MITIGATION OF SOLAR ULTRAVIOLET-B RADIATION INDUCED PHOTOINHIBITION IN PHOTOCHEMISTRY AND PHOTOSYNTHESIS OF RICE *(Oryza sativa* L.).

By

SHAFEEQA T.

(2014-11-194)

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2016

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THESIS

Submitted in partial fulfilment of the requirements for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF HORTICULTURE, VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

2016

DECLARATION

I hereby declare that this thesis entitled **"Mitigation of solar ultraviolet-B radiation induced photoinhibition in photochemistry and photosynthesis of rice (*Oryza sativa* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date:

SHAFEEQA T.

(2014-11-194)

CERTIFICATE

Certified that this thesis entitled **"Mitigation of solar ultraviolet-B radiation induced photoinhibition in photochemistry and photosynthesis of rice (*Oryza sativa* L)"** is a record of research work done independently by Ms. Shafeeqa T. (2014-11-194) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Vellanikkara,

Date:

Dr. K. Nandini
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**.** Shafeeqa, T., a candidate for the degree of **Master of Science in Agriculture**, with major in **Plant Physiology**, agree that the thesis entitled **"Mitigation of solar ultraviolet-B radiation induced photoinhibition in photochemistry and photosynthesis of rice (*Oryza sativa* L.)"**may be submitted by Ms. Shafeeqa, T., in partial fulfilment of the requirement for the degree.

Dr. K. Nandini
(Chairman, Advisory Committee)
Professor and Head
Department of Plant Physiology,
College of Horticulture
Vellanikkara

Dr. T. Girija
(Member, Advisory Committee)
Professor
Department of Plant Physiology
College of Horticulture
Vellanikkara

Dr. Beena, R.
(Member, Advisory Committee)
Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani

Dr. U. Jaikumaran
(Member, Advisory Committee)
Professor and Head
Agriculture Research Station,Mannuthy

EXTERNAL EXAMINER

(Name and Address)

ACKNOWLEDGMENT

I bow to the lotus feet of ***Almighty*** *whose grace had endowed me the inner strength and confidence and blessed me with a helping hand at each step during my life.*

At this moment of accomplishment, my heart is overwhelmed with gratitude and I wish if these words could convey the subtle feelings.

I am ineffable in expressing my deep sense of gratitude to my major advisor ***Dr. K. Nandini****, Professor and Head, Dept. of Plant Physiology, College of Horticulture, Vellanikkara, for her inspiring guidance, critical comments, constant supervision, support and encouragement throughout the course of my research work. I am really grateful for the keen interest taken by her in the prompt correction of the manuscript.*

My sincere thanks are due to, ***Dr. T. Girija*** *Professor, Department Of Plant Physiology, College Of Horticulture, Vellanikkara, member of my advisory committee for the valuable guidance, timely suggestions and help rendered in the conduct of the experiment.*

I also owe my deep sense of gratitude and sincere thanks to ***Dr. U. Jaikumaran****, Professor and Head, Agricultural Research Station, Mannuthy, member of my advisory committee for the his timely help, critical scrutiny of the manuscript and valuable guidance for the successful completion of my research work.*

Iam extremely thankful to ***Dr. R. Beena,*** *Assistant Professor, Dept. Of Plant Physiology, Vellayani, member of my advisory committee for her support, suggestions and guidance throughout the study.*

I take this opportunity to express my indebtedness with veneration and devotion to ***Dr. S. Krishnan****, Professor and Head, Dept. of Agricultural statistics, College of Horticulture, Vellanikkara, for his constant support, suggestions, valuable guidance and timely help rendered in the conduct of the experiment.*

I am thankful to ***Dr. Jidhu Vaishnavi****, Teaching Assistant, Dept. of Plant Physiology, College of Horticulture, Vellanikkara, for her support and help throughout the study.*

I wish to express my sincere thanks to all the non-teaching staff members ***Sheena chechi, Ammini chechi, Jerin, Jinu chechi, Sandhya chechi, Food Security Army*** *and* ***labourers*** *for their whole hearted cooperation and timely assistance. I also appreciate and acknowledge the facilities provided at* ***ARS****, Mannuthy and* ***RARS****, Pattambi for the successful completion of my research work.*

My heartfelt gratitude cannot be captured in words for the unflinching support, constant encouragement of my friends ***Sushna, Nabeela, Uma, Smitha, and Aswany****. I express my sincere thanks to my classmate* ***Nithya N.*** *for her affection and kind help offered during my thesis work.*

My duty is incomplete if I forget my senior and junior friends ***Gayathri, Suada, Yogesh, Ramanarayana, Garggi, Sreepriya, and Vishnu*** *for their love and affection and helping hand throughout my PG programme.*

I owe special thanks to Library, College of horticulture. ***Dr. Francis*** *and all other staff members of Library, who guided me in several ways, which immensely helped for collection of literature for writing my thesis.*

I express my deep sense of gratitude to Kerala Agricultural University for financial and technical support for persuasion of my study and research work.

I am in dearth of words to express my love towards ***my beloved parents, Brother, sisters, grandparents*** *and other* ***family members*** *for their boundless affection, moral support, eternal love, deep concern, prayers and personal sacrifices which sustains peace in my life.*

It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I once again express my heartful thanks to all those who helped me in completing this venture in time.

Shafeeqa T.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ANNEXURES

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.), with its wider adaptability in varied ecological zones is the staple food for large population at global level. Twenty three percentage gross cropped area in India is contributed by rice and provides 45% of total food grain production. Rice cultivation in Kerala is1.8 lakh ha and produces 5.62 lakh tonnes. Many reports have shown rice is sensitive towards changing climate especially to high temperature and high concentration of UV-B radiation. Global warming, stratospheric ozone depletion and acid rain are the different environmental stresses under climatic change. Increase in green house gases like CO_2, CFC, NO, N_2O, CH_4 are major gas pollutants for the destruction of ozone layer so it leads to elevation of surface UV-B radiation. Biological consequences of enhanced UV-B radiation on plants and animals are the great concern in current situation. In the tropical region the emission of UV-B radiation is high in full sunlight condition compared to temperate regions. Hence, rice as a tropical cereal crop receives higher level of ambient UV-B radiation (Tomen *et al*., 2003).

Even though UV contributes a small portion in solar radiation at the range (280-320nm) it causes a deleterious effect to the plants and leads to photobiological stress. UV-B exclusion studies undertaken at Vellanikkara have shown that rice was sensitive to solar UV-B radiation (Wagh, 2015). Yield was very low under natural solar UV-B radiation when compared to UV-B reduced condition.

Observations on diurnal variation of UV-B radiation at Vellanikkara, in Thrissur revealed that maximum UV-B (1.5 to 3wm^{-2}) was reached at crop canopy between 12 and 2pm during January to April 2014. UV-B related studies have shown that current level of solar UV-B radiation affected photosynthesis, stomatal conductance, chlorophyll content, flavanoid and phenol content in rice variety Jyothi and Uma (Wagh, 2015). Secondary compounds like phenol, flavanoid, and xanthophylls accumulated to protect the plants from high UV-B radiation (Teramura,

et al.,1992). Yield decline in rice during puncha season in Kole lands of Kerala was also associated with increasing level of UV-B radiation (Nandini *et al.,* 2014).

Abiotic stresses like drought, salinity, high temperature and UV-B radiation elicit Reactive Oxygen Species (ROS) like singlet oxygen (O_2^1), Superoxide (O_2^-), hydroxyl peroxyl (HO_2) and hydrogen peroxide (H_2O_2) in plants. Excess ROS directly react with macromolecules and oxidize membrane lipid, nucleic acid and protein. So it damages all the cellular functions and finally reduces the growth and productivity of plants.

Plants develop various kind of defense mechanism against UV-B damage either activation of repair mechanisms or by stimulation of protection mechanisms. However, when compared to other crops, this adaptation was less in rice indicating photoinhibition in photosynthesis of rice. Studies to protect rice crops from enhanced UV-B radiation through manipulative attempts are very few. Breeding and genetic improvements are the long term approach for UV-B tolerance. Exogenous application of stress alleviating ecofriendly chemicals is short and easy viable approach. Many reports describe the damage that occur in cell membrane due to UV-B stress partially mitigated by plant growth regulators or organic solutes (Sharma and Dubey, 2005)

Significant advances have been made in mitigating the inhibitory effects of environmental stresses by exogenous application of compatible solutes like glycine betaine and proline, antioxidants like ascorbic acid and growth regulator like ABA, cytokinin, triazole and strobilurin group of fungicides.

Too high doses of UV-B radiation and Photosyntheticaly Active Radiation (PAR) interact with other stresses leading to photooxidative destruction of photosynthetic apparatus and inhibition of photosynthesis (Solhaag and Gauslaa, 2004). Under normal condition, glycine betaine induces UV-B stress tolerance by protecting photosystem II and Rubisco enzymes (Foolad and Ashraf, 2007). Application of glycine betaine may reduce the activity of ROS under elevated UV-B

radiation. Mohammed *et al*. (2013) reported that under elevated UV-B radiation exogenous application of glycine betaine increased yield by 18% in rice.

Ascorbic acid is an important antioxidant in the plants for scavenging the reactive oxygen species developed during stress and also it can induce the stress responsive genes to enhance plant protection against UV-B radiation. Hence it is a promising target to achieve higher yield under abiotic stresses like UV-B, drought, salinity and temperature (Khan *et al*., 2011).

Combination fungicide, 25 WG trifloxystrobin+ 50 WG tebuconazole (Trade name-Nativo 75 WG, Bayer Crop Science Ltd. Mumbai) is used for imparting tolerance to both biotic and abiotic stress. The active substance trifloxystrobin and tebuconazole present in *Nativo* can influence physiological alteration in plants causing longer retention of green leaf tissue, inhibition of ethylene biosynthesis, increase in level of endogenous cytokinin and auxin, better nitrogen assimilation, increase in CO_2 assimilation, increase in water use efficiency and harvest index (Nagajothi, 2013).

Towards augmenting rice production in the state, possibilities of taking a second crop of rice in puncha season is explored, but constrained by low productivity. One among the several environmental factors limiting productivity is the abundance of UV-B radiation during this season. Hence, detailed field investigation to verify the potential of the above reported ecofriendly chemicals towards mitigating UV-B stress is needed. The efficacies of these chemicals are to be evaluated for providing recommendation in situations of UV-B stress in rice crop. With this background, the present study was formulated in rice with the following objectives

1. To evaluate the effect of glycine betaine, ascorbic acid and combination fungicide 25 WG trifloxystrobin+ 50 WG tebuconazole (*Nativo*) on morphological and physiological changes in rice under two levels of UV-B radiation.

2. To evaluate the effect of glycine betaine, ascorbic acid and combination fungicide 25 WG trifloxystrobin + 50 WG tebuconazole (*Nativo*) on biochemical characters in rice under two levels of UV-B radiation.

3. To evaluate the effect of glycine betaine, ascorbic acid and combination fungicide 25 WG trifloxystrobin+ 50 WG tebuconazole (*Nativo*) on yield and yield parameters under two levels of UV-B radiation.

Review of Literature

2. REVIEW OF LITERATURE

Sun is the source of all kind of radiation which include ultraviolet radiation (100nm-400nm), photosyntheticaly active radiation (400nm-700nm) and infra red (above 700nm). All these components are involved in many biological processes under natural condition. Green plants prefer PAR to carryout photosynthesis, but sometimes the harmful radiation, mainly UV radiation causes deleterious effect to the plant kingdom. According to its wavelength ultra violet radiation having three category UV-A (320 to 400 nm), UV-B (280 to 320 nm) and UV-C (100 to 280 nm). Among these UV-C which is most damaging is absorbed completely in the atmosphere and hence not reaching in earth's surface but the amount of UV-B radiation which can also cause biological damage is increased in earth surface due to the depletion of stratospheric ozone layer (Russell *et al*., 1996).

Wide range of damaging effect studied in plants due to high concentration of UV-B. The effects of UV-B radiation on plants have been well reviewed by Tevini and Teramura (1989). It affects photochemistry in photosystem II, destruction of DNA, protein, nucleic acid, pigments, enzymes and growth regulators. UV-B also affects physiological process such as photosynthetic rate, stomatal conductance, transpiration rate, etc. Pigments like chlorophyll, carotenoid, xanthophyll, flavanoid vary according to the level of UV-B and finally it reduces total yield and yield characters (Pal *et al*., 1997)

In the current scenario of changing climatic condition, abiotic stresses become more challenging and have reported several negative impacts on rice production and productivity. UV-B stress is an important issue nowadays affecting the rice crop growth, metabolism and yield. Scientists have reported that damage to cell membrane by UV-B stress could be alleviated by organic solutes or plant growth regulators (Sharma and Dubey, 2005)

Plants need to change their internal homeostasis according to changing external environment to acclimatize for the present situation. Accumulation of compatible solutes (Osmolyte) is the primary action that happens in the plant body. Evidences from various studies of plant physiology, biochemistry and plant genetics showed the impact of various growth promoting substance on plant metabolic process.

Glycine betaine, the amphoteric quarternary amine, plays a major role as a compatible solute in the plant to overcome various stresses like drought, salinity and high temperature. Now latest approach in genetic engineering is to manipulate the effect of glycine betaine as osmolyte for the plants which are very sensitive to different kind of stresses (Sakamoto and Murata, 2000). Glycine betaine increases the tolerance of plant towards the abiotic stress through various processes like protection of photosynthetic machinery, induction of genes to produce various compatible solutes to protect plants from stress, reduce the harmful effect of ROS and maintain the ion channel protein.

Reactive oxygen species (ROS) are the byproduct of normal metabolic process like photosynthesis, respiration and photorespiration. Under various stresses like drought, salinity, high temperature and high UV radiation increases the amount of reactive oxygen species, which cause serious damage to the cell such as lipid peroxidation, destruction in membrane permeability, inactivation of enzymes and protein. This can be efficiently quenched and scavenged by ascorbic acid.

Various experiments proved the photo protective potential of triazole group of fungicide under elevated UV-B radiation (Agati and Tattini, 2010). Triazole is largest and most important group of systemic fungicide which have both fungicidal and growth regulator property which influences hormonal changes, enzyme activities, photosynthetic rates and yield components. Also protect plants from various abiotic stresses like drought, salinity and UV-B radiation. Uniconazole, triadimefon and

paclobutrazole are the example for triazole fungicide it reduces the enzyme activity of Cyt P450. *Nativo* is a protectant and curative fungicide with active ingredient trifloxystrobin and tebuconazole which enhance plants ability to tolerate abiotic stress also. Apart from plant growth stimulating properties, triazoles protects plants from biotic and abiotic stresses like pathogen, drought, high temperature, UV-B radiation and air pollutants (Voesenek *et al*., 2003)

In this chapter, national and international literatures on different aspects pertinent to the current study are reviewed under the following captions

2.1 Effect of glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on morphological and phenological traits under UV-B condition

2.2 Effect of glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on physiological traits under UV-B condition

2.3 Effect of glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on biochemical characters under UV-B condition

2.4 Effect of glycine betaine, ascorbic acid and combination fungicides 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on yield parameters under UV-B condition

2.1 Effect of glycinebetaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on morphological and phenological traits under UV-B condition

2.1.1 Plant height

Reduction in plant height has often been used as an index to assess the degree of UV-B radiation sensitivity in crops (Biggs and Kossuth,1978)

Study conducted to evaluate the response of rice varieties Uma and Jyothi to UV-B radiation at Vellanikkara reported that UV excluded condition recorded highest plant height (42.2%) than UV- supplemented condition (Wagh, 2015).

Decrease in plant height was observed as a UV-B damage in rice (Fiscus and Booker, 1995). Rice plants grown under 10 KJ UV-B recorded 12% decrease in plant height and 23% decrease in shoot dry weight (Mohammed and Tarpley, 2011)

Ebrahim *et al.* (2014) conducted a study in which plant height found significantly higher under plants treated with ascorbic acid and nitrogen fertilizer. Very less yield reported in plants grown under control condition without foliar application of ascorbic acid and nitrogen fertilizer.

Chinoy (1984) reported that exogenous application of ascorbic acid to growing root will increase the nitrogen fixation in root nodules. Ascorbic acid treatment increased root and shoot length but decreased root-shoot ratio (Roy and Srivastava, 1999).

In many cases there is a significant reduction in plant height (3.18% and 2.87%) observed in rice and tomato by foliar application of triazole fungicide which contain mixture of strobilurin and tebuconazole (Nagajothi, 2013)

Triazole is a group of fungicide with a property to regulate plant growth acts as growth retardant also. Some studies showed that tebuconazole and triadimefon

reduced the plant growth. Triazole fungicide has a property to increase the number of branches by decreasing the height (Setia *et al*., 1995). Reduction in plant height in crops treated with triazole fungicide is due to poor induction of gibberellic acid which is the hormone responsible for intermodal elongation (Fletcher *et al*., 2000)

Triadimefon treatments promote the synthesis of cytokinin which enhances the synthesis of chlorophyll content (Fletcher *et al.*, 2000). Gopi *et al.* (2007) found that triazole compounds increase the cytokinin activity and leads to cell division and root length.

Watson and Himelick (2004) reported that triazole treated Oak plants showed some morphological changes such as increased root to shoot ratio, increased chlorophyll levels, elevated level of epicuticular wax formation, enlarged chloroplast and decreased intermodal elongation.

2.1.2 Number of tillers

Study conducted in 188 rice cultivars collected from different ecosystems to check the interspecific variation in sensitivity of rice under UV-B enhanced condition revealed that among 188 rice cultivars 41 cultivars reduced its tiller count because of the enhanced level of UV-B radiation (Dai *et al*., 1994).

Wagh (2015) observed 33.9 percent increment in tiller number for the plants growing under UV-B excluded condition. Decrease in tiller number as a result of supplemental UV-B radiation were observed in rice during the tillering stage (Kumagai *et al*., 2001)

2.1.3 Phenological characters

Invitro regenerated rice (*Oryza sativa* cv. ADT 43) grown under two level of UV-B radiation low (10 min d^{-1}) and high (30 min d^{-1}) could not show any difference

for days to heading for UV treated and UV untreated plants (Ramakrishnan and Kulandaivelu, 2014)

Study conducted in green gram (*Vigna radiate* L.) to check the effect of UV-B radiation revealed that initiation of flowering and 50% flowering are delayed in UV elevated condition (12.2 KJ $m^{-2}d^{-1}$) when compared to the ambient level (10 $KJm^{-2}d^{-1}$) (Rajendiran and Ramanujan, 2004)

Davey *et al.* (2000) reported that ascorbic acid has an important role in flowering time and senescence. Exogenous application of ascorbic acid and salicylic acid provide resistance to the plants under chilling stress (Kumar *et al.,* 2010)

Shikuku *et al.* (2010) reported that under stress condition especially water stress condition plants need more days to attain the harvestable maturity than unstressed well watered plants

2.2 Effect of glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on physiological traits under UV-B condition.

2.2.1 Photosynthetic rate

The prime action site of UV-B damage is the photosynthetic apparatus (Vass *et al*., 2005). Kataria *et al.* (2014) reviewed a deleterious effect of UV-B radiation on D1and D2 reaction centre proteins, which affect the productivity of plants.

Xiaoquin and Qing (2006) Reported that maple seedling exposed to enhanced UV-B (14.33 KJ $m^{-2}day^{-1}$) caused a notable decline in net photosynthetic rate

Under high temperature and salinity high accumution of glycine betain stabilizes the oxygen evolving photosystem II, sustain and protect the photosynthetic machinery (Murata, *et al*., 2007)

Nagajothi (2013) reported that foliar application of *Nativo* increased the yield about 12.83% and 20.49% in rice and tomato. Similar reports also introduced Zhang *et al.* (2010), that is combination of strobilurin and triazole improved the photosynthetic rate in wheat. In another study triazole fungicide increased the photosynthetic rate which leads to better yield (Qiu *et al*., 2005)

Yan and Pan (1992) reported that photosynthetic rates, chlorophyll content and activity of RuBP carboxylase increased in Pea nut and Foxtail millet when it is treated with triazole fungicides like paclobutrazole and triadimefon.

2.2.2 Stomatal conductance

Stomatal closure by enhanced UV-B radiation and increased leaf diffusive resistance was explained by Tevini and Teramura in 1989. Later Nogues *et al.* (1998), observed substantial decrease of adaxial and abaxial stomatal conductance in Pea plants grown under enhanced UV-B radiation.

Afzal *et al*. (2005 a) conducted a study in wheat (*Triticum aestivum*) revealed that growth and physiological responses increased by exogenous application of ascorbic acid also mitigate detrimental effect due to salinity.

Navarro *et al.* (2007) studied that triazole treated plants having higher stimulation in stomatal regulation due to the increase of photosynthetic pigments.

2.2.3 Transpiration rate

Liu *et al.* (2003) reported that decreased transpiration rate due to stomatal diffusion resistance occurred in *Trichosanthes kirilowii* exposed to higher UV-B level.

Studies show that salt stressed plants posses a significant reduction in the transpiration rate, which can be enhanced by the application of 20 mM glycine

betaine. The results were obtained within 3 to 4 days after application (Hu and Hu, 2012)

In another experiment, transpiration rate reduced at an extend of 8.82% and 17.5% in rice and tomato due to the application of strobilurin fungicide, which contain trifloxystrobin and tebuconazole (Nagajothi, 2013)

2.2.4 Chlorophyll fluorescence

The chlorophyll fluorescence parameter widely used to evaluate the efficiency of photosynthetic system in chloroplast membrane under different abiotic atress condition (Chen *et al*., 2010)

Long *et al.* (1994) reported that strong light intensity induce photoinhibition by decreasing photochemical efficiency of chloroplast in C_3 crops like rice soybean etc.

Various chlorophyll fluorescence parameters are derived by the theory of energy fluxes of desired changes in absorbed and dissipated light energy fluxes (Force *et al*., 2003)

Poontariga *et al.* (1996) reported that quantum yield of non cyclic electron transport (Fm-Fs/Fm) was reduced in plants under salt stress but it is partially alleviated by exogenous application of glycine betaine i.e., 27% increment in quantum yield of non cyclic electron transport observed in plants treated with glycine betaine.

Bjorkman and Demming (1987) proposed that chlorophyll fluorescence ratio (Fv/Fm) has a positive correlation towards leaf photosynthesis. Propiconazole and tetraconazole improved the Fv/Fm ratio in wheat (Gilley and Fletcher, 1997)

In some studies the plants treated with paclobutrazole increased its photosynthetic efficiency about 11-13% under stress condition and also paclobutrazole has a capacity to alter fluorescence ratio (Berova *et al*., 2002).

2.3 Effect of glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on biochemical characters under UV-B condition.

2.3.1 Chlorophyll content

Pal *et al.* (1999) reported that 10% reduction in chlorophyll content by the exposure of UV-B radiation and the reduction more among dicot species (10-78%) compared to monocot (0-33%)

The amount of leaf chlorophyll is reduced in the plants grown under saline condition which can be alleviated by the exogenous application of glycine betaine. Under normal condition glycine betaine do not improve the leaf chlorophyll but it had a significant effect on stressed plant (Hu and Hu, 2012).

In another study Michaela *et al.* (2000), found that foliar application of glycine betaine on tomato plants under drought or salinity condition increased the chlorophyll content. Study conducted in cotton plant to check the influence of glycine betaine on chlorophyll content revealed that 800ppm of glycine betaine increased Chl a , Chl b and total Chl in cotton plants.

Khalil *et al.* (2012) studied the effect of ascorbic acid in *Osimum basilicum* plant under water stress, they observed that foliar application of ascorbic acid increased the photosynthetic pigments Chlorophyll a, Chlorophyll b, and total Chlorophyll, also the higher concentration of ascorbic acid decreased the content of photosynthetic pigments.

Under 40% field capacity a significant decrease in chlorophyll pigments was observed in soybean. Improvement in chlorophyll content could be observed after foliar application of ascorbic acid (100ppm) but decline in photosynthetic pigment was found under higher concentration of ascorbic acid (Amira and Abdul Qados, 2014)

Khodary (2004) observed an improvement on chlorophyll b content in maize due to the application of ascorbic acid and salicylic acid. Application of antioxidants increased the chlorophyll content in canola under saline stress (Sakr and Arafa, 2009)

Gortz *et al.* (2008) found that triazole compound has a positive effect on relative chlorophyll content in barley which is measured by SPAD. Some other systemic fungicide like cyproconazole and propiconazole was also increased chlorophyll index (Hennouni *et al.,* 2012)

Foliar application of a mixture of trifloxystrobin and tebuconazole increased the chlorophyll index in rice and tomato up to 16.91% and 18.08% respectively (Nagajothi and Jeyakumar, 2014).

Neda and Payman (2013) conducted study in tomato and found that under drought stress plants reduced chlorophyll and carotenoid pigment. Exogenous application of triadimefon enhanced the chlorophyll and carotenoid pigment

In tapioca pigment composition was found increased by the application of triadimefon and hexaconazole (Gomathinayagam *et al.,* 2007). Triazole treatment enhanced the chlorophyll and xanthophylls content in the leaves of *Catharanthus roseus* (Abdul jaleel *et al.,* 2007)

2.3.2 Flavanoid content

Flavanoids are the light absorbing protective pigments which is seen in leaves and stems especially those plant parts exposed to UV-B radiation and this pigments

mainly situated in epidermal cell to protect internal cell layers (Braun and Tevini,1993) . Cladwell *et al.* (1983) and Beggs *et al.* (1980) reported that plants alleviates negative effect of UV-B radiation by accumulation of the UV-B absorbing pigments like flavanoid and phenol.

Plants acclimatize to UV-B by improved photo repair mechanism and accumulation of UV-B absorbing pigments like flavanoid and phenol. UV-B absorbing pigments not only accumulated in the tissues where direct exposure of UV-B occur but also it is present in subcellular domains like vacuoles, roots, leaves, nucleolus and chloroplast (Jansen *et al*., 2012 ; Schreines *et al*., 2014)

Agati and Tattini (2010) reported that UV-B regulates the genes of flavanoid biosynthesis and protective role of phenolics is based on their antioxidative capabilities. Study conducted in rice cultivar revealed that the content of UV-B absorbing pigments positively correlated to UV-B radiation (Teramura *et al*., 1992)

Grossmann *et al*. (1987) reported that triazole compounds stimulate or retard plant hormones by inhibiting ergosterole and gibberellins and also it regulates endogenous cytokinin and ABA. Primary effect of triazole fungicide is to inhibit kaurene oxidase activity and gibberellins biosynthesis and promote ABA synthesis (Graebe, 1984)

2.3.3. Phenol content

Wallace and Fry (1994) reported that phenols are secondary metabolites which involved in various physiological processes and also modulate the cell wall plasticity. Hence, an increase in phenolic concentration, protect the photosynthetic tissue from enhanced UV-B radiation (Mohammed and Tarpley, 2011a)

UV radiation act as mild stimulus for the synthesis of phenolic compounds in plants. Accumulation of penolic compounds occurs through phenyl propanoid pathway (Logemann *et al*., 1999)

Alexieva *et al.* (2001) reported that plants grown under low PAR/UV-B accumulate more UV-B absorbing phenolic compound

Afzal *et al.* (2005b) and Dolatabadian *et al.* (2008) reported that nowadays more attention has been given to application of plant growth regulators exogenously. Physiological and biochemical responses increased by soaking seed in plant growth regulators before planting (Ashraf and Rauf, 2001)

Application of strobilurin fungicide increased phenol content in rice (22.10%) and phenol content in tomato (31.91%) due to the activation of phenyl alanine lyase (Nagajothi, 2013)

Significant increase in phenol content was observed in turmeric (Jaleel *et al*., 2008) and in *plectranthus* (Lakshmanan *et al*., 2007) by triazole fungicide application. Sakhabutdinova *et al.* (2003) reported that phenolic plant growth regulators like salicylic acid have a capacity to alter physiological process in plants.

Jaleel *et al*. (2008) reported that phenol content reduced and auxin content increased in plants treated with triadimefon. In another study catalase activity also increased due to application of triadimefon in banana (Coolbaugh *et al*., 1978)

2.3.4 Xanthophyll content

Xanthophyll acts on light harvesting photosynthetic membranes and protect photosynthetic apparatus from excessive light energy by quenching chlorophyll and singlet oxygen (Siefarmann-Harms, 1987)

Under harmful light condition the sudden appearance of xanthophyll in the thylakoid membrane is actually a protective mechanism to reduce the adverse effect due to stress by re- enforcing the protective compounds like α-tochopherol (Sarry *et al*., 1994)

Khan (2007) reported that ascorbic acid has a role in photochemistry of plants which regulate redox reaction of photosynthetic electron carriers also act as co factor for violaxanthin de-epoxidase present in xanthophylls cycle which help plants for photo protection.

2.3.5 Catalase activity

UV-B induced oxidative burst of H_2O_2 (Karpenski *et al*., 1997) increased the activities of catalase which acts as antioxidant enzyme (Asada, 1999)

Reshmi and Rajalakshmi (2012) reported an increase in the catalase activity in UV-treated medicinal plant *Spilanthes acmella* (Tooth ache plant)

Hu and Hu (2012) conducted a study in perennial rye grass and observed an increase in catalase activity by 22% and 20% by application of 20 and 50 mM glycine betaine respectively under salinity stress.

Tijen and Ismail (2004) observed that under salt stress condition exogenous application of glycine betaine reduced the activities of catalase and superoxide dismutase in leaves, because the external feeding of glycine betaine reduced the injury due to ROS during stress.

Batool *et al.* (2012) conducted a pot culture experiment in *Saccharum spp*. Hybrid cv. HSF.240 under salt stress. They applied different concentration of ascorbic acid (0.1, 0.5 and 1mM) with or without NaCl (100mM) application and found that treatments not only reduced the salt content in the plants but also it increased the growth and improved the antioxidant enzymes and proline content.

Ijaz. (2012) in an experiment with hybrid maize revealed that exogenous application of ascorbic acid and salicylic acid increased fresh and dry weights of roots and shoots, chlorophyll b, increased membrane stability index, higher catalase

and peroxidase activity, leaf relative water content and ultimately increased the yield and yield parameters

Appu and Muthukrishnan (2014) reported that exogenous application of low concentration of ascorbic acid and salicylic acid improved the activity of antioxidant under abiotic stress.

Study conducted in pumpkin found that plants treated with 30 mgL^{-1} salicylic acid and 15mgL^{-1} ascorbic acid increased shoot and root biomass (Rafique *et al.,* 2011). Dolatabadian *et al.* (2008) conducted study in *Brassica napus* found that ascorbic acid enhances antioxidant enzyme system so that it will reduce the deleterious effect of salinity.

Marshal *et al.* (2000) reported that triazole fungicides increased ABA, proline and antioxidant system and reduced the shoot growth of the plants. Triadimefon increased proline content and SOD in tomato under drought stress. Catalase activity has no significant effect when the plants treated with triadimefon under stress (Neda and Payman, 2013)

2.4 Effect of glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) on yield and yield parameters under UV-B condition.

Yield characters refer to the components of rice crop which directly correlate in to yield. These are number of panicle per plant, number of spikelet per panicle, 1000 grain weight and grain yield. Study conducted in Japanese rice cultivars under UV-B supplemented condition found that elevated UV, reduced the yield and grain development (Kumagai *et al*., 2001). In another study conducted in rice revealed a reduction in panicle number, tiller number, dry mass, grain size and grain yield due to the supplemental UV-B radiation (Hidema *et al*., 2005)

Hassen *et al.* (2013) conducted a study in apricot (*Prunus armeniaca* L.cv.shahrouds) flowers found that application of 200mg/L ascorbic acid reduced damage of flower organs and reduced electrolyte leakage when compared to control. But higher concentration of ascorbic acid has a negative effect under chilling stress.

Zeevaart *et al.* (1993a) reported that triazole compounds considered as inhibitors of gibberellins biosynthesis block three separate gibberellins biosynthesis pathway. Even though gibberellins production inhibited the cell division occur but the cell do not elongate that leads to shorter internodes but more leaves (Quinlan, 1981)

Ebrahim *et al.* (2014) found that foliar application of ascorbic acid with N_2 fertilizer had a significant variation in 1000 grain weight in Quinoa (*Chenopodium quinoa*)

Caleb and David.(1999) reported that exogenous application of ascorbic acid on root nodules increases the nitrogenous activity and total nodule number which also enhances the yield of leguminous crop. In soybean (*Glycine max*) 0.5 to 1mM application of ascorbic acid exogenously improved the root weight.

Mohammed and Tarpley (2011b) found that under stress condition (Salinity stress) exogenous application of glycine betaine on leaves improved the grain yield of soybean due to the induction of lateral branch, pod number per plant and thousand grain weight . In another study exogenous application of glycine betaine increased 20-30% yield of vegetables under stress condition (Makela *et al*., 1998). In tomato also 27-39% yield increased by the foliar application of glycine betaine at mid flowering stage under heat and salt stress.

Foliar application of glycine betaine under drought stress in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) increased the yield up to 25% and 11% respectively. This is because of the increment in net photosynthesis, stomatal conductance, reduction in rate of photorespiration and improvement of more efficient gas exchange

(Makela *et al.,* 1996) and thus prevents photoinhibition due to better availability of carbon for photosynthetic process.

Research on effect of UV-B on yield parameters of rice revealed that more number of filled grain and spikelets were observed under UV excluded condition compared to natural solar UV-B condition (Wagh, 2015)

Rice plant treated with 16 $KJm^{-2}day^{-1}$ UV-B showed increase in spikelet sterility and a decrease in harvest index (Yun *et al*., 2010)

Materials and Methods

3. MATERIALS AND METHODS

The aim of the present study was to understand the photoprotective potential of eco friendly stress mitigating chemicals on photo inhibition in photochemistry and photosynthesis of rice (*Oryza sativa* L.) under natural solar UV-B radiation. A pot culture experiment was conducted at the Department of Plant Physiology, College of Horticulture, Vellanikkara, during November 2015 to March 2016. A brief account of the materials used and methodologies adopted in this study are given below.

3.1 General details

3.1.1 Location

The pot culture experiment was carried out at College of Horticulture Vellanikkara. The geographical co-ordinates of the location of the experimental plot are 10º 32'N and 76º 16' E with an altitude of 22.5m above MSL.

3.1.2 Season

The crop period was from November 2015 to March 2016

3.2.1. Plant material

Rice variety Uma (Mo. 16) developed by Rice Research Station, Moncompu was used in this study. It is a popular medium duration (115-120 days) rice variety of Kerala. In Kerala this red kernelled, medium bold grain variety is usually cultivated for three seasons. In Kuttanad under favourable condition it is cultivated as an additional crop and yield is more than 5t/ha. Uma is resistant to BPH and also it is a non lodging variety.

3.2.2 The experiment details are given below

Design: Completely Randomized Design (CRD)

Treatments: -

Chemicals : 3 at 2 concentrations

Control : a) Water spray

b) Absolute control

Total :8 (2x3+2)

Replication : 3

Number of pots per treatment :3

Variety : Uma (Mo.16)

Levels of UV-B radiation: Two

1. Open condition- Where crop exposed to 100% natural solar UV - B radiation

2. Poly house condition - Which include 20 percent reduced ambient UV-B radiation

Treatment details:

1. T_1C_1: Glycine betaine -10ppm

2. T_1C_2: Glycine betaine - 20ppm

3. T_2C_1: Ascorbic acid - 50ppm

4. T_2C_2: Ascorbic acid -100ppm

5. T_3C_1: Nativo 75 WG - 50 ppm

6. T_3C_2: Nativo 75 WG - 70 ppm

7. T_4: Water spray

8. T_5: Absolute control

The treatments were given as foliar spray at 30th and 60th day after transplanting.

3.2.3 Details of UV-B environment

After transplanting the crop was exposed to the following two levels of UV-B radiation

1. Open condition - 100% natural solar UV-B radiation by placing the experiment pots in an open field.
2. Polyhouse condition - Ventilated polyhouse of size 21.37 m² was used to expose the crop to 80% natural solar radiation which allows 20% reduction of UV-B from ambient level for plant growth. The polyhouse roof was cladded with UV limiting polyethylene sheet (0.25mm thickness) which contain UV-B filter that transmit 80 percent full spectrum and exclude 20% of UV-B at ambient level.

3.2.4 Raising of crop and management

Earthern pots of (27cm length and 29cm diameter,) were filled with clay loam soil collected from paddy field of Agriculture Research Station, Mannuthy (ARS, Mannuthy). FYM applied in each pot at the time of pot filling@ 5t/ha .The seedlings were raised in mat nursery. All necessary care was given to nursery till it attained the required age for transplanting. After 20 days seedlings were transplanted in 120 pots at the rate of two seedlings per hill and two hills per pot. The pots were then arranged in two different UV level. One set of pots (60 nos) were placed under open condition and another set of 60 pots were placed under reduced UV-B condition in a polyhouse. Nutrient fertilizers such as nitrogen in the form of urea (46% N) Phosphorus as factomphos (20% P) and potassium as muriate of potash (58% K) was applied at the rate of 90:45:45 kg/ha respectively. Full dose of P and K, 33 percent N were applied

Open condition - 100% natural solar radiation

Polyhouse condition - 80% natural solar radiation

Plate 1: Rice grown under different levels of UV-B

Chlorophyll fluorometer

Portable photosynthesis system

Quantum light meter

UV-B meter

Plate 2: Instruments used during the study

Measurement of UV radiation

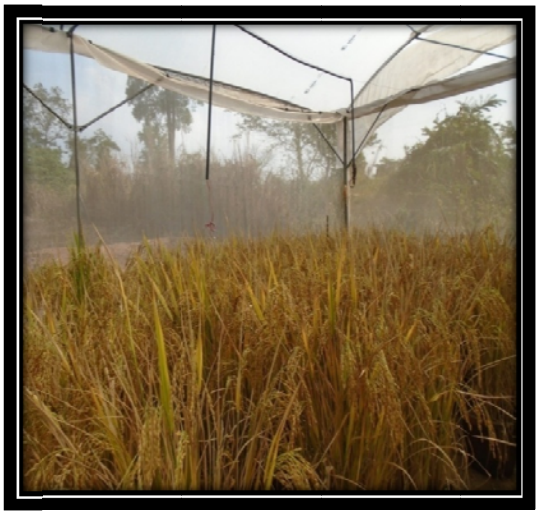
Measurement of gas exchange parameter

Plate 3: Taking observations using various instruments

Seedling stage

Tillering stage

Flowering stage



Harvesting stage

Plate 4: Various growth stages of rice

as basal and remaining doses of N was given in two equal doses at tillering and maximum tillering stage.

3.2.5 UV-B and PAR (Photosyntheticaly Active Radiation) measurement

Daily variation in ultraviolet-B (UV-B) radiation at plant canopy was monitored throughout the growing period of rice using UV-B meter (Model- 3414F, Field Scout, Spectrum technology, Icn. USA). Measurements were taken between 8 am to 2 pm (8 am,10 am, 12noon, and 2pm) at two hours interval and UV radiation was expressed as Wm^{-2}

Photosynthetic photon flux density (PPFD) is the intensity of photosynthetically active radiation (PAR) which is expressed in $\mu molm^{-2}s^{-1}$. The incident photosynthetically active radiation (PAR) in two growing condition was also measured using quantum light meter (Model- 3415 F, Field scout, spectrum technology, Icn. USA) from 8am to 2pm daily.

3.3 Observations recorded

3.3.1 Morphological and phenological observation

a) Plant height

Nine plants were selected randomly and tagged from each treatment to measure height of the plant at 45^{th} day after transplanting (15 days after 1^{st} treatment application). It was measured from the base of the plant to tip of longest leaf of the plant and expressed in centimeter.

b) Number of tillers per hill

Number of tillers were counted by selecting two hills from each replication at maximum tillering stage and expressed as tiller per hill.

e) Days to heading

The days when 50% panicle tip emerged from the flag leaf sheath in each pot is recorded as days to heading and this was counted from date of transplanting.

d) Days to 50% percent flowering

Flowering stage was considered when stamens of spikelet came out and yellowish whitish structures was seen in spikelet of mother tillers. Number of days taken to complete 50 percent flowering in each treatment was counted from transplanting and the mean value expressed in days.

e) Days to harvest

Number of days taken for the crop to harvest was counted from transplanting date and mean value expressed as days to harvest

f) Grain filling period

Grain filling period was derived by counting the number of days from 50% flowering to harvest for different treatments.

3.3.2 Physiological observation

Physiological parameters were recorded at 15 days after 1st and 2nd treatment application that is at 45th and 75th days after transplanting.

3.3.2.1 Leaf gas exchange parameters

Photosynthetic rate ($\mu molCo_2 m^{-2} s^{-1}$), transpiration rate ($\mu mol\ H_2O\ m^{-2} s^{-1}$), and stomatal conductance ($mol\ H_2O\ m^{-2} s^{-1}$) were recorded using portable photosynthesis system (PPS- Model – LI-6400 of Licor Inc. Lincoln, Nebraska, USA) during 45th and 75th days after transplanting. The measurements were made on upper most fully expanded leaf (3rd leaf from top) and totally 3 measurements were taken from the

same leaf .Three plants from each treatment selected for measurement of photosynthetic characters. Observations were recorded between 8.30am to 10.30 am

3.3.2.2 Chlorophyll fluorescence

The leaf used for photosynthetic parameter measurements were used for chlorophyll fluorescence measurement also. The chlorophyll fluorescence was measured using the instrument portable modulated chlorophyll fluorometer (OPTI-SCIENCES: OSIP Hudson, USA). Before measuring chlorophyll fluorescence parameter, leaf was put in dark adapted state for 30 minutes using light exclusion clips. The following chlorophyll fluorescence parameters were measured. Minimum chlorophyll fluorescence in dark adapted state (F_o) and Maximum chlorophyll fluorescence in dark adapted state (Fm). Using these two parameter the maximum photo chemical efficiency of photo system II was calculated using the formula Fv/Fm = (Fm-F_o)/Fm.

3.3.3 Biochemical characters

The biochemical parameters recorded at 45th and 75th DAT (15 days after each chemical application) was as detailed below

a) Flavanoid content

The content was examined spectro photometricaly according to Mirecki and Teramara (1984). Five hundred mg leaf sample put in 80% acidified ethanol (methanol: water: HCl 79: 20: 1) and kept overnight in dark. Absorbance was read at a wavelength of 300 nm and flavanoid content was calculated using the following formula and expressed as $A_{300}g^{-1}$ fr.wt. of plant sample

$$Y= 16.05xA$$

Where, Y- concentration of UV-B absorbing compound equivalent to Coumaric acid

A - Absorbance at 300nm

b) Xanthophyll

Xanthophyll estimation was done by the method of Neogy *et al.* (2001). Two hundred and fifty mg leaf sample collected from third leaf was macerated with 10 ml of 80% acetone at 4ºC using pestle and mortar. Centrifuged the content for 15 minutes at 3000 rpm, the residue re extracted twice and collected the supernatant. The acetone extract was shaken in separating funnel by adding equal volume of hexane. The xanthophyll was extracted from hexane fraction by repeated washing with 90% ethanol. The ethanol fraction containing xanthophylls was measured at 450 nm in a UV- VIS Spectrophotometer (Spectroquant, Pharo 300, Merck KGaA, Germany). Calculation done by the following formula and expressed as A_{450} µg g^{-1}

$$\frac{\text{Absorbance of sample at 450 nm (µg)}}{\text{Weight of the sample (g)}}$$

c) Chlorophyll content

The total chlorophyll, chlorophyll a and chlorophyll b were estimated in a fully expanded young leaf by the method suggested by Hiscox and Israelstam (1979) using DMSO as extraction reagent. Chlorophyll extracted in DMSO was estimated in UV- VIS Spectrophotometer (Spectroquant, Pharo 300, Merck KGaA, Germany) at two wavelengths 645 and 663 nm. The formulae used for the chlorophyll calculation is given below and the results were expressed in mg g^{-1} fr. wt.

Chlorophyll a = [(12.7xA663) - (2.69xA645)] x V/1000xW

Chlorophyll b = [(22.9xA645) - (4.68xA663)] x V/1000xW

Total chlorophyll = [(20.2xA645) + (8.02xA663)] x V/1000xW

Where, A - Absorption at given wavelength

V - Volume of supernatant solution made

W- Weight of the sample

d) Phenol content

The procedure given by Malick and Singh (1980) was followed for the estimation of phenol. Five hundred mg leaf sample ground and homogenized in 10 ml of 80% ethanol. Ethanol extract centrifuged at 10000 rpm for 20 minutes. The residue was re-extracted five times with 80% ethanol until the colour disappeared from the residue. The supernatant was pooled and evaporated to dry. The residue dissolved in 5ml of distilled water and different aliquots (0.2 to 2ml) were pipetted in to test tubes and made up the volume in to 3ml with water. Folin - Ciocatteau reagent 0.5 ml was added to the test tube and after 3 minute 2ml of 20% Na_2CO_3 solution added. Reading was taken at 650nm using UV- VIS Spectro photometer (Spectro quant @ Pharo, 300). Catechol was used for standard preparation and the phenol content expressed as mg g^{-1} of fr. wt. Calculation of phenol content was done by the following formula.

$$\frac{\text{Test sample absorbance}}{\text{Standard solution absorbance}} \times \frac{\text{Concentration of standard solution}}{\text{weight of sample}}$$

e) Catalase activity

Catalase activity was analysed by the method suggested by Barber (1980). Five hundred mg fresh leaf sample collected, macerated with 10 ml of cold phosphate buffer and centrifuged the content at 3000 rpm for 10 minute. Each 1ml extract taken in 5 different beakers to that 5ml of 1.5% sodium perborate and 1.5ml of phosphate buffer added. To each beaker ten ml of 2N sulphuric acid was poured at the time interval of 1 minute, 2 minute, 3 minute, 4 minute after adding enzyme extact in 1st four beakers respectively. In the last beaker 10 ml H_2O_2 added before addition of

enzyme extract. This beaker kept as blank for comparison. The content in the beaker titrated against 0.05 $KMnO_4$. The end point noted by the development of pink colour which persist for 30 Seconds. The amount of H_2O_2 scavenged by catalase was calculated by the following formula.

$$\frac{25x0.85}{2} \times \frac{V}{W}$$

Where, W= weight of sample

V=Volume of $KmnO_4$ utilized (Titer value)

The activity expressed in terms of enzyme unit. One unit of enzyme (catalase) defined as that amount of enzyme, which break down 1µ mol.of H_2O_2/ min g^{-1} fr. wt.

3.3.4 Yield parameters

a) Number of panicle per hill

Number of panicle was counted from all the pots in each replication after harvest and the mean value noted and expressed as number of panicle per hill.

b) Number of spikelet per panicle

Eighteen panicles were selected randomly from each replication for counting the number of spikelets. Mean value for single panicle was worked out and expressed as spikelet per panicle.

c) 1000 grain weight

Randomly selected 1000 grains were weighed using electronic balance (Model- CB-10, ConTech, Instrument Company, Mumbai, India) from each treatment and recorded as 1000 grain weight in gram

d) Fertility co-efficient (%)

Grains were selected from randomly selected 18 panicles and separated in to filled grains and chaff grains. Fertility co-efficient (%) was calculated by the ratio of filled grains to the total number of spikelet in 18 panicles.

e) Grain yield

Manual harvest was done from each pot and separated straw and grain. Grain weight was taken using electronic balance (Model- CB-10, ConTech, Instrument Company, Mumbai, India) and recorded as grain yield per pot in grams.

3.4 Stastical analysis

Stastical analysis was done using WASP 2.0, developed by ICAR, GOA. Pair wise comparisons of the treatments were done using critical difference.

Results

4. RESULTS

The pot culture experiment was conducted to investigate the photo protective potential of stress alleviating chemicals against UV-B stress in rice at the College of Horticulture, Vellanikkara. The maximum and minimum temperature recorded during the study period was 37.4ºC and 19.6ºC.

The data recorded on morphological, phenological, physiological, biochemical and yield attributes were calculated and analyzed statistically. The mean data of all observations are presented in appropriate tables. The results of the study conducted are detailed below.

4.1 UV-B analysis

The mean value of UV-B radiation recorded at different times of the day (8am, 10am, 12pm, 2pm) throughout the growing period (November- 2015 to March-2016) is given in Table 1.

Significantly higher UV-B radiation was recorded under 100% solar radiation when compared to 80% solar radiation from November-2015 to March-2016. Daily observation under both conditions revealed diurnal variation in UV-B radiation. The UV-B radiation gradually increased from 8am to 12pm and decreased from 12pm to 2pm under both conditions. The maximum UV-B radiation was recorded at 12 noon.

Temporal observations of the UV-B radiation showed that highest UV-B was recorded in the month of February and March 2016 and in all months 12 noon recorded highest UV-B radiation in both conditions. At 100% solar radiation the UV-B near the top of the canopy was in the range of 1.82 to 2.18 Wm^{-2} at 12 pm where as at 80% solar radiation it was 0.87 to 0.94 Wm^{-2} at the same time.

Table 1: Diurnal variation in UV-B radiation (Wm^{-2}) during the growth period.

November-2015	**8 AM**	**10 AM**	**12 PM**	**2 PM**	**CD**
100% solar radiation	0.56	1.21	1.82	1.23	**0.20**
80% solar radiation	0.27	0.49	0.87	0.58	**0.08**
December-2015					
100% solar radiation	0.60	1.29	1.83	1.39	**0.18**
80% solar radiation	0.20	0.66	0.91	0.47	**0.18**
January-2016					
100% solar radiation	0.60	1.35	1.83	1.35	**0.07**
80% solar radiation	0.20	0.66	0.92	0.47	**0.18**
February-2016					
100% solar radiation	0.54	1.75	2.14	0.87	**0.35**
80% solar radiation	0.30	0.59	0.91	0.65	**0.07**
March-2016					
100% solar radiation	0.66	1.69	2.18	1.05	**0.15**
80% solar radiation	0.30	0.62	0.94	0.67	**0.05**

4.2 Photosyntheticaly active radiation (PAR) analysis

The mean value of monthly PAR at different times (8am, 10am, 12pm, 2pm) throughout the growing period (November- 2015 to March- 2016) is given in Table 2.

Significantly higher PAR was recorded under 100% solar radiation when compared to 80% solar radiation from November 2015 to March 2016. Daily observations revealed that PAR gradually increased from 8am to 12pm but it decreased from 12 to 2pm under both conditions. In all months 12 noon recorded highest PAR in both conditions. At 12 noon under open condition PAR was in the

range of 1508.32 to1786.92 μmolm^{-2}s^{-1}and it was 997.19 to 1261.42 μmolm^{-2}s^{-1} under UV-B reduced condition.

Table 2: Photosyntheticaly active radiation (μmolm^{-2}s^{-1}) recorded at different time.

November-2015	**8 AM**	**10 AM**	**12 PM**	**2 PM**	**CD**
100% solar radiation	687.75	1221.78	1508.32	905.25	**307.84**
80% solar radiation	365.85	569.02	997.19	462.17	**146.26**
December-2015					
100% solar radiation	501.50	1134.40	1687.95	1118.40	**84.39**
80% solar radiation	348.80	555.16	1116.13	850.72	**219.48**
January-2016					
100% solar radiation	535.46	1157.94	1685.65	1217.41	**291.68**
80% solar radiation	337.67	914.33	1008.71	724.25	**281.89**
February-2016					
100% solar radiation	633.17	1409.67	1665.12	1115.92	**380.93**
80% solar radiation	309.65	889.70	1124.20	918.75	**60.68**
March-2016					
100% solar radiation	537.53	1323.48	1786.92	1441.63	**219.23**
80% solar radiation	339.65	857.15	1261.42	1001.65	**164.50**

4.3 Morphological and phenological characters

Data on morphological and phenological observations recorded at two growing conditions (100% and 80% solar radiation) are given below.

4.3.1 Plant height

The mean data of plant height (Table.3) measured at the tillering stage showed significant variation between two growing conditions viz. 100% natural solar radiation and 80% solar radiation. Crop grown at 80% solar radiation recorded maximum height (71.06 cm) when compared to plants grown at 100% natural solar radiation (58.84 cm).

Table3: Effect of two levels of UV-B radiation on plant height(cm), number of tillers per hill , days to heading, days to 50% flowering and days to harvest.

Condition	Plant height(cm)	No of tillers $hill^{-1}$	Days to heading	Days to 50% flowering	Days to harvest
100% solar radiation	58.84	18	73.96	76.64	115.92
80% solar radiation	71.06	15	66.83	71.13	111.63
CD(0.05)	**0.49**	**0.67**	**1.81**	**0.70**	**0.69**

Mean data on the effect of chemical treatments on plant height are given in Table.4 Significant variation in plant height was observed with different chemical treatments under two UV-B conditions. Under 100% solar radiation, among the chemical treatments T_1C_1 recorded maximum height (60.98 cm) which was on par with T_1C_2, T_2C_1, T_2C_2, and T_4 (water spray). Treatments with both concentration (50ppm and 70ppm) of *Nativo* (T_3) recorded lowest height (57.39 cm) among the three chemicals used in the study, which was significantly lower when compared to T_4 (water spray) and significantly higher when compared to T_5 (absolute control).

Under 80% natural solar UV-B condition T_3C_2 (Nativo-70ppm) and T_4 (water spray) only recorded significantly higher plant height than other chemical treatments and absolute control. The treatments T_1C_1, T_1C_2, T_2C_1, T_2C_2, and T_3C_1 were on par

with absolute control. They could not enhance plant height under reduced UV-B condition.

Table 4: Effect of treatments on plant height (cm) under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	60.98	71.06
T_1C_2	60.72	71.12
T_2C_1	59.78	70.91
T_2C_2	60.11	70.65
T_3C_1	57.39	70.71
T_3C_2	57.39	71.61
T_4	59.17	71.58
T_5	55.22	70.83
CD(0.05)	**1.93**	**0.43**

T_1C_1 - Glycine betaine - 10ppm T_1C_2- Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray T_5- Absolute control

4.3.2 Number of tiller per hill

The mean values of number of tillers per hill at two growing conditions are given in Table.3. Tiller count was significantly more under ambient UV-B radiation (18 tillers $hill^{-1}$) when compared to 20% reduced ambient UV-B radiation (15 tillers $hill^{-1}$) irrespective of treatments.

Among treatments T_2C_2 and T_4 recorded significantly higher number of tiller count (19 tillers hill^{-1}) which was on par with T_1C_2, T_2C_1, T_3C_1 and T_5. The lowest tiller count was observed in treatment T_1C_1 (16 tillers hill^{-1}) and T_3C_2 (15 tillers hill^{-1})

Under 80% UV-B condition T_2C_1 (Ascorbic acid 50ppm) recorded significantly maximum tiller count (19.0 tillers hill^{-1}) when compared to all other treatments. The lowest tiller number was observed in T_5 (12.0 tillers hill^{-1}) which was on par with T_1C_2, T_2C_2, T_3C_1 (14.0 each tillers hill^{-1}). T_4 and T_1C_1 were 2nd in producing maximum number of tillers (16 tillers hill^{-1}) which was on par with T_3C_2 (15 tillers hill^{-1})

Table 5: Effect of treatments on number of tillers hill^{-1} under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	16.0	16.0
T_1C_2	17.0	14.0
T_2C_1	18.0	19.0
T_2C_2	19.0	14.0
T_3C_1	18.0	14.0
T_3C_2	15.0	15.0
T_4	19.0	16.0
T_5	18.0	12.0
CD(0.05)	**1.40**	**2.43**

T_1C_1 - Glycine betaine - 10ppm

T_1C_2- Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 – Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray

T_5 - Absolute control

4.3.3 Days to heading

Mean data on days to heading under two levels of UV-B radiation are presented in Table.3.Two levels of UV-B radiation revealed significant variation in days to heading. Plants grown under 100% ambient solar radiation took 74 days for heading where as plants under 80% solar radiation headed within 67 days. Early heading was observed under reduced level of ambient UV-B radiation.

Mean data on effect of treatments on days to heading are given in Table.6.The different treatments could not make significant effect on days to heading under 100% natural solar radiation whereas treatments expressed significant effect under reduced ambient UV-B condition (80% UV-B condition).

Early heading was observed under T_5 (62 days) which was on par with T_1C_1, T_2C_2, T_3C_2 and T_4 where the number of days for heading was recorded as 67, 66, 64 and 66 days respectively. Among treatments more number of days to heading was recorded by T_3C_1 (72 days) followed by T_2C_1 (68 days) and T_1C_2 (67 days)

Table 6: Effect of treatments on days to heading under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	72.00	67.00
T_1C_2	75.00	68.00
T_2C_1	74.00	68.00
T_2C_2	71.00	66.00
T_3C_1	76.00	72.00
T_3C_2	70.00	64.00
T_4	76.00	66.00
T_5	76.00	62.00
CD(0.05)	**NS**	**5.02**

T_1C_1 - Glycine betaine - 10ppm T_1C_2 - Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray T_5 - Absolute control

4.3.4 Days to 50% flowering

Mean data on effect of two levels of UV-B radiation on days to flowering are given in Table.3 Significant difference was observed in days to 50% flowering under two growing condition (100% and 80% solar radiation). Early completion of 50% flowering was observed under 80% solar radiation (71 days) and late completion of 50% flowering occurred under100% solar radiation (76 days).

Mean data on effect of treatments on days to 50% flowering are given in Table.7. All chemical treatments could contribute a significant effect on days to 50% flowering under both ambient and reduced solar radiation. Under 100% solar radiation T_1C_1, T_3C_2 and T_2C_2 completed 50% flowering at 75, 73 and 75 days

respectively where as all other treatments took 78 days to complete 50% flowering. The absolute control also took more days (78) to complete 50% flowering which was on par with T_1C_2, T_2C_1, T_3C_1, T_4 and T_5.

Under 80% solar radiation all the treatment effect was on par except T_3C_1 which recorded significantly more number of days (74 days) to complete 50% flowering.

Table7: Effect of treatments on days to 50% flowering under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	75.00	71.00
T_1C_2	78.00	71.00
T_2C_1	78.00	71.00
T_2C_2	75.00	70.00
T_3C_1	78.00	74.00
T_3C_2	73.00	70.00
T_4	78.00	71.00
T_5	78.00	72.00
CD(0.05)	**2.67**	**2.74**

T_1C_1 - Glycine betaine - 10ppm T_1C_2 - Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 -Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray T_5 - Absolute control

4.3.5 Days to harvest

The mean value of days to harvest at two growing conditions are given in Table.3.Plants under reduced solar radiation attained early harvestable maturity and

harvested early (112 days) but the plants grown under open condition harvested at 116 DAT.

Mean data on effect of treatments on days to harvest are given in Table.8. Though the two growing conditions revealed a significant effect on days to harvest, the different treatment effect was non significant for this phenological character.

Table 8: Effect of treatments on days to harvest under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	115.00	111.00
T_1C_2	116.00	111.00
T_2C_1	117.00	112.00
T_2C_2	116.00	112.00
T_3C_1	116.00	113.00
T_3C_2	116.00	113.00
T_4	116.00	112.00
T_5	115.00	111.00
CD(0.05)	**NS**	**NS**

T_1C_1 - Glycine betaine – 10ppm T_1C_2- Glycine betaine – 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 – Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray T_5 - Absolute control

5.4 Physiological characters

The results of the physiological characters observed 15 days after each treatment application i.e. 45^{th} and 75^{th} DAT are given below.

5.4.1 Photosynthetic rate

The mean value of photosynthetic rate at two growing conditions at two different stages of growth are given in Table 9.Photosynthetic rate was recorded after 45th DAT and 75th DAT from plants grown under 100% and 80% solar radiation. Photosynthetic rate increased from 45th DAT to 75th DAT under both conditions. At 45th DAT there was no significant difference in photo synthetic rate under both conditions, but at 75th DAT the mean data of photosynthetic rate showed a significant variation between two growing conditions. At this stage higher photosynthetic rate was recorded under 80% solar radiation (26.07 µmol $CO_2 m^{-2} s^{-1}$) and lower photosynthetic rate was recorded under 100% solar radiation (24.98 µmol $CO_2 m^{-2} s^{-1}$).

Table 9: Effect of two levels of UV-B radiation on photosynthetic rate (µmol CO_2 $m^{-2} s^{-1}$), transpiration rate (mmol H_2O $m^{-2} s^{-1}$), stomatal conductance (mol H_2O $m^{-2} s^{-1}$) and chlorophyll fluorescence (Fv/Fm) at different growth stages.

Growing condition	Photosynthetic rate(µmol CO_2 $m^{-2} s^{-1}$)		Transpiration rate (mmol H_2O $m^{-2} s^{-1}$)		Stomatal conductance (mol H_2O $m^{-2} s^{-1}$)		Chlorophyll fluorescence (Fv/Fm)	
	45th DAT	75th DAT	45th DAT	75th DAT	45th DAT	75th DAT	45th DAT	75th DAT
100% solar radiation	23.50	24.98	2.09	2.19	0.15	0.16	0.66	0.71
80% solar radiation	22.50	26.07	2.03	2.40	0.14	0.20	0.69	0.72
CD(0.05)	**NS**	**0.81**	**NS**	**0.17**	**NS**	**0.02**	**0.01**	**NS**

Mean data on effect of treatments on photosynthetic rate at 45th DAT and 75thDAT are given in Table.10.The different chemical treatment recorded a significant difference in photosynthetic rate under two conditions at 45th DAT and 75th DAT. The overall photosynthetic rate increased from 45th DAT to 75th DAT under both condition.

At 45th DAT significantly higher photosynthetic rate (26.67 µmol CO_2 m^{-2} s^{-1}) was recorded with the treatment T_3C_2 when compared to all other treatments. The lowest photosynthetic rate was recorded by T_5 (17.32 µmol CO_2 m^{-2} s^{-1}) which was on par with T_1C_1 (22.85 µmol CO_2 m^{-2} s^{-1}). All other treatments T_1C_2, T_2C_1, T_2C_2, T_3C_1 and T_4 were on par and significantly superior to T_1C_1 and T_5.

Under reduced UV-B radiation (80% solar radiation) all the treatments were significantly superior to T_5 which recorded the lowest photosynthetic rate (16.30 µmol CO_2 m^{-2} s^{-1}) at 45th DAT. The highest photosynthetic rate of 27.12 µmolCO_2 m^{-2} $s^{-1}$was recorded by the crop treated with T_3C_2 which was significantly higher to all other chemical treatments. The photosynthetic rate observed in other treatments was in the order of T_4 (25.58 µmol CO_2 m^{-2} s^{-1})> T_3C_1 (24.77 µmol CO_2 m^{-2} s^{-1})> T_2C_2 (22.81 µmol CO_2 m^{-2} s^{-1})>T_1C_1 (22.23 µmol CO_2 m^{-2} s^{-1}) > T_2C_1 (21.27 µmol CO_2 m^{-2} s^{-1}) > T_1C_2 (19.92 µmol CO_2 m^{-2} s^{-1}).

At 75th DAT under 100% solar radiation all the treatments recorded significantly higher photosynthetic rate when compared to control T_5 (19.17 µmol CO_2 m^{-2} s^{-1}). Among the chemical treatments T_3C_2 recorded significantly higher photosynthetic rate (29.42 µmol CO_2 m^{-2} s^{-1}). The photosynthetic rate observed in other treatments in the order of T_1C_1 (22.62 µmol CO_2 m^{-2} s^{-1})> T_3C_1 (24.69 µmol CO_2 m^{-2} s^{-1})> T_4(25.34 µmol CO_2 m^{-2} s^{-1})> T_2C_2(25.96 µmol CO_2 m^{-2} s^{-1})> T_2C_1(26.32 µmol CO_2 m^{-2} s^{-1})> T_1C_2(26.36 µmol CO_2 m^{-2} s^{-1}).

Crops grown at 80% solar radiation also showed significant variation in photosynthetic rate due to different treatments. T_5 recorded the lowest rate (22.89 µmol CO_2 m^{-2} s^{-1}) which was on par with T_1C_2 (23.52 µmol CO_2 m^{-2} s^{-1}). All chemical treatments were superior to T_1C_2 and T_5 treatments. Among the chemicals T_3C_1 (27.09 µmol CO_2 m^{-2} s^{-1}) , T_2C_1 (25.32 µmol CO_2 m^{-2} s^{-1}), T_2C_2 (26.05 µmol CO_2 m^{-2} s^{-1}), and T_1C_1 (25.46 µmol CO_2 m^{-2} s^{-1}) were on par but significantly higher to T_1C_2 and T_5. Highest photosynthetic rate was recorded by T_3C_2 (29.91 µmol CO_2 m^{-2} s^{-1}) which was on par with T_4 (28.31 µmol CO_2 m^{-2} s^{-1}).

Table10: Effect of treatments on photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) under two levels of UV-B radiation at different growth stages.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	22.85	22.23	22.62	25.46
T_1C_2	25.21	19.92	26.36	23.52
T_2C_1	24.49	21.27	26.32	25.32
T_2C_2	24.23	22.81	25.96	26.05
T_3C_1	23.20	24.77	24.69	27.09
T_3C_2	26.67	27.12	29.42	29.91
T_4	24.06	25.58	25.34	28.31
T_5	17.32	16.30	19.17	22.89
CD(0.05)	**2.85**	**0.19**	**2.44**	**2.19**

T_1C_1- Glycine betaine - 10ppm T_1C_2 - Glycine betaine -20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 -Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray T_5 - Absolute control

5.4.2 Transpiration rate

The mean value of transpiration rate at two growing conditions at two different stages of growth are given in Table.9

Transpiration rate recorded at 45th DAT was non significant just like photosynthetic rate but there was a significant difference at 75th DAT between 100% and 80% solar radiation. Plants exposed to 80% solar radiation recorded higher transpiration rate (2.40 mmol H_2O $m^{-2}s^{-1}$) when compared to crop grown under 100% solar radiation (2.19 mmol H_2O $m^{-2}s^{-1}$)

Mean data on effect of treatments on transpiration rate at 45th DAT and 75thDAT are given in Table.11. Different treatments recorded significant variation in transpiration rate at 45th and 75th DAT under two growing conditions. Generally there was an increment in transpiration rate from 45th to 75th DAT. Lowest transpiration rate in the range of 1.2 to 1.5 mmol H_2O $m^{-2}s^{-1}$ was observed for T_5 whereas highest transpiration rate was recorded by T_3C_2 ranging from 3.07 to 3.59 mmol H_2O $m^{-2}s^{-1}$. The lowest and highest transpiration rate was observed in accordance with photosynthetic rate under the same growing condition.

Table11: Effect of treatments on transpiration rate (mmol H_2O $m^{-2}s^{-1}$) under two levels of UV-B radiation at different growth stages.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	1.57	1.78	1.52	2.22
T_1C_2	2.49	1.62	2.52	2.12
T_2C_1	2.42	2.42	2.16	2.14
T_2C_2	2.18	2.26	2.14	2.30
T_3C_1	1.73	1.74	1.71	2.57
T_3C_2	3.07	3.26	3.58	3.59
T_4	1.88	2.38	1.77	2.77
T_5	1.37	1.24	1.50	1.47
CD(0.05)	**0.35**	**0.72**	**0.57**	**0.41**

T_1C_1 - Glycine betaine - 10ppm

T_1C_2- Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1- Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray

T_5 - Absolute control

5.4.3 Stomatal conductance

The mean value of stomatal conductance at two growing conditions at two different stages of growth is given in Table.9. There is no significant difference in stomatal conductance at 45th DAT but significant variation was observed at 75th

DAT. More stomatal conductance (0.20 mol H_2O $m^{-2}s^{-1}$) recorded under 80% solar radiation than 100% solar radiation (0.16 mol H_2O $m^{-2}s^{-1}$).

Mean data on effect of treatments on stomatal conductance at 45th DAT and 75thDAT are given in Table 12. At 45th DAT and 75th DAT under both 100% and 80% solar radiation, different treatments showed significant differences in stomatal conductance. Plants without any chemical treatments (T_5) had significantly lower stomatal conductance under both conditions at 45th and 75th DAT (0.06, 0.05, 0.04, 0.07 mol H_2O $m^{-2}s^{-1}$ respectively). Significantly higher stomatal conductance was noted for the treatment T_3C_2 under 100% and 80% solar radiation at 45th and 75th DAT (0.17, 0.18, 0.20, 0.23 mol H_2O $m^{-2}s^{-1}$ respectively).

Under 100% solar radiation treatments T_1C_1 (0.16 mol H_2O $m^{-2}s^{-1}$), T_2C_2 (0.16mol H_2O $m^{-2}s^{-1}$),T_3C_1 (0.15 mol H_2O $m^{-2}s^{-1}$) and T_4 (0.14 mol H_2O $m^{-2}s^{-1}$) were on par and significantly superior to all other treatments at 45th DAT

At 75th DAT at the same level of UV-B significantly highest stomatal conductance was recorded in T_3C_2 followed by T_1C_2 (0.14 mol H_2O $m^{-2}s^{-1}$) and T_2C_1 (0.13 mol H_2O $m^{-2}s^{-1}$). Treatments T_1C_1 (0.09 mol H_2O $m^{-2}s^{-1}$), T_2C_2 (0.12 mol H_2O $m^{-2}s^{-1}$), T_3C_1 (0.11mol H_2O $m^{-2}s^{-1}$) and T_4 (0.11mol H_2O $m^{-2}s^{-1}$) were on par but significantly superior to T5.

Table12: Effect of treatments on stomatal conductance (mol H_2O $m^{-2}s^{-1}$) under two levels of UV-B radiation at different growth stages.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	0.16	0.15	0.09	0.20
T_1C_2	0.17	0.10	0.14	0.21
T_2C_1	0.16	0.13	0.13	0.20
T_2C_2	0.16	0.15	0.12	0.21
T_3C_1	0.15	0.16	0.11	0.22
T_3C_2	0.17	0.18	0.20	0.23
T_4	0.14	0.17	0.11	0.19
T_5	0.06	0.05	0.04	0.07
CD(0.05)	**0.03**	**0.05**	**0.03**	**0.05**

T_1C_1 - Glycine betaine - 10ppm T_1C_2- Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray T_5 - Absolute control

5.4.4 Chlorophyll fluorescence (Fv/Fm)

The mean values of chlorophyll fluorescence at two growing conditions at two different stages of growth are given in Table.9. The chlorophyll fluorescence parameter given as Fv/Fm ratio represents photochemical efficiency of photosystem II (PS II). Measurement of minimal fluorescence (F_0) and maximal fluorescence (Fm)

yields made after 30 minutes dark adaptation was used for determining the ratio of variable to maximal fluorescence (Fv/Fm).

Chlorophyll fluorescence was recorded at 45th and 75th DAT from plants grown under 100% and 80% solar radiation. The Fv/Fm ratio increased from 45th to 75th DAT under both condition, but at 45th DAT the mean data of Fv/Fm showed a significant variation between two growing condition. Maximum quantum efficiency of photosystem II was under 80% solar radiation (0.69 Fv/Fm) and lower values was recorded under 100% solar radiation (0.66 Fv/Fm).

Mean data on effect of treatments on chlorophyll fluorescence (Fv/Fm) at 45th and 75th DAT are given in Table 13. At 45th and 75th DAT under 100% and 80% solar radiation, treatments showed significant differences. In general T_3 (Nativo75 WG) at both concentration maintain more Fv/Fm ratio than all other chemicals like glycine betaine and ascorbic acid. Plants without any chemical treatments (T_5) recorded significantly lower photochemical efficiency (Fv/Fm) under both conditions at 45th and 75th DAT (0.63, 0.66, 0.63, 0.65). Significantly higher Fv/Fm ratio was noted for the treatment T_3C_2 under 100% and 80% solar radiation at 45th and 75th DAT (0.72, 0.73, 0.78, and 0.79). The reduction of Fv/Fm was more significant at vegetative phase when compared with reproductive phase.

Table13: Effect of treatments on chlorophyll fluorescence (Fv/Fm) under two levels of UV-B radiation at different growth stages.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	0.64	0.66	0.66	0.72
T_1C_2	0.72	0.67	0.73	0.68
T_2C_1	0.66	0.66	0.70	0.71
T_2C_2	0.66	0.69	0.72	0.72
T_3C_1	0.65	0.71	0.68	0.73
T_3C_2	0.72	0.73	0.78	0.79
T_4	0.65	0.72	0.69	0.73
T_5	0.63	0.66	0.63	0.65
CD(0.05)	**0.03**	**0.04**	**0.07**	**0.04**

T_1C_1 - Glycine betaine - 10ppm

T_1C_2- Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray

T_5 - Absolute control

5.5 Biochemical characters

The biochemical characters observed at 45th DAT and 75th DAT are explained under the following heading.

5.5.1 Chlorophyll content

The mean values of chlorophyll content at two growing conditions at two different stages of growth are given in Table 14. Chlorophyll a, chlorophyll b and total chlorophyll were found significantly different at 45th and 75th DAT under both conditions. At 45th DAT chlorophyll a, chlorophyll b and total chlorophyll were found significantly higher under 80% solar radiation. The values were 2.13, 0.64, 2.76 mg g^{-1} fr. wt respectively. At 75th DAT also it was significantly higher under 80% solar radiation where the values were 1.54, 0.33 and 1.86 mg g^{-1} fr. wt for Chlorophyll a, Chlorophyll b and total Chlorophyll respectively.

Table14: Effect of two levels of UV-B radiation on chlorophyll content (mg g^{-1} fr. wt.).

Condition	45th DAT			75th DAT		
	Chl a (mg g^{-1} fr. wt.)	Chl b (mg g^{-1} fr. wt.)	Total Chl (mg g^{-1} fr. wt.)	Chl a (mg g^{-1} fr. wt.)	Chl b (mg g^{-1} fr. wt.)	Total Chl (mg g^{-1} fr. wt.)
100% solar radiation	1.81	0.48	2.28	1.12	0.28	1.39
80% solar radiation	2.13	0.64	2.76	1.54	0.33	1.86
CD(0.05)	**0.12**	**0.05**	**0.14**	**0.07**	**0.04**	**0.08**

Mean data on effect of treatments on chlorophyll content at 45th DAT and 75thDAT are given in Table 15 and Table 16.Treatment effect on chlorophyll a, chlorophyll b, total chlorophyll under two levels of UV-B radiation at two growth stages (45th and 75th DAT) found significant. At 45th DAT under 100% solar radiation T_3C_2 recorded maximum chlorophyll a (2.31 mg g^{-1} fr. wt), chlorophyll b (0.68 mg g^{-1} fr. wt) and total chlorophyll (2.98 mg g^{-1} fr. wt) and lower values were recorded by T_5 (chl a-1.05, chl-b – 0.18, total chl-1.24 mg g^{-1} fr. wt). Under 80% solar radiation at

the same stage chlorophyll b was found non- significant, whereas Chlorophyll a and total chlorophyll was significantly higher for T_3C_2 (2.41 and 3.10 mg g^{-1} fr. wt) . Lowest chlorophyll a and total Chlorophyll content were noted for absolute control (1.53 and 2.06 mg g^{-1} fr. wt).

At 75th DAT under 100% solar radiation the treatment effect for Chlorophyll b was found non significant. Chlorophyll a and total Chlorophyll was significantly higher for T_3C_2 (1.24 and 1.56 mg g^{-1} fr. wt). Significantly lower content of chlorophyll was observed for absolute control T_5 (0.76 and1.04 mg g^{-1} fr. wt). Under 80% solar radiation Chlorophyll a was higher for T_1C_1 (1.66 mg g^{-1} fr. wt) which was on par with T_3C_2 (1.56 mg g^{-1} fr. wt). All other chemical treatments had significantly lower values than absolute control T_5 recorded lowest chlorophyll a (0.54 mg g^{-1} fr. wt). Significantly higher level of chlorophyll b and total chlorophyll was observed for T_3C_2 (0.56 and 2.12 mg g^{-1} fr. wt), and lowest for absolute control (0.27 and 0.81 mg g^{-1} fr. wt)

Table15: Effect of treatments on chlorophyll a (mg g^{-1} fr. wt.), chlorophyll b (mg g^{-1} fr. wt.) and total chlorophyll (mg g^{-1} fr. wt.) under two levels of UV-B radiation at 45th DAT.

Treatments	45th DAT					
	100% solar radiation			80% solar radiation		
	Chl a (mg g^{-1} fr. wt.)	Chl b (mg g^{-1} fr. wt.)	Total Chl (mg g^{-1} fr. wt.)	Chl a (mg g^{-1} fr. wt.)	Chl b (mg g^{-1} fr. wt.)	Total Chl (mg g^{-1} fr. wt.)
T_1C_1	2.07	0.58	2.64	2.09	0.60	2.70
T_1C_2	2.22	0.63	2.84	2.26	0.69	2.95
T_2C_1	1.21	0.41	1.63	2.19	0.63	2.82
T_2C_2	2.15	0.56	2.72	2.16	0.62	2.78
T_3C_1	1.90	0.38	2.27	2.02	0.62	2.64
T_3C_2	2.31	0.68	2.98	2.41	0.69	3.10
T_4	1.59	0.44	2.04	2.38	0.71	3.09
T_5	1.05	0.18	1.24	1.53	0.54	2.06
CD(0.05)	**0.37**	**0.17**	**0.40**	**0.35**	**NS**	**0.45**

T_1C_1 - Glycine betaine – 10ppm

T_1C_2- Glycine betaine – 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray

T_5 - Absolute control

Table 16: Effect of treatments on chlorophyll a (mg g^{-1} fr. wt.), chlorophyll b (mg g^{-1} fr. wt.) and total chlorophyll (mg g^{-1} fr. wt.) under two levels of UV-B radiation at 75th DAT

Treatments	75th DAT					
	100% solar radiation			80% solar radiation		
	Chl a (mg g^{-1} fr. wt.)	Chl b (mg g^{-1} fr. wt.)	Total Chl (mg g^{-1} fr. wt.)	Chl a (mg g^{-1} fr. wt.)	Chl b (mg g^{-1} fr. wt.)	Total Chl (mg g^{-1} fr. wt.)
T_1C_1	1.11	0.29	1.40	1.66	0.24	1.91
T_1C_2	0.86	0.22	1.08	1.08	0.21	1.29
T_2C_1	1.17	0.30	1.47	1.30	0.30	1.60
T_2C_2	1.21	0.29	1.49	1.45	0.29	1.74
T_3C_1	1.17	0.17	1.34	1.34	0.28	1.62
T_3C_2	1.36	0.35	1.71	1.56	0.56	2.12
T_4	1.24	0.32	1.56	0.72	0.51	1.23
T_5	0.76	0.26	1.04	0.54	0.27	0.81
CD(0.05)	**0.19**	**NS**	**0.22**	**0.25**	**0.12**	**0.25**

T_1C_1 - Glycine betaine - 10ppm

T_1C_2 - Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 -Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray

T_5 - Absolute control

5.5.2 Flavanoid content

The mean value of flavanoid content at two growing conditions at two different stages of growth are given in Table 17. Changes in flavanoid content was observed only at 75th DAT. Significantly high flavanoid content (27.01 $A_{300}g^{-1}$ fr. wt.) was observed under 100% solar radiation when compared to 80% solar radiation (24.92 $A_{300}g^{-1}$ fr. wt.).

Table 17: Effect of two levels of UV-B radiation on flavanoid ($A_{300}g^{-1}$ fr. wt.), xanthophyll (µg g^{-1} fr. wt.), phenol (mg g^{-1} fr. wt.) and catalase (µmol of H_2O_2 per min g^{-1} fr. wt.).

Condition	Flavanoid ($A_{300}g^{-1}$ fr. wt.)		Xanthophyll (µg g^{-1} fr. wt.)		Phenol (mg g^{-1} fr. wt.)		Catalase (µmol of H_2O_2 per min g^{-1} fr. wt.)	
	45th DAT	75th DAT	45th DAT	75th DAT	45th DAT	75th DAT	45th DAT	75th DAT
100% solar radiation	25.77	27.01	5.93	4.39	1.13	3.53	4.55	6.21
80% solar radiation	23.71	24.92	6.92	6.15	0.80	2.19	11.74	17.13
CD(0.05)	**NS**	**1.53**	**0.73**	**0.89**	**0.04**	**0.35**	**0.34**	**0.29**

Various treatment effect on flavanoid content was analysed at 45th DAT and 75th DAT under 100% and 80% solar radiation and mean values presented in Table 18. At 45th DAT, under 100% solar radiation flavanoid content was higher for T_5 (34.24 $A_{300}g^{-1}$ fr. wt) which was on par with T_1C_2 (23.60 $A_{300}g^{-1}$ fr. wt), T_2C_1 (32.64 $A_{300}g^{-1}$ fr. wt), T_3C_1(24.40 $A_{300}g^{-1}$ fr. wt) and T_4 (32.30 $A_{300}g^{-1}$ fr. wt) . The lowest flavanoid content of 15.95 $A_{300}g^{-1}$ fr. wt was recorded by T_2C_2. Under 80% solar radiation flavanoid content was more under T_5 (31.71 $A_{300}g^{-1}$ fr. wt) which was significantly different from T_1C_2 (20.95 $A_{300}g^{-1}$ fr. wt), T_2C_1 (24.78 $A_{300}g^{-1}$ fr. wt),

T_4(25.34 $A_{300}g^{-1}$ fr. wt), T_3C_1 (25.16 $A_{300}g^{-1}$ fr. wt), T_3C_2 (20.77 $A_{300}g^{-1}$ fr. wt), T_2C_2 (20.73 $A_{300}g^{-1}$ fr. wt) and T_1C_1 (20.22 $A_{300}g^{-1}$ fr. wt).

At 75th DAT, under 100% solar radiation T_5 recorded highest flavanoid content (30.81 $A_{300}g^{-1}$ fr. wt) which was on par with T_1C_1(29.75 $A_{300}g^{-1}$ fr. wt) , T_1C_2(28.52 $A_{300}g^{-1}$ fr. wt)and T_4(28.70 $A_{300}g^{-1}$ fr. wt) , also was significantly different from T_2C_1, T_2C_2 , T_3C_1 and T_3C_2which recorded 24.94, 20.21, 26.47, 22.68 $A_{300}g^{-1}$ fr. wt respectively. Under 80% solar radiation also T_5 recorded highest flavanoid content (28.76 $A_{300}g^{-1}$ fr. wt) which was on par with T_2C_1 (28.18 $A_{300}g^{-1}$ fr. wt) and significantly superior to T_3C_2 and T_4 which recorded 21.83 and 21.20 $A_{300}g^{-1}$ fr. wt respectively .

Table18: Effect of treatments on flavanoid content ($A_{300}g^{-1}$ fr. wt.) under two levels of UV-B radiation at different growth stages.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	21.99	20.22	29.75	25.49
T_1C_2	23.60	20.95	28.52	25.30
T_2C_1	32.64	24.78	24.94	28.18
T_2C_2	15.95	20.73	20.21	25.93
T_3C_1	24.40	25.16	26.47	27.64
T_3C_2	21.03	20.77	22.68	21.83
T_4	32.30	25.34	28.70	21.20
T_5	34.24	31.71	30.81	28.76
CD(0.05)	**11.43**	**6.26**	**4.21**	**4.60**

T_1C_1 – Glycine betaine – 10ppm

T_1C_2- Glycine betaine – 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 – Ascorbic acid - 100 ppm

T_3C_1 – Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray T_5 – Absolute control

5.5.3 Xanthophyll

The mean values of xanthophyll content at two growing conditions at two different stages of growth are given in Table 17.

There was significant difference in xanthophylls content between two conditions at 45th and 75th DAT, irrespective of treatments. Decline in xanthophylls content was observed to the tune of 5.93 to 4.39 µg g^{-1} fr. wt and 6.92 to 6.15 µg g^{-1} fr. wt from 45th to 75th DAT under 100% and 80% solar radiation respectively. Under ambient condition the xanthophylls content was low when compared to 80% reduced UV-B levels.

Various treatment effect on xanthophylls content was calculated at 45th DAT and 75th DAT under 100% and 80% solar radiation and mean values presented in Table 19.There was significant difference between treatments for xanthophylls content which was significant only under 80% solar radiation at 45th DAT and 75th DAT. At 45th DAT under 80% solar radiation T_2C_2 recorded highest value (8.29 µgg^{-1} fr. wt) followed by T_5 (8.16 µg g^{-1} fr. wt) which was on par. Treatments could not produce any significant difference for xanthophylls content under 100% solar radiation at this stage of crop.

The highest xanthophylls content of 9.48 µg g^{-1} fr. wt was observed in T_5 followed by T_4 (8.58 µg g^{-1} fr. wt) at 75th DAT under 80% solar radiation which was significantly different from all other chemical treatments. Under 100% solar radiation treatment effect was non significant.

Table 19: Effect of treatments on xanthophyll content (µg g^{-1} fr. wt.) under two levels of UV-B radiation at different growth stages.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	7.03	6.73	4.06	5.34
T_1C_2	7.10	7.37	3.41	3.77
T_2C_1	5.21	6.85	4.70	5.40
T_2C_2	5.62	8.29	6.06	4.78
T_3C_1	5.34	4.80	4.10	5.97
T_3C_2	5.31	5.71	4.28	5.87
T_4	4.99	7.48	4.96	8.58
T_5	6.85	8.16	3.57	9.48
CD(0.05)	**NS**	**2.12**	**NS**	**2.53**

T_1C_1 - Glycine betaine - 10ppm

T_1C_2- Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray

T_5 - Absolute control

5.5.4 Phenol

The mean values of Phenol content at two growing conditions at two different stages of growth are given in Table 17. Phenol content was increased from 45th DAT to 75th DAT. The high phenol content was observed under 100% solar radiation at 45th DAT (1.13 mg g^{-1} fr. wt) and 75th DAT (3.53 mg g^{-1} fr. wt), where as it was 0.80 and 2.19 mg g^{-1} fr. wt respectively for crops grown under 80% solar radiation.

Effect of various ameliorative treatments on phenol content was estimated at 45th DAT and 75th DAT under 100% and 80% solar radiation and mean values presented in Table 20. Treatment effect on phenol content under two levels of UV-B radiation at 45th and 75th DAT showed significant difference only under 100% solar radiation at 45th DAT, where glycine betaine at both concentration recorded highest phenol content (1.2 mg g^{-1} fr. wt) followed by T_3C_1(1.19 mg g^{-1} fr. wt) , T_3C_2 (1.15 mg g^{-1} fr. wt) and T_5(1.14 mg g^{-1} fr. wt). Lowest phenol content recorded by the application of ascorbic acid T_2C_1 (0.99 mg g^{-1} fr. wt).Treatment effect were non significant under 80% solar radiation at 45th DAT and under 100% and 80% solar radiation at 75th DAT.

Table 20: Effect of treatments on phenol content (mg g^{-1} fr. wt.) under two levels of UV-B radiation at different growth stages.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	1.21	0.91	3.56	3.30
T_1C_2	1.21	0.85	2.88	1.81
T_2C_1	0.99	0.80	3.43	2.13
T_2C_2	1.06	0.82	3.27	2.57
T_3C_1	1.19	0.75	3.51	2.16
T_3C_2	1.15	0.82	3.77	1.91
T_4	1.12	0.74	4.15	2.08
T_5	1.14	0.71	3.66	1.55
CD(0.05)	**0.13**	**NS**	**NS**	**NS**

T_1C_1 – Glycine betaine – 10ppm

T_1C_2- Glycine betaine – 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 – Ascorbic acid - 100 ppm

T_3C_1 – Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray T_5 – Absolute control

5.5.5 Catalase

The mean value of catalase at two growing conditions at two different stages of growth are given in table.17

Catalase activity was worked out at 45th and 75th DAT. Catalase activity found more under 80% solar radiation (11.74 and17.13 µmol of H_2O_2 per min g^{-1} fr. wt) compared with plants grown under 100% solar radiation (4.55 and 6.20 µmol of H_2O_2 per min g^{-1} fr. wt) at both stages. Catalase activity increased from 45th DAT to 75th DAT, under two growing conditions.

Mean data on effect of treatments on catalase activity at 45th DAT and 75thDAT are given in Table 23.Ttreatment effect was non significant in catalase activity under two levels of UV-B radiation at vegetative stage (45th DAT) but it was significant at reproductive phase (75th DAT), under 100% and 80% solar radiation. Plants showed more catalase activity in T_3C_2(7.28 µmol of H_2O_2 per min g^{-1} fr. wt) and was significantly different from T_1C_1,T_2C_1, T_2C_2, T_4 and T_5.Very low activity was noticed in absolute control (5.36 µmol of H_2O_2 per min g^{-1} fr. wt).

Under 80% solar radiation catalase activity was higher for T_3C_2 (18.98 µmol of H_2O_2 per min g^{-1} fr. wt) which was on par with T_4 and T_5. Significantly lower catalase activity was noted for T_2C_2 (15.51 µmol of H_2O_2 per min g^{-1} fr. wt)

Table 21: **Effect of treatments on catalase activity (µmol of H_2O_2 per min g^{-1} fr. wt.) under two levels of UV-B radiation at different growth stages**.

Treatments	45th DAT		75th DAT	
	100% solar radiation	80% solar radiation	100% solar radiation	80% solar radiation
T_1C_1	4.39	11.62	6.29	16.84
T_1C_2	5.24	11.48	6.48	16.04
T_2C_1	4.25	11.76	6.14	17.01
T_2C_2	4.39	11.90	4.99	15.51
T_3C_1	3.83	11.62	6.96	17.23
T_3C_2	4.68	11.05	7.28	18.98
T_4	4.61	12.21	6.14	17.76
T_5	5.04	12.31	5.36	17.70
CD(0.05)	**NS**	**NS**	**0.94**	**1.43**

T_1C_1 - Glycine betaine - 10ppm T_1C_2 - Glycine betaine – 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1- Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray T_5 - Absolute control

5.6 Yield characters

Yield and yield parameters were recorded from crop grown under 100% and 80% solar radiation and described under the following heading.

5.6.1 Number of panicle per hill

Mean data on number of panicle per hill under two levels of UV-B radiation are presented in Table.22. Significant difference in number of panicle per hill under 100% and 80% solar radiation was observed irrespective of treatments. Number of panicle were more under open condition (14 panicle per hill) when compared to plants grown under reduced solar radiation (12 panicle per hill)

Table 22: Effect of two levels of UV-B radiation on yield characters

condition	Number of panicle per hill	Number of spikelet per panicle	1000 grain weight (g)	Grain yield per pot (g)
100% solar radiation	14.00	101.43	21.98	80.37
80% solar radiation	12.00	156.01	21.99	123.31
CD(0.05)	**0.58**	**8.09**	**NS**	**10.94**

Mean data on effect of treatments on number of panicle per hill under two levels of UV-B radiation are presented in Table 23. Even though number of panicles were higher under open condition different treatments could not produce any significant variation under 100% and 80% solar radiation.

Table 23: Effect of treatments on number of panicles per hill under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	12.00	12.00
T_1C_2	13.00	12.00
T_2C_1	14.00	12.00
T_2C_2	14.00	11.00
T_3C_1	14.00	12.00
T_3C_2	15.00	12.00
T_4	14.00	13.00
T_5	14.00	11.00
CD(0.05)	**NS**	**NS**

T_1C_1 - Glycine betaine - 10ppm T_1C_2- Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray T_5 - Absolute control

5.6.2 Number of spikelet per panicle

Mean data on number of spikelet per panicle under two levels of UV-B radiation are presented in Table.22

Significant difference in number of spikelet per panicle was observed under two growing conditions. More number of spikelets were seen under 80% solar radiation (156 spikelet per panicle) when compared to crops grown under 100% solar radiation (101 spikelet per panicle)

Mean data on effect of treatments on number of spikelet per panicle under two levels of UV-B radiation are presented in Table 24. Treatment effect on spikelet per panicle under 100% solar radiation found to be non significant. Remarkable treatment effect in number of spikelet per panicle was found in plants grown under 80% solar radiation. Treatment T_3C_2 contributed highest spikelet per panicle (180 spikelet per panicle) which was on par with T_1C_1, T_2C_2, T_3C_1 and T_4. Significantly lower spikelet per panicle observed in T_1C_2 (150.56) and T_2C_1 (144.06) and lowest number observed in absolute control (121 spikelet/panicle).

Table 24: Effect of treatments on number of spikelet per panicle under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	94.78	162.25
T_1C_2	120.67	150.56
T_2C_1	104.56	144.04
T_2C_2	102.11	161.11
T_3C_1	91.33	161.78
T_3C_2	121.67	179.83
T_4	100.65	167.32
T_5	75.65	121.16
CD(0.05)	**NS**	**19.17**

T_1C_1 - Glycine betaine – 10ppm

T_1C_2- Glycine betaine – 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2- Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray

T_5 - Absolute control

5.6.3 Thousand grain weight

Mean data on thousand grain weight under two levels of UV-B radiation are presented in Table.22. Thousand grain weight is found non- significant under different levels of UV-B radiation

Mean data on effect of treatments on thousand grain weight under two levels of UV-B radiation are presented in Table 25.

Treatment effect on 1000 grain weight significantly varied under both condition. In 100% solar radiationT_3C_2 recorded highest 1000 grain weight (24.81g) followed by T_2C_1 (22.67 g) and T_1C_1 (22.56 g). The lowest 1000 grain weight (19.5g) was observed in plant without any chemical treatments (T5).

Under 80% solar radiation also T_3C_2 recorded significantly higher 1000 grain weight (24.23g) where as the lowest 1000 grain weight (19.44g) was observed in T_5 which was on par with T_1C_1(20.85) and T_1C_2(20.89).

Table 25: Effect of treatments on 1000 grain weight (g) under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	22.56	20.85
T_1C_2	22.55	20.89
T_2C_1	22.67	22.58
T_2C_2	22.37	23.43
T_3C_1	21.13	22.51
T_3C_2	24.81	24.23
T_4	22.27	22.05
T_5	19.50	19.44
CD(0.05)	**1.20**	**1.82**

T_1C_1 – Glycine betaine – 10ppm T_1C_2- Glycine betaine – 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm T_3C_2 - Nativo 75 WG - 70 ppm

T_4- Water spray T_5-Absolute control

5.6.4 Grain yield

Mean data on grain yield under two levels of UV-B radiation are presented in Table.22. Grain yield per pot recorded significant difference between crops grown under 80% and100% solar radiation. More grains were harvested from crop grown at 80% solar radiation (123.3gpot^{-1}) and less from100% solar radiation (80.3g pot^{-1})

Mean data on effect of treatments on grain yield under two levels of UV-B radiation are presented in Table 26. Effect of treatments on grain yield found significant under two levels of solar radiation.

Under 100% solar radiation plants treated with T_3C_2 yielded 110.85g pot^{-1} which was significantly different from all other chemical treatments. The second highest yield per pot was recorded in treatments T_1C_2 (89.24 g pot^{-1}) followed by T_2C_1 (88.05 g pot^{-1}) and T_2C_2 (83.28 g pot^{-1}) which were significantly different from T_5 (61.57 g pot^{-1}) all other treatments were on par with T_5.

Under 80% solar radiation all treatments except T_1C_2 recorded significantly higher yield when compared to T_5 (95.5 g pot^{-1}). Among the treatments T_3C_2 recorded significantly higher yield (142.85 g pot^{-1}) followed by T_4 (138.40 g pot^{-1}) >T_3C_1 (127.33 g pot^{-1}) >T_2C_2 (121.51 g pot^{-1}) >T_1C_1 (118.42 g pot^{-1}) >T_2C_1 (116.98 g pot^{-1}).

Table 26: Effect of treatments on grain yield (g pot^{-1}) under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	66.19	118.42
T_1C_2	89.24	108.56
T_2C_1	88.05	116.98
T_2C_2	83.28	121.51
T_3C_1	71.05	127.33
T_3C_2	110.85	142.85
T_4	74.02	138.40
T_5	61.57	95.50
CD(0.05)	**21.24**	**13.95**

T_1C_1 - Glycine betaine - 10ppm

T_1C_2 - Glycine betaine - 20 ppm

T_2C_1 - Ascorbic acid - 50 ppm

T_2C_2 - Ascorbic acid - 100 ppm

T_3C_1 - Nativo 75 WG - 50 ppm

T_3C_2 - Nativo 75 WG - 70 ppm

T_4 - Water spray

T_5 - Absolute control

Discussion

5. DISCUSSION

The present research work was conducted to understand the photo protective potential of ecofriendly stress mitigating chemicals on photoinhibition in photochemistry and photosynthesis of rice (*Oryza sativa* L.) under solar ultraviolet B (UV-B) radiation. The rice variety Uma grown in pots under two levels of UV-B radiation and treated with three chemicals with two concentrations.

The results of various morphological, physiological, biochemical and yield characters are discussed in this chapter.

5.1 Ultraviolet-B (UV-B) and Photosyntheticaly active radiation (PAR) measurements

Significantly higher UV-B and PAR recorded under ambient solar condition when compared to 80% solar condition. Higher UV-B (2.18 Wm^{-2}) and PAR (1786 $\mu molm^{-2}s^{-1}$) were recorded in the month of March-2016 and lower value were during November – 2015 (1.82 Wm^{-2} and 1508 $\mu molm^{-2}s^{-1}$ respectively) at Vellanikkara, Thrissur (Fig.1 and Fig.2). At the same time the mean solar irradiance (Annexure. II) measured was maximum during March-2016 (21.5Wm^{-2}) and the minimum was in November-2015 (14.46Wm^{-2}). This clearly indicated that an increase or decrease in solar radiation causes a corresponding increase or decrease in UV-B radiation also. Gradual increment in both UV-B and PAR was noted from 8am to 12pm and decreased from 12pm to 2pm in both ambient and reduced solar condition. The present observation indicated that under natural or ambient condition relatively high or low intensities of UV-B is accompanied with moderate or high intensities of visible spectral range, which is in line with observation of Teramura and Sullivan (1994). High levels of visible light (400-700 nm) can cause photoinhibition and primary damage to PS II.

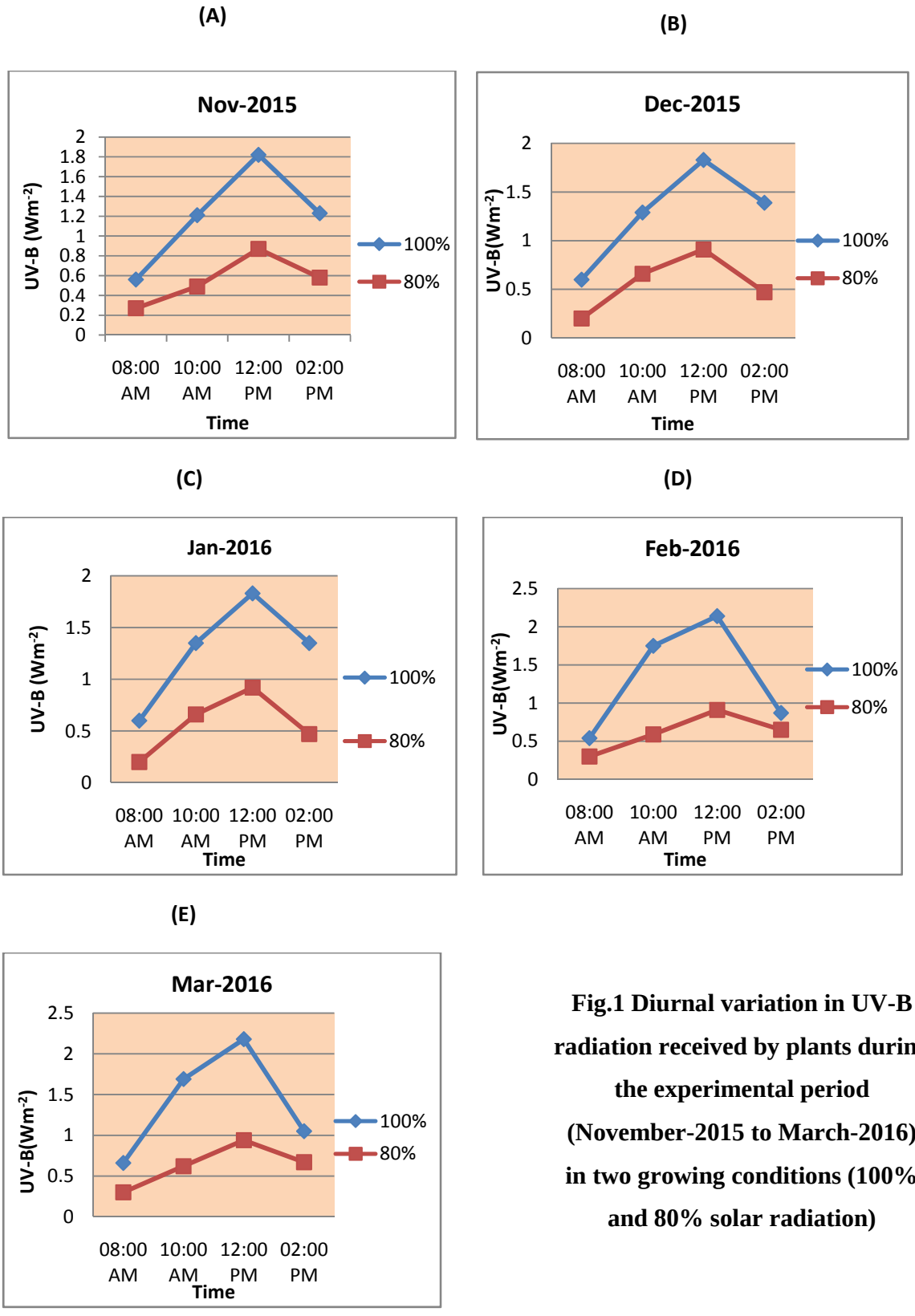
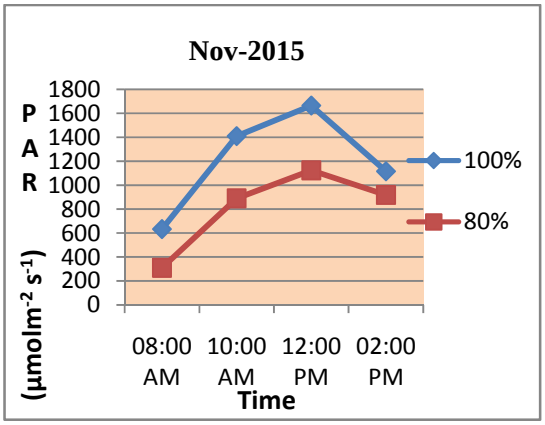


Fig.1 Diurnal variation in UV-B radiation received by plants during the experimental period (November-2015 to March-2016) in two growing conditions (100% and 80% solar radiation)

(A)



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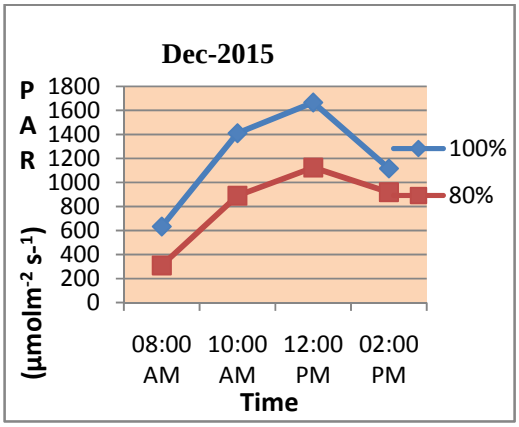
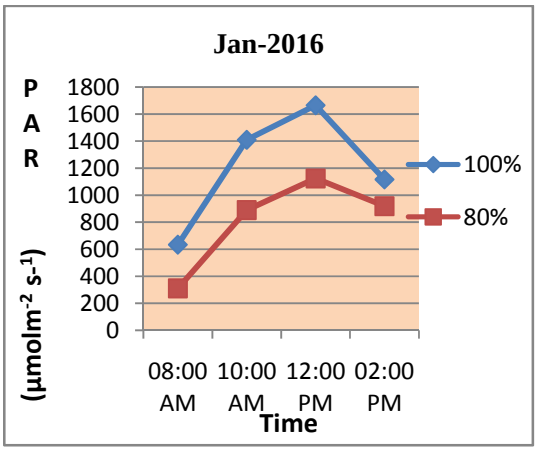


(C)



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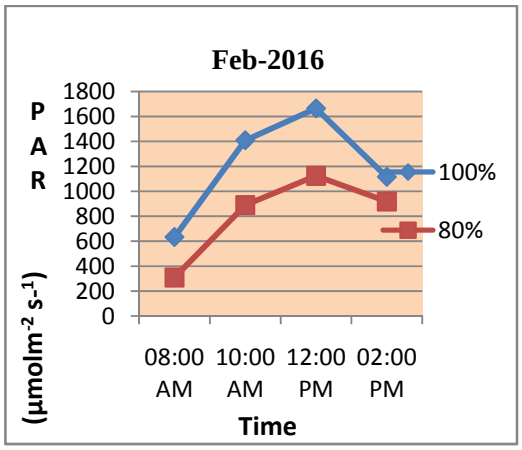


(E)

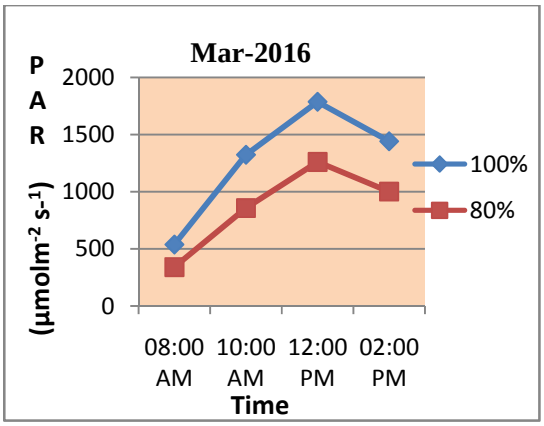


Fig.2 Diurnal variation in PAR received by plants during the experimental period (November-2015 to March-2016) in two growing condition (100% and 80% solar radiation)

The same trend in diurnal variation of UV-B was reported from December-2013 to April-2014 by Wagh (2015), however the UV-B values were higher in the range 2.82 to 3.58 Wm^{-2} when compared to UV-B recorded in the present study during 2015-2016.

5.2 Morphological characters as influenced by glycine betaine, ascorbic acid, and combination fungicide 25WG trifloxystrobin+ 50WG tebuconazole (*Nativo*) under two levels of UV-B radiation.

The results indicated that the ambient level of UV-B radiation significantly reduced the plant height of rice variety Uma. This may be due to the reduced intermodal elongation under UV-B stress as reported earlier by Teramura and Sullivan (1994), which could be due to photooxidative destruction of auxin followed by reduced cell wall extensibility as observed in sun flower (Ros and Tevini, 1995). Plants grown under reduced solar radiation (80% solar radiation) attained 17% higher plant height when compared to plants grown under 100% solar radiation, where as there was 20% increase in tiller number recorded under ambient solar condition. UV-B exclusion studies conducted at Vellanikkara (Wagh, 2015) revealed that plant height and tiller number were higher under UV-B excluded condition in comparison with 100% and 80% solar radiation

In the present study among ameliorative chemical treatments, glycine betaine at both concentration (10ppm and 20ppm) and ascorbic acid 50 and 100ppm could enhance the plant height which was on par with water spray under 100% solar radiation. Glycine betaine as an osmolyte might have decreased the cell osmotic potential and thus maintained cell turgor pressure which is necessary for growth through cell elongation. Increment in plant height in glycine betaine treated plants may be due to the translocation of exogenously applied glycine betain to actively growing and expanding portions of crops which was previously explaind by Makela *et al.* (1996).

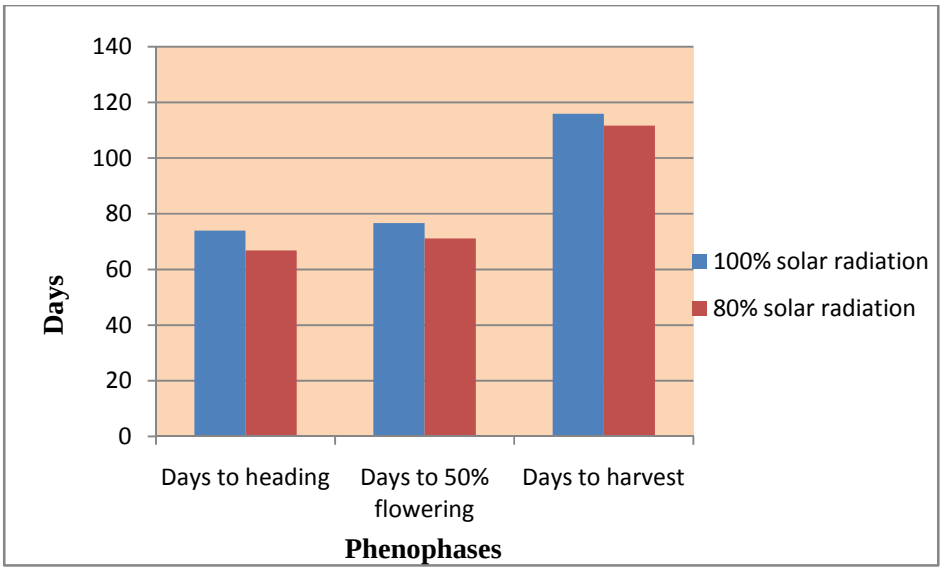


Fig.3 Variation in phenophases of rice under two levels of UV-B radiation

Though glycine betaine enhanced the height, it could not enhance tiller number which was the lowest and on par with Nativo 50 and 70ppm. *Nativo* at both concentrations showed the lowest height under 100% solar radiation but it could cause 19% increase in height under 80% solar radiation. More over 70ppm *Nativo* caused maximum height under reduced UV level when compared to all treatments. This enhancement in height may be due to co- occurrence of other environmental factors like RH and temperature under reduced UV-B radiation in polyhouse.

Crop received with treatment glycine betaine 10 ppm and *Nativo* 70ppm recorded lowest tiller number under 100% solar radiation indicating the ability of this chemical to cause moderate tillering which is an important trait associated with yield improvement. Plants received *Nativo* 70ppm exhibited moderate tillering ability under both condition.

5.3 Phenological characters affected by glycine betaine, ascorbic acid and and combination fungicides 25 WG trifloxystrobin+ 50WG tebuconazole (*Nativo*) under two levels of UV-B radiation

In the present study under ambient condition UV-B altered the different phenophases and prolonged time to achieve days to heading, days to 50% flowering and days to harvest. Plants attained all the phenophases 4- 5days earlier under UV-B reduced condition (80% solar radiation) (Fig.3). The earliness to achieve all the phenophases under reduced solar condition may be due to the high chlorophyll content which in turn induce high photosynthetic rate at tillering stage and there by early ripeness to flowering. Similar studies to evaluate crop response under abiotic stresses revealed that plants grown under stress condition took more days for flowering and maturity when compared to plants grown under controlled conditions (Shikuku *et al*., 2010).

Under 100% solar radiation the treatment effect for days to heading was non-significant. Crops which received Nativo 50 ppm (T_3C_1), ascorbic acid 50ppm (T_2C_1)

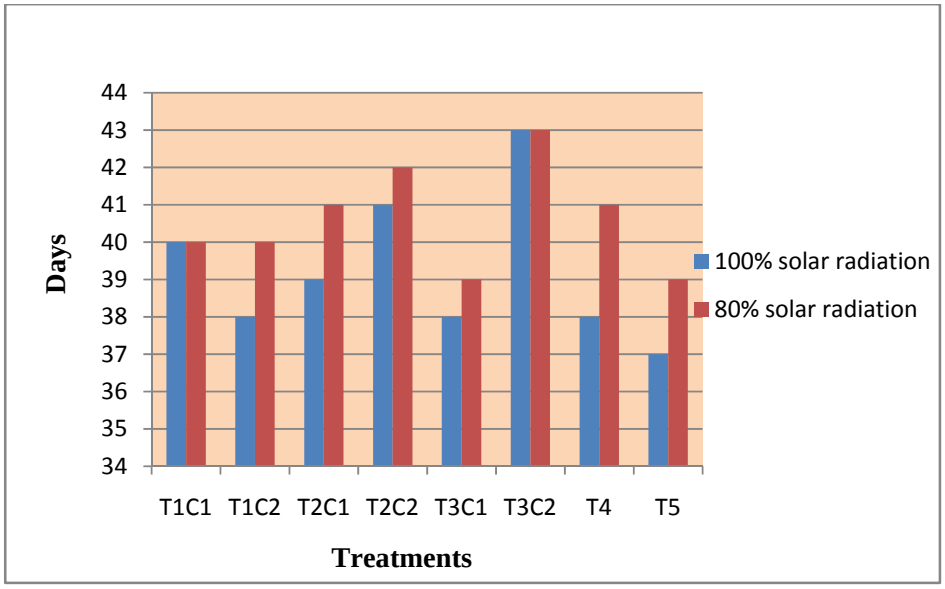


Fig.4 Variation in grain filling period of rice among different chemical treatments under two levels of UV-B radiation

T_1C_1 - Glycine betaine - 10ppm **T_1C_2- Glycine betaine - 20 ppm**

T_2C_1 - Ascorbic acid - 50 ppm **T_2C_2 - Ascorbic acid - 100 ppm**

T_3C_1 - Nativo 75 WG - 50 ppm **T_3C_2 - Nativo 75 WG - 70 ppm**

T_4 - Water spray **T_5 - Absolute control**

and glycine betaine 20ppm (T_1C_2) prolonged the days to heading compared to control (T_5) under 80% solar radiation.

Difference in days between harvest and 50% flowering indicates the grain filling period which is an important parameter deciding the thousand grain weight and yield. In the present study the grain filling period decreased under 100% solar radiation when compared to 80% solar radiation (Fig.4). This may be one of the reasons for contributing the higher yield in crops grown under 80% solar radiation.

Variation in grain filling period due to foliar appliacation of stress alleviating chemicals was also observed. Treatment T_3C_2 (Nativo 70ppm) could enhance the grain filling period up to 43 days under both condition which contributed maximum grain yield also. Control plants took only 37 days for grain filling under 100% solar radiation where as it was prolonged to 39 days under 80% solar radiation. The ability of *Nativo* at high concentration to prolong grain filling period may be due to its ethylene inhibition activity to decrease senescence and retain greenness in crops. Previously Grossmann *et al.* (1999), also linked strobilurin with delayed senescence of leaves which consequently prolonged photosynthetic activity of green tissue and there by better management of crop stress. This may be also due to the property of Nativo to alter the physiological characters like retention of green leaf tissue, increase in endogenous cytokinin and auxin level, inhibition of ethylene biosynthesis, increase in CO_2 assimilation, better N assimilation, increase in water use efficiency and harvest index as reported by Nagajothi and Jeyakumar(2014).

5.4 Physiological characters affected by glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin + 50WG tebuconazole (*Nativo*) under two levels of UV-B radiation

Photosynthesis is the most important physiological character that influences total growth and development of plants. Comparison of the photosynthetic rate of crop grown under two different growing conditions revealed that at 45th DAT, which

coincide with the maximum vegetative growth phase, could not give a significant difference to this parameter.

At reproductive phase (75^{th} DAT) the photosynthetic rate, stomatal conductance and transpiration rate varied significantly between two conditions. Significantly higher rate of photosynthesis was recorded under 80% solar radiation than 100% solar radiation. This phenophase coincided with the highest UV-B radiation received during the month of Feb-March 2016. This may be the reason for decreased photosynthetic rate under 100% solar radiation when compared to 80% solar radiation. Moreover, the chlorophyll pigment of photosynthetic apparatus is reduced by UV-B with concomitant loss of the photosynthetic rate. The reduction in photosynthetic rate due to increase in UV-B radiation was previously reported in rice (Wagh, 2015).

Plants grown under 100% solar radiation showed a decrease of 20% stomatal conductance thereby increasing stomatal limitations for CO_2 uptake to the tune of 4.18%. Usually stomatal imposed limitation on photosynthetic rate is severe under abiotic stress condition. Stomatal closure and increased leaf diffusive resistance by enhanced UV-B radiation was explained by Tevini and Teramura in 1989. Later Nogues *et al.* (1998), observed substantial decrease of adaxial and abaxial stomatal conductance in pea plants grown under enhanced UV-B radiation.

Present study also revealed that stomatal conductance was correlated with transpiration rate which leads to CO_2 influx for assimilatory metabolism. Previously Feng *et al.* (2007), also reported that photosynthesis can be decreased by reduction in stomatal conductance or by reduction in chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll). So change in chlorophyll content in turn cause change in photosynthetic rate also. Increased photosynthetic rate lead to increased growth and dry matter accumulation in UV-B reduced condition (80% solar condition) .The inhibition in photosynthetic rate may be due to cumulative effect of

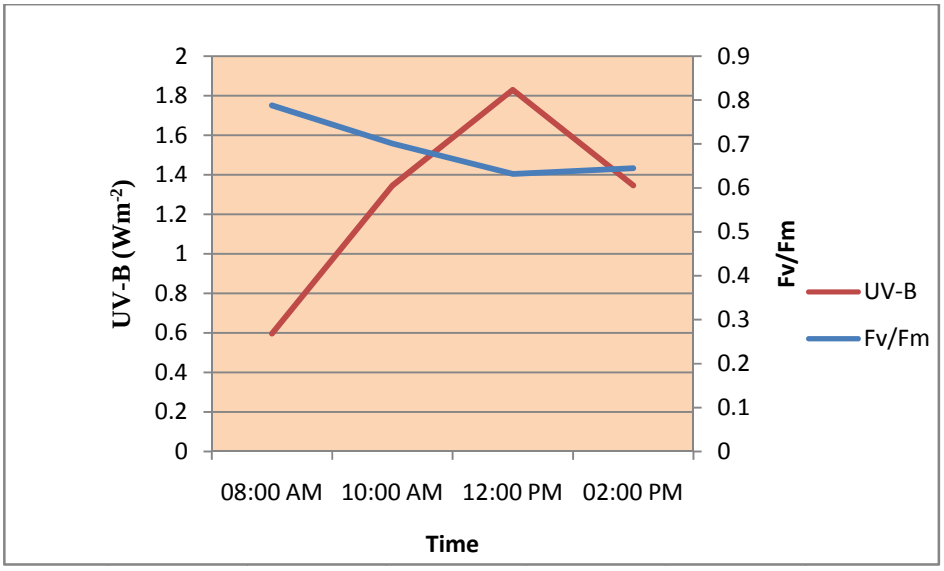


Fig.5 Diurnal variation in photoinhibition of photochemical efficiency of PS II

(A) **(B)**

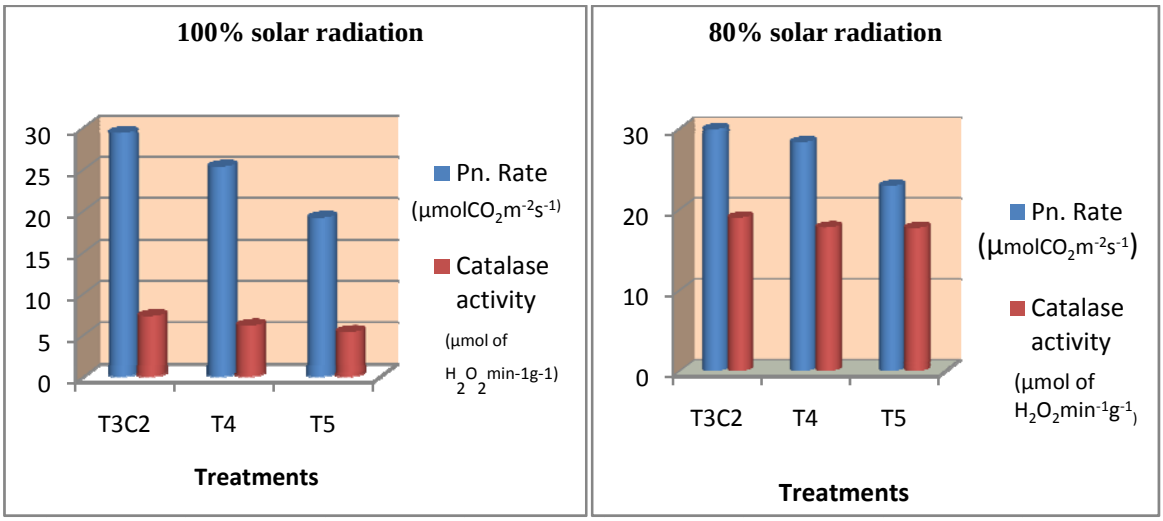


Fig.6 Effect of combination fungicide *Nativo* (25WG trifloxystrobin+50 WG tebuconazole) 70ppm (T_3C_2) on photosynthetic rate and catalase acitivity under two levels of UV-B radiation compared to water spray (T_4) and absolute control (T_5).

UV-B radiation on thylakoid membrane, photosystem II and compounds like Rubisco (Nedunchezhian and Kulandaivelu,1991).

Among chemical treatments *Nativo* 70ppm increased the photosynthetic rate. It was in the tune of 35% and 34% under 100% solar radiation at 45th and 75th DAT respectively. Under reduced level of solar radiation the increase was 39% and 23% at 45th and 75th DAT respectively. Increase in CO_2 assimilation due to foliar application of *Nativo* in rice and tomato irrespective of season was reported earlier by Nagajothi and Jeyakumar (2014). The present observation also is in line with the report on positive effect of strobuilurin and triazole group of chemicals on plant photosynthesis observed in wheat and soybean (Zhang *et al*., 2010). The increased rate of photosynthesis by the application of *Nativo* at 70ppm may be due to alleviation of stomatal limitations by the fungicide under stress condition as previously reported in bhindhi (Sujatha *et al*., 1999), tomato and rice (Nagajothi and Jeyakumar, 2014). This may also be related to increase in chlorophyll content due to the application of *Nativo* in this study. The data also revealed that rate of transpiration was increased by the application of *Nativo* at two stages under both condition. This may be associated with enhanced stomatal conductance by this chemical. This observation is contradictory to the report of Sairam *et al.* (1995), who observed a reduction in transpiration rate due to inhibition of stomatal conductance by *Nativo*.

Analysis of data on chlorophyll fluorescence parameter (Fv/Fm) indicated UV-B induced reduction in the activity of photosystem II photochemistry by low Fv/Fm values under 100% UV-B radiation. This might have caused a photoinhibition in photosynthesis under natural condition of UV-B when compared to reduced level (80% solar UV-B radiation)(Fig.5). Chlorophyll fluorescence ratio (Fv/Fm) has a positive correlation with leaf photosynthesis. The decrease in Fv/Fm ratio indicates chlorophyll fluorescence emission with increasing PPFD and UV-B at 100% full sun light. In addition to this if plant stress is more few reaction centers are open in PS II and Fv/Fm ratio is lowered. Thus the chlorophyll fluorescence parameter is widely

used to evaluate the efficiency of photosynthetic system in chloroplast membrane under different abiotic atress condition (Chen *et al*., 2010). Strong light intensity induces photoinhibition by decreasing photochemical efficiency of chloroplast in C_3 crops like rice soybean etc. (Long *et al*., 1994). Similarly the present investigation also revealed that UV-B induced damage can be further enhanced due to additional photo damage induced by PPFD included in the visible spectrum of light.

Among chemical treatments combination fungicide Nativo (25 WG trifloxy strobin + 50 WG tebuconazole) recorded significantly higher Fv/Fm values compared to other treatments. Triazole compounds like propiconazole and tetraconazole improved the Fv/Fm ratio in wheat (Gilley and Fletcher, 1997) under stress condition. Scientist observed that plants treated with paclobutrazole, a plant growth regulator increased its photosynthetic efficiency about 11-13% under stress condition and also paclobutrazole has a capacity to alter fluorescence ratio (Berova *et al*., 2002).

5.5 Biochemical characters affected by glycine betaine, ascorbic acid and combination fungicide 25 WG trifloxystrobin+ 50WG tebuconazole (*Nativo*) under two levels of UV-B radiation

Chlorophyll content

Plants received more UV-B radiation at ambient solar condition (100% solar radiation) which reduced the content of chlorophyll pigment (chlorophyll a, chlorophyll b, and total chlorophyll). UV-B radiation reduced the chlorophyll content in pea due to reduction in chlorophyll a/b binding protein (Dai *et al*., 1995). Chlorophyll content and chlorophyll flourescence parameter might indicate the influence of environmental stress on growth because these parameters are closely related with the rate of carbon assimilation.

Reduction in total chlorophyll, chlorophyll a and chlorophyll b at100% solar radiation indicates potential photobiological consequences of UV-B radiation.

Decrease in chlorophyll content under 100% natural solar radiation may be due to photooxidation and degradation under high irradiance. Decrease in total chlorophyll content was 17.1% and 32.6% under two growing conditions from 45th to 75th DAT. This may be also due to reduced synthesis of chlorophyll protein phycobilin, which is easily destroyed by UV-B radiation (Nedunchezhian and Kulandaivelu.,1991).

Among the chemical treatments Nativo 70ppm recorded highest chlorophyll content under two growing conditions at 45th and 75th DAT. At 45th DAT increment in total chlorophyll content was 58.3% and 33.5% under 100 and 80% solar radiation respectively when compared to absolute control, where as it was 39.1% and 61.7% at 75th DAT.

The present findings on chlorophyll content are in line with the report that mixture of trifloxystrobin and tebuconazole increased the chlorophyll index in rice and tomato up to 16.91% and 18.08% respectively (Nagajothi and Jeyakumar, 2014). Some other systemic fungicide like cyproconazole and propiconazole also increased chlorophyll index (Hennouni *et al.*, 2012). Gortz *et al.* (2008) found that triazole compound has a positive effect on relative chlorophyll content in barley which is related to SPAD values.

Flavanoid content

The present study revealed that 20% reduction in ambient UV-B level resulted in reduction of flavanoid content which is considerd as a UV-B absorbing compound. Hence it indicates that flavanoid content enhanced in response to increased level of UV-B under 100% solar radiation. Though the effect was non significant at 45th DAT it was significant at 75th DAT

The photo protective pigments vary according to the level of UV-B radiation. Highest flavanoid was recorded for the plants grown under 100% solar radiation when compared to 80% solar radiation. It may be inferred that the UV-B penetration

was partially blocked by flavanoid pigment which protect the photosynthetic apparatus to some extent based on the threshold level of UV-B radiation in rice (Teramura *et al*., 1992; Yuan *et al*., 2014)

Various ameliorative chemicals had significant effect on reducing the flavanoid content under 100% solar radiation at 45th and 75thDAT(Table.18), but this effect was not so pronounced at different phenophases under reduced level of solar radiation. This indicates the ability of three chemicals to alleviate the stress effect under both conditions. Absolute control and water spray maintained highest flavanoid content at 45th and 75th DAT under both growing condition.

Among chemicals *Nativo* at 70ppm concentration caused a reduction in flavanoid content to the tune of 38.5% under 100% and 34.5% under 80% solar radiation during vegetative growth phase (at 45th DAT). During reproductive stage this reduction was 26% and 24% under 100% and 80% solar radiation levels respectively. This may be due to the ameliorative effect of chemicals in reducing UV-B stress and channelizing the carbon pool from the secondary metabolic pathways to primary photosynthetic pathway which finally contributed to the plant yield.

Xanthophyll content

Even in the absence of substantial UV-B irradiance UV-B absorbing substances were formed in leaf epidermis. Accumulation of such compounds when the plants were exposed to reduced level of UV-B radiation indicated that it is not only dependent on UV-B exposure. Biosynthesis of photoprotective pigments like flavones or xanthophylls are up regulated even under excess light stress, irrespective of the relative proportions of solar wave bands (Alessio *et al*., 2011)

Though scientist have indicated the photo protective role of xanthophyll pigment in UV stress condition (Eskling *et al*., 1997) the present study could not show such a relation. Comparison of xanthophylls content under two level of UV-B

radiation indicated high amount of this pigment under reduced level of UV-B radiation at both phenophases. An increment of 14.3% at 45th DAT and 28.6% at 75th DAT may be due to the inhibition of the activity of enzyme violaxanthin de-epoxidases under UV stress condition as this enzyme is involved in the synthesis of different xanthophylls compounds.

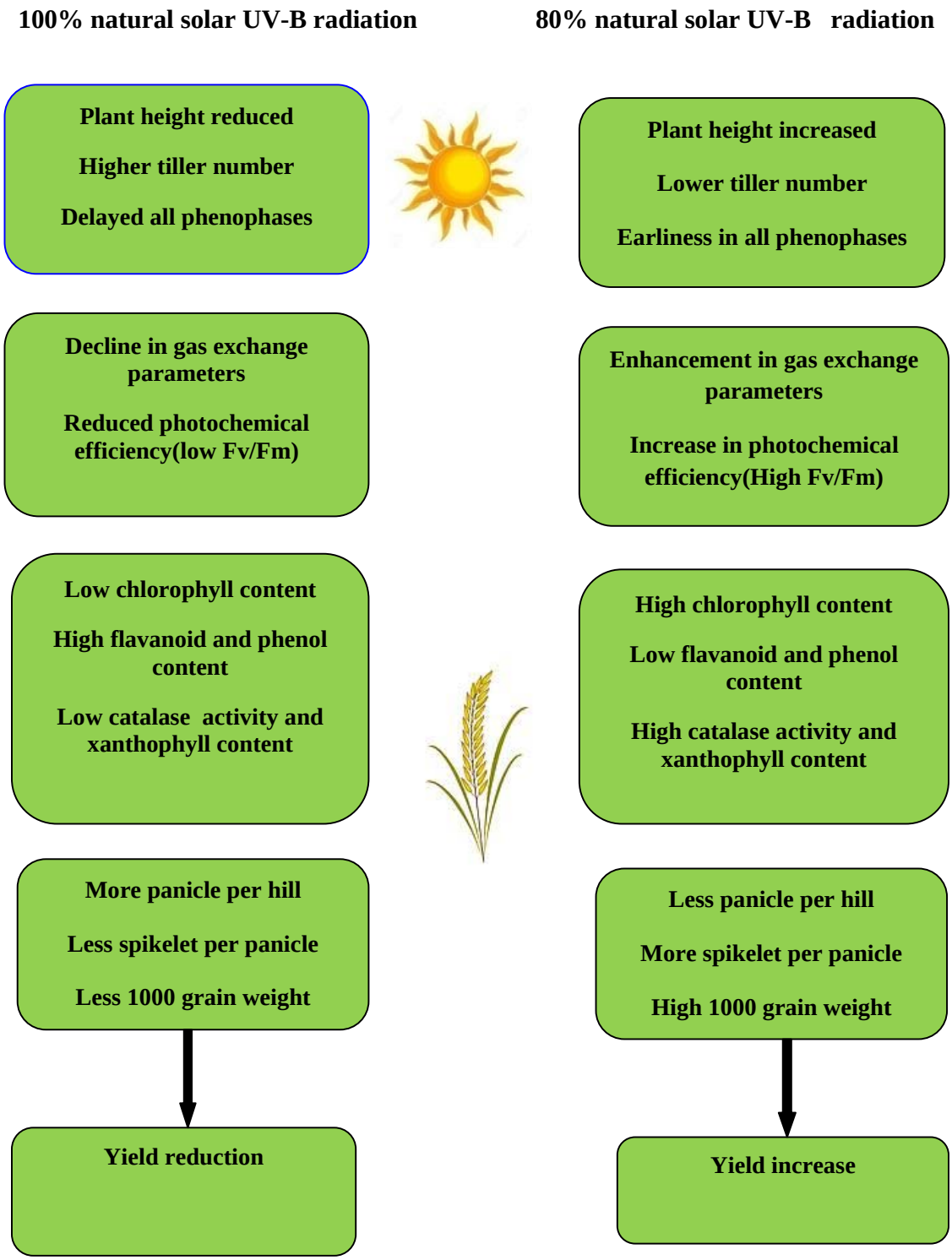
Under ambient level of UV radiation the ameliorative chemicals could not produce significantly pronounced effect on xanthophylls content at 45th and 75th DAT. The absolute control caused a remarkable increase (62%) in xanthophyll content under reduced level of radiation when compared to 45th DAT (16%). Among the three chemicals both concentration of glycine betaine and ascorbic acid reduced xanthophylls content at 75th DAT where as *Nativo* could improve this to the tune of 19.5% and 2.7% at C_1 andC_2 concentration respectively

Phenol content

Analysis of data on phenol content revealed that phenolic compounds increased when exposed to 100% solar radiation compared to 80% solar radiation at two growth stages (Table.17). There was 67.9% increment in phenol content from 45th to 75th DAT under 100% solar radiation where as this increment was 63.5% under 80% solar radiation. Previous report also suggests increase in flavanoid and phenol contents in response to supplemental UV-B in rice (Wagh, 2015).

Among the chemicals significantly higher phenol content was recorded for plants treated with both concentration of glycine betaine followed by *Nativo*. Glycine betaine at both concentrations had 6.14% more phenol than control, where as this increment was 4.38% and 0.8% by *Nativo* 50ppm and 70 ppm concentration respectively. At the same time the ascorbic acid treatment showed reduction to the tune of 13.16% and 7.01% by 50 and 100ppm concentration.

Fig.7 Variation in morphological, physiological and biochemical parameters contributing yield under 100% and 80% natural solar UV-B radiation



The crop without any treatment recorded more phenolic compound which was further enhanced by the application of glycine betaine and *Nativo*. This observation is in line with the previous report on phenolic enhancement by foliar spray of glycine betain in rice (Farooq *et al.,* 2008) and maize (Anjum *et al*., 2011) under drought condition. Further Sundarvadana e*t al.* (2007), also observed an enhancement of phenolic content in rice by foliar spray of azoxystrobin, a strobilurin group of fungicide like *Nativo*. This may be due to fungicidal property which leads to the activation of lignification related enzymes like PAL, PPO in phenyl propanoid pathway as well as phenolic content.

Catalase activity

Catalase is the important antioxidant enzyme in plants which can efficiently scavenge the free radicals and H_2O_2. Present study indicated that activity of the enzyme catalase enhanced from vegetative to reproductive stage under both level of solar radiation .This may be associated with enhanced photosynthetic activity which need more ROS scavenging molecule or antioxidant enzymes like catalase to remove ROS species, especially H_2O_2 during photorespiration from peroxisomes (Fig.6)

The various ameliorative treatments including Nativo enhanced the catalase activity in the range of 59% to 67.6% under 80% solar radiation at reproductive phase. At the same time the absolute control also enhanced the activity of this antioxidant enzyme to 69.7% which could enhance the yield to 35.5% more under reduced level of UV-B radiation.

5.6 Yield and yield characters affected by glycine betaine, ascorbic acid and combination fungicide 25WG trifloxystrobin+ 50WG tebuconazole (*Nativo*) under two levels of UV-B radiation

Current level of UV-B at ambient solar radiation reduced the overall yield and yield parameters in rice crop compared to reduced solar condition (Fig.7). This might

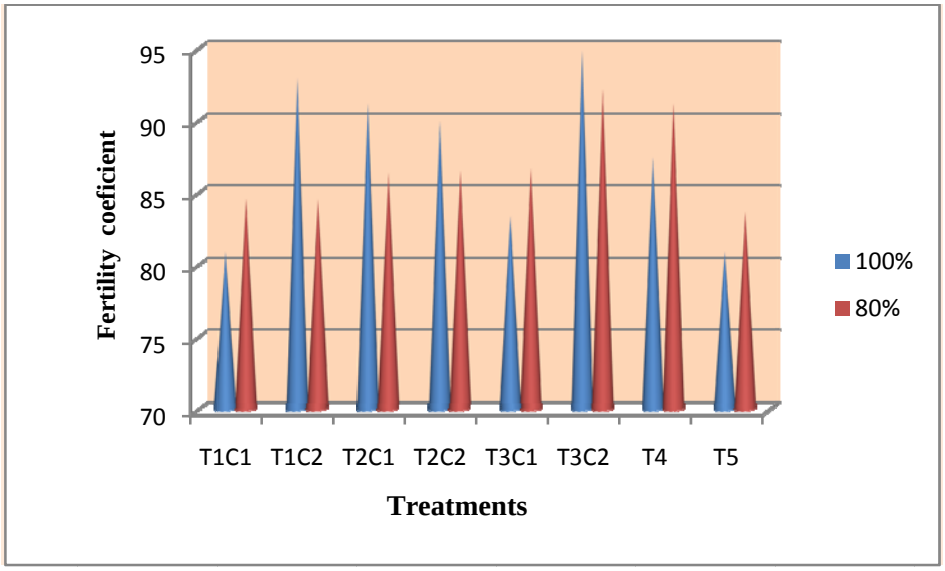


Fig.8 Variation in fertility co-efficient of rice among different chemical treatments under two levels of UV-B radiation

T_1C_1 - Glycine betaine - 10ppm **T_1C_2- Glycine betaine - 20 ppm**

T_2C_1 - Ascorbic acid - 50 ppm **T_2C_2 - Ascorbic acid - 100 ppm**

T_3C_1 - Nativo 75 WG - 50 ppm **T_3C_2 - Nativo 75 WG - 70 ppm**

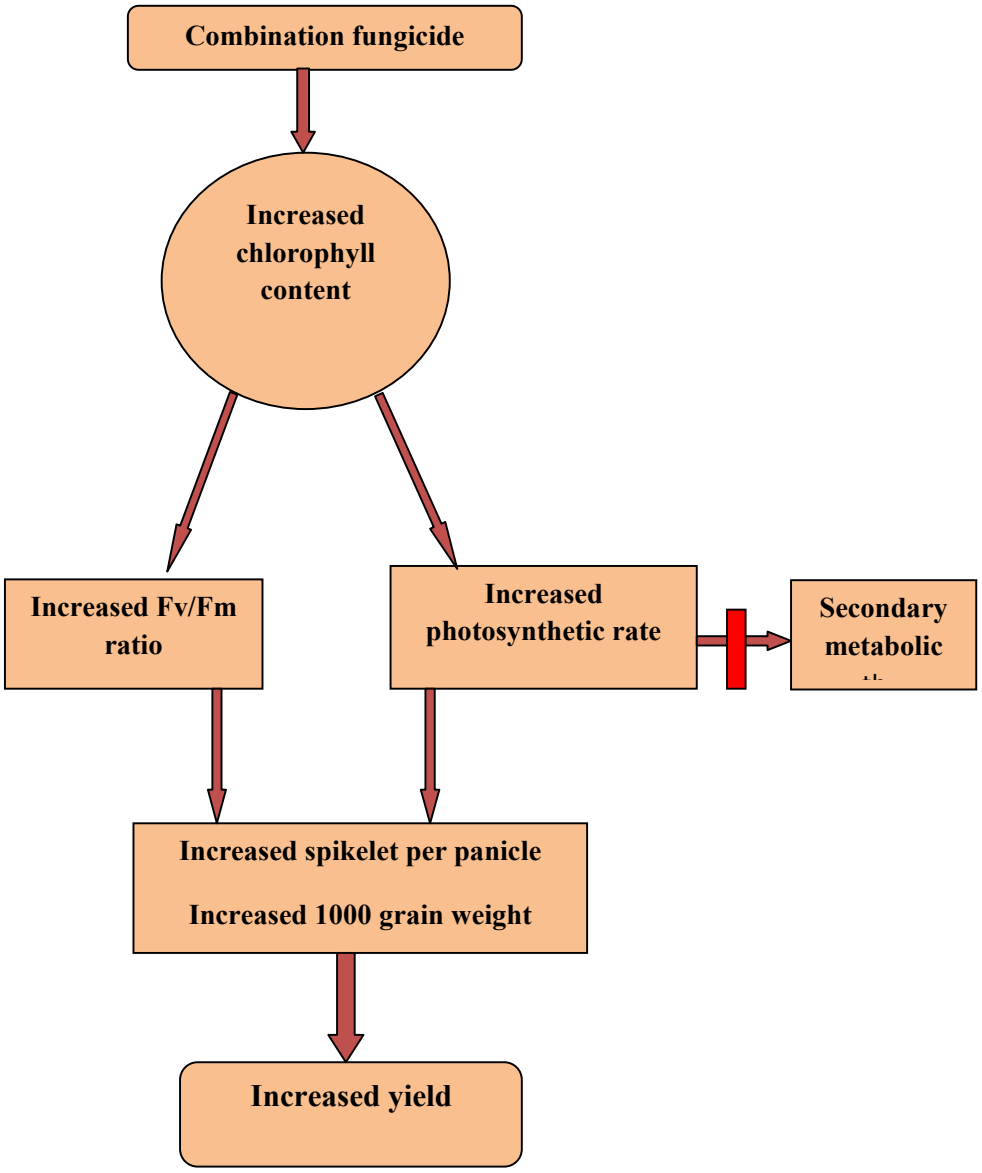
T_4 - Water spray **T_5 - Absolute control**

be due to reduced chlorophyll content, photosynthetic rate, stomatal conductance and transpiration rate. Yield characters depend on optimum level of climatic factors during the reproductive phase. High level of UV-B radiation on reproductive phase reduced the yield due to less partitioning of photoassimilates in to reproductive organs rather than vegetative sink especially for the formation of secondary metabolites. Higher level of phenol and flavanoid under 100% solar radiation indicates the partitioning of photoassimilates for the synthesis of secondary metabolites. Study conducted in Japanese rice cultivars under UV-B supplemented condition showed that elevated UV reduced the yield and grain development (Kumagai *et al*., 2001). In another study conducted in rice, panicle number, tiller number, dry mass, grain size and grain yield reduced due to the supplemental UV-B radiation (Hidema *et al*., 2005)

Effect of treatments on yield and yield characters under both condition revealed that 70 ppm *Nativo* (T_3C_2) recorded maximum number of spikelets under both condition which correspondingly contributed to higher yield. Among chemical treatments, combination fungicide proved to be superior by having maximum fertility co-efficient compared to other treatments under both condition (Fig.8). Nativo (Trifloxystrobin+Tebuconazole) has a property to alter the physiological characters of plants like retention of green leaf tissue, increase in endogenous cytokinin and auxin, inhibition of ethylene biosynthesis, increase in CO_2 assimilation, better N assimilation, increase in water use efficiency and harvest index (Nagajothi and Jeyakumar 2014). *Nativo* treated plant retained chlorophyll content that lead to higher photosynthesis and less ethylene production which in turn reduced the aging of crop and increased the grain filling period as physiological advantage for higher yield.

The biochemical compound flavanoid, which is synthesized through shikimic acid pathway from precursor derived from carbohydrate metabolism, can cause a control on carbon fluxes in primary metabolic pathway like photosynthesis. The yield reduction under 100% solar radiation can be explained due to deviation of carbon

Fig.9 Flow chart showing the UV-B stress mitigating effect of combination fungicide *Nativo* (25 WG trifloxystrobin+ 50WG tebuconazole).



derived compounds from photosynthetic pathway to secondary metabolic pathway to synthesis secondary photo protecting compounds like flavanoid, phenol and xanthophylls.

The present study revealed that among stress alleviating chemicals combination fungicide 25 WG trifloxystrobin+ 50WG tebuconazole at 70ppm enhanced yield under 100 and 80% solar radiation (Fig.9). This is associated with more number of panicles per plant and number of spikelet per panicle. The high photosynthetic rate, more photochemical efficiency of PS II, less stomatal limitation, more chlorophyll content and enhanced activity of antioxidant enzyme catalase might have favoured more number of spikelet and thereby higher yield by foliar application of Nativo 70ppm under UV-B stress condition.

Summary

6. SUMMARY

Photosynthesis, performed by green plants through sunlight energy, is arguably the most important metabolic reaction to sustain life on earth. Unavoidably plants are exposed to solar radiation present in the sun light spectrum reaching on earth surface and is divided in to UV-A (320-400nm), UV-B(280-320nm), UV-C (100 to 280nm) and Photosyntheticaly Active Radiation, PAR (400-700nm). Among this, UV-C is biologically more dangerous and is mostly absorbed in the atmosphere hence not reaching the earth surface. Nowadays the environmental implication of increasing solar UV-B radiation on agricultural production has gained concern due to stratospheric ozone decrease occurring over both northern and southern hemispheres. From 1979 onwards, thinning of ozone is about 5% per decade when averaged over entire year. Sensitivity to UV-B radiation varies within and between plant species although the mechanisms underlying this different behavior are far from fully understood. Reports showed that enhanced UV-B radiation negatively affected one third of the plant species studied. There are two mechanisms in which plant respond to high doses of UV-B either, activation of repair mechanisms or by stimulation of protection mechanisms. Sensitivity of crop plants to UV-B radiation varies depending on species, cultivars and growth condition. Rice, the most important staple food, is affected by increased level of UV-B radiation. When compared to other crops, the photoprotective mechanism is less in rice.

Various protective mechanisms have been adopted to avoid the deleterious effects of high dose UV-B radiation by plant growth regulators or organic solutes such as ascorbic acid (antioxidant), glycine betaine (osmolyte) and Nativo 75 WG (Strobilurin fungicide). Hence, the present study was undertaken at College of Horticulture, Vellanikkara to understand the photo protective potential of ecofriendly stress alleviating chemicals like glycine betaine (10ppm and 20ppm), ascorbic acid (50ppm and 100ppm) and Nativo 75 WG (50ppm and 70ppm) with two concentration and its effect on photo inhibition in photochemistry and photosynthesis of rice (*Oryza*

sativa L.) under two levels of solar ultraviolet-B radiation (100% and 80% solar radiation)

The main objective of the study was:

- To understand the photoprotective potential of strees alleviating ecofriendly chemicals like glycine betaine, ascorbic acid and Nativo 75 WG in various morphological, phenological, physiological and biochemical changes in rice under two levels of UV-B radiation (100% and 80% solar radiation).

The study revealed that high level of UV-B recorded during February- March 2016 coincides with the 3rd cropping season (puncha). It caused morphological, physiological, phenological and biochemical changes in rice crop. This damaging effect can be partially alleviated by the foliar application of growth stimulating ecofriendly chemicals like glycine betaine (an osmolyte), ascorbic acid (an antioxidant) and Nativo 75 WG (a strobilurin fungicide). The effect of these chemicals was also compared with water spray and absolute control without any other treatments.

The salient findings of the study are as follows

- Significantly higher UV-B (2.18 Wm^{-2}) and PAR (1786 $\mu molm^{-2}s^{-1}$) was recorded during March-2016 under 100% solar radiation compared to 80% solar radiation
- Observations on various phenophases of growth indicated that grain filling period (difference between days to harvest and days to 50% flowering) decreased under 100% UV-B radiation. But, the application of Nativo 70ppm could enhance the grain filling period which consequently contributed to more yield.

- UV-B stress alleviating chemicals could enhance the plant height compared to absolute control under both 100 and 80% solar radiation. Among the chemicals, glycine betaine 10ppm contributed to maximum height under two levels of radiation.
- Gas exchange parameters like photosynthetic rate, stomatal conductance and transpiration rate decreased during reproductive phase under 100% solar radiation, where the crop experienced high UV-B radiation during this phenophase. Among the chemicals, Nativo 70 ppm enhanced the above parameters due to the positive effect of strobilurin group of chemicals on plant photosynthesis; these positive effects may also be due to enhancement in chlorophyll content observed in this study.
- Crops exposed to ambient levels of solar radiation experienced chlorophyll fluorescence emission indicated by lower Fv/Fm ratio. The UV-B radiation might induced phoinhibition by decreasing photochemical efficiency of chloroplast as induced by lower photosynthetic rate and Fv/Fm ratio under 100% solar radiation. Among the chemicals, Nativo 70ppm favoured more Fv/Fm values indicating improvement in photosynthetic efficiency.
- High chlorophyll content, catalase activity and xanthophyll content was observed under open condition where it needs more photoprotection from photoxidative stress.
- Among the chemical treatments, significantly higher chlorophyll content was recorded for the plants treated with 70ppm Nativo and this may be the reason for the increased photosynthetic rate in plants treated with T_3C_2 .
- Catalase activity was significant only at flowering stage where, increased metabolic activity of plants treated with T_3C_2 demanded higher scavenging enzyme (catalase) under both condition (100 and 80% solar radiation).
- Xanthophyll content was significant only under 80% solar radiation at both 45^{th} and 75^{th} DAT. Absolute control maintained high content of xanthophyll

which indicated that the plant experienced stress due to UV-B. Nativo could cause a 30% decrease in xanthophyll content when compared to control, thereby reduced carbon partitioning to secondary metabolic pathway.

- Flavanoid content was more under 100% solar radiation when compared to reduced level of radiation. In both growing condition T4 and T5 maintained high flavanoid content. Among the chemicals, Nativo 70ppm caused maximum reduction in flavanoid content under both condition.
- Significant difference in phenol content was observed in plant grown under 100% solar radiation at vegetative stage (45^{th} DAT). Among the chemicals glycine betaine at both concentration and Nativo 50ppm improved the phenol content but it was on par with absolute control.
- Among the yield contributing factors, thousand grain weight and number of spikelet per panicle were enhanced due to the application of Nativo 75 WG at 70ppm ; which in turn caused highest yield under both level of UV-B radiation.
- In general, combination fungicide Nativo (25 WG trifloxystrobin+50 WG tebuconazole) at the concentration of 70ppm with its substrate property caused changes in phenological, physiological and biochemical parameters which could alleviate UV-B stress effect.
- The maximum yield observed when *Nativo* 70 ppm was used as the mitigating treatment may be due to the enhancement in thousand grain weight and number of spikelet per panicle, more photosynthetic rate and less fluorescence emission, more chlorophyll content, catalase activity and reduction in synthesis of secondary metabolites like flavanoid and xanthophylls. This study revealed that UV-B stress direct the primary metabolites towards the production of photoprotective secondary metabolites thereby reduced the yield under 100% natural solar radiation. Ameliorative chemicals could reduce the synthesis of these secondary products as a physiological advantage

for diverting the carbon for biomass accumulation which ultimately results in the enhancement of crop yield. Among the chemicals, combination fungicide *Nativo* 70ppm was superior in enhancing the above parameters followed by glycine betaine and water spray.

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Annexure

ANNEXURE I

Table 1: Data on Temperature (^{0}C) and Relative Humidity (%) under two levels of solar radiation (80% and 100% solar radiation)

		80% solar radiation		100% solar radiation	
November-2015		Temperature	RH	Temperature	RH
1st week	1 Nov-7 Nov	31.89	75.56	30.54	67.00
2nd week	8 Nov-14 Nov	29.05	84.00	28.05	73.00
3rd week	15 Nov-21 Nov	30.76	72.85	29.82	66.00
4th week	22 Nov-28 Nov	30.66	68.13	29.54	58.00
December-2015					
1st week	29 Nov-5 Dec	30.10	70.50	29.80	58.00
2nd week	6Dec-12Dec	29.95	66.00	29.25	63.00
3rd week	13 Dec-19 Dec	29.27	65.50	28.66	55.00
4th week	20 Dec-26 Dec	29.40	74.00	28.30	44.00
January-2016					
1st week	27Dec-2 Jan	31.50	62.50	30.75	41.00
2nd week	3 Jan-9 Jan	29.27	53.63	27.98	39.00
3rd week	10 Jan-16 Jan	28.80	44.50	28.60	39.00
4th week	17 Jan-23 Jan	29.70	38.00	29.45	48.00
5th week	24 Jan-30 Jan	29.30	43.75	28.40	39.00
February-2016					
1st week	31 jan-6 Feb	30.10	67.92	29.79	35.00
2nd week	7 Feb-13 Feb	31.09	55.63	29.48	42.00
3rd week	14 Feb-20 Feb	30.21	43.38	29.51	32.00
4th week	21 Feb-27 Feb	30.82	60.50	30.03	45.00
March-2016					
1st week	28 Feb-5 Mar	31.75	43.50	30.83	34.00
2nd week	6 Mar-1Mar	32.35	43.75	31.82	45.00
3rd week	13 Mar-19 Mar	32.15	42.25	31.15	52.00
4th week	20 Mar-26 Mar	30.70	43.25	30.05	53.00

ANNEXURE II

Table 2: Monthly mean solar irradiance (Wm^{-2}) received at Vellanikkara during Nov-2015 to Mar-2016

Month	Solar radiation (Wm^{-2})
November	14.46
December	17.11
January	19.86
February	20.65
March	21.53

ANNEXURE III

Table 3: Variation in fertility co-efficient (%) among various chemical treatments under two levels of UV-B radiation

Treatments	100% solar radiation	80% solar radiation
T_1C_1	80.87	84.57
T_1C_2	92.91	84.53
T_2C_1	91.15	86.35
T_2C_2	89.91	86.51
T_3C_1	83.33	86.66
T_3C_2	94.81	92.18
T_4	87.43	91.16
T_5	80.87	83.65
CD(0.05)	4.58	NS

MITIGATION OF SOLAR ULTRAVIOLET-B RADIATION INDUCED PHOTOINHIBITION IN PHOTOCHEMISTRY AND PHOTOSYNTHESIS OF RICE (*Oryza sativa* L.).

By

SHAFEEQA T.

(2014-11-194)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

(PLANT PHYSIOLOGY)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF HORTICULTURE, VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2016

Abstract

Sun light contains ultraviolet (UV) radiation which is separated in to UV-C (100-280nm), UV-B (280-320 nm) and UV-A (320-400nm). Evidences from data collected from both satellite and field experiments indicated an increase in UV-B radiation reaching on the earth surface, due to decrease in ozone layer. Hence, UV-B has become more challenging nowadays causing threat to agriculture production in tropics. UV-B exclusion and enhancement studies conducted in the Department of Plant Physiology, College of Horticulture, Vellanikkara have revealed a decline in rice yield due to UV-B radiation in rice varieties Jyothi and Uma (Wagh, 2015).

The present study entitled "Mitigation of solar ultraviolet-B radiation induced photoinhibition in photochemistry and photosynthesis of rice (*Oryza sativa* L.)" was conducted during 2014-2016 in the Department of Plant Physiology, College of Horticulture, Vellanikkara with the objective to understand the photo protective potential of ecofriendly stress mitigating chemicals on photoinhibition and photosynthesis of rice *(Oryza sativa* L.) under solar ultraviolet-B radiation.

The pot culture experiment was conducted during November 2015 to March 2016 with rice variety Uma under two growing conditions *viz*. open condition- where the crop exposed to 100% solar radiation and polyhouse condition- which transmit 20% reduced full spectrum solar radiation including UV-B. Three ameliorative chemical treatments with two concentrations such as glycine betaine (10ppm and 20ppm), ascorbic acid (50ppm and 100 ppm) and combination fungicide 25WG trifloxystrobin + 50 WG tebuconazole (Nativo 75 WG- 50ppm and 70ppm) were given as foliar application at 30^{th} and 60^{th} DAT and observations were taken 15 days after each chemical spraying. The experiment was laid out as completely randomized design (CRD). The UV-B and Photosyntheticaly Active Radiation (PAR) in both conditions were monitored regularly throughout the crop period.

The data on UV-B and PAR revealed significantly higher UV-B (2.18 Wm^{-2}) and PAR (1786 $\mu molm^{-2}s^{-1}$) during March-2016 under ambient condition. All growth phenophases were delayed under 100% solar radiation. Combination fungicide 25 WG trifloxystrobin+50WG

tebuconazole 70ppm enhanced grain filling period under both the growing condition compared to other chemical treatments; the ultimate realization being a relatively good yield.

Gas exchange parameters like photosynthetic rate, stomatal conductance and transpiration rate decreased during reproductive phase under 100% solar radiation, where the crop experienced high UV-B radiation. Among chemicals, 25 WG trifloxystrobin+50WG tebuconazole 70ppm enhanced the above parameters by alleviating the photoinhibition in photosynthesis and PSII activities. Photochemical efficiency as indicated by high Fv/Fm ratios was enhanced by foliar application of 25 WG trifloxystrobin+50WG tebuconazole 70ppm.

High chlorophyll content, catalase activity and xanthophyll content were observed under reduced UV-B condition. Among chemical treatments significantly higher chlorophyll content was recorded for the rice plants treated with 25 WG trifloxystrobin+50WG tebuconazole 70ppm and this might be the reason for the increased photosynthetic rate in plants due to its application.

The maximum yield contributed by 25 WG trifloxystrobin+50WG tebuconazole 70ppm may be due to the enhancement in thousand grain weight and number of spikelet per panicle, more photosynthetic rate and less fluorescence emission / increased photochemical efficiency of PS II, more chlorophyll content, catalase activity and reduction in the synthesis of secondary metabolites like flavanoid and xanthophylls. The ameliorative effect of this chemical has to be explored under field level for better results and recommendation to farmers for raising 3rd crop during puncha season.