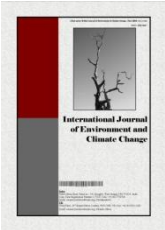

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Effect of Glyphosate on Physiology and Biochemical Properties of Purple Nutsedge (*Cyperus rotundus* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to understand the influence of herbicide on the changes in physiology and biochemical properties of purple nutsedge weed (*Cyperus rotundus* L.). Laboratory and pot culture study were conducted at the Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore during 2020-21. Glyphosate was tested with different concentrations (500, 600, 700, 800, 900 and 1000 ppm) and various soaking duration (6, 12, 24 and 48 hrs) with three replication. The extent of weed damage is based on the site of herbicide action, inhibition of enzymes activity and other metabolic functions. It resulted in chlorophyll content and membrane stability index of the weeds were affected. Proline level of treated weeds were found to be increased at initial stage of herbicide application and followed by declined gradually. It causes the weeds to kill by exhibiting oxidative stress. Phytotoxic effect of herbicide application was visualized until 15-20 DAHA (days after herbicide application) and thereafter weeds were died due to cessation of all the metabolic activities. The findings of the soaking experiment confirmed the negative impact of herbicide on phenol and starch content in tubers. Phenol degradation or dilution was observed to be increased

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with increasing duration of soaking period and however starch content decreases simultaneously. The results showed that application of glyphosate @ 1000 ppm is more effective to arrest the population of *Cyperus rotundus*.

Keywords: Purple nutsedge; glyphosate; proline; phenol; starch content.

1. INTRODUCTION

The demand for food crops is steadily increasing and need to feed the growing population. However, weeds are considered as biotic constraints which affect crop production. Proper management of weeds will undoubtedly increase crop production besides preserving natural resources. Purple nutsedge (*Cyperus rotundus* L.) is considered as one of the world's worst weeds which disseminated throughout the tropics and subtropics in 52 distinct crops and 92 countries [1]. It devastates farmlands rapidly and cause severe yield losses, even up to 100 per cent in some cases [2]. The purple nutsedge compete with crop for resources and reduces the cotton yield by 23 to 89 per cent, when compared to no purple nutsedge infestation [3]. Perennial habit of purple nutsedge adds advantage to persist in fields for many years and become difficult to control [4]. The management of *Cyperus* spp., were attempted using different cultural and chemical methods, however the results have been not promising [5]. Accumulation of phenolic compounds during stress or unfavourable condition hinders tubers germination from soil and survival. Moreover, foliar applied herbicides kill only primary tubers and leave the rest of the chain intact [5]. Weed seed propagules which unaffected act as reserve and establish as the season progresses [6]. As a result, nutsedge management measures must entail a long-term commitment to prevent the fresh tuber formation, as well as breaking the dormancy and eliminating viable tubers.

The presence of phenolic compounds acts as a tuber germination inhibitor [7], protecting tubers from unfavourable environmental conditions and helps to remain viable for long period in the soil. The stored carbohydrate (starch) serves as a food reserve for the tubers and allows the weed to establish continuously under favourable conditions [8,9]. This study was looked at the effect of different concentrations of glyphosate with different soaking period for the degradation of phenols and exhaustion of the food reserve in purple nutsedge tubers. It was based on the previous work on control methods used in the recent past, as well as management options for perennial sedge weed. Pot culture experiment was also conducted to assess the effect of herbicide on the physiology and biochemical properties of the plant to optimize the herbicide dose for desired weed control activity.

2. MATERIALS AND METHODS

A laboratory and pot culture study was conducted at the Department of Agronomy, Tamil Nadu Agricultural University during 2020-21. Tubers of purple nutsedge (*Cyperus rotundus*) were obtained in bulk from the garden lands of Moongilpatti, (11°01'75"N and 78°18'89"E), Trichy district, Tamil Nadu used as the base material for the study.

2.1 Laboratory Study

The tubers were cleaned and washed with water before soaking at different concentrations of glyphosate with different duration. The following are the experimental details. Total phenol and starch content were estimated in tubers after the soaking.

2.1.1 Factor 1: Glyphosate @ different concentration

T_1 - Soaking in Glyphosate @ 500 ppm
T_2 - Soaking in Glyphosate @ 600 ppm
T_3 - Soaking in Glyphosate @ 700 ppm
T_4 - Soaking in Glyphosate @ 800 ppm
T_5 - Soaking in Glyphosate @ 900 ppm
T_6 - Soaking in Glyphosate @ 1000 ppm
T_7 - Control (water)

2.1.2 Factor 2: Different duration of soaking

D_1 - Soaking duration @ 6 hrs
D_2 - Soaking duration @ 12 hrs
D_3 - Soaking duration @ 24 hrs
D_4 - Soaking duration @ 48 hrs

2.2 Pot Culture Study

For the pot culture experiment, medium sized plastic pots with the dimension of 18 x 15 cm were used. Each pot was filled with 5 kgs of pot mixture (2:1 mixture of red soil and FYM). The

collected *C. rotundus* tubers were sorted based on their size and weight to maintain uniformity in the study. Ten tubers were sown in each pot. They were watered regularly and monitored. Glyphosate was prepared at a concentration of 500, 600, 700, 800, 900 and 1000 ppm. Herbicide with different concentrations were sprayed on the respective pots along with 1 per cent ammonium sulphate and tween 20 solution at 30 DAS (Days after sowing).

2.2.1 Membrane Stability Index (MSI)

Cell membrane stability was studied by observing the leakage of the membrane under stress. Leaf bits of 0.1 g were taken in a test tube and 10 ml of distilled water was added and kept for half an hour @ 40^{o}C in a water bath after which the initial EC of that solution was taken after removing the leaf bits. Finally, the leaf bits were immersed in the same solution and boiled at 100^{o}C for 10 minutes in a hot water bath. The leaf bits were removed from the solution and final EC was measured. The membrane stability index was calculated using the method suggested by Sairam et al. [10].

2.2.2 Chlorophyll content

The top fully developed third leaf was selected for extracting the chlorophyll pigments. Chlorophyll a, b and total chlorophyll content were estimated using spectrophotometer.

To calculate chlorophyll a, b and total, the following formulas were used, which are expressed in mg g $^{-1}$ fresh weight [11].

$$\text{Chlorophyll 'a'} = \frac{(12.7 \times \text{OD at } 663) - (2.69 \times \text{OD at } 645)}{W} \times V \text{ mg g}^{-1}$$

$$\text{Chlorophyll 'b'} = \frac{(22.9 \times \text{OD at } 645) - (4.68 \times \text{OD at } 663)}{W} \times V \text{ mg g}^{-1}$$

$$\text{Total Chlorophyll} = \frac{(20.2 \times \text{OD at } 645) - (8.02 \times \text{OD at } 663)}{W} \times V \text{ mg g}^{-1}$$

Where,
W - Weight of the leaf sample (mg),
V - Volume of supernatant solution (ml) and
O.D. - Optical Density.

2.2.3 Chlorophyll a/b ratio

The chlorophyll ‘a’ to ‘b’ ratio was calculated by dividing the chlorophyll ‘a’ content by chlorophyll ‘b’ content.

2.2.4 Proline

The proline was determined based on the standard procedure of Bates et al. [12], which is expressed as μg g^{-1} of fresh weight.

2.3 Starch Estimation

Starch was extracted using 15 ml of 80 per cent ethanol by boiling 100 mg powdered sample for 30 min at 80°C followed by centrifugation @ 10,000 rpm for 30 mins. The extraction was repeated thrice until there was no colour change with anthrone reagent. The extract after evaporating off in a water bath at 80ºC, was treated with 52 per cent perchloric acid for starch extraction and the process was repeated thrice. The starch content was measured using anthrone reagent [13].

2.4 Total Phenol

Total phenol content was estimated by Folin-ciocalteau method which was described by Vinson et al [14]. A sample of 500 mg (treated tubers) was taken and macerated into small pieces. Then 5 ml of 80% ethanol was added and the mixture was boiled for 10 mins to prevent oxidation of phenols by polyphenol oxidase. After that, the sample was cooled and macerated with 80 per cent ethanol and the final volume was made up to 5 ml. Then it was centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected, from which 1 ml was taken in a test tube and 2 ml of 20 per cent sodium carbonate, 1 ml of folin reagent were added to that solution. Water was used to make up the volume up to 10 ml. The solution was kept 30 minutes for colour development at room temperature. Then, read at 660 nm in UV-Vis spectrophotometer.

2.5 Statistical Analysis

The data collected from experiments were subjected to analysis of variance (ANOVA) as single and two factor analysis. The critical difference at the 5 per cent level of significance of different treatments was compared as reported by Gomez and Gomez [15].

3. RESULTS AND DISCUSSION

3.1 Effect of Different Herbicide Concentration and Soaking Duration on Total Phenol Content of *C. rotundus* Tubers

The laboratory results revealed that phenol content of tubers was increased with herbicide concentration and treatment duration (Table 1). Significant differences between treatments were observed at different duration of observation. Higher herbicide concentration recorded highest phenol content among different concentrations. The minimum content of phenol was observed in untreated tubers. Interaction between duration of soaking and herbicide concentrations was highly significant. The highest phenol concentration of 28.81 mg g-1 (utmost phenol content of tuber) was recorded with the tubers soaked for 12 hours at herbicide concentration of 1000 ppm followed by lower herbicide concentration of 900, 800, 700 ppm with tubers soaked for 24 hrs. The utmost phenol content was achieved in lower concentration of herbicide (500 and 600 ppm) with tubers soaked for 48 hrs only. However, the phenol content was not altered significantly in the untreated tubers with increasing soaking duration.

The results indicated that phenol content of tubers varied with different herbicide concentration and soaking period. The utmost phenol content of 28.81 mg g^{-1} was observed in tubers treated with higher concentration of herbicides and soaked for shorter duration. The phenol content of the tubers depends on the herbicide doses and its absorption however, the phenol content increased gradually with the soaking duration increased. It might be due to higher concentration of herbicide increased the phenolic content of tubers as a mechanism to inhibit the sprouting of buds under induced xenobiotic stress [16]. Similarly, increased duration might be increasing the absorption rate of herbicide into the tubers and dilute or degrade the phenol content. Thus, herbicide induced changes in phenol content supports the differential modulation of phenol metabolism [17,18]. The untreated tubers (control treatment) recorded lower phenol content. The range in total phenol recorded in this study was compared with the report of Parikh and Patel [19].

Table 1. Effect of different doses of herbicide on Total phenol content (mg g^{-1} of tubers) of Purple nutsedge tubers

Treatments	D_1	D_2	D_3	D_4	Mean
T_1	3.58	8.13	9.61	28.81	12.53
T_2	3.83	8.51	11.81	28.81	13.24
T_3	8.42	9.51	28.81	28.81	18.89
T_4	9.61	14.94	28.81	28.81	20.54
T_5	9.92	18.96	28.81	28.81	21.63
T_6	10.75	28.81	28.81	28.81	24.30
T_7	3.51	7.03	9.16	11.24	7.74
Mean	19.16	13.70	20.83	26.30	
	T	**D**		**T x D**	
SEd	0.321	0.242		0.641	
CD(P = 0.05)	0.652	0.506		1.304	

3.2 Effect of Different Herbicide Concentration and Soaking Duration on Starch Content of *C. rotundus* Tubers

The data associated with the effect of herbicide on the starch content of purple nutsedge tubers in different duration was presented in Table 2. Glyphosate applied @ 1000 ppm recorded lower starch content followed by 900, 800, 700, 600 and 500 ppm. With respect to different duration of soaking, the minimum content of starch was recorded in 48 hrs after treatment followed by 24, 12 and 6 hrs. Among the interaction effect, the lower starch content was observed in tubers treated with glyphosate @ 1000 ppm for 42 hrs. Application of herbicide at higher concentration reduced the starch content in tubers than their corresponding lower doses. The mean starch content at different sampling duration over different treatments showed significant differences. Starch content decreased progressively with increased soaking period. The similar trend was also noticed with all the herbicide doses for starch content except untreated control, which significantly increased throughout the experimental period. Mishra et al. [20] reported that herbicides degraded the starch content of nutsedge tubers and converted into simple sugars. Depletion of food reserve may lead to reduction in germination, viability and multiplication of tubers [21].

Table 2. Effect of different doses of herbicide on starch content (mg g^{-1} of tubers) of Purple nutsedge tubers

Treatments	D_1	D_2	D_3	D_4	Mean
T_1	75.2	70.3	62.3	42.1	62.5
T_2	72.1	67.0	59.0	39.4	59.4
T_3	69.4	65.9	59.9	38.7	58.5
T_4	67.3	61.1	52.9	31.1	53.1
T_5	63.0	58.3	51.0	27.2	49.9
T_6	61.1	48.2	35.9	22.9	42.0
T_7	88.4	82.0	76.2	69.6	79.1
Mean	70.9	64.7	56.7	38.7	57.8
	T	**D**		**T x D**	
SEd	0.587	0.443		1.174	
CD (P = 0.05)	1.196	0.907		2.452	

3.3 Effect of Different Doses of Herbicide on Physiology and Biochemical Properties of *rotundus* Plants

Plant growth is the result of biochemical and physiological process as well as herbicides interfere with this process and affect weeds growth [22]. An analysis of variance showed that herbicidal treatments had a significant effect on membrane stability index (MSI), which is presented in Fig. 1. There was no significant difference between the control and other treatments @ 1 DAHA (Days after herbicide application). However, there was a significant difference recorded between control and other treatments due to increase in soaking duration.

There was a declining trend of MSI in plant from one day after herbicide application to plant death. Treatment imposed after one day recorded higher MSI than later stages (5, 10 and 15 DAHA). Membrane electrolyte permeability induced by xenobiotic stresses resulted in stability reduction [23, 24]. Herbicide induced xenobiotic stress due to imbalanced water and metabolite solution (e.g. amino acid) enhancement reduced MSI [25]. The past research findings had also indicated that any stress increased the plant electrolyte leakage and thus, led to chlorophyll degradation and subsequent loss in yield and death of plant [26]. Control treatment recorded higher MSI than other treatments due to no herbicide application to the *C. rotundus* plant which might have allowed the leaves to maintain the balanced electrolyte. MSI was decreased in *C. rotundus* due to herbicide treatments and showed herbicide toxicity. It produced free radicals which caused higher permeability and membrane instability. Because of the critical role of cell membranes in metabolism regulation, membrane stability index could be a suitable index for investigating levels of membrane damage and the presence of oxidative stress [27].

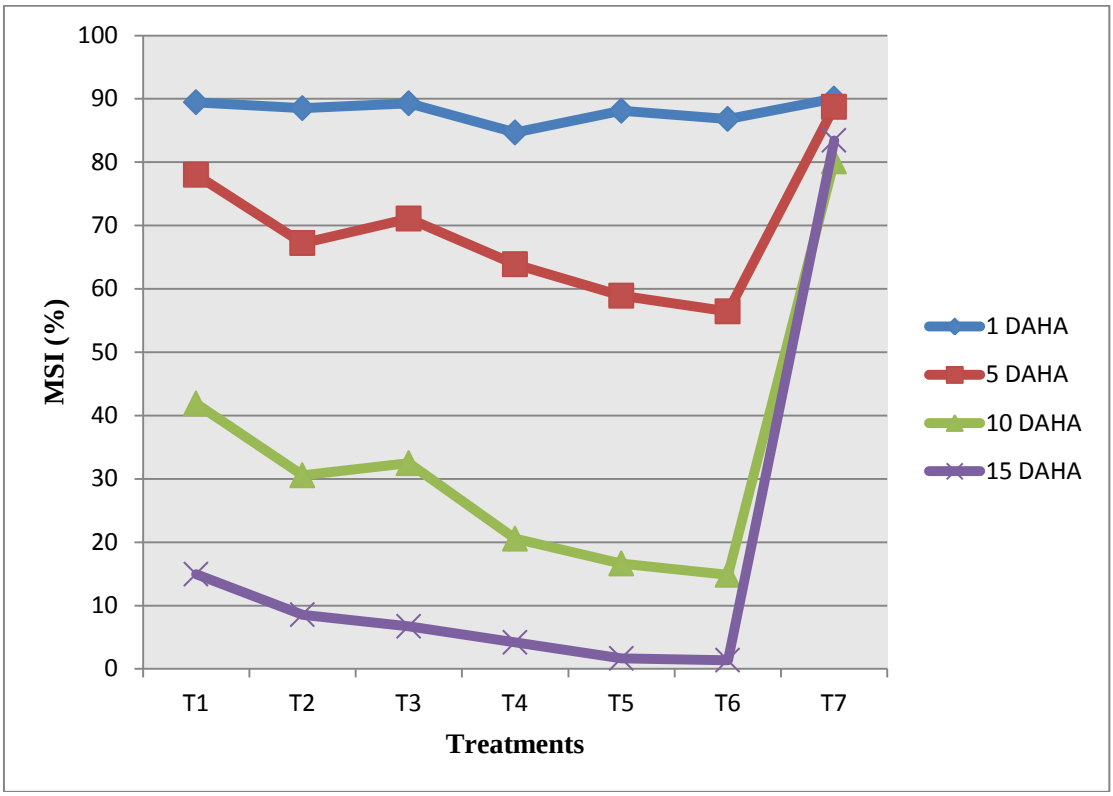

Fig. 1. Effect of different doses of herbicide on MSI (%) of purple nutsedge

Chlorophyll content was decreased with increase in application rate of herbicide. Chlorophyll a, b and total chlorophyll content were observed to be decreased from 1 DAHA to 15 DAHA (Tables 3 & 5). The lower chlorophyll content revealed that herbicide interfered with photosynthesis and inhibit EPSP synthase activity. EPSPs synthase are taken place in the chloroplast of most plant species and participate in the formation of aromatic amino acids. In control plants, chloroplast content was remaining unaffected and grana and thylakoids were intact [16]. However, herbicide treated plants were observed with disorganized and swollen and chlorophyll concentration was also decreased [28]. Similarly, chlorophyll a/b ratio was decreased from one day after herbicide application to death of the plants due to herbicide application. Visual symptoms recorded from 5 DAHA confirmed these research findings.

Table 3. Effect of different doses of herbicide on Chlorophyll a and Chlorophyll b (mg g^{-1}) of purple nutsedge

Treatments	Chlorophyll a				Chlorophyll b			
	1 DAHA	5 DAHA	10 DAHA	15 DAHA	1 DAHA	5 DAHA	10 DAHA	15 DAHA
T_1	3.43	3.44	2.06	1.19	1.22	0.98	0.59	0.37
T_2	3.61	3.27	2.20	1.32	1.18	0.87	0.64	0.31
T_3	3.51	3.54	2.88	1.21	1.21	0.91	0.61	0.29
T_4	3.66	3.52	2.26	1.18	1.01	0.93	0.63	0.32
T_5	3.58	2.95	2.22	1.05	1.09	0.85	0.58	0.28
T_6	3.71	2.60	2.12	0.94	1.28	0.89	0.54	0.27
T_7	3.36	3.66	3.17	3.01	1.32	1.01	1.04	1.01
SEd	0.065	0.062	0.030	0.028	0.027	0.017	0.014	0.015
CD(P=0.05)	0.140	0.130	0.070	0.059	0.059	0.037	0.029	0.033

Table 4. Effect of different doses of herbicide on Total chlorophyll (mg g^{-1}) and Chlorophyll a/b of purple nutsedge

Treatments	Total chlorophyll				Chlorophyll a/b			
	1 DAHA	5 DAHA	10 DAHA	15 DAHA	1 DAHA	5 DAHA	10 DAHA	15 DAHA
T_1	4.65	4.42	2.65	1.56	2.81	3.51	3.49	3.22
T_2	4.79	4.14	2.84	1.63	3.06	3.76	3.43	4.26
T_3	4.72	4.45	3.49	1.50	2.90	3.89	4.72	4.17
T_4	4.67	4.45	2.89	1.50	3.62	3.78	3.59	3.69
T_5	4.67	3.80	2.80	1.33	3.28	3.46	3.83	3.75
T_6	4.99	3.49	2.66	1.21	2.90	2.92	3.93	3.48
T_7	4.68	4.67	4.21	4.02	2.55	3.62	3.05	2.98
SEd	0.100	0.086	0.053	0.030	0.040	0.069	0.054	0.092
CD (P = 0.05)	0.214	0.186	0.113	0.064	0.086	0.148	0.116	0.196

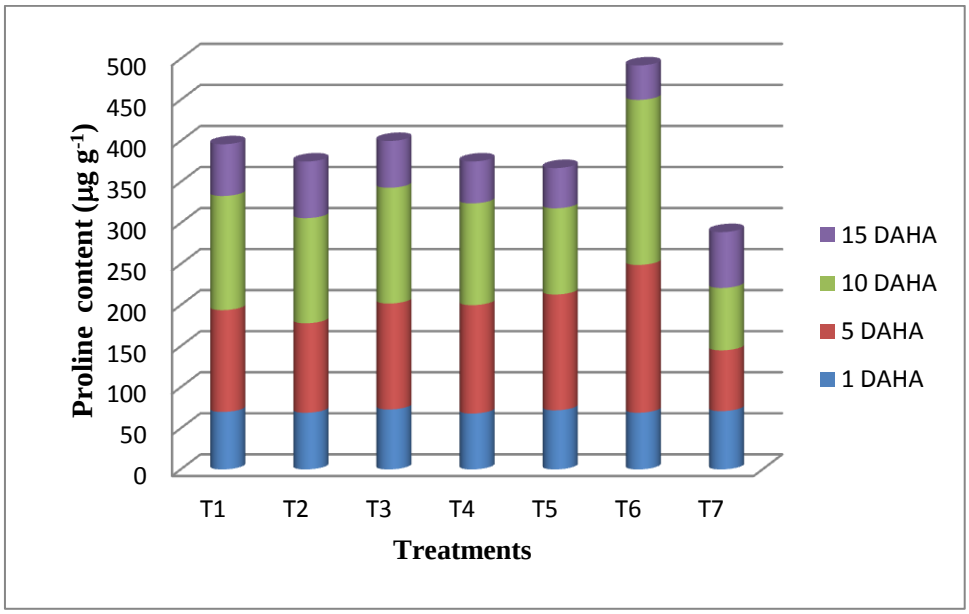

Fig. 2. Effect of different doses of herbicide on proline (µg g^{-1}) of purple nutsedge

Moreover, there was no significant difference in proline content between the control and other treatments @ 1 DAHA. However, plants treated with higher dose of herbicides recorded higher proline content when compared to other treatments at the later stages of herbicide application (Fig. 2). Proline accumulation is an indicator of plant water stress. Release of proline is the best way to judge the vulnerability of plants to stress [29]. Herbicide stress as xenobiotic due to imbalanced water and metabolite solution (e.g. amino acid) [25]. Many researchers have highlighted that plants under environmental stress had high secondary metabolites content [30-32]. Xenobiotic stress had triggered the production of free amino acids such as proline. Proline is an indicator and osmolyte which required for osmotic adjustment under stress environment [33]. There was lesser production of proline in the control treatment.

4. CONCLUSION

The present study indicated that magnitude of weed control is directly influenced by rate of herbicide application. Herbicide enter into a target site and disturb the enzymatic activity and other physiological functions which resulted in the reduction of chlorophyll content, membrane stability index, starch content and increased phenol content in plants and tubers. The findings of this study revealed that glyphosate @ 1000 ppm have greater detrimental effect on physiology and biochemical properties of purple nutsedge. Therefore, it is concluded that glyphosate applied @ 1000 ppm could be an

optimum dose for effective management of *Cyperus rotundus* weeds.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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