. T_4 (0.270) showed maximum proline content followed by T_6 (0.252) and T_2 (0.243). Hand weeded plot (T_7, 0.143) showed less proline content followed by T_1 (0.165). At the time of flowering, proline content of T_6 (0.278) increased and became on par with T_4 (0.270). Minimum proline content was recorded in T_7 (0.145) followed by T_1 (0.175). At flowering, proline content of treatment plots increased compared to one week of observation.

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Table. 15 Effect of herbicides on proline content of rice plant (mg g^{-1})

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	0.165^{e}	0.175^{e}
T_2 (2,4-D, 2X)	0.243^{b}	0.250^{b}
T_3 (Almix®, X)	0.205^{cd}	0.222^{d}
T_4 (Almix®, 2X)	0.270^{a}	0.270^{a}
T_5 (Penox., X)	0.193^{d}	0.233^{c}
T_6 (Penox., 2X)	0.252^{b}	0.278^{a}
T_7 (HWC)	0.143^{f}	0.145^{f}
T_8 (UWC)	0.210^{c}	0.237^{c}
CD (0.05)	0.014	0.09

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.4 Nitrate reductase enzyme activity

Data on effect of herbicides on nitrate reductase enzyme activity are given in Table 16. One week after herbicides application, there were no significant difference in nitrate reductase enzyme activity. At flowering stage, T_7 (486.67) showed maximum value followed by T_1 (430). Application of twice the normal dose of herbicides (T_6, T_4, T_2) (190, 203.33, 203.33) significantly reduced the nitrate reductase enzyme activity.

Table. 16 Effect of herbicides on nitrate reductase enzyme activity of rice plant (μ mol of NO_2^- formed g^{-1} FW h^{-1})

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	500.00	430.00^{b}
T_2 (2,4-D, 2X)	433.33	203.33^{e}
T_3 (Almix®, X)	446.67	386.67^{c}
T_4 (Almix®, 2X)	410.00	203.33^{e}

T_5 (Penox., X)	460.00	363.33^{d}
T_6 (Penox., 2X)	463.33	190.00^{e}
T_7 (HWC)	506.67	486.67^{a}
T_8 (UWC)	313.33	363.33^{d}
CD (0.05)	NS	14.74

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.5 IAA content

Data on effect of herbicide on IAA content of rice plant are given in Table 17. Hand weeded plot (T_7, 0.300) showed the highest IAA content followed by T_1 (0.250) after one week of herbicides application. T_3 (0.200) was on par with T_8 (0.200). Lowest IAA content was recorded in T_4 (0.142) and T_6 (0.150). At flowering stage, T_6 (0.050) showed a decline in IAA content and became the least value among the herbicides applied plots. Maximum IAA content was recorded in T_7 (0.308) followed by T_1 (0.250). T_3 (0.217) was on par with T_5 (0.200) and T_8 (0.200).

Table. 17 Effect of herbicides on IAA content of rice plant (mg of unoxidised auxin g^{-1} FW)

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	0.250^{b}	0.250^{b}
T_2 (2,4-D, 2X)	0.175^{d}	0.108^{d}
T_3 (Almix®, X)	0.200^{c}	0.217^{c}
T_4 (Almix®, 2X)	0.142^{e}	0.075^{e}
T_5 (Penox., X)	0.200^{c}	0.200^{c}
T_6 (Penox., 2X)	0.150^{e}	0.050^{f}
T_7 (HWC)	0.300^{a}	0.308^{a}
T_8 (UWC)	0.200^{c}	0.200^{c}

CD (0.05)	0.009	0.023

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.6 Net photosynthesis

Data on effect of herbicides on net photosynthesis of rice plant are given in Table 18. One week after herbicide application, T_7 (31.90) showed the highest net photosynthesis followed by T_1 (31.53). The values were significantly lesser in T_4 (24.24) followed by T_6 (26.07). At flowering stage, hand weeded plot (T_7, 9.11) was superior to herbicides applied plots. Net photosynthesis of T_6 (4.47) reduced and became lower than T_4 (5.21). Due to heavy rains during 2018, the estimation of net photosynthetic rate was delayed which might have control to the lower values, however the treatment effect was evident in the observations.

Table. 18 Effect of herbicides on net photosynthesis of rice plant (μ mol CO_2 m^{-2} s^{-1})

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	31.53^b	8.34^b
T_2 (2,4-D, 2X)	27.47^e	5.81^e
T_3 (Almix®, X)	30.05^c	6.88^c
T_4 (Almix®, 2X)	24.24^g	5.21^f
T_5 (Penox., X)	30.27^c	6.26^d
T_6 (Penox., 2X)	26.07^f	4.47^g
T_7 (HWC)	31.90^a	9.11^a
T_8 (UWC)	29.76^d	6.15^d
CD (0.05)	0.276	0.175

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.7 Stomatal conductance

Data on effect of herbicides on stomatal conductance of rice plant are given in Table 19. After seven days of application, T_7 (1.77) had relatively higher stomatal conductance compared to other treatments followed by T_1 (1.63). Twice the normal dose of herbicides reduced the stomatal conductance and the values were lower than unweeded condition. T_4 (0.680) recorded the lowest value followed by T_6 (1.04). At flowering, T_7 (0.523) showed maximum stomatal conductance followed by T_1 (0.467). T_6 (0.209) had the least stomatal conductance followed by T_4 (0.295). The stomatal conductance was lower during the flowering stage.

Table. 19 Effect of herbicides on stomatal conductance of rice plant (mol H_2O_2 m^{-2} s^{-1})

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	1.63^b	0.467^b
T_2 (2,4-D, 2X)	1.14^e	0.306^f
T_3 (Almix®, X)	1.52^d	0.394^c
T_4 (Almix®, 2X)	0.680^g	0.295^g
T_5 (Penox., X)	1.57^c	0.359^d
T_6 (Penox., 2X)	1.04^f	0.209^h
T_7 (HWC)	1.77^a	0.523^a
T_8 (UWC)	1.50^d	0.313^e
CD (0.05)	0.017	0.001

2,4-D, X and 2X-0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X-0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X-0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.8 Catalase enzyme activity

Data on effect of herbicides on catalase enzyme activity of rice plant are given in Table 20. Seven days after herbicides application, catalase enzyme activity of T_4 (27.31) was higher compared to other treatment plots. T_7 (13.43) showed the lowest catalase enzyme activity followed by T_1 (14.96). Twice the normal dose of herbicides showed higher catalase activity compared to its normal doses and unweeded plot (18.68) showed catalase activity higher than normal dose of herbicides applied plots and lower than twice the normal doses of herbicides applied plots.

At the time of flowering, the catalase enzyme activity was found to be increased in all the treatments. However, the treatment T_7 (47.83) had significantly lower values followed by T_1 (50.77). T_6 (73.21) and T_5 (56.89) plots showed an increase in catalase enzyme activity compared to one week of herbicides application. Highest catalase enzyme activity was observed in T_6 (73.21) followed by T_4 (69.81). In T_8 (60.97) the catalase enzyme activity was higher than T_1, T_3, T_5 and lower than T_2, T_4, T_6.

Table. 20 Effect of herbicides on catalase enzyme activity of rice plant (Catalase enzyme units g^{-1})

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	14.96^{ef}	50.77^{g}
T_2 (2,4-D, 2X)	20.09^{c}	64.83^{c}
T_3 (Almix®, X)	18.57^{cd}	53.95^{f}
T_4 (Almix®, 2X)	27.31^{a}	69.81^{b}
T_5 (Penox., X)	17.33^{de}	56.89^{e}
T_6 (Penox., 2X)	23.93^{b}	73.21^{a}
T_7 (HWC)	13.43^{f}	47.83^{h}
T_8 (UWC)	18.68^{cd}	60.97^{d}
CD (0.05)	2.586	2.610

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.9 Total aminoacid content

Data on effect of herbicides on total aminoacid content of rice plant are given in table 21. One week after herbicides application, significant change was observed between herbicides applied plots and handweeded control. Hand weeded plot (T_7, 10.77) showed higher aminoacid content which was on par with T_1 (9.89). Lowest was recorded in plots in which twice the normal dose of herbicides applied (T_2, 8.70), (T_6, 8.56), (T_4, 8.50). At flowering, no significant change was noted among treatments.

Table. 21 Effect of herbicides on total amino acid content (mg g^{-1})

Treatment	7 DAH application	At flowering
T_1 (2,4-D, X)	9.89^{ab}	8.84
T_2 (2,4-D, 2X)	8.70^{c}	8.09
T_3 (Almix®, X)	8.97^{bc}	8.67
T_4 (Almix®, 2X)	8.50^{c}	7.82
T_5 (Penox., X)	9.11^{bc}	8.43
T_6 (Penox., 2X)	8.56^{c}	7.62
T_7 (HWC)	10.77^{a}	9.07
T_8 (UWC)	$8.77b^{c}$	8.26
CD (0.05)	1.163	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.3.10 Total carbohydrate

Data on effect of herbicides on total carbohydrates of rice plant are given in Table 22. At the time of flowering, twice the normal dose (T_6, 46.93) showed an increase in total carbohydrate content followed by normal dose of T_5 (43.47), T_4 (44.53), T_2 (42.93) and T_8 (43.47). The least value was recorded in T_7 (39.47) and T_1 (41.07).

Table. 22 Effect of herbicides on total carbohydrate content of rice plant (mg g^{-1})

Treatment	At flowering
T_1 (2,4-D, X)	41.07^{de}
T_2 (2,4-D, 2X)	42.93^{bcd}
T_3 (Almix®, X)	41.60^{cde}
T_4 (Almix®, 2X)	44.53^{b}
T_5 (Penox., X)	43.47^{bc}
T_6 (Penox., 2X)	46.93^{a}
T_7 (HWC)	39.47^{e}
T_8 (UWC)	43.47^{bc}
CD (0.05)	2.378

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.4 Yield attributes

4.4.1 Productive tillers/m^2

The productive tillers/m^2 of T_7 (294.33) was higher compared to other treatments, however T_1 (293.67) was on par with T_7 (294.33). Normal dose of herbicides showed relatively higher productive tillers compared to its twice the normal doses. The lowest productive tillers/ m^2 was noted in T_6 (279.50) which was on par with T_4 (281.33).

4.4.2 Spikelets/ panicle

The number of spikelets/ panicle of rice did not show any significant variation between the treatments. It ranged from 105.20-113.13.

4.4.3 Filled grains/ panicle

There were no significant difference in filled grains/ panicle of hand weeded plot, unweeded plot and herbicide applied plots and it ranged from 99-105.27.

4.4.4 Chaff %

Highest chaff percentage was noted in T_6 (10.26) which was on par with T_4 (9.98). T_2 (9.07) and T_8 (8.55) also showed comparatively higher chaff percentage, while normal dose of herbicides recorded relatively low chaff percentage which was on par with T_7 (6.00). However, the chaff% was significantly higher for T_5 (7.73) as compared to T_7 (6.00).

4.4.5 Thousand grain weight (g)

There was no significant difference among the treatments and it ranged from 29.37-29.55g.

Table. 23 Effect of herbicides on yield attributes

Treatment	Productive tillers/m^2	Spikelets/ panicle	Filled grains/ panicle	Chaff %	Thousand grain weight (g)
T_1 (2,4-D, X)	293.67^{a}	105.67	103.87	6.65^{ef}	29.53
T_2 (2,4-D, 2X)	282.83^{de}	107.00	100.33	9.07^{abc}	29.42
T_3 (Almix®, X)	289.50^{b}	112.33	103.07	7.39^{def}	29.50
T_4 (Almix®, 2X)	281.33^{e}	111.53	99.93	9.98^{ab}	29.40
T_5 (Penox., X)	288.33^{bc}	111.07	102.67	7.73^{cde}	29.48
T_6 (Penox., 2X)	279.50^{e}	105.20	99.00	10.26^{a}	29.37
T_7 (HWC)	294.33^{a}	113.13	105.27	6.00^{f}	29.55

T_8 (UWC)	285.00[cd]	108.60	101.53	8.55[bcd]	29.45
CD (0.05)	3.558	NS	NS	1.655	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.4.6 Grain yield

The grain yield of the herbicides applied plots was lower than the hand weeded plot (T_7, 4.49). However in treatments T_1 (4.35) and T_3 (4.22) grain yield was on par with hand weeded plot, while it was significantly lower in T_5 (4.10), T_8 (3.78), T_2 (3.49) and T_4 (3.43). The lowest value was observed in T_6 (3.01) which was significantly lower than the unweeded control.

4.4.7 Straw yield

In case of straw yield, T_7 (5.65) recorded maximum straw yield which was on par with T_1 (5.55). All other treatments showed significantly lower values. Twice the normal dose of herbicides application significantly reduced the straw yield and lowest straw yield was recorded in T_6 (4.36) and T_4 (4.48) followed by T_2 (4.56) which was lower than unweeded control (4.77).

Table. 24 Effect of herbicides on rice yield (t ha^{-1})

Treatment	Grain yield	Straw yield
T_1 (2,4-D, X)	4.35[ab]	5.55[a]
T_2 (2,4-D, 2X)	3.49[d]	4.56[e]
T_3 (Almix®, X)	4.22[ab]	5.34[b]
T_4 (Almix®, 2X)	3.43[d]	4.48[ef]
T_5 (Penox., X)	4.10[bc]	5.03[c]
T_6 (Penox., 2X)	3.01[e]	4.36[f]
T_7 (HWC)	4.49[a]	5.65[a]
T_8 (UWC)	3.78[cd]	4.77[d]

CD (0.05)	0.370	0.164

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.5 Observations on weeds

The major weeds observed on the experimental plots were broadleaved weeds and grasses. The only grass species observed was *Echinochloa crusgalli,* which was removed by hand weeding at weekly interval. *Eclipta alba, Ludwigia parviflora* and *Cyanotis axillaris* were the major broad leaved weeds.

4.5.1 Weed count

Data on weed count before and one week after herbicide application are given in Table 25. Before herbicide application there was no significant difference among treatments. One week after herbicide application, T_7 (0) showed the lowest weed count which was on par with T_2 (0.167). Application of twice the normal dose of herbicides reduced the weeds considerably compared to its normal dose application. Maximum weeds were noted in unweeded plot (T_8, 17) followed by T_3 (2.00), T_5 (2.67) and T_6 (1.67).

Weed count at one month after herbicide application is given in Table 26. Among the different weed species *Cyanotis axillaris* was controlled in herbicides applied plots while it was high in unweeded plot (2.17). *Ludwigia parviflora* was observed in all the treatments. T_8 (16.50) recorded the highest count followed by T_5 (7.50). T_7 (0.667) was on par with T_2 (0.833) and had the least value. Weed population was least in treatment where twice the normal dose of herbicides was applied. *Eclipta alba* was observed in T_8 (3.33) and T_3 (1.33). In all other treatments it was controlled. *Cyperus iria* was observed at one month after herbicide application in the unweeded and handweeded plots.

Table. 25 Total weed count before and one week after herbicide application (no m^{-2})

Treatment	Before herbicide application	7 DAH application
T_1 (2,4-D, X)	4.73 (22.83)	1.15^{cd} (0.833)
T_2 (2,4-D, 2X)	4.32 (18.83)	0.800^{ef} (0.167)
T_3 (Almix®, X)	4.23 (18.33)	1.58^{b} (2.00)
T_4 (Almix®, 2X)	4.62 (21.67)	1.08^{de} (0.667)
T_5 (Penox., X)	4.42 (19.83)	1.76^{b} (2.67)
T_6 (Penox., 2X)	4.48 (20.17)	1.47^{bc} (1.67)
T_7 (HWC)	5.31 (29.00)	0.710^{f} (0.00)
T_8 (UWC)	4.25 (18.17)	4.18^{a} (17.00)
CD (0.05)	NS	0.332

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control. *$\sqrt{x+0.5}$ transformed value, original value in parentheses.

Table. 26 Species wise weed count at one month after herbicide application (no m^{-2})

Treatment	*Cyanotis axillaris*	*Ludwigia parviflora*	*Eclipta alba*	*Cyperus iria*
T_1 (2,4-D, X)	0.707^b (0.00)	1.99^c (3.50)	0.707^c (0.00)	0.707 (0.00)
T_2 (2,4-D, 2X)	0.707^b (0.00)	1.15^d (0.833)	0.707^c (0.00)	0.707 (0.00)
T_3 (Almix®, X)	0.707^b (0.00)	2.30^c (4.83)	1.344^b (1.33)	0.707 (0.00)
T_4 (Almix®, 2X)	0.707^b (0.00)	2.08^c (3.83)	0.707^c (0.00)	0.707 (0.00)
T_5 (Penox., X)	0.707^b (0.00)	2.83^b (7.50)	0.707^c (0.00)	0.707 (0.00)
T_6 (Penox., 2X)	0.707^b (0.00)	2.34^c (5.00)	0.707^c (0.00)	0.707 (0.00)
T_7 (HWC)	0.707^b (0.00)	1.05^d (0.667)	0.707^c (0.00)	0.805 (0.170)
T_8 (UWC)	1.631^a (2.17)	4.09^a (16.50)	1.957^a (3.33)	0.977 (0.500)
CD (0.05)	0.054	0.460	0.132	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control. *$\sqrt{x+0.5}$ transformed value, original value in parentheses.

4.5.2 Weed dry weight

Data on effect of herbicides on weed dry weight at one week after herbicide application are given in Table 27. After one week, highest dry weight was recorded in T_8 (8.87) followed by T_5 (1.82) which was on par with T_3 (1.10). Hand weeded plot recorded the least dry weight (0) which was on par with T_2 (0.052). Twice the normal dose of herbicides showed relatively less weed dry weight compared to its normal doses.

Effect of herbicides on weed dry weight at one month after herbicide application are given in Table 28. In case of *Cyanotis axillaris*, herbicide application removed the weed completely and it was seen only in T_8 (36.20) which was the unweeded control. Maximum dry weight of *Ludwigia parviflora* was observed in T_8 (185.78) followed by T_5 (135.93). Minimum was noted in T_7 (8.20) which was on par with T_2 (14.06). Twice the normal dose of herbicides resulted in less weed dry weight compared to normal doses. In case of *Eclipta alba*, treatment T_8 (58.27) and T_3 (21.24) indicate the presence of the weed, while it was completely controlled in other herbicide treatments. One month after herbicide application, *Cyperus iria* was observed in hand weeded and unweeded plots.

Table. 27 Weed dry weight one week after herbicide application (kg ha^{-1})

Treatment	7 DAH application
T_1 (2,4-D, X)	0.885^{de} (0.290)
T_2 (2,4-D, 2X)	0.741^{e} (0.052)
T_3 (Almix®, X)	1.262^{bc} (1.10)
T_4 (Almix®, 2X)	0.877^{de} (0.273)
T_5 (Penox., X)	1.49^{b} (1.82)
T_6 (Penox., 2X)	1.12^{cd} (0.767)
T_7 (HWC)	0.707^{e} (0.00)

T_8 (UWC)	3.05[a] (8.87)
CD (0.05)	0.345

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control. *$\sqrt{x+0.5}$ transformed value, original value in parentheses.

Table. 28 Species wise dry weight one month after herbicide application (kg ha^{-1})

Treatment	*Cyanotis axillaris*	*Ludwigia parviflora*	*Eclipta alba*	*Cyperus iria*
T_1 (2,4-D, X)	0.707[b] (0.00)	8.15[d] (66.18)	0.707[c] (0.00)	0.707 (0.00)
T_2 (2,4-D, 2X)	0.707[b] (0.00)	3.79[e] (14.06)	0.707[c] (0.00)	0.707 (0.00)
T_3 (Almix®, X)	0.707[b] (0.00)	10.12[c] (102.58)	4.60[b] (21.24)	0.707 (0.00)
T_4 (Almix®, 2X)	0.707[b] (0.00)	8.83[cd] (78.18)	0.707[c] (0.00)	0.707 (0.00)
T_5 (Penox., X)	0.707[b] (0.00)	11.68[b] (135.93)	0.707[c] (0.00)	0.707 (0.00)
T_6 (Penox., 2X)	0.707[b] (0.00)	10.12[c] (101.87)	0.707[c] (0.00)	0.707 (0.00)
T_7 (HWC)	0.707[b] (0.00)	2.62[e] (8.20)	0.707[c] (0.00)	1.37 (2.27)
T_8 (UWC)	5.985[a] (36.20)	13.64[a] (185.78)	7.65[a] (58.27)	2.26 (5.95)
CD (0.05)	0.712	1.378	0.709	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control. *√x+0.5 transformed value, original value in parentheses.

4.5.3 Weed control efficiency

Effect of herbicides on weed control efficiency are given in Table 29. The plots applied with twice the normal dose of herbicides showed more weed control efficiency than that of their normal dose. One week after herbicide application, T_7 (100) showed maximum weed control efficiency which was on par with all other herbicide treatments except T_5 (77.17). At one month after herbicide application, T_7 (96.63) recorded maximum weed control efficiency which was on par with T_2 (95.37). Lowest value was noted in T_5 (53.87) which was on par with T_3 (57.52).

Table. 29 Effect of herbicides on weed control efficiency (%)

Treatment	7 DAH application	One month after herbicide application
T_1 (2,4-D, X)	96.85^{a}	77.81^{b}
T_2 (2,4-D, 2X)	99.42^{a}	95.37^{a}
T_3 (Almix®, X)	87.64^{ab}	57.52^{de}
T_4 (Almix®, 2X)	96.53^{a}	73.79^{bc}
T_5 (Penox., X)	77.17^{b}	53.87^{e}
T_6 (Penox., 2X)	91.24^{a}	65.28^{cd}
T_7 (HWC)	100^{a}	96.63^{a}
T_8 (UWC)	-	-
CD (0.05)	12.909	10.748

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.5.4 Weed persistence index

Effect of herbicides on weed persistence index are given in Table 30. One week after herbicide application, T_5 (1.27) showed highest weed persistence index which was on par T_3 (1.07) and T_6 (1.03). T_7 (0) showed the lowest WPI which was on par with T_2 (0.202). One month after herbicide application, there was no significant difference among treatments.

Table. 30 Effect of herbicides on weed persistence index

Treatment	7 DAH application	One month after herbicide application
T_1 (2,4-D, X)	0.664^{bc}	1.47
T_2 (2,4-D, 2X)	0.202^{cd}	1.36
T_3 (Almix®, X)	1.07^{ab}	1.56
T_4 (Almix®, 2X)	0.786^{b}	1.31
T_5 (Penox., X)	1.27^{a}	1.43
T_6 (Penox., 2X)	1.03^{ab}	1.60
T_7 (HWC)	0.00^{d}	0.51
T_8 (UWC)	-	-
CD (0.05)	0.472	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}, HWC- hand weeded control, UWC- unweeded control.

4.6 Net house study

4.6.1 *Marsilea quadrifoliata*

When normal dose of 2,4-D was applied, plants showed drooping symptom on the 4th day, but they recovered from the symptom by 6th day. Application of twice the normal dose of 2,4-D also lead to drooping and yellowing of leaf on the 4th day after herbicide application, however it took more days (8 days) for recovery.

Application of both the doses of Almix® showed 40% yellowing of leaves on 4th day after herbicide application. On 6th day, weed showed 60% of yellowing symptom for normal dose and 70% yellowing symptom for twice the normal dose of Almix®. Yellowing of plants increased on 10th day, 80% symptom was observed for normal dose of Almix® and 90% for twice the normal dose of Almix®. Twice the normal dose of Almix® showed complete drying by 10th day, while normal dose took 12 days after herbicide application for complete drying of the weed.

On 4th day after herbicide application, 20% of the plants showed yellowing symptoms on application of both the doses of penoxsulam. Symptoms increased and became 40% by 10th day for normal dose, there after symptoms persisted. When twice the normal dose of herbicide was applied plants showed 60% yellowing by 14th day and there after no further symptoms was observed.

Table. 31 Effect of herbicides on weed *Marsilea quadrifoliata* (days)

Treatment	DAH application									
	2	4	6	8	10	12	14	16	18	20
T_1 (2,4-D, X)	0	1	0	0	0	0	0	0	0	0
T_2 (2,4-D, 2X)	0	1	1	0	0	0	0	0	0	0
T_3 (Almix®, X)	0	2	3	3	4	5	5	5	5	5
T_4 (Almix®, 2X)	0	2	3	4	5	5	5	5	5	5
T_5 (Penox., X)	0	1	1	1	2	2	2	2	2	2
T_6 (Penox., 2X)	0	1	1	1	2	2	3	3	3	3
T_7 (Control)	0	0	0	0	0	0	0	0	0	0

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.1.1Chlorophyll stability index

There was no significant difference among the treatments except for T_4 (54.93).

2,4-D (X) | 2,4-D (2X)

0

0

1-4th day

1-4th day

0-12th day

0-12th day

Plate. 6. Effect of 2,4-D (normal, X and twice the normal, 2X) on *Marsilea quadrifoliata*

Almix® (X) **Almix® (2X)**

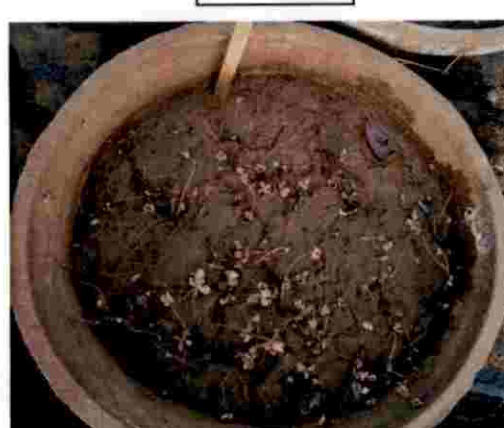
0

0

4-10th day

4-8th day

5- 12th day



5-10th day

Plate. 7. Effect of Almix® (normal, X and twice the normal, 2X) on *Marsilea quadrifoliata*

Penoxsulam (X)	Penoxsulam (2X)
	
0	0
	
1-4th day	**1**-4th day
	
2-14th day	**3**-14th day

Plate. 8. Effect of penoxsulam (normal, X and twice the normal, 2X) on *Marsilea quadrifoliata*

4.6.1.2 Catalase enzyme activity

Catalase enzyme activity was recorded highest in T_2 (65.28) which was on par with T_1 (53.72) and T_5 (47.83). Lowest was recorded in T_7 (26.29) which was on par with all other herbicides.

4.6.1.3 SOD enzyme activity

Herbicides applied plots showed higher SOD enzyme activity except T_4 (0.559), which was on par with T_7 (0.543).

4.6.1.4 Wax content of leaf

There was no significant difference in wax content of leaf due to herbicide application and it ranged from 0.002-0.006 mg.

Table. 32 Effect of herbicides on biochemical parameters of weed *Marsilea quadrifoliata*

Treatment	CSI	Catalase enzyme activity (enzyme units g^{-1})	SOD enzyme activity (enzyme units mg^{-1} FW)	Wax content of leaf (mg)
T_1 (2,4-D, X)	96.03^{a}	53.72^{ab}	0.663^{ab}	0.006
T_2 (2,4-D, 2X)	88.21^{ab}	65.28^{a}	0.746^{a}	0.002
T_3 (Almix®, X)	66.14^{ab}	$33.55b^{c}$	0.562^{ab}	0.002
T_4 (Almix®, 2X)	54.93^{b}	28.10^{c}	0.559^{b}	0.002
T_5 (Penox., X)	82.31^{ab}	47.83^{abc}	0.653^{ab}	0.002
T_6 (Penox., 2X)	70.34^{ab}	33.77^{bc}	0.576^{ab}	0.002
T_7 (Control)	-	26.29^{c}	0.543^{b}	0.002
CD (0.05)	40.84	24.26	0.186	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.2 *Ludwigia parviflora*

Ludwigia parviflora showed 43% and 50% yellowing symptom for normal and twice the normal dose of 2,4-D respectively on 2nd day after herbicide application. On 4th day after herbicide application, normal dose of 2,4-D caused 50 % yellowing of leaves while twice the normal dose resulted 90% yellowing of leaves. Twice the normal dose of application caused complete drying of the plant by 8th day, while normal dose caused 90% drying. On 10th day after herbicide application, complete drying of plant occurred for normal dose of 2,4-D applied pots.

Observation taken on the 4th day after Almix® application showed 20% and 40% yellowing of leaves with normal and twice the normal doses respectively. On 8th day after herbicide application, 50% yellowing of plants was seen in normal dose of Almix® applied plants and 80% yellowing of plants for twice the normal dose. Complete drying of plant was seen on 10th day after herbicide application only for twice the normal dose of Almix® while 70% yellowing persisted for normal dose of Almix®.

Both the doses of penoxsulam recorded only 10% yellowing of leaves on 8th day after herbicide application and symptom persisted.

Table. 33 Effect of herbicides on *Ludwigia parviflora* (days)

Treatment	DAH application							
	2	4	6	8	10	11	12	14
T_1 (2,4-D, X)	3	3	3	5	5	5	5	5
T_2 (2,4-D, 2X)	3	5	5	5	5	5	5	5
T_3 (Almix®, X)	0	1	1	3	4	4	4	4
T_4 (Almix®, 2X)	0	3	3	4	5	5	5	5
T_5 (Penox., X)	0	0	0	1	1	1	1	1

T_6 (Penox., 2X)	0	0	0	1	1	1	1	1
T_7 (Control)	0	0	0	0	0	0	0	0

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.2.1 Chlorophyll stability index

Chlorophyll stability index did not show significant variation among treatments except T_2 (46.39).

4.6.2.2 Catalase enzyme activity

There was no significant difference in catalase activity of different treatments and it ranged from 46.39-67.66 enzyme units g^{-1}.

4.6.2.3 SOD enzyme activity

There was no significant difference in SOD enzyme activity of different treatments and it ranged from 1.28- 1.71 enzyme units mg^{-1} FW.

4.6.2.4 Wax content of leaf

Wax content of leaf showed no significant difference among treatments and it ranged from 0.001-0.006 mg.

2,4-D (X) | **2,4-D (2X)**

0

0

3-4th day

3-2nd day

5-8th day

5-4th day

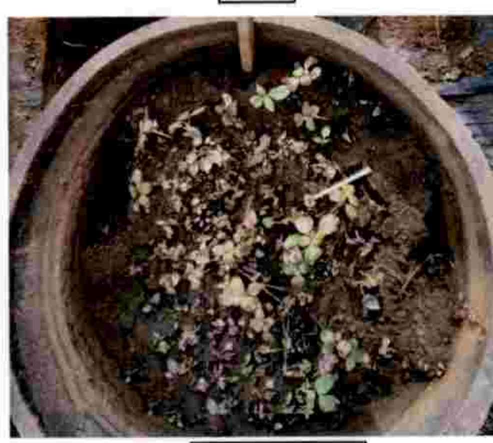
Plate. 9. Effect of 2,4-D (normal, X and twice the normal, 2X) on *Ludwigia parviflora*

Almix® (X) | **Almix® (2X)**

0

0

1-4th day



4-8th day

4-10th day

5-10th day

Plate. 10. Effect of Almix® (normal, X and twice the normal, 2X) on *Ludwigia parviflora*

Penoxsulam (X)

0

1-8th day

1-14th day

Penoxsulam (2X)

0

1-8th day

1-14th day

Plate. 11. Effect of penoxsulam (normal, X and twice the normal, 2X) on *Ludwigia parviflora*

Table. 34 Effect of herbicides on biochemical parameters of weed *Ludwigia parviflora*

Treatment	CSI	Catalase enzyme activity (enzyme units g^{-1})	SOD enzyme activity (enzyme units mg^{-1} FW)	Wax content of leaf (mg)
T_1 (2,4-D, X)	46.86[ab]	59.61	1.33	0.002
T_2 (2,4-D, 2X)	46.39[b]	60.75	1.29	0.001
T_3 (Almix®, X)	54.61[ab]	61.65	1.49	0.004
T_4 (Almix®, 2X)	53.83[ab]	61.20	1.34	0.003
T_5 (Penox., X)	67.66[a]	61.88	1.70	0.004
T_6 (Penox., 2X)	62.35[ab]	62.33	1.71	0.006
T_7 (Control)	-	56.67	1.28	0.003
CD (0.05)	21.140	NS	NS	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.3 *Fimbristylis miliacea*

Application of both the doses of 2,4-D caused 10% yellowing of *Fimbristylis miliacea* on 2nd day after herbicide application, while by the 4th day, symptoms increased and became 20% and 50% respectively for normal and twice the normal dose. On 6th day, normal dose recorded 50% yellowing of plants, while twice the normal dose recorded 90% yellowing. Complete drying of the plants was observed by the 8th day for twice the normal dose and 10th day for normal dose.

Application of normal and twice the normal dose of Almix® recorded 50% yellowing of plants on 6th day after herbicide application. However, regrowth of plant occurred later.

In case of penoxsulam yellowing symptom was observed in the lower leaves. 50 per cent yellowing of plants was recorded for normal dose and twice the normal dose on 6th and 4th day after herbicide application respectively. Complete drying of plant was noted on 6th day for twice normal dose and 10th day for normal dose.

Table. 35 Effect of herbicides on weed *Fimbristylis miliacea* (days)

Treatment	DAH application				
	2	4	6	8	10
T_1 (2,4-D, X)	1	1	3	5	5
T_2 (2,4-D, 2X)	1	3	5	5	5
T_3 (Almix®, X)	0	0	3	3	3
T_4 (Almix®, 2X)	0	0	3	3	3
T_5 (Penox., X)	0	0	3	3	5
T_6 (Penox., 2X)	0	3	5	5	5
T_7 (Control)	0	0	0	0	0

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.3.1 Chlorophyll stability index

There was no significant difference among different treatments in case of chlorophyll stability index and it ranged from 46.46-67.18.

4.6.3.2 Catalase enzyme activity

Catalase enzyme activity showed no significant difference among treatments and it ranged from 68.91-76.16 enzyme units g^{-1}.

4.6.3.3 SOD enzyme activity

SOD enzyme activity was higher in all herbicide applied pots compared to control (T_7, 0.879).

2,4-D (X) | **2,4-D (2X)**

0 | **0**

1-2nd day | **1**-2nd day

5-8th day | **5**-6th day

Plate. 12. Effect of 2,4-D (normal, X and twice the normal, 2X) on *Fimbristylis miliacea*

Almix® (X) | **Almix® (2X)**

0

0

0- 4th day

0- 4th day

3-6th day

3-6th day

Plate. 13. Effect of Almix® (normal, X and twice the normal, 2X) on *Fimbristylis miliacea*

Penoxsulam (X) **Penoxsulam (2X)**

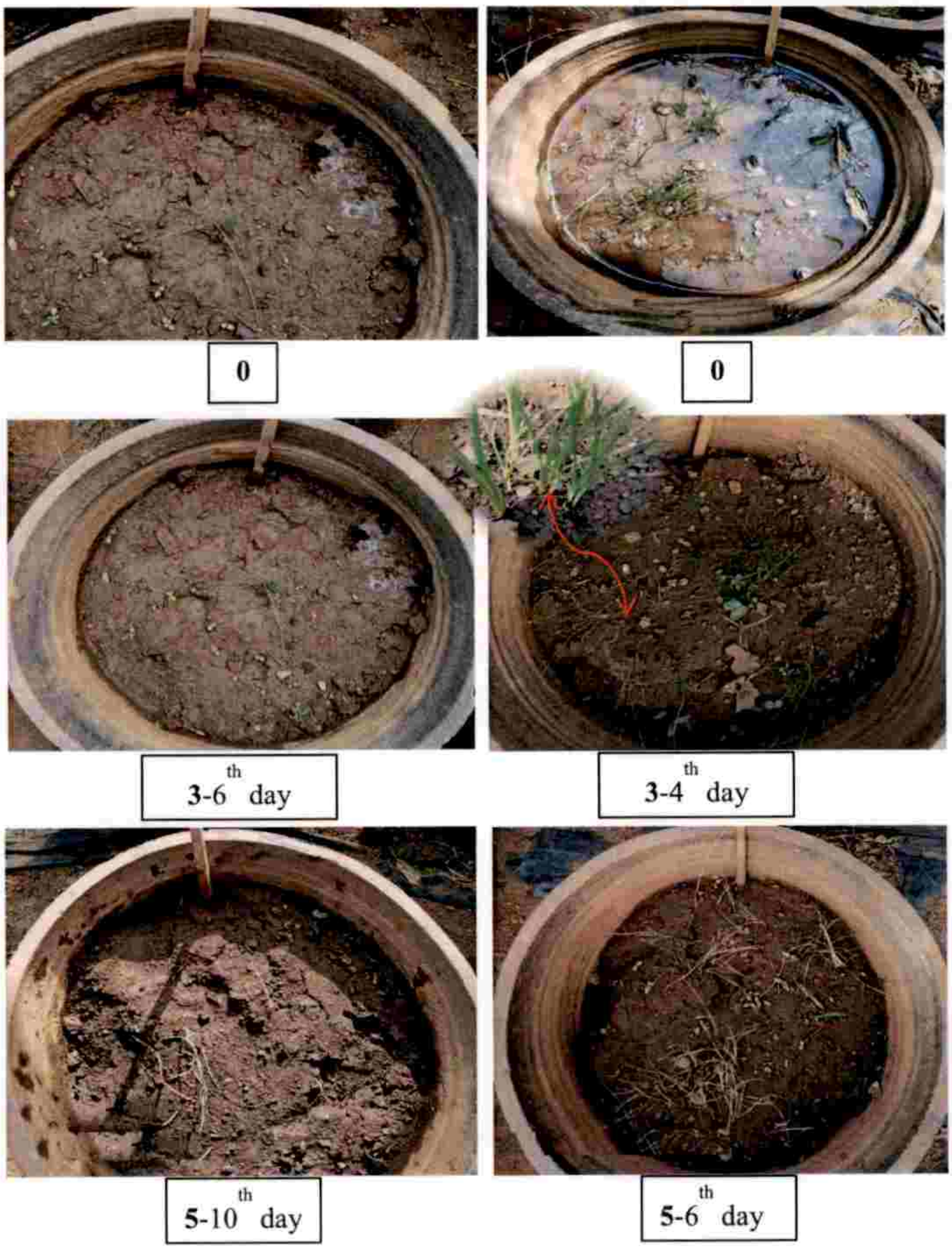


Plate. 14. Effect of penoxsulam (normal, X and twice the normal, 2X) on *Fimbristylis miliacea*

4.6.3.4 Wax content of leaf

There was no significant difference in wax content of leaf among different treatments and it ranged from 0.002-0.067 mg.

Table. 36 Effect of herbicides on biochemical parameters of weed *Fimbristylis miliacea*

Treatment	CSI	Catalase enzyme activity (enzyme units g^{-1})	SOD enzyme activity (enzyme units mg^{-1} FW)	Wax content of leaf (mg)
T_1 (2,4-D, X)	52.61	73.44	1.10^{ab}	0.005
T_2 (2,4-D, 2X)	48.72	73.44	1.04^{ab}	0.003
T_3 (Almix®, X)	59.84	75.71	1.21^{ab}	0.005
T_4 (Almix®, 2X)	67.18	76.16	1.53^{a}	0.067
T_5 (Penox., X)	59.72	74.35	1.11^{ab}	0.005
T_6 (Penox., 2X)	46.46	70.95	1.01^{ab}	0.002
T_7 (Control)	-	68.91	0.879^{b}	0.003
CD (0.05)	NS	NS	0.603	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.4 *Cyperus iria*

When 2,4-D was applied 20% of the plants showed yellowing symptoms by the 2nd day in case of twice the normal dose of the chemical. Yellowing symptom symptoms was visible in the normal dose only by the 6th day while twice the normal dose recorded 40% yellowing of leaves. By the 14th day 60% of the plants in the normal dose of 2,4-D showed yellowing while it was 90% for twice the normal dose. Complete drying of *Cyperus iria* with 2,4-D was seen only by the 18th day for normal dose and 16th day for twice the normal dose of herbicide.

In case of Almix®, only 10% of plants showed yellowing symptoms in both normal and twice the normal dose on the 14th day and left plant survived from toxicity.

Application of normal dose of penoxsulam showed no symptoms on *Cyperus iria*, 20% of the plants showed yellowing symptoms for twice the normal dose on 14th day and plant recovered from the symptom on 16th day after herbicide application.

Table. 37 Effect of herbicides on weed *Cyperus iria* (days)

Treatment	DAH application								
	2	4	6	8	10	12	14	16	18
T_1 (2,4-D, X)	0	0	2	2	2	2	3	3	5
T_2 (2,4-D, 2X)	1	0	2	2	2	2	5	5	5
T_3 (Almix®, X)	0	0	0	0	0	0	1	0	0
T_4 (Almix®, 2X)	0	0	0	0	0	0	1	0	0
T_5 (Penox., X)	0	0	0	0	0	0	0	0	0
T_6 (Penox., 2X)	0	0	0	0	0	0	1	0	0
T_7 (Control)	0	0	0	0	0	0	0	0	0

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.4.1 Chlorophyll stability index

T_5 (61.41) was recorded with maximum CSI which was on par with all the treatments except T_2 (28.95).

4.6.4.2 Catalase enzyme activity

There was no significant different difference in catalase enzyme activity among treatments and it ranged from 61.77-70.27 enzyme units g^{-1}.

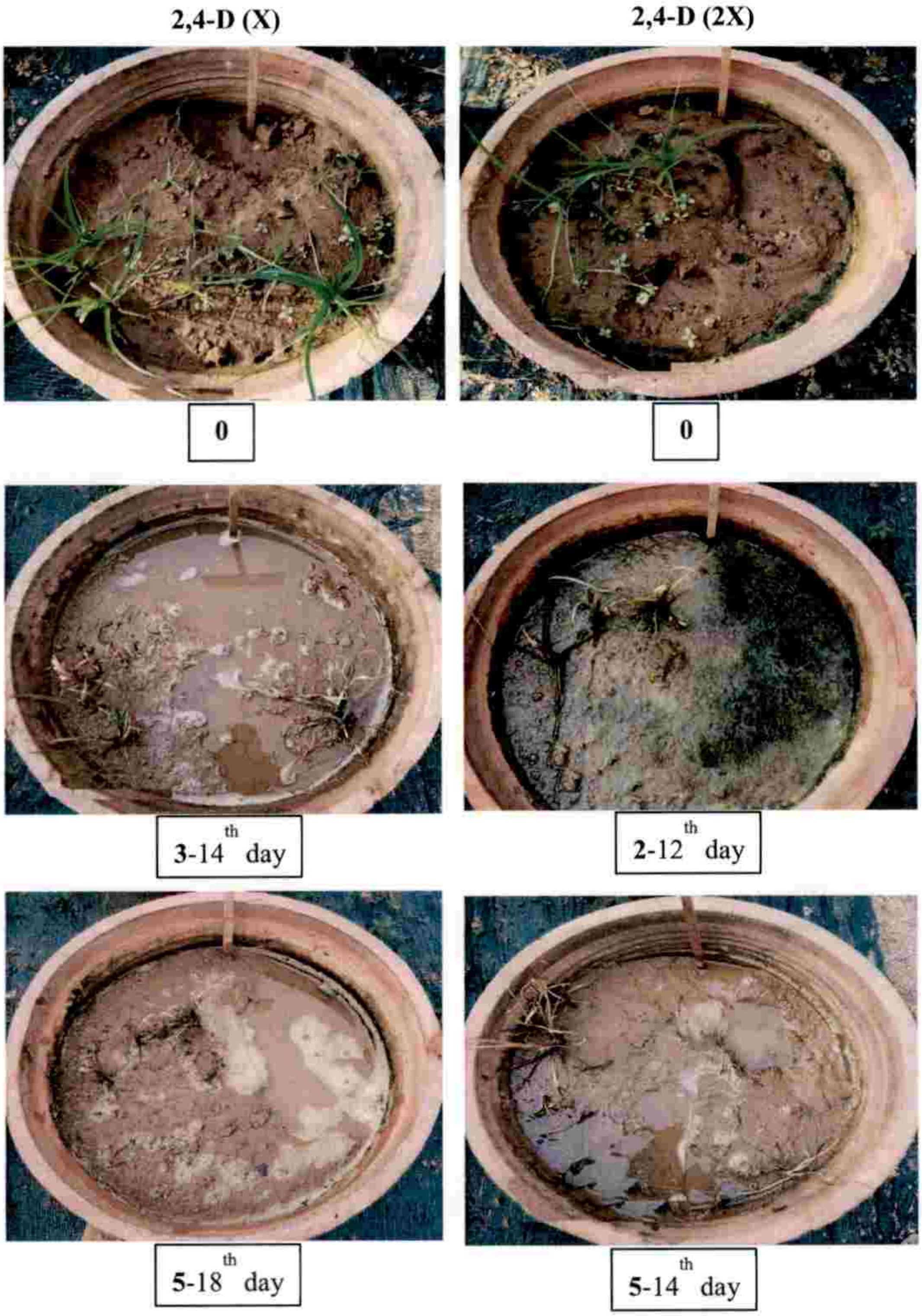
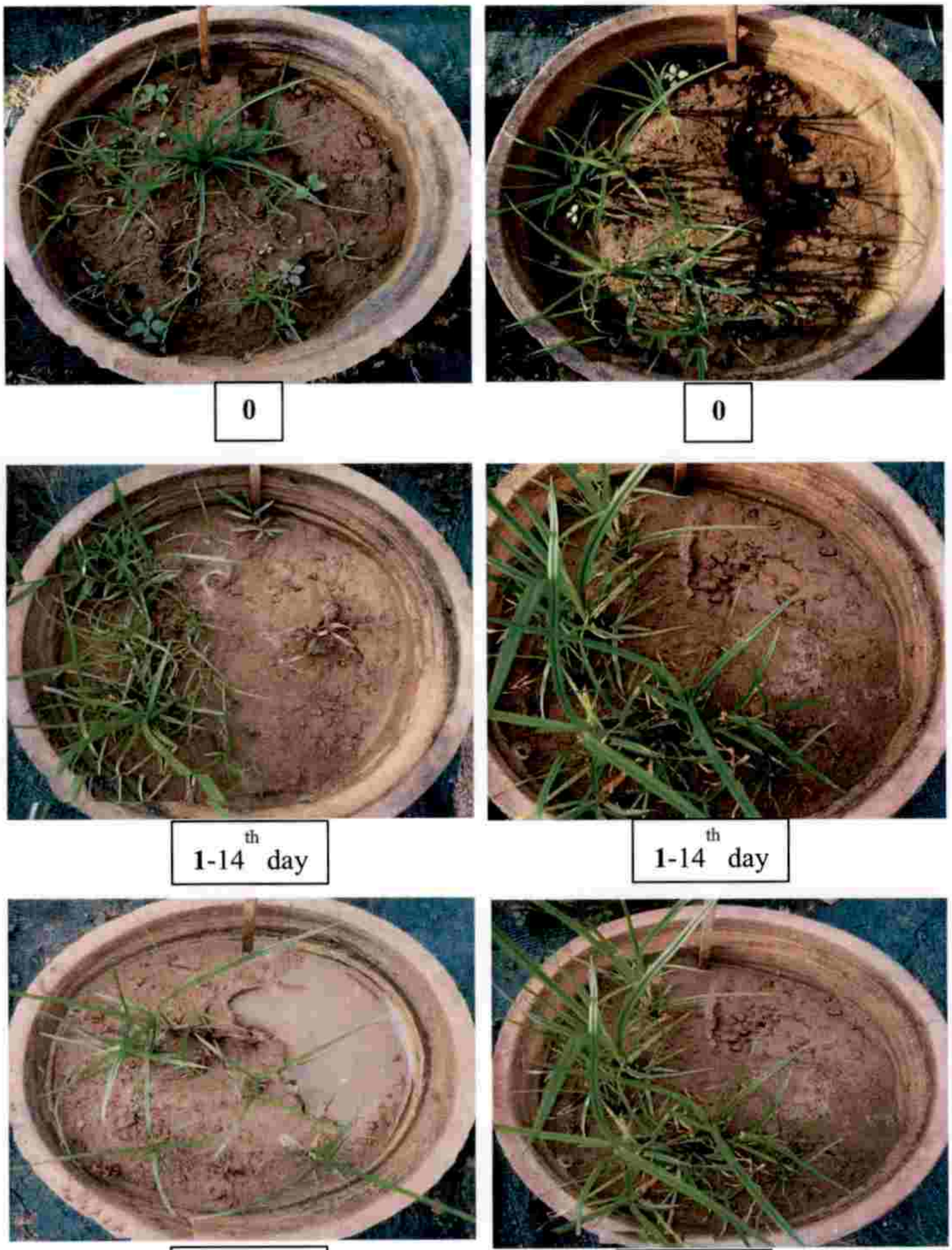


Plate. 15. Effect of 2,4-D (normal, X and twice the normal, 2X) on *Cyperus iria*

Almix® (X) **Almix® (2X)**



0 0

1-14th day 1-14th day

0-16th day 0-16th day

Plate. 16. Effect of Almix® (normal, X and twice the normal, 2X) on *Cyperus iria*

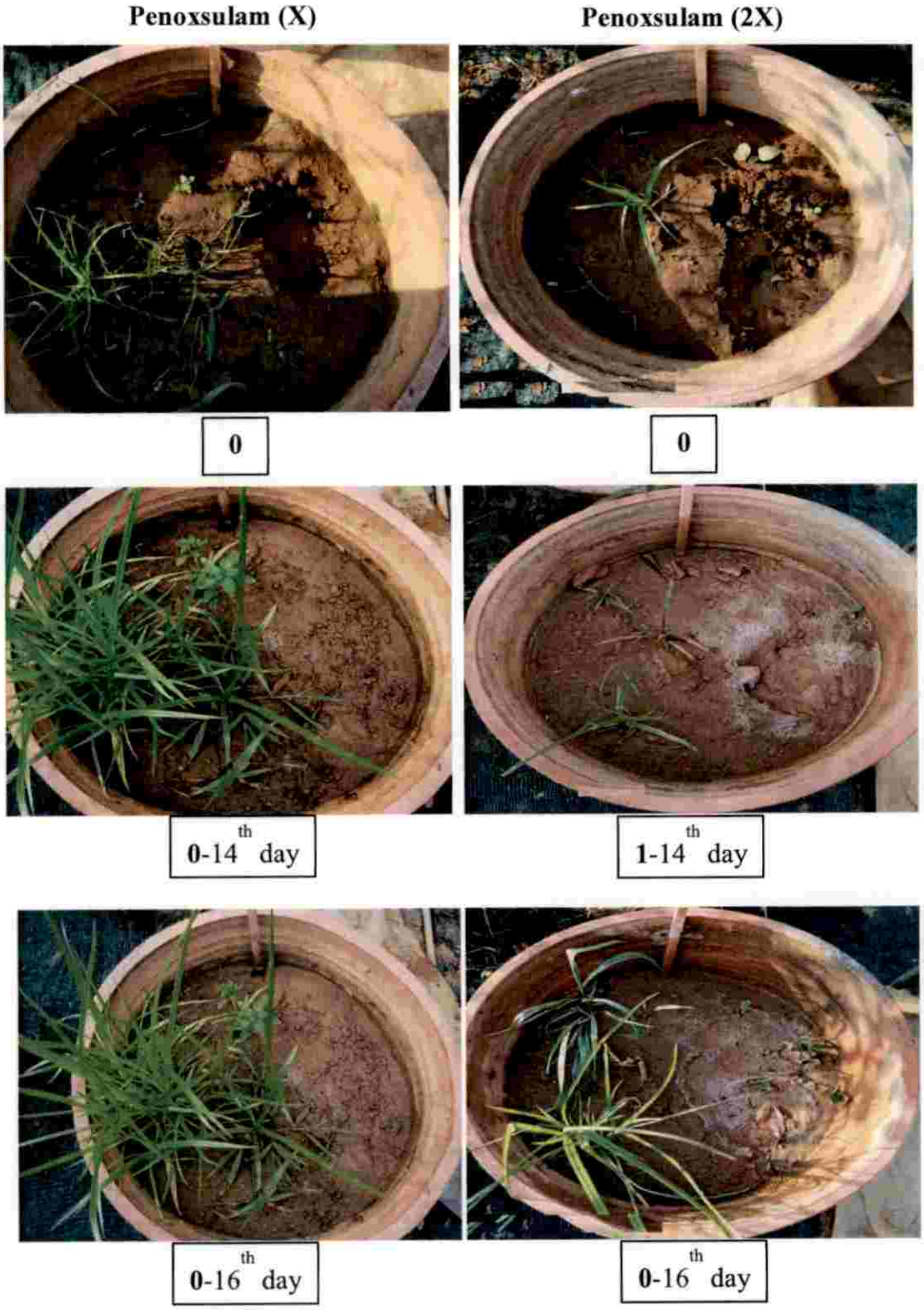


Plate. 17. Effect of penoxsulam (normal, X and twice the normal, 2X) on *Cyperus iria*

4.6.4.3 SOD enzyme activity

Herbicides applied pots recorded higher SOD enzyme activity compared to control (T_7, 0.170) except T_2 (0.242), which was on par with control.

4.6.4.4 Wax content of leaf

There was no significant difference in wax content among treatments and it ranged from 0.002-0.016 mg.

Table. 38 Effect of herbicides on biochemical parameters of weed *Cyperus iria*

Treatment	CSI	Catalase enzyme activity (enzyme units g^{-1})	SOD enzyme activity (enzyme units mg^{-1} FW)	Wax content of leaf (mg)
T_1 (2,4-D, X)	39.13^{ab}	63.47	0.756^{ab}	0.003
T_2 (2,4-D, 2X)	28.95^{b}	62.90	0.242^{b}	0.002
T_3 (Almix®, X)	57.19^{a}	63.47	0.774^{ab}	0.008
T_4 (Almix®, 2X)	39.95^{ab}	66.30	0.806^{ab}	0.010
T_5 (Penox., X)	61.41^{a}	70.27	1.51^{a}	0.016
T_6 (Penox., 2X)	60.16^{a}	68.57	1.20^{a}	0.014
T_7 (Control)	-	61.77	0.170^{b}	0.004
CD (0.05)	27.62	NS	0.892	NS

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.5 *Sphenoclea zeylanica*

In case of herbicide 2,4-D, normal dose recorded 20% yellowing of plant and 40% yellowing of plant for twice the normal dose on 4th day after herbicide application. On the 6th day, normal dose showed 60% of symptoms and twice the normal dose showed 80% of symptoms respectively. Normal dose recorded 80%

yellowing of plant on 8th day, while twice the normal dose recorded 90% yellowing. Complete drying of plant was observed on 10th day after herbicide application for twice the dose while normal dose recorded 90% drying of plant. On 12th day, normal dose 2,4-D showed complete drying of plant.

In case of Almix®, twice the normal dose showed 20% yellowing of plant on 4th day after herbicide application. On 6th day, normal dose showed 20% yellowing of plant, while twice the normal dose recorded 40% destruction. On 8th day, normal dose showed no progression in yellowing and twice the normal dose showed 50% yellowing of plant. On 10th day, 80% yellowing of plant was seen for twice the normal dose and 50 % yellowing for normal dose applied plant, while complete drying of plant was recorded in twice the normal dose and 80% yellowing for normal dose recorded by 12th day. Complete drying of plant was seen for normal dose on 14th day after herbicide application.

In case of penoxsulam, 10% of yellowing of plant was noted for normal and twice the normal dose applied plant by 4th day. On 6th, 60% yellowing of plant was recorded in normal dose and twice the normal dose. Twice the normal dose showed 60% of symptom at 8th day, while 90% of yellowing by the normal dose. On 12th day, complete drying of plant was seen for the normal dose and 80% yellowing of plant was recorded for twice the normal dose of herbicide application. Complete drying of plant occurred on 12th day for twice the normal dose.

Table. 39 Effect of herbicides on weed *Sphenoclea zeylanica* (days)

Treatment	DAH application						
	2	**4**	**6**	**8**	**10**	**12**	**14**
T_1 (2,4-D, X)	0	1	3	4	5	5	5
T_2 (2,4-D, 2X)	0	2	4	5	5	5	5
T_3 (Almix®, X)	0	0	1	1	3	4	5
T_4 (Almix®, 2X)	0	1	3	4	4	5	5
T_5 (Penox., X)	0	1	4	5	5	5	5

10U

T_6 (Penox., 2X)	0	1	3	3	4	5	5
T_7 (Control)	0	0	0	0	0	0	0

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

4.6.5.1 Chlorophyll stability index

Effect of herbicides on chlorophyll stability index of weed *Sphenoclea zeylanica* is given in Table 40. The highest value was recorded in T_3 (48.17) followed by T_4 (28.51). Lowest value was noted in T_2 (10.62) which was on par with T_5 (15.45).

4.6.5.2 Catalase enzyme activity

There was no significant difference in catalase enzyme activity of different treatments and it ranged from 27.20-39 enzyme units g^{-1}.

4.6.5.2 SOD enzyme activity

Effect of herbicides on SOD enzyme activity of weed *Sphenoclea zeylanica* is given in Table 40. Herbicide applied pots showed higher SOD enzyme activity compared to control. The highest was recorded in T_3 (0.665) which was on par with T_4 (0.571). The lowest SOD enzyme activity noted in T_7 (0.193) which was on par with T_2 (0.281) and T_5 (0.348).

4.6.5.3 Wax content of leaf

Effect of herbicides on wax content of leaf is given in Table 40. Among herbicide applied pots, T_3 (0.038) showed the highest wax content which was on par with T_4 (0.024).

2,4-D (X)	**2,4-D (2X)**

0 | **0**

1-4th day | **2**-4th day

5-10th day | **5**-8th day

Plate. 18. Effect of 2,4-D (normal, X and twice the normal, 2X) on *Sphenoclea zeylanica*

Almix® (X) | **Almix® (2X)**

0

0

1-6th day

1-4th day

5-14th day

5-12th day

Plate. 19. Effect of Almix® (normal, X and twice the normal, 2X) on *Sphenoclea zeylanica*

Penoxsulam (X) | **Penoxsulam (2X)**

0

0

1-4th day

1-4th day

5-8th day

5-12th day

Plate. 20. Effect of penoxsulam (normal, X and twice the normal, 2X) on *Sphenoclea zeylanica*

Table. 40 Effect of herbicides on biochemical parameters of weed *Sphenoclea zeylanica*

Treatment	CSI	Catalase enzyme activity (enzyme units g^{-1})	SOD enzyme activity (enzyme units mg^{-1} FW)	Wax content of leaf (mg)
T_1 (2,4-D, X)	20.54^{cd}	32.30	0.411^{bc}	0.006^{bc}
T_2 (2,4-D, 2X)	10.62^{e}	27.20	0.281^{cd}	0.004^{c}
T_3 (Almix®, X)	48.17^{a}	39.10	0.665^{a}	0.038^{a}
T_4 (Almix®, 2X)	28.51^{b}	39.00	0.571^{ab}	0.024^{ab}
T_5 (Penox., X)	15.45^{de}	32.30	0.348^{cd}	0.005^{bc}
T_6 (Penox., 2X)	22.57^{bc}	34.00	0.458^{bc}	0.008^{bc}
T_7 (Control)	-	27.77	0.193^{d}	0.004^{c}
CD (0.05)	6.071	NS	0.182	0.019

2,4-D, X and 2X- 0.8 and 1.6 kg ai ha^{-1}, Almix®, X and 2X- 0.004 and 0.008 kg ai ha^{-1}, Penoxsulam, X and 2X- 0.025 and 0.05 kg ai ha^{-1}.

Discussion

5. DISCUSSION

Chemical weed control is a cost effective method to control weeds in the rice ecosystem. 2,4-D, penoxsulam and Almix® are popular post emergent herbicides commonly used for control of broad leaved weeds in rice.

The main focus of the present study is to identify broad leaved weeds and sedges that can be selectively controlled by Almix®, penoxsulam and 2,-D as well as the dose of herbicides required for this. It also aims to evaluate the effect of these herbicides on morphological, physiological and biochemical parameters of rice plant. An attempt is made from the result obtained to discuss the effect of these parameters on yield and yield attributes of rice.

5.1 Effect of herbicides on morphological characters of rice

Application of chemical herbicides was found to affect the morphological attributes of rice such as plant height and tiller number and phenophases of the crop such as days to flowering.

In the present study normal doses of the herbicides *viz.*, 2,4-D, Almix® and penoxsulam applied on the 20th day after broadcasting resulted in 3-6% reduction in plant height at the time of harvest. When twice the normal dose of the herbicide was applied it contributed to 10-12% reduction. Among the herbicides penoxsulam showed maximum height reduction followed by Almix® while 2,4-D had the least effect on rice crop. Penoxsulam and Almix® are both ALS inhibiting herbicides while 2,4-D is a synthetic Auxin. Menon (2012) stated that post emergent herbicides such as Almix®, fenaxaprop p-ethyl and bispyribac- sodium showed a slight reduction in plant height of rice compared with hand weeded plot, though it did not show any phytotoxicity symptom.

Tiller number of rice crop was found to be affected by herbicide application. During early tillering phase (40 DAS) tiller number was significantly lower in herbicide applied plots when compared with hand weeded plot. At the time of harvest tiller number of 2,4-D (normal dose) treatment was on par with hand weeded plot while in case of Almix® and penoxsulam reduction of 4 and 5 % respectively was noticed. Twice the normal dose of herbicide adversely affected tiller number in all three herbicide treatments and it was significantly lower than unweeded control. In twice the normal dose of ALS inhibiting herbicides, it was less than unweeded control, while unweeded control was on par with twice the normal dose of 2,4-D. The reduction in plant height and tiller number in Almix® and penoxsulam where the twice the recommended dose was applied may be due to the inhibition of acetolactose synthase enzyme which is involved in production of branched chain amino acids. Koschnick *et al.* (2007) had also reported inhibition of protein synthesis and enzymes by ALS inhibiting herbicides as the cause for growth reduction.

The number of days taken for flowering was found to be affected by herbicide application. It was delayed by 3 days in case of twice the normal dose of penoxsulam compared to handweeded control. Ramanarayana (2014) has reported that ALS inhibiting and ACCase herbicides caused a reduction in tiller number, plant height and delay in flowering compared to hand weeded plot.

5.2 Effect of herbicide on growth indices

In the present study, the maximum growth rate of the rice plant was observed during the 20-40 DAS. Hand weeded control and the plants in the plots where normal dose of 2,4-D herbicide was applied showed on par RGR values while there was a 3.3 to 3.8% reduction in the RGR values in those plants where twice the normal dose of herbicides was applied indicating that excess herbicides application has a negative influence on the growth rate of the rice plant. Similar results were reported by Nithya (2016) and Ramanarayana (2014) in rice. Throughout the growth stages of the crop, the trend followed for RGR was similar.

Cumulative increase in dry matter production per unit area (CGR) was found to be significantly different among treatments after the critical period of growth (45-75 DAS). Similar findings have been reported by Singh *et al.* (2014) in rice. They reported that application of herbicides 0-30 DAS did not contribute to change in CGR during the initial growth but at the later stages of growth there was significant reduction in CGR values as compared to handweeded control. NAR is a product of LAI and CGR. In the present study NAR also showed the same trend as that of CGR. Twice the normal dose of herbicide applied plots showed more reduction in CGR and NAR compared to normal dose applied plots. Channappagaudar *et al.* (2008) reported that herbicide application caused a reduction in NAR and CGR compared to handweeded control. Early reduction in LAI might have contributed to the reduction in dry matter accumulation in the later stages.

Herbicide application significantly reduced the LAI of the rice plant in all stages of the crop. However, among the treatment 2,4-D normal dose was on par with control during 40^{th}, 60^{th}, and 80^{th} day and there after it showed significant reduction. Normal dose of penoxsulam and Almix® were on par and showed significantly lower values than control. Maximum reduction was observed in the treatments where twice the normal dose of herbicides were applied. Muhammad *et al.* (2015) observed that LAI of herbicide applied plot was less than hand weeded plot in direct seeded rice.

5.3 Phytotoxicity screening of rice plant

Phytotoxicity screening of the rice crop was done on third and fifth day after herbicide application. Among the herbicide treatments symptoms such as tip burn and aerial root development was seen only in the 2,4-D applied plots. The recovery of the crop was faster in the treatments where normal herbicide dosage was given while the plants took more time to recover in those plots where twice the normal dose of 2,4-D was applied. This is in confirmation with the findings of Okfor (1986). He stated that post emergent application of 2,4-D amine showed slight burn and yellowing of leaves, however the plant recovered from the injury. ALS inhibiting herbicides such as penoxsulam and Almix® showed no visual phytotoxicity symptoms. This was validated by Ramanarayana (2014).

5.4 Effect of herbicides on physiological parameters of rice

5.4.1 Chlorophyll content

Estimation of chlorophyll content of the rice plants at one week after herbicide application and flowering stage revealed that there was significant reduction in chlorophyll a in both the dosages of herbicides as compared to handweeded control. This was reflected in the total chlorophyll content also. However, chlorophyll b content did not show significant variation in the treatments where normal dose of herbicides was applied. Twice the normal dose of the herbicides significantly reduced both chlorophyll a and chlorophyll b content. Sahoo *et al.* (1993) reported that the effect of post emergence herbicide on total chlorophyll content was dose dependent especially during the early stages of the crop.

At flowering, chlorophyll b content was found to be significantly lower than the handweeded control in all the treatments. However the values of chlorophyll a was found to be on par with the handweeded control in case of treatments where normal dose of the herbicides was applied. This has been validated by the findings of Ralph *et al.* (2000). He observed that higher doses of simazine herbicide showed a reduction in chlorophyll b content while chlorophyll a showed no difference among treatments. Application of twice the normal dose of herbicides reduced the chlorophyll content in all the three chemicals tested. This might be because herbicides in general inhibit the common enzyme between the pathway of chlorophyll and cytochrome synthesis and caused the formation of an intermediate tetrapyrroline which prevented the formation of chlorophyll pigment (Matringe *et al.*, 1989). Among the herbicide tested, normal dose of 2,4-D had the least damage on chlorophyll content. Ivanov *et al.* (2002) stated that lower concentration of 2,4-D showed less reduction in chlorophyll content compared to higher concentration.

5.4.2 IAA content

IAA is a plant growth regulator produced in the shoot tip and moves to the root activating cell division and elongation. It is also a signalling molecule

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necessary for plant growth and development (Taiz and Zeiger, 1991). In the current study IAA content of the rice plant was significantly reduced by herbicides which is evident from Table 17 and this did not improve even after one month. The reduction was higher in the plants where twice the normal dose of the herbicide was applied. Similar results were reported by Ramanarayana (2014) in the case of ALS inhibitors such as Almix® and Azimsulfuron. Machackova and Matschke 2002) observed 30% reduction in IAA content after application of 2,4-D and 15% after application of glyphosate in oak trees.

Treatment wise correlation of IAA content with rice yield is given in (Fig. 2). This indicates the significance of IAA to productivity and brings out the adverse effect of excess herbicide usage on the crop.

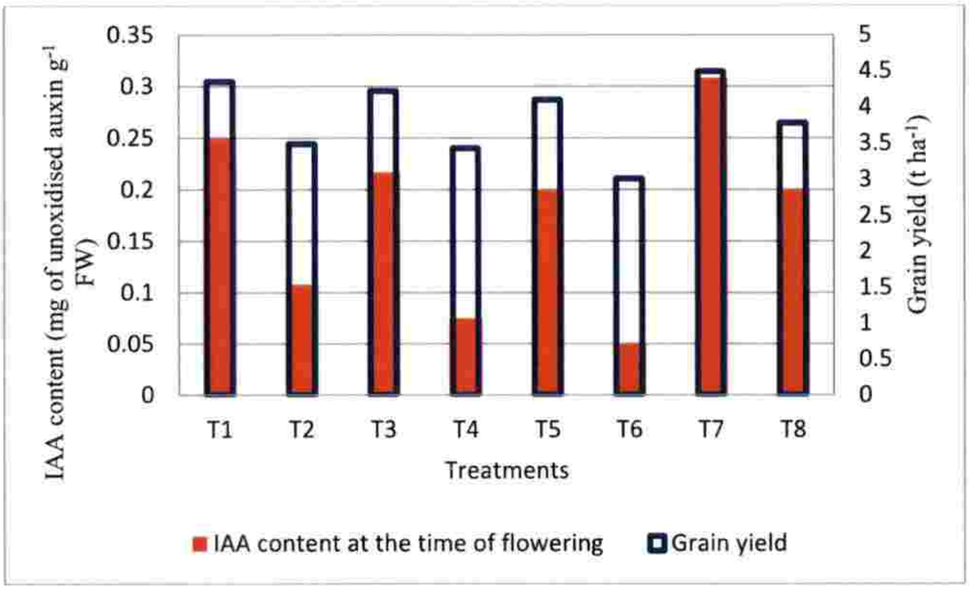

Fig. 2. Effect of treatments on IAA content and grain yield of rice

T_1- 2,4-D @ O.8 kg ai ha^{-1} **T_2- 2,4-D @ 1.6 kg ai ha^{-1}**

T_3- Almix® @ 0.004 kg ai ha^{-1} **T_4- Almix® @ 0.008 kg ai ha^{-1}**

T_5- Penoxulam @ 0.025 kg ai ha^{-1} **T_6- Penoxsulam @ 0.050 kg ai ha^{-1}**

T_7- Control- hand weeded **T_8- Control- unweeded**

5.4.3 Net photosynthesis

Net photosynthetic rate was found to be affected by herbicide application. As compared to hand weeded control all the herbicides significantly reduced the net photosynthesis one week after herbicides application and also at flowering. The effect was more pronounced in the case of twice the normal dose of the chemicals. This may be also due to the effect of the herbicides on the total chlorophyll content of the crop (Table 12&13). Tejada *et al.* (2013) reported that net photosynthesis reduced when the plant was affected with chlorosis, indicating chlorophyll content as an indicator of net photosynthesis. Zhou *et al*. (2007) reported that the application of ALS inhibiting herbicides causes inhibition of acetolactose synthase enzyme which interrupts synthesis of aminoacids like valine, leucine, isoleucine. This in turn results in the decline of photosynthesis.

The influence of treatments on net photosynthesis and grain yield is depicted in (Fig. 3). The negative effect of excess herbicide application on photosynthesis and consequently on yield of the crop is evident from the study.

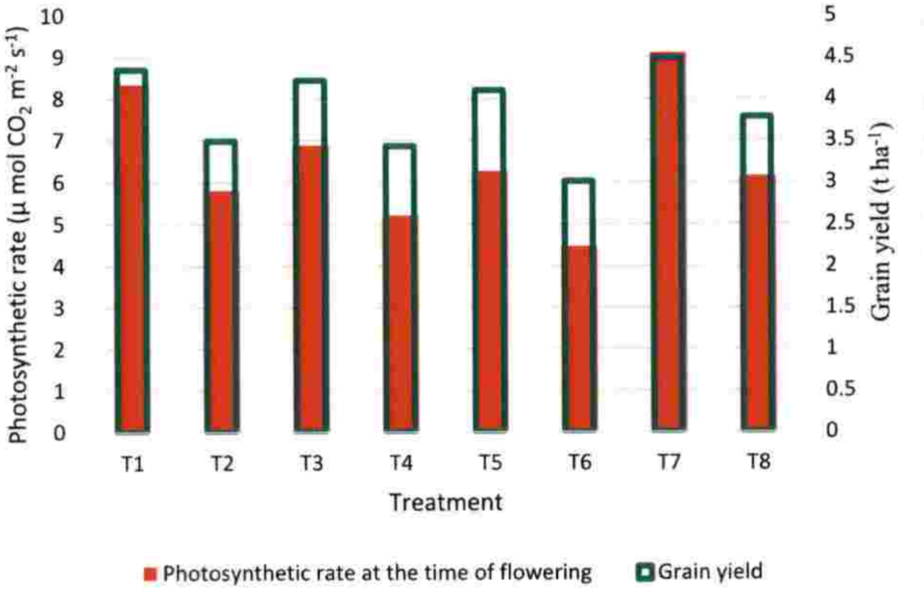

Fig. 3. Effect of treatments on photosynthetic rate and grain yield of rice

T_1- 2,4-D @ O.8 kg ai ha^{-1} **T_2- 2,4-D @ 1.6 kg ai ha^{-1}**

T_3- Almix® @ 0.004 kg ai ha^{-1} **T_4- Almix® @ 0.008 kg ai ha^{-1}**

T_5- Penoxulam @ 0.025 kg ai ha^{-1} **T_6- Penoxsulam @ 0.050 kg ai ha^{-1}**

T_7- Control- hand weeded **T_8- Control- unweeded**

5.4.4 Stomatal conductance

Stomatal conductance is the inflow and outflow of CO_2 and H_2O through stomata. Under stress condition, the closure of stomata occurs as a defence mechanism against loss of water, increasing stomatal resistance consequently decreases the stomatal conductance (Taiz and Zeiger, 2009). Stomatal conductance was adversely affected by herbicide application compared to handweeded control. Among the herbicides, normal dose of 2,4-D showed a better value compared to handweeded control. Twice the normal dose reduced the stomatal conductance which was lower than unweeded control. Agostinetto *et al.* (2016) stated that post emergent herbicides application caused a decrease in photosynthetic rate and stomatal conductance. Zabalza *et al.* (2006) reported that application of ALS inhibiting herbicide (imazethapyr) reduced stomatal conductance, which caused a reduction in nitrogen uptake by roots. Fig. 4 denotes the effect of treatments on stomatal conductance and grain yield of rice.

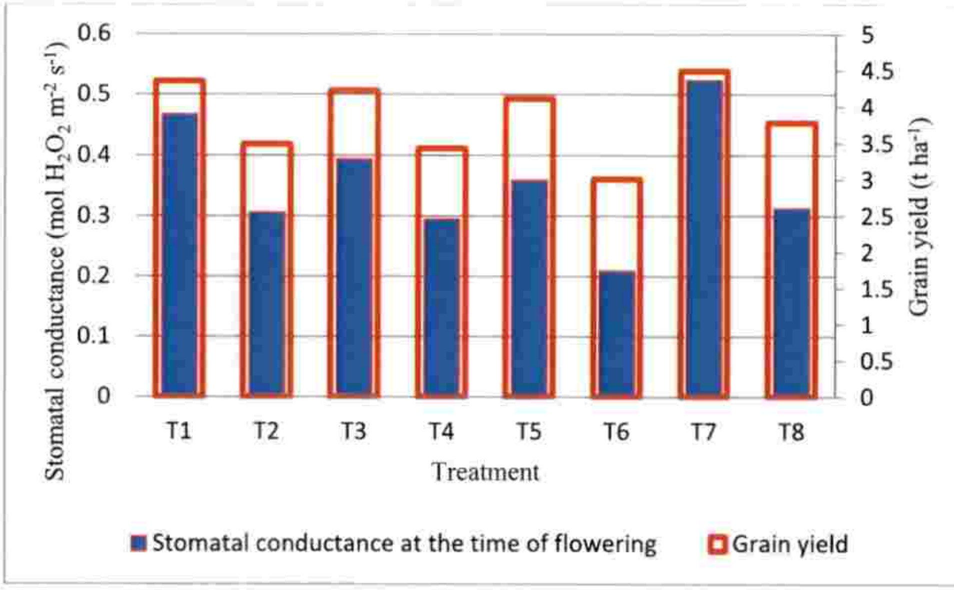

Fig. 4 Effect of treatments on stomatal conductance and grain yield of rice

T_1- 2,4-D @ O.8 kg ai ha^{-1} **T_2- 2,4-D @ 1.6 kg ai ha^{-1}**

T_3- Almix® @ 0.004 kg ai ha^{-1} **T_4- Almix® @ 0.008 kg ai ha^{-1}**

T_5- Penoxulam @ 0.025 kg ai ha^{-1} **T_6- Penoxsulam @ 0.050 kg ai ha^{-1}**

T_7- Control- hand weeded **T_8- Control- unweeded**

5.5 Effect of herbicides on biochemical parameters of rice

5.5.1 Proline content

Herbicide application increased the proline content in the rice crop. Increase was significantly higher in all the doses of herbicides both 7 days after herbicide application and at the time of flowering. However among the herbicides normal dose of 2,4-D only marginally increased the proline content (3.3%) while twice the normal dose of Almix® showed the highest (47%) increase in the 7th day. Increase in proline content by herbicide application has also been reported by Fayez and Kristen (1996) and Serantus *et al*. (2002). A similar trend was seen even at the time of flowering, indicating that the stress experienced by the plant by herbicide application was not completely recovered. Protein degradation will also lead to proline accumulation in plants (Langaro *et al.*, 2016). Proline act as a stress marker and can be part of a defence mechanism of the plant. Fig.5 denotes the effect of treatments on proline content one week and at the time of flowering.

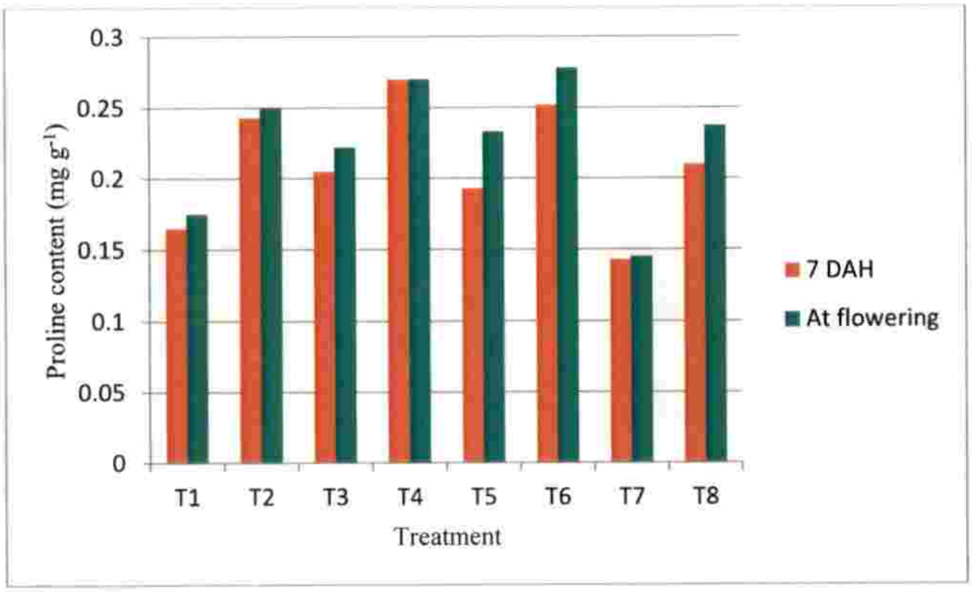

Fig. 5. Effect of treatments on proline content at 7 DAH application and flowering stage

T_1- 2,4-D @ O.8 kg ai ha^{-1} **T_2- 2,4-D @ 1.6 kg ai ha^{-1}**

T_3- Almix® @ 0.004 kg ai ha^{-1} **T_4- Almix® @ 0.008 kg ai ha^{-1}**

T_5- Penoxulam @ 0.025 kg ai ha^{-1} **T_6- Penoxsulam @ 0.050 kg ai ha^{-1}**

T_7- Control- hand weeded **T_8- Control- unweeded**

5.5.2 Nitrate reductase enzyme activity

Nitrate reductase activity showed no significant difference among treatment at 7 days after herbicide application while at flowering, twice the normal dose applied plots showed the least value which was less than unweeded control. Handweeded control showed the maximum nitrate reductase enzyme activity. This result was in concurrence with the findings of Beevers *et al.* (1963). They observed that prolonged application of 2,4-D caused a reduction in nitrate reductase enzyme activity of cucumber. Zabalza *et al.* (2006) stated that application of ALS inhibiting herbicide imazethapyr caused inhibition of nitrate reductase enzyme activity in roots and leaves. Reduction of nitrate reductase enzyme indicates inhibition of N metabolism in the plants which in turn will have a negative impact on yield.

5.5.3 Total aminoacid content

There was significant reduction in the total aminoacid content of rice crop when estimated on the 7th day after herbicide application. The reduction was more in the case of ALS inhibiting herbicides such as Almix® and penoxsulam as compared to 2,4-D. The ALS inhibiting herbicides affect the synthesis of branched chain aminoacid (Alla *et al.*, 2008). Twice the normal dose of herbicides significantly reduced the aminoacid content in the leaves. However the amino acid content showed lower values even at flowering stage, but there was no significant variation between treatments. This might be due to the incessant rainfall experienced by the plant during the growth period. Reduction in aminoacid content immediately after herbicide application has been reported by Nithya (2016). However they also observed recovery in synthesis of aminoacid at flowering stage. In the present study, this recovery observed was not significant. This might be the effect of incessant rainfall experienced by the crop during the growth period.

5.5.4 Total soluble protein

Significant reduction in total soluble protein content was observed one week after herbicide application and even at flowering stage. Among the herbicides twice the normal dose of herbicides applied plots recorded higher reduction compared to normal dose applied plots and control. This reduction in soluble protein content might have contributed to the reduction in growth of the plant. This result was validated by Langaro *et al.* (2016). They observed that both 24 and 12 hours after spray, a decline in soluble protein of rice was observed when treated with post emergent herbicide carfentrazone-ethyl. This reduction may be due to the hydrolysis of protein and formation of aminoacid and proline. Ashraf and Murtaza (2017) stated that an increase in the concentration of 2,4-D showed a reduction in soluble protein content of rice crop. Zulet *et al*. (2013) reported that application of ALS inhibiting herbicides caused a decrease in the level of protein in the plant. Zabalza *et al.* (2006) stated that ALS inhibiting herbicide application reduced the plant protein content and decreased N assimilation. Fig 6. denotes the effect of treatments on total soluble protein content one week and at flowering stage of rice crop.

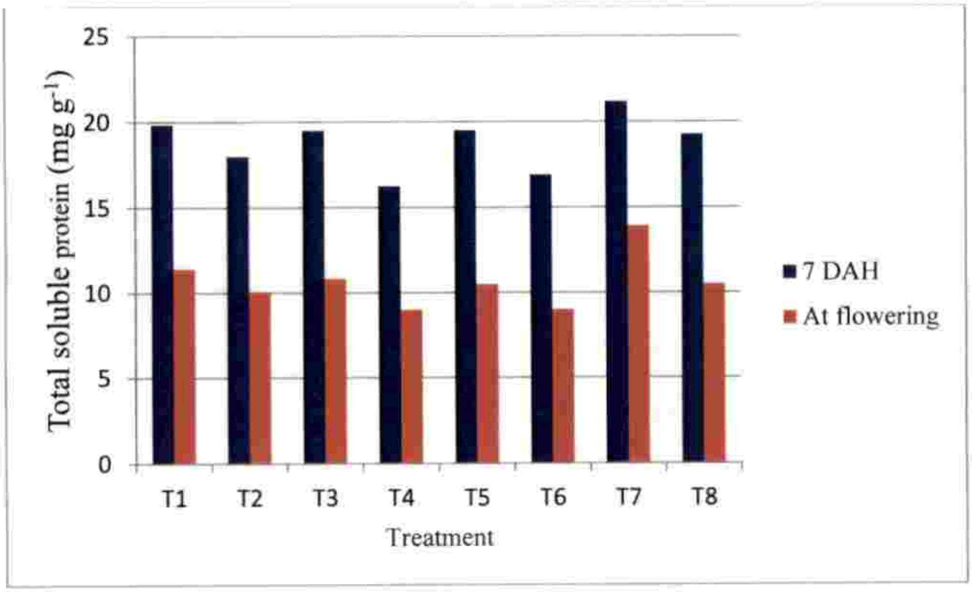

Fig. 6. Effect of treatments on soluble protein content at 7 DAH application and flowering stage

T_1- 2,4-D @ O.8 kg ai ha^{-1} | **T_2- 2,4-D @ 1.6 kg ai ha^{-1}**

T_3- Almix® @ 0.004 kg ai ha^{-1} | **T_4- Almix® @ 0.008 kg ai ha^{-1}**

T_5- Penoxulam @ 0.025 kg ai ha^{-1} | **T_6- Penoxsulam @ 0.050 kg ai ha^{-1}**

T_7- Control- hand weeded | **T_8- Control- unweeded**

5.5.5 Catalase enzyme activity

In the present study, catalase enzyme activity was found to increase in the herbicide applied plots compared to hand weeded plot. Catalase is an enzyme that coverts H_2O_2 produced by SOD to H_2O and O_2. The increase in catalase activity by herbicide application might be due to the presence of H_2O_2 in the plant (Song *et al.*, 2007). At one week after herbicide application 2,4-D showed low catalase content which was on par with handweeded control, while at flowering stage all the treatment plots showed an increase in the catalase enzyme activity compared to hand weeded control. This result was validated by the findings of Qian *et al.* (2011). They reported that application of ALS inhibiting herbicide imazethapyr showed an increase in catalase enzyme activity in *Arabidopsis thaliana* compared to control. In potato cell culture an increase in catalase and decrease in SOD was observed by the application of 2,4-D (Piexoto *et al.*, 2008). Among the herbicides, twice the normal dose of penoxsulam showed higher catalase enzyme activity at flowering. The result was in concurrence with the findings of Nohatto *et al.* (2016). They observed that herbicides like penoxsulam and bentazon had greater potential to cause oxidative stress in rice plant. This effect is mitigated by the production of catalase and SOD in the plant as a defence mechanism.

As catalase enzyme is involved in stress mitigation it was found to be negatively correlated with grain yield (Fig. 7). Higher dose of the herbicides contributed to higher catalase enzyme activity resulting in lower yield (Fig. 8).

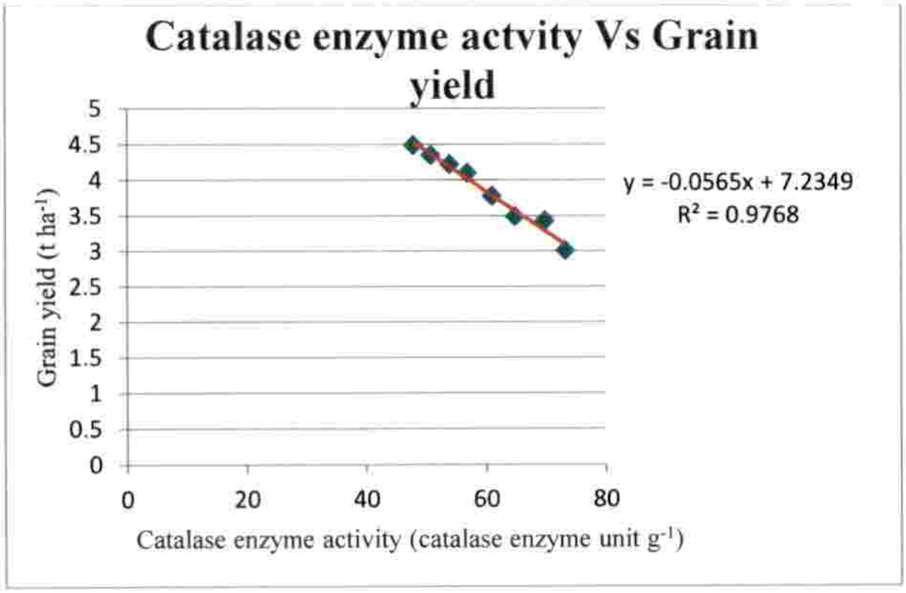

Fig. 7. Relationship between catalase enzyme activity and grain yield

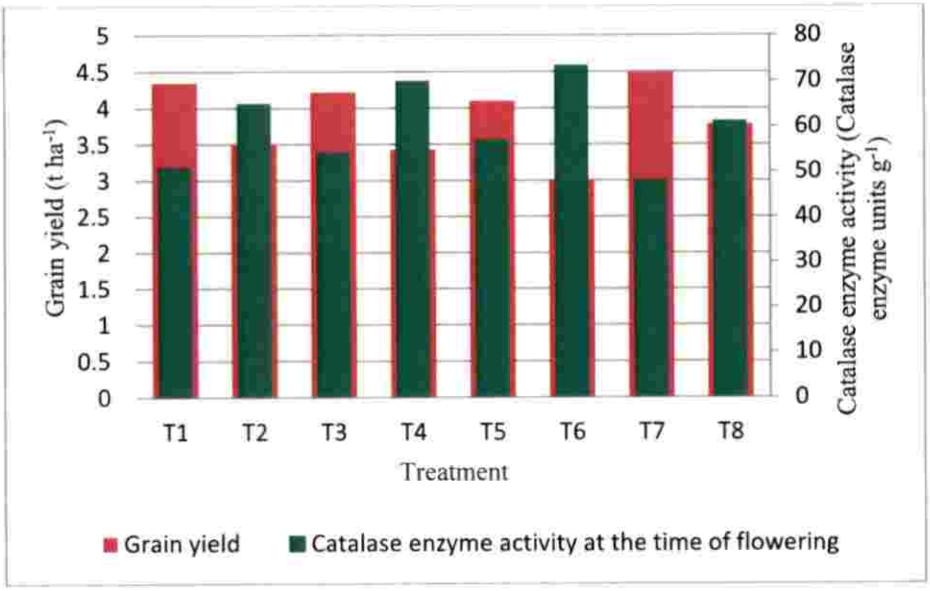

Fig. 8. Effect of treatments on catalase enzyme activity and grain yield of rice

T_1- 2,4-D @ O.8 kg ai ha^{-1} **T_2- 2,4-D @ 1.6 kg ai ha^{-1}**

T_3- Almix® @ 0.004 kg ai ha^{-1} **T_4- Almix® @ 0.008 kg ai ha^{-1}**

T_5- Penoxulam @ 0.025 kg ai ha^{-1} **T_6- Penoxsulam @ 0.050 kg ai ha^{-1}**

T_7- Control- hand weeded **T_8- Control- unweeded**

5.5.6 Total carbohydrate content

The total carbohydrate in the leaves at flowering was found to be significantly higher in twice the normal dose of penoxsulam applied plot. Herbicide application generally increased the soluble sugar of the leaf as compared to control and the increase was influenced by dosage of the herbicides, indicating that this might be a stress response. The result was validated by the findings of Royuela *et al.* (2000). They reported that total soluble sugar and starch content showed an increase in the leaves and roots of pea plant after ALS inhibiting herbicide application. The high carbohydrate content in the penoxsulam treated plot might be the reason for high susceptibility of the crop to bacterial leaf blight. As per the findings of Altman and Campbell (1977), herbicide application induced foliar and soil borne diseases in plants. It may be due to the production of sugar exudates which attracted the pathogens.

5.6 Effect of herbicides on yield and yield attributes of rice

Yield parameters such as number of productive tillers showed a significant reduction in all the herbicide applied plots except in normal dose of 2,4-D compared to handweeded control. Twice the normal dose of herbicides showed lower productive tillers/m^2 and higher chaff%. Among herbicides applied at twice the normal dose, penoxsulam showed lower value of productive tillers and higher chaff%. This result was in concurrence with the findings of Katara *et al.* (2012). They observed that application of ALS inhibiting herbicide chlorimuron-ethyl recorded less effective tillers/m^2 and1000 grain weight compared to weed free check in wheat.

Grain yield was highest in hand weeded plot which was on par with normal dose of 2,4-D and Almix®. This was validated by Dhakshayani *et al.* (2017). They reported that application of metsulfuron methyl + chlorimuron-ethyl showed a similar grain yield as that of handweeded plot. Straw yield was maximum in hand weeded plots, which was on par with normal dose of 2,4-D. Antralina *et al.* (2015) reported that among the herbicides penoxsulam-cyhalofop butyl, bispyribac sodium and 2,4-D+methyl metsulfuron, application of 2,4-D+methyl metsulfuron recorded a similar grain and straw yield with hand weeded control. In the present study twice the normal dose of herbicides contributed to 22-33% reduction in grain and 19-23% reduction in straw yield respectively. Lowest yield was recorded with application of twice the normal dose of penoxsulam. These results are in conformity with the findings of Ray (1984). He reported that application of ALS inhibiting herbicides in rice plant caused a reduction in yield and yield attributes. This may be due to the inhibition of synthesis of aminoacid and reduction of photosynthetic rate.

The biochemical parameters which contribute to yield such as soluble protein, nitrate reductase enzyme activity, IAA content, net photosynthesis, stomatal conductance and chlorophyll content were reduced at flowering stage of rice crop. It may be due to the flood along with herbicide toxicity. During this period, stress parameters such as proline and catalase activity increased. Compared to normal dose, twice the normal dose of herbicides resulted in higher decline in yield and yield parameters. Andrese *et al.* (2013) reported that increase in the dosage of post emergence herbicide caused injury to the rice crop.

5.7 Observation on weeds

5.7.1 Effect of herbicides on weed population and weed dry matter

Due to unexpected flood, weed count was comparatively less and only few weeds were noted before herbicide application. At one week after herbicide application the hand weeded plot had least number of weeds and weed dry weight which was on par with twice the normal dose of 2,4-D. Twice the normal dose of herbicides recorded lower weed count and weed dry weight compared to normal dose of herbicides. Highest weed count and dry matter was recorded in unweeded control followed by normal dose of penoxsulam. This result was validated by Chauhan and Abugho (2012). They reported that penoxsulam was ineffective in controlling some rice weeds such as southern crabgrass. This may be due to lower herbicide uptake and translocation. In the current study, *Ludwigia parviflora* was observed in all the plots one month after herbicide application, however the weed count was higher in normal dose of penoxsulam applied plot. *Cyanotis axillaris* was completely controlled in all the herbicide applied plots while *Eclipta alba* was noted in normal dose of Almix® applied plot. Pal and Banerjee (2007) reported that application of different doses of penoxsulam caused an increase in weed biomass and density in 30 to 90 days after transplanting.

5.7.2 Weed control efficiency

One week after herbicide application, weed control efficiency of herbicide applied plots and hand weeded plot showed similar values except for normal dose of penoxsulam which had a higher population of *Ludwigia parviflora*. One month after herbicide application, twice the normal dose of herbicides showed higher weed control efficiency compared to its normal dose. Among the herbicides, twice the normal dose of 2,4-D showed maximum weed control efficiency and normal dose of penoxsulam showed the least weed control efficiency. This was validated by Singh (2005). They reported that post emergent application of 2,4-D at 500g/ha recorded highest weed control efficiency. Pal and Banerjee (2007) reported that penosxulam application reduced weed control efficiency from 30-90 days.

5.7.3 Weed persistence index

Application of ALS inhibiting herbicides showed higher weed persistence index compared to synthetic auxin type, while lowest value was observed in handweeded control which was on par with twice the normal dose of 2,4-D. This might be due to the persistence of *Ludwigia parviflora* in penoxsulam applied plots which was not controlled by the herbicide. The presence *of Eclipta alba* in Almix® applied plots contributed to the higher values of WPI in this treatment. Since *Marsilea quadrifoliata* was not seen in the rice field, all the other dicot weeds were controlled by 2,4-D which had the lowest value.

5.8 Net house study

5.8.1 Effect of herbicides on *Marsilea quadrifoliata*

In case of *Marsilea quadrifoliata* the most effective herbicide was Almix® which completely controlled the weeds by 12^{th} day after herbicide application for normal dose and 10^{th} day after herbicide application for twice the normal dose. In the case of penoxsulam only 40% and 60% control was observed for normal and twice the normal dose while, 2,4-D did not produce any impact on the weed.

According to Somboon *et al.* (2019), herbicide application leads to the production of ROS. So plants produce antioxidant enzymes like catalase and SOD and peroxidase to detoxify these chemicals. This was evident in the current study where herbicide application led to an increase in production of catalase and SOD enzymes in the plants. However, the increase in these enzymes was significantly higher in the case of 2,4-D applied plants compared to Almix® and penoxsulam. The lowest value was observed in the case of Almix® applied plants. Hence activation of the detoxifying capacity might be attributed as one of the causes for resistance of *Marsilea quadrifoliata* to 2,4-D.

5.8.2 Effect of herbicides on *Ludwigia parviflora*

Ludwigia parviflora was controlled by the application of both the doses of 2,4-D and twice the normal dose of Almix®. Normal dose of Almix® recorded 70% drying of plant while it was ineffective against both the doses of penoxsulam. To evaluate the resistance of the weed to the herbicides, parameters such as CSI, wax content of leaf, SOD and catalase enzyme activity were estimated and the results indicated that these parameters did not have any role in resistance mechanism. This indicated that some other mechanism would have contributed to the resistance of *Ludwigia parviflora* for against herbicides. Cavalcanti *et al.* (2004) reported that herbicide application showed an increase in catalase and SOD content, but it may not be the reason for resistance against herbicides.

5.8.3 Effect of herbicides on *Fimbristylis miliacea*

Fimbristylis miliacea was controlled in both the doses of penoxsulam and 2,4-D while both the doses of Almix® showed only 50% phytotoxicity symptom on plants and from which later they recovered. Holt *et al.* (1993) reported that repeated application of sulfonylureas herbicides caused resistance among weeds by the method of metabolic inactivation. Almix® belongs to this category of herbicides and the recovery of weeds might be attributed to the resistance to the chemical. In the present study, plants which survived from phytotoxicity recorded higher SOD enzyme activity, while parameters such as catalase enzyme activity, wax content did not have any role in the resistance mechanism and CSI was less in twice the normal dose of 2,4-D applied plants, indicated its earlier death. However, increase in SOD enzyme activity may be one of the reasons for the survival of the weed against both the doses of Almix®.

5.8.4 Effect of herbicides on *Cyperus iria*

The result showed that *Cyperus iria* was completely controlled in normal and twice the normal dose of 2,4-D by 18^{th} and 16^{th} day respectively while ALS inhibiting herbicides were ineffective. This result was in concurrence with the findings of LeBaron (1989). Resistance mechanism of weeds against ALS inhibiting herbicides may be due to the alteration of the ALS enzyme. In the present study SOD increased in ALS inhibiting herbicides compared to synthetic auxin type. This may be one of the reasons for the resistance of *Cyperus iria* against ALS inhibiting herbicides and CSI was lower in twice the normal dose of 2,4-D applied plants, indicated the earlier death. Wang and Zhou (2006) reported that change in antioxidant enzymes such as SOD and peroxidase was noted in wheat when applied with chlorimuron-ethyl. However, catalase enzyme activity and wax content were not involved in the resistance mechanism.

5.8.5 Effect of herbicides on *Sphenoclea zeylanica*

Effect of herbicides on *Sphenoclea zeylanica* showed that it was controlled in all herbicide applied pots. But the days taken for the complete death was different in each treatment. Normal dose of Almix® took more days for complete death (14 days) while twice the normal dose of 2,4-D and normal dose of penoxsulam showed minimum days for complete death (10 days). SOD enzyme activity, wax content and CSI were recorded highest in Almix® application. This increase in SOD enzyme activity and wax content may be the reason for delay in death of weeds.

The present study revealed that *Marsilea quadrifoliata* was not controlled by both the doses of 2,4-D and penoxsulam while both the doses of Almux® could effectively control the weed. *Ludwigia parviflora* was controlled by both the doses of 2,4-D while only twice the normal dose of Almix® could control the weed. Penoxsulam did not have any effect on the weed. *Fimbristylis miliacea* was controlled by penoxsulam and 2,4-D but Almix® was not effective for this weed. *Cyperus iria* could be controlled only by 2,4-D while *Sphenoclea zeylanica* was effectively controlled by all the 3 herbicide formulations. Twice the normal dose of herbicides showed higher weed control efficiency compared to its normal dose while it effected the physiological and biochemical parameters of rice, hence a reduction in yield.

Summary

6. SUMMARY

Use of herbicides to control weed infestation is an important aspect of modern agriculture. Post emergent herbicides such as 2,4-D, Almix®, penoxsulam are the important herbicides widely used in rice ecosystem. Farmers have reported inhibition of growth in rice plant after herbicide application. Higher dosage of herbicides showed better weed control efficiency. However some weeds were found to escape even higher doses of herbicides. The main focus of the study has been the morphological, physiological and biochemical changes in rice plant by the application of different doses of post emergent herbicides such as 2,4-D, Almix® and penoxsulam and also to identify the weeds which are resistant against these herbicides.

The experiment was conducted at ARS, mannuthy using the rice variety, Jyothi. The plot size was 15 m^2 (5m X 3m) and herbicides used were normal and twice the normal dose of 2,4-D, Almix® and penoxsulam. Control for effective comparison includes handweeded and unweeded condition. The post emergent herbicides were applied 20DAS using knapsack sprayer and hand weeding was done on the same day. Visual phytotoxicity scoring was done on the third and fifth day after spraying. Rice was harvested when it was fully matured. After manual threshing, cleaning and drying yield was taken and expressed in t/ha.

Total weed count and dry weight, species wise weed count and dry weight weed persistence index and weed control efficiency were recorded at 7 days after herbicide application and 60 DAS. Height, tillers/m2, RGR, CGR, LAI, NAR of rice plant were taken at 20 days interval up to harvest. Yield attributes like productive tillers/m^2, panicle length, spikelets per panicle, chaff%, 1000 grain weight were recorded. The straw and grain yield were taken and expressed in t/ha.

Weeds such as *Marsilea quadrifoliata, Ludwigia parviflora, Cyperus iria, Fimbristylis miliacea* and *Sphenoclea zeylanica* were collected from different places and grown in pots. The herbicides mentioned above were applied at 20 DAS and days taken for the complete death of plants were recorded and biochemical parameters calculated one week after herbicide application.

The salient features of study are as follows

- The rice plant in the herbicide applied plots showed significant reduction in height and tiller number compared to hand weeded plots. Twice the normal dose applied plots showed more reduction compared to normal dose applied plot.
- Growth indices such as CGR and NAR showed significant reduction in herbicide applied plots only after 60 days. 2,4-D normal dosage showed values on par with hand weeded control.
- LAI and RGR showed reduction in herbicide applied plots from the initial stage onwards.
- Effect of herbicides on physiological parameters indicated that chlorophyll content, IAA content, stomatal conductance and net photosynthesis showed a reduction in herbicide applied plots compared to hand weeded control.
- Biochemical parameters such as total soluble protein, nitrate reductase enzyme activity, total aminoacid content showed a significant reduction in herbicide applied plots compared to hand weeded control.
- Stress indicating parameters such as proline and catalase enzyme activity was higher in twice the normal dose of herbicide applied plots and lower in handweeded control.
- Maximum grain and straw yield was recorded in hand weeded control which was on par with normal dose of 2,4-D and Almix®. Twice the normal dose of herbicides significantly reduced the straw and grain yield.
- Higher weed control efficiency and lower weed persistence index, weed count and weed dry weight was recorded in twice the normal dose of herbicides applied plots.
- *Marsilea quadrifoliata* was completely controlled in both normal and twice the normal dose of Almix®. Penoxsulam normal and twice the dose showed 40% and 60% control. 2,4-D both the dosages failed to control the weed.

- *Ludwigia parviflora* was completely controlled in normal, twice the normal dose of 2,4-D and twice the normal dose of Almix® while 70% control was observed in normal dose. Penoxsulam normal and twice the normal doses recorded only 10% control.
- *Fimbristylis miliacea* was completely controlled in normal and twice the normal doses of 2,4-D and penoxsulam. Normal and twice the normal doses of Almix® showed 50% control.
- *Cyperus iria* was completely controlled in both the doses of 2,4-D, while Almix® normal and twice the normal dose applied pots showed only 10% control. Penoxsulam normal dose showed no symptom and twice the normal dose showed 20% control.
- *Sphenoclea zeylanica* was controlled by the normal dose of all the three herbicides.
- In most of the resistant weeds the content of stress detoxifying enzymes such as SOD and catalase was found to increase but mode of action needs further elucidation.

Conclusion

Higher dose of herbicide application caused stress to the plant which in turn resulted in growth suppression. Normal dose of herbicides affected physiological and biochemical parameters such as chlorophyll content, IAA content, stomatal conductance, net photosynthesis, soluble protein, nitrate reductase enzyme activity, total aminoacid content of rice without affecting yield. Twice the normal dose of herbicides recorded higher weed control efficiency compared to its normal doses, but it reduced the yield significantly which was even lower than the unweeded control. The normal dose of 2,4-D, Almix® and penoxsulam were less harmful to the plant and the plants were able to recover within a month of application so that the yield was not significantly affected. Bioefficacy studies of herbicides on selected weeds revealed that 2,4-D was ineffective against *Marsilea quadrifoliata*. Almix® was ineffective against *Cyperus iria* and *Fimbristylis miliacea*. Penoxsulam was ineffective against *Marsilea quadrifoliata, Ludwigia parviflora* and *Cyperus iria*, while *Sphenoclea zeylanica* was controlled by all the herbicides. Dosage of chemical influenced the time taken for complete drying of the plant.

Future line of work

The current study has been proposed to identify the effect of different doses of herbicides such as 2,4-D, Almix® and penoxsulam on physiological and biochemical parameters of rice that contribute to yield reduction in rice and also to identify the efficacy of these herbicides against broad leaved weeds and sedges. Though catalase and SOD enzyme activity showed increase in the resistant weeds, further studies are required to elucidate the resistance mechanism.

- To investigate possible role of different herbicides to regulate the chlorophyll pigment composition in rice.
- Comparative transcriptome analysis can yield possible discrete role of certain herbicides on specific weeds.

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PHYSIOLOGICAL EVALUATION OF HERBICIDAL EFFECTS ON RICE, BROADLEAVED WEEDS AND SEDGES

By

Linu C

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ABSTRACT OF THE THESIS

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DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF HORTICULTURE,

VELLANIKKARA, THRISSUR – 680656

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Abstract

The study on "Physiological evaluation of herbicidal effects on rice, broadleaved weeds and sedges" was conducted during the period from July 2018 to November 2018 at Agricultural Research Station, Mannuthy, Thrissur. The objectives of the study were identification of broadleaved weeds and sedges that are selectively controlled by Almix®, penoxsulam and 2,4-D and also to evaluate the effect of these herbicides on the growth, physiology and yield of rice.

The experiment was laid out in RBD with three replications and six herbicidal treatments. The rice variety selected for the study was Jyothi. Treatments included normal and twice the normal doses of 2,4-D, Almix® and penoxsulam and two controls (hand weeded and unweeded). The herbicides were sprayed 20 DAS. Morphological parameters were observed at 20 days interval. Biochemical parameters were estimated before herbicidal application, one week after herbicidal application and at the time of flowering. Yield attributes and yield were recorded during the harvest of the crop.

Twice the normal doses of herbicides resulted in 10-12% reduction in plant height of rice while normal doses of herbicides resulted in 3-6% reduction in plant height at the time of harvest. Tiller number of rice crop was found to be affected by herbicide application.At the time of harvest, tiller number of 2,4-D (normal dose) applied plot was on par with hand weeded plot while in case of Almix® and penoxsulam the reduction was 4 and 5 % respectively. Twice the normal doses of herbicides adversely affected tiller number in all three herbicide treatments and it was significantly lower than unweeded control. Number of days taken for flowering was found to be affected by herbicide application compared to hand weeded control. Effect of herbicides on growth indices *viz.*, crop growth rate (CGR) and net assimilation rate (NAR) indicated that only after critical period of growth, there was a significant variation among the treatments. In case of relative growth rate

(RGR) and leaf area index (LAI) there was a significant reduction in herbicide applied plots compared to hand weeded control throughout the growth stages. Twice the normal doses of herbicides showed greater reduction in the growth attributes.

Biochemical parameters such as soluble protein, total amino acid and nitrate reductase enzyme activity showed a decline in herbicide treatments compared to hand weeded control. Proline content and catalase enzyme activity showed an increase with herbicide application while physiological parameters such as IAA content, cholorophyll content, stomatal conductance and net photosynthesis showed a decline. Twice the normal doses of herbicides significantly affected these parameters compared to the recommended doses of these chemicals.

Weed count, dry weight, weed control efficiency and weed persistence index were taken one week after herbicide application and at 60 DAS. Lower weed count, dry weight, weed persistence index and higher weed control efficiency were recorded in twice the normal doses of herbicides as compared to the normal doses.

In the present study, grain yield was highest in handweeded plot which was on par with normal doses of 2,4-D and Almix®. Since disease infestation was higher in penoxsulam treatment the yield was also affected. Twice the normal doses of herbicides contributed to 22-33% reduction in grain and 19-23% reduction in straw yield respectively. Among the herbicides 2,4-D (synthetic auxin type) showed a better performance compared to Almix® and penoxsulam (ALS inhibiting type).

A net house experiment was conducted to understand the bioefficacy of the herbicides on broadleaved weeds and sedges, *viz. Marsilea quadrifoliata, Ludwigia parviflora, Cyperus iria, Fimbristylis miliacea and Sphenoclea zeylanica.* The study revealed that *Marsilea quadrifoliata* was not controlled by both the doses of 2,4-D and penoxsulam while both the doses of Almix®

could effectively control the weed. *Ludwigia parviflora* was controlled by both the doses of 2,4-D while only twice the normal dose of Almix® could control the weed. Penoxsulam did not have any effect on the weed. *Fimbristylis miliacea* was controlled by penoxsulam and 2,4-D but Almix® was not effective for this weed. *Cyperus iria* could be controlled only by 2,4-D while *Sphenoclea zeylanica* was effectively controlled by all the three herbicide formulations. The dosage of chemical influenced the time taken for complete drying of the plant. Though catalase and superoxide dismutase enzyme activity showed increase in the resistant weeds, further studies are required to elucidate the resistance mechanism.