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Physiological response of rice to herbicide application

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ABSTRACT

The experiment was conducted at Agricultural Research Station, Mannuthy in the year 2018 with the rice variety '*Jyothi*'. The experiment was laid out in RBD with three replications. Treatments included recommended and double the recommended doses of 2,4-D, metsulfuron-methyl + chlorimuron-ethyl and penoxsualm and two controls (hand weeded and unweeded). Herbicides were sprayed at 20 days after sowing (DAS). Biochemical parameters were estimated at one week after herbicidal application and at the time of flowering. Biochemical parameters such as soluble protein, total amino acid and nitrate reductase enzyme activity showed a decline in herbicide treatments compared to hand weeded control. Proline content and catalase enzyme activity showed an increase with herbicide application while other physiological parameters like Indole acetic acid (IAA) content, chlorophyll content, stomatal conductance and net photosynthesis showed a decline. Double the recommended dose of herbicides affected these parameters more adversely as compared to the recommended dose of herbicides. Even though double the recommended dose of herbicide exhibited higher weed control efficiency, it reduced the yield of rice plant.

Rice is the third most cultivated cereal in the world and it is the staple food for more than half of the world's population. Due to rapid growth of population, the production has to be increased further. To achieve this goal, it is important to reduce crop loss caused by weed competition. Weeds not only reduce production but also reduce the grain quality of rice (Ramanarayana 2014). Different post-emergence herbicides are used to control already emerged weeds which compete with the developing crop. 2,4-D, penoxsulam and ready-mix formulations of metsulfuron-methyl 10% + chlorimuron-ethyl 10% are some popular post-emergence herbicides currently used in Kerala. 2,4-D is the most widely used auxinic herbicide that is used for the control of broadleaved weeds and sedges without affecting monocots. Penoxsulam and metsulfuron-methyl + chlorimuron-ethyl are the herbicides which inhibit the synthesis of essential enzyme acetolactate synthase (ALS). Selectiveness of herbicides to certain weeds accounts for the usage of high dosages of the herbicides by farmers. The present study aimed at understanding the impact of excess usage of herbicides on physiology of rice crop.

The study was conducted in the year 2018 during the *Kharif* season in Agricultural Research Station, Mannuthy, Thrissur. The experiment was laid out in randomized bock design with eight treatments replicated three times. Plot size was 5 x 3 m (15 m^2). The rice variety selected for the study was '*Jyothi*'. The treatments included recommended and double the recommended doses of 2,4-D (0.8 and 1.6 kg/ha), metsulfuron-methyl + chlorimuron-ethyl (0.004 and 0.008 kg/ha) and penoxsulam (0.025 and 0.05 kg/ha) and two hand weeding (HW) at 20 and 60 DAS and without any weeding operation cited as unweeded (UW) in the text. The herbicides were sprayed at 20 DAS. Plant samples were collected one week after herbicide application and at the time of flowering for biochemical analysis.

The content of chlorophyll a, chlorophyll b and total chlorophyll were measured by the method adopted by Hiscox and Israelstam (1979). While the photosynthetic rate and stomatal conductance were calculated by using infrared gas analyzer (Model LI-6400 of ICOR Inc. Licoln, Nebraska, USA). The reading was taken from 8 am to 10 am. Indole acetic acid (IAA) was estimated by the method proposed by Parthasarathy *et al.* (1970) using Garden weber reagent.

Total soluble protein was estimated using the method suggested by Lowry *et al.* (1951). The content of total aminoacid was estimated using ninhydrin method proposed by Mooreand Stein (1948). The activity of catalase enzyme (CAT) was estimated by permanganate titration method of Barber (1980). Proline content was estimated by the method of Bates *et al.* (1973). The content nitrate reductase enzyme in the leaf was estimated by the method given by Hageman and Flesher (1960). The nitrite formed was estimated by the method described by Nicholas *et al.* (1976), by measuring the absorbance at 540 nm using spectrophotometer. Weed control efficiency calculated using the formula suggested by Mani *et al.* (1973). The crop harvested from each replication was threshed, winnowed and weighed separately. The straw and grain weights were recorded separately and expressed in t/ha. The data were subjected to statistical package WASP and SPSS. Multiple comparison among treatment means, where the F test was significant (at 5% level) were done with Duncan's Multiple Range Test.

Estimation of chlorophyll content of the rice plants at one week after herbicide application and at flowering stages revealed that there was significant reduction in chlorophyll a for all the dosages of herbicides as compared to hand weeded control (**Table 1** and **2**). This was also reflected in the total chlorophyll content. However, the chlorophyll b content did not show significant variation in the treatments where recommended dose of herbicides was applied. Application of higher doses of herbicides significantly reduced both chlorophyll a and chlorophyll b content. Earlier, Sahoo *et al.* (1993) reported that the effect of post emergence herbicide on total chlorophyll content was dose dependent, especially during the early stages of crop growth.

At flowering, chlorophyll b content was found to be significantly lower than the hand weeded control in all the treatments. However, the values of chlorophyll a was found to be at par with the hand weeded control in case of treatments where normal dose of the herbicides was applied. This confirmed to the findings of Ralph *et al.* (2000). Application of double the recommended dose of herbicides reduced the chlorophyll content in 2,4-D, metsulfuron-methyl + chlorimuron-ethyl and penoxsulam. This might be due to the fact that herbicides in general inhibit the common enzyme between the pathway of chlorophyll and cytochrome synthesis and cause the formation of an intermediate tetrapyrroline which prevented the formation of chlorophyll pigment (Matringe *et al.* 1989). Among the herbicides tested,

Table 1. Effect of herbicides on chlorophyll content of rice plant at 7 days after herbicide (DAH) application (mg/g of fresh leaf)

Treatment	Chlorophyll		Total chlorophyll
	a	b	
2,4-D (800 g/ha)	3.55^{b}	1.05^{ab}	4.60^{ab}
2,4-D (1600 g/ha)	3.07^{d}	0.814^{cd}	3.88^{e}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	3.23^{c}	0.951^{abcd}	4.18^{cd}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	2.39^{f}	0.740^{d}	3.13^{g}
Penoxsulam (25 g/ha)	3.42^{b}	0.970^{abc}	4.39^{bc}
Penoxsulam (50 g/ha)	2.81^{e}	0.799^{cd}	3.60^{f}
Two hand weeding at 20 and 60 DAS	3.72^{a}	1.12^{a}	4.84^{a}
Unweeded	3.15^{cd}	0.873^{bcd}	4.02^{de}
LSD (p=0.05)	0.148	0.215	0.237

Table 2. Effect of herbicides on chlorophyll content of rice plant at flowering (mg/g of fresh leaf)

Treatment	Chlorophyll		Total chlorophyll
	a	b	
2,4-D (800 g/ha)	2.25^{ab}	1.12^{b}	3.38^{b}
2,4-D (1600 g/ha)	1.88^{c}	0.75^{cd}	2.63^{d}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	2.25^{ab}	1.10^{b}	3.34^{b}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	1.55^{d}	0.70^{d}	2.25^{e}
Penoxsulam (25 g/ha)	2.21^{ab}	1.06^{b}	3.27^{b}
Penoxsulam (50 g/ha)	1.13^{e}	0.522^{e}	1.65^{f}
Two hand weeding at 20 and 60 DAS	2.33^{a}	1.90^{a}	4.22^{a}
Unweeded	1.99^{bc}	0.903^{c}	2.89^{c}
LSD (p=0.05)	0.287	0.157	0.191

recommended dose of 2,4-D had the least damage on chlorophyll content and the result corroborated well the early report of Ivanov *et al.* (2002) who stated that lower concentration of 2,4-D showed less reduction in chlorophyll content compared to higher concentration.

IAAis a plant growth regulator produced in the shoot tip and moves to the root activating cell division and elongation. It is also a signalling molecule necessary for plant growth and development (Taiz and Zeiger 1991). In the present study, IAA content of the rice plant was significantly reduced by herbicides (**Table 3)** and this did not improve even at the time of flowering. The reduction was higher in the plants where double the recommended dose of the herbicide was applied. Similar results were reported by Ramanarayana (2014) in the case of ALS inhibitors such as metsulfuron-methyl + chlorimuron-ethyl and azimsulfuron. Machackova and Matschke (2002) observed 30% reduction in IAA content after application of 2,4-D and 15% after application of glyphosate in oak trees. Among the herbicides, normal dose of 2,4-D showed least reduction in IAA content at 7 DAH application and flowering (0.250, 0.250).

Table 3. Effect of herbicides on IAA content of rice plant (mg of unoxidized auxin/g fresh weight)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	0.250^{b}	0.250^{b}
2,4-D (1600 g/ha)	0.175^{d}	0.108^{d}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	0.200^{c}	0.217^{c}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	0.142^{e}	0.075^{e}
Penoxsulam (25 g/ha)	0.200^{c}	0.200^{c}
Penoxsulam (50 g/ha)	0.150^{e}	0.050^{f}
Two hand weeding at 20 and 60 DAS	0.300^{a}	0.308^{a}
Unweeded	0.200^{c}	0.200^{c}
LSD (p=0.05)	0.009	0.023

The net photosynthetic rate was found to be affected by herbicide application (**Table 4**). As compared to hand weeded control (31.90 and 9.11µ mol CO_2/ m^2/ s, respectively, at 7 DAH application and at flowering) all the herbicides significantly reduced the net photosynthesis. The effect was more pronounced as the dose was increased. Among the herbicides, recommended dose of 2,4-D had the least effect on the photosynthesis of the rice plant and this was consistent with the effect being recorded in case of chlorophyll. Tejada *et al.* (2013) reported that net photosynthesis reduced when the plant was affected with chlorosis, indicating chlorophyll content as an indicator of net photosynthesis. Zhou *et al.* (2007) reported that the application of ALS inhibiting herbicides caused an inhibition of acetolactose synthase enzyme that interrupts synthesis of aminoacids like valine, leucine, isoleucine and this, finally resulted in the decline of photosynthetic rate.

Table 4. Effect of herbicides on net photosynthesis of rice plant (µ mol $CO_2/m^2/s$)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	31.53^{b}	8.34^{b}
2,4-D (1600 g/ha)	27.47^{e}	5.81^{e}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	30.05^{c}	6.88^{c}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	24.24^{g}	5.21^{f}
Penoxsulam (25 g/ha)	30.27^{c}	6.26^{d}
Penoxsulam (50 g/ha)	26.07^{f}	4.47^{g}
Two hand weeding at 20 and 60 DAS	31.90^{a}	9.11^{a}
Unweeded	29.76^{d}	6.15^{d}
LSD (p=0.05)	0.276	0.175

Stomatal conductance indicates the inflow and outflow of CO_2 and H_2O through stomata. Under stress condition, the closure of stomata occurs as a defence mechanism against loss of water and increasing stomatal resistance consequently decreases the stomatal conductance (Taiz and Zeiger 2009). In the present experiment, the stomatal conductance was adversely affected by herbicide application compared to handweeded control (**Table 5**). Double the recommended dose reduced the stomatal conductance which became lower even than the unweeded control. Agostinetto *et al.* (2016) also observed that post-emergence herbicides application caused a decrease in photosynthetic rate and stomatal conductance. Zabalba *et al.* (2006) reported that application of ALS inhibiting herbicide (imazethapyr) reduced stomatal conductance, which caused a reduction in nitrogen uptake by roots.

Table 5. Effect of herbicides on stomatal conductance of rice plant (mol $H_2O_2/m^2/s$)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	1.63^{b}	0.467^{b}
2,4-D (1600 g/ha)	1.14^{e}	0.306^{f}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	1.52^{d}	0.394^{c}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	0.680^{g}	0.295^{g}
Penoxsulam (25 g/ha)	1.57^{c}	0.359^{d}
Penoxsulam (50 g/ha)	1.04^{f}	0.209^{h}
Two hand weeding at 20 and 60 DAS	1.77^{a}	0.523^{a}
Unweeded	1.50^{d}	0.313^{e}
LSD (p=0.05)	0.017	0.001

Herbicide application increased the proline content in the rice crop (**Table 6**). The increase was significantly higher in all the doses of herbicides both 7 DAH application and at the time of flowering. However among the herbicides, recommended dose of 2,4-D (0.165) only marginally increased the proline content (3.3%) while double the recommended dose of metsulfuron-methyl + chlorimuron-ethyl (0.270) led to an increase of 47%. Double the recommended dose of herbicides recorded maximum increase compared to recommended doses. Proline act as a stress marker and can be part of a defence mechanism of the plant and hence excess dosage of 2,4-D leads to increase in proline content of plants (Ivanov *et al.* 2002).

Table 6. Effect of herbicides on proline content of rice plant (mg/g)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	0.165^{e}	0.175^{e}
2,4-D (1600 g/ha)	0.243^{b}	0.250^{b}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	0.205^{cd}	0.222^{d}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	0.270^{a}	0.270^{a}
Penoxsulam (25 g/ha)	0.193^{d}	0.233^{c}
Penoxsulam (50 g/ha)	0.252^{b}	0.278^{a}
Two hand weeding at 20 and 60 DAS	0.143^{f}	0.145^{f}
Unweeded	0.210^{c}	0.237^{c}
LSD (p=0.05)	0.014	0.09

Nitrate reductase activity showed no significant difference among treatment at 7 DAH application, while at flowering, higher dose of herbicides showed the least value which was even less than unweeded control (363.33 μ mol of NO_2^- formed/gfresh weight/h). Hand weeded control (486.67 μ mol of NO_2^- formed/g Fresh weight/h) showed the maximum nitrate reductase activity. Beevers *et al.* (1963) observed that prolonged application of 2,4-D herbicide caused a reduction in nitrate reductase enzyme activity of cucumber. While, Zabalza *et al.* (2006) observed similar phenomenon with ALS inhibiting herbicides. Hence, Inhibition of N metabolism is a major impact of excess herbicide usage.

There was significant reduction in the content of total aminoacid of rice crop when estimated on the 7 DAH application in the present study (**Table 8**). The reduction was more in the case of ALS inhibiting herbicides such as metsulfuron-methyl + chlorimuron-ethyl (8.97 mg/g) and penoxsulam (9.11 mg/g) as compared to 2,4-D (9.89 mg/g). When the herbicides were applied at double the recommended dose, they further reduced the aminoacid content in the leaves.

Table 7. Effect of herbicides on activity of nitrate reductase enzyme of rice plant (μ mol of NO_2^- formed/g fresh weight/h)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	500.00	430.00^{b}
2,4-D (1600 g/ha)	433.33	203.33^{e}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	446.67	386.67^{c}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	410.00	203.33^{e}
Penoxsulam (25 g/ha)	460.00	363.33^{d}
Penoxsulam (50 g/ha)	463.33	190.00^{e}
Two hand weeding at 20 and 60 DAS	506.67	486.67^{a}
Unweeded	313.33	363.33^{d}
LSD (p=0.05)	Non-significant	14.74

Table 8. Effect of herbicides on total aminoacid content of rice plant (mg/ g)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	9.89^{ab}	8.84
2,4-D (1600 g/ha)	8.70^{c}	8.09
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	8.97^{bc}	8.67
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	8.50^{c}	7.82
Penoxsulam (25 g/ha)	9.11^{bc}	8.43
Penoxsulam (50 g/ha)	8.56^{c}	7.62
Two hand weeding at 20 and 60 DAS	10.77^{a}	9.07
Unweeded	8.77b^{c}	8.26
LSD (p=0.05)	1.163	Non-significant

The reduction in nitrate reductase enzyme activity and total aminoacid content might be the major reasons for the reduction in total soluble protein content of rice plants (**Table 9**). The data indicated the reduction in soluble protein content as compared to the hand weeded control (21.17 and 13.92 mg/g) both at one week after herbicide application and at the time of flowering. Excess dosage of the herbicides caused higher inhibition as compared to the recommended dosages.

In the present study, the activity of catalase enzyme was found to increase in the herbicide applied plots compared to handweeded plot and the data has been presented in **Table 10**. Catalase is an enzyme that coverts H_2O_2 produced by superoxide dismutase (SOD) to H_2O and O_2. At one week after herbicide application 2,4-D (14.96 units/g) showed low catalase content which was at par with hand weeded control (18.68 units/g), while at flowering stage all the treatment plots showed an increase in the catalase enzyme activity compared to hand weeded control (47.83 units/g). The oxidative stress imparted by the

Table 9. Effect of herbicides on soluble protein content of rice plant (mg/g)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	19.83^{b}	11.42^{b}
2,4-D (1600 g/ha)	18.00^{e}	10.08^{e}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	19.50^{c}	10.83^{c}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	16.25^{f}	09.00^{f}
Penoxsulam (25 g/ha)	19.50^{d}	10.50^{d}
Penoxsulam (50 g/ha)	16.91^{f}	09.00^{f}
Two hand weeding at 20 and 60 DAS	21.17^{a}	13.92^{a}
Unweeded	19.25^{d}	10.50^{d}
LSD (p=0.05)	0.400	0.232

Table 10. Effect of herbicides on activity of catalase enzyme of rice plant (Units of enzyme/g)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	14.96^{ef}	50.77^{g}
2,4-D (1600 g/ha)	20.09^{c}	64.83^{c}
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	18.57^{cd}	53.95^{f}
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	27.31^{a}	69.81^{b}
Penoxsulam (25 g/ha)	17.33^{de}	56.89^{e}
Penoxsulam (50 g/ha)	23.93^{b}	73.21^{a}
Two hand weeding at 20 and 60 DAS	13.43^{f}	47.83^{h}
Unweeded	18.68^{cd}	60.97^{d}
LSD (p=0.05)	2.586	2.610

herbicides led to such increase in activity of scavenging enzyme in the plant (Piexoto *et al.* 2008).

One week and one month after herbicide application, double the recommended dose of herbcides reported higher weed control efficiency (**Table 11**). Among the herbicides, double the recommended dose of 2,4-D showed maximum weed control efficiency and recommended dose of penoxsulam showed the least weed control efficiency. This was validated by Singh (2005). They reported that post-emergence application of 2,4-D at 500g/ha recorded highest weed control efficiency.

Grain yield was highest in hand weeded plot which was at par with recommended dose of 2,4-D and metsulfuron-methyl + chlorimuron-ethyl (**Table 12**). In the present study, double the recommended dose of herbicides contributed to 22-33% reduction in grain and 19-23% reduction in straw yield, respectively. Antralina *et al.* (2015) reported that among the herbicides penoxsulam-cyhalofop-butyl, bispyribac-sodium and 2,4-D + methyl metsulfuron, application of 2,4-D + methyl metsulfuron recorded a similar grain and straw yield with hand weeded control.

Table 11. Effect of herbicides on weed control efficiency (%)

Treatment	7 DAH application	At flowering
2,4-D (800 g/ha)	96.85[a]	77.81[b]
2,4-D (1600 g/ha)	99.42[a]	95.37[a]
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	87.64[ab]	57.52[de]
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	96.53[a]	73.79[bc]
Penoxsulam (25 g/ha)	77.17[b]	53.87[e]
Penoxsulam (50 g/ha)	91.24[a]	65.28[cd]
Two hand weeding at 20 and 60 DAS	100[a]	96.63[a]
Unweeded	-	-
LSD (p=0.05)	12.909	10.748

Table 12. Effect of herbicides on rice yield (t/ha)

Treatment	Grain yield	Straw yield
2,4-D (800 g/ha)	4.35[ab]	5.55[a]
2,4-D (1600 g/ha)	3.49[d]	4.56[e]
Metsulfuron-methyl + chlorimuron-ethyl (4 g/ha)	4.22[ab]	5.34[b]
Metsulfuron-methyl + chlorimuron-ethyl (8 g/ha)	3.43[d]	4.48[ef]
Penoxsulam (25 g/ha)	4.10[bc]	5.03[c]
Penoxsulam (50 g/ha)	3.01[e]	4.36[f]
Two hand weeding at 20 and 60 DAS	4.49[a]	5.65[a]
Unweeded	3.78[cd]	4.77[d]
LSD (p=0.05)	0.370	0.164

Our study indicated that plants are subjected to stress by the application of these chemicals and to overcome this, stress relieving mechanism are activated which operate at the expense of primary metabolism. Double the recommended dose of herbicides recorded higher weed control efficiency compared to its recommended doses, but it reduced the yield significantly which was even lower than the unweeded control. The recommended dose of 2,4-D, metsulfuron-methyl + chlorimuron-ethyl and penoxsulam were less harmful to the plant and the plants were able to recover within a month of application so that the yield was not significantly affected.

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