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Effect of herbicide application on biochemical changes in weeds commonly infesting tuber crops growing fields

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Abstract
A field experiment was conducted at ICAT CTCRI, Thiruvananthapuram during 2017-18 to study on "Effect of post emergence herbicide application on biochemical changes in weeds commonly infesting tuber crops growing fields". The study focused on two major weeds commonly seen in the tuber crops fields at the Thiruvananthapuram *i.e* one narrow leaved weed/monocot- *Pennisetum pedicellatum* and one broad leaved weed/dicot- *Indigofera hirsuta*. Experiment consists of three replications, each replication with 7 treatments. Three post-emergence herbicides (Propaquizafop, glyphosate and clodinofop propargyl) were sprayed to each treatment plot of elephant foot yam crop. Biochemical parameters such as chlorophyll, carotene and protein content of weeds of treatments were estimated on 3rd, 7th and 10th day after spraying the herbicide. The study revealed that the variation in biochemical parameters with the application of herbicides. Chlorophyll, carotene and protein content of weeds showed gradual reduction in each interval of time. From the study it was observed that the herbicide glyphosate drastically reduced the chlorophyll, carotene and protein content of weeds after its application and quickly killed both weeds. Clodinofop propargyl, Propaquizafop were effective in controlling grassy weed than broad leaved weed.

Keywords: Weed, herbicide, chlorophyll, protein, yield

Introduction
Weeds are ubiquitous and continue to be a major constraint in the production of root and tuber crops. When crop plants and weeds grow in close proximity weeds compete with crops for space, light, water and nutrients and their root or shoot system overlaps and attribute for reduction in crop productivity and quality of agricultural produce (Rao and Nagamani, 2010; Rao *et al*., 2015) [1, 2]. Some weeds exhibit allelopathic reactions inhibiting the growth of the crops (Demon *et al.*, 1975; Bhowmick and Doll, 1982; Einhelling, 1985; Weston and Duke 2003; Batish *et al*., 2007) [3, 4, 5, 6, 7]. The prevailing agro-climatic conditions such as temperature, humidity, day length and edaphic factors particularly soil moisture and nutrient status, etc. determines the composition of weed species, weed population and their competitive ability. Traditionally weed control in India is largely dependent on manual weeding, to some extent mechanical weeding in large farm lands. Nevertheless, labour scarcity and high wages compel farmers to opt for alternative options (Rao *et al*., 2015) [2].

The subsistence farmer of the tropics spends more time, money and energy on weed control than on any other aspect of crop production due to more incidences of weed and high labour wages. Nevertheless, the farmers continue to experience heavy losses in crop yield due to weed interference. A conservative estimate of about 10% loss in the tropics would amount to a total loss of about 25 million tons of food grains, valued at approximately Rs. 65000 crore (USD 13 billion) (Yaduraju, 2012) [8]. The total economic losses will be much higher if indirect effect of weeds on health, loss of biodiversity, nutrient depletion, grain quality, etc. is taken into consideration. Losses of similar magnitude would occur in root and tuber crops. Special attention is required for research on weed management in root and tuber crops owing to the initial slow growing nature of these crops (Moody and Ezumah, 1974; Srinivasan and Maheswarappa, 1993; Nedunchezhiyan and Misra, 2008; Nedunchezhiyan *et al*., 2013; Ravindran *et al*., 2010) [9, 10, 11, 12, 13].

The yield loss due to presence of weeds in tuber crops is reported by many workers

Table 1: The yield loss due to presence of weeds in tuber crops - report of different workers

Crop	Yield loss (%)	
Cassava	100	Moody and Ezumah, 1974; Akobundu, 1980; Hahn and Keyser, 1985; Ambe *et al*., 1992 [9, 14, 15, 16]
	50	Lebot, 2009 [17]
Sweet potato	91	Nedunzhiyan *et al*., 1998 [18]
	50-60	Stall, 2010 [19]
Yam	69-91	Moody and Ezumah, 1974 [9]
Taro	60	Nedunzhiyan *et al*., 1996 [20]
Elephant foot yam	100	Suresh *et al*., 2019 [21]

Chemical method (herbicides application) of weed control can reduce the dependency on manual weeding. Furthermore, weed control through herbicide application will be faster than manual weeding. Herbicides are likely to become inevitable method of weed control in tuber crops where labour is scarce or expensive or farm size is large (Agahiu *et al*., 2011; Suresh *et al*., 2019) [22, 21].

Chemical weed control has many advantages; the use of pre-emergence herbicides provides control of strong competing weeds during crop establishment and thus helps in weed control. Time of herbicides application is important in determining the effectiveness and length of weed control duration (Carter *et al*., 2007; James *et al*., 2007) [23, 24]. Selective post emergence herbicides are effective in the cropped field where they kill the weeds without harming the crop.

The herbicide demand in India is rising sharply and could double in the next few years as an acute labour shortage makes them a cheaper option and a rally in farm goods prices prompts farmers to grow crops with extra care (Mukherjee, 2011) [25]. Usage of herbicides occupies 44% of the total agrochemicals globally and 30% in India (Sondhia, 2014) [26]. In our country 60 herbicides of different modes of action are registered. More than 700 formulations of herbicides are available in the market (Choudhury *et al*., 2016) [27]. Nowadays combination formulations of two different herbicides are also becoming popular amongst farmers for broad-spectrum weed control. Even, proposal for combination formulation of more than two active principles has been suggested to the Registration Committee to combat resistant weeds (Choudhury *et al*., 2016) [27]. In this high adoption scenario of herbicide the present experiment was carried out to find the effectiveness of post emergence herbicides on weed control at phenotypic level and biochemical changes within the weed plant.

Materials and Methods

The study entitled "Effect of herbicide application on biochemical changes in weeds commonly infesting tuber crops growing fields" was carried out in elephant foot yam field at Central Tuber Crops Research Institute (ICAR-CTCRI), Sreekariyam, Thiruvananthapuram during the period of April 2017- January 2018. The study focused on two major weeds i.e one narrow leaved weed/monocot- *Pennisetum pedicellatum;* and one broad leaved weed/dicot- *Indigofera hirsuta*. The experiment was conducted in Randomized Block Design with three replications with different herbicide combinations viz., T_1- Application of propaquizafop @ 0.075 kg ai ha^{-1}, T_2- Application of propaquizafop @ 0.10 kg ai ha^{-1}, T_3- Application of clodinofop propargyl @ 0.075 kg ai ha^{-1}, T_4- Application of clodinofop propargyl @ 0.10 kg ai ha^{-1}, T_5- Application of glyphosate @ 1.0 kg ai ha^{-1}, T_6-Application of glyphosate @ 1.25 kg ai ha^{-1}, T_7- No chemical application (control). Details of herbicides used in the study are mentioned here under:

Table 2: Details of herbicides used in the study

Herbicide name	Formulation	Recommended dosage (a.i = active ingredient)	Trade name	Manufacturer/supplier in India
Propaquizafop	10% EC	75 a.i gha^{-1}	Agil	Adama Agricultural Solutions
Clodinofop propargyl	15%WP	75 a.i gha^{-1}	Security	National pesticides and chemicals
Glyphosate	41% SL	2000 a.i gha^{-1}	Glydon	Sumitomo Chemical India Pvt. Ltd.

The required amount of herbicides for the experiment was calculated by using the following formula.

$$\text{Required chemical} = \frac{\text{a.i. ha-1} \times 100}{\text{EC\%}}$$

Post emergence herbicides at different concentrations were applied after the emergence of weeds. The weed leaf samples were collected on 3days, 7days and 10 days after application of herbicides. The phenotypic changes were observed at different intervals (3, 7, 10 days after application).

Quantitative estimation of protein

Protein content of weeds (Broad leaved weeds, grass) samples were estimated by Bradford (1976) [28] method. The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible colour change. The assay is useful since the extinction co-efficient of a dye albumin complex solution is constant over a 10 fold concentration range.

Total protein extraction for protein quantification

0.2g of fresh leaf sample (weeds) was weighed out and grated using a pre chilled motor and pestle and made into a fine paste with the help of 10 ml ice cold phosphate buffer (pH 7.4). The homogenate was then transferred into a 20 ml centrifuge tube. The sample was centrifuged for 5 minutes at 5000 rpm. 0.1 ml of protein extract was pipette out into a test tube. To this 5ml Bradford reagent was added. The contents were thoroughly mixed and kept for 5 minutes at room temperature. The absorbance was read after 5 minutes at 595 nm. Two replications from each sample were used for the assay. The protein content was estimated using a standard curve.

$$\text{Quantity of protein in micro g/g} = \frac{\text{Factor from graph (µg/ml) x sample absorbance (OD) x volume of buffer (ml)}}{\text{Weight of the sample (g) x volume of the sample taken (ml)}}$$

Estimation of total chlorophyll and carotenoids

The total chlorophyll and carotenoids content of the leaves (weeds) was estimated using acetone. Fresh leaf sample (weeds) (0.1 g) was taken and grinded thoroughly using 80% acetone. The homogenate was filtered using filter paper. After filtration, the volume of the liquid was made up to 50 ml using 80% acetone. The absorbance was measured at 663.2, 646.8, 470 nm using a spectrophotometer. The chlorophyll content was measured by substituting the absorbance values in the given formula:

Mg chlorophyll A/g tissue = 12.1 ($A_{663.2}$) –2.81($A_{646.8}$) x 1 W x V

Mg chlorophyll B/g tissue = 20.13 ($A_{646.8}$) – 5.03 ($A_{663.2}$) x 1 W x V mg total chlorophyll (per g tissue) = Chlorophyll A + Chlorophyll B

$$\text{Total carotenoids} = \frac{(1000 \times A_{470}) - (1.82 \times \text{chlorophyll a}) - (85.02 \times \text{chlorophyll b}) \times V}{198 \times 1000 \times W}$$

Where,

A = Absorbance at specific wave lengths

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of the tissue extracted

Statistical analysis

The analysis was computed by using computer software program OPSTAT, CCS HAU. http://www.202.141.47.5/opstat/index.asp [29].

Results and Discussions

The present study revealed that weeds (broad leaved and grassy weeds) had variation in the biochemical parameters such as chlorophyll, carotene and protein content to the different herbicides application. Biochemical parameters of weeds showed gradual reduction in each interval of time (3, 7, 10 days) after the herbicide application.

In broad leaved weed at 3 days after herbicides application chlorophyll A content was significantly reduced with high concentrations of clodinofop (T_4- 0.01 kg ai ha^{-1}), glyphosate (T_6- 1.25 kg ai ha^{-1}). Whereas cholorphyll B and total chlorophyll contents were significantly reduced with high concentrations of three herbicides used in the experiment (T_2, T_4, T_6). At 7 days after herbicides application chlorophyll A, B contents and total chlorophyll content was significantly reduced with high concentrations of propaquizafop (T_2- 0.01 kg ai ha^{-1}), both the concentrations of glyphosate (T_5- 1.0 kg ai ha^{-1}, T_6- 1.25 kg ai ha^{-1}). Whereas at 10 days after application chlorophyll A was significantly reduced with the treatment with both the concentrations of glyphosate (T_5- 1.0 kg ai ha^{-1}, T_6- 1.25 kg ai ha^{-1}). In narrow leaved weed chlorophyll A, B, total chlorophyll contents were significantly reduced by glyphosate at both the concentrations.

In both the weeds at 3, 7, 10 days after herbicides application chlorophyll A, B, total chlorophyll contents were reduced with the days passed after herbicides application (Table 1, 2). The key enzymes associated with lipid biosynthesis were affected by Clodinofop, Propaquizafop [ACCase (enzyme Acetyl CoA Carboxylase) inhibitors] on cell division, growth and amino acid biosynthesis; Glyphosate [EPSP synthase (enzyme enolpyruvylshikimate-3-phosphate) inhibitor] caused chlorophyll degeneration, inhibit ion of chlorophyll biosynthesis, bleaching and starvation leads to drying and death of weeds. Similar results were reported with glyphosate application by previous workers (Sreenivasulu *et al*., 2015; Luiz *et al*., 2012; Zobiole *et al*., 2010; Mateos-Naranjo *et al*., 2009; Zobiole *et al*., 2011; Huang *et al*., 2012) [30, 31, 32, 33, 34, 35]. The incorporation of magnesium (Mg) by Mg chelates in the porphyrin structure is a necessary step leading to the synthesis of chlorophyll molecules (Tanaka and Tanaka, 2007) [36]. Foliar application of glyphosate will decrease concentrations of cations (Cakmak *et al*. 2009) [37]. Glyphosate may prevent chlorophyll synthesis indirectly by decreasing the Mg content in leaves Cakmak *et al*. (2009) [37], which lead to a decreased chlorophyll content and photosynthetic rate (Zobiole *et al*., 2012) [38]. Similary by inducing iron (Fe) deficiency, glyphosate may prevent the biosynthesis of δ- aminolevulinic acid (ALA), a component of the chlorophyll biosynthetic pathway (Marsh et al., 1963) [39]. Catalase and peroxidase, both enzymes implicated in ALA biosynthesis, are highly sensitive to Fe deprivation (Marsh et al., 1963) [39]. Glyphosate is a strong cation chelator, due to its carboxyl and phosphonate groups, forming complexes with nutrients in plant tissues, thus making them unavailable for biological process, including photosynthesis (Cakamk et al., 2009) [37]. In addition, glyphosate has been proposed to interfere with ALA biosynthesis by controlling the conversion of alpha-ketoglutarate to ALA and /or the condensation of glycine with succinyl-CoA to form ALA and CO_2 (Kitchen, 1980) [40].

Leaf carotene content of both the weeds was adversely affected due to application of herbicides (Table 3). At three days after application there was no significant difference in the leaf carotene content of broad leaved weed. But at 7 and 10 days after the application the leaf carotene content was reduced drastically in all the herbicide treatments. In the grassy weed also similar trend was noticed at 7 and 10 days after application.

Leaf protein content of both the weeds was adversely affected due to application of herbicides (Table 4). All herbicide treatments showed a significant decrease in protein content over untreated check at 3, 7, 10 days after spraying. The key enzymes associated with synthesis of amino acids and proteins are targeted by the herbicides which may be one of the possible reasons for decreased protein content under herbicide treatments (Singh *et al*., 2013) [41].

Table 3: Effect of post emergence herbicides on chlorophyll content (mg/g fresh weight) of broad leaved weeds

S. No	Chlorophyll - a			Cholophyll - b			Total chlorophyll		
	3 DAS	7 DAS	10 DAS	3 DAS	7 DAS	10 DAS	3 DAS	7 DAS	10 DAS
T1	1.06	0.79	0.14	0.29	0.21	0.06	1.35	1.00	0.20
T2	1.04	0.60	0.11	0.23	0.14	0.05	1.27	0.74	0.16
T3	1.03	0.86	0.10	0.25	0.22	0.07	1.28	1.08	0.17
T4	0.92	0.83	0.07	0.23	0.22	0.04	1.15	1.05	0.11
T5	1.05	0.27	0.06	0.24	0.15	0.11	1.29	0.42	0.17
T6	1.01	0.23	0.05	0.24	0.13	0.08	1.25	0.36	0.13
T7	1.17	0.96	0.90	0.30	0.23	0.15	1.46	1.19	1.05
S.E(m)	0.01	0.02	0.002	0.02	0.01	0.01	0.01	0.03	0.01

DAS – Days after spraying the herbicide

Table 4: Effect of post emergence herbicides on chlorophyll content (mg/g fresh weight) of grassy weeds

S. No	Chlorophyll - a			Cholophyll - b			Total chlorophyll		
	3 DAS	7 DAS	10 DAS	3 DAS	7 DAS	10 DAS	3 DAS	7 DAS	10 DAS
T1	0.85	0.53	0.42	0.23	0.20	0.10	1.08	0.73	0.52
T2	0.69	0.46	0.24	0.17	0.14	0.08	0.86	0.60	0.32
T3	0.89	0.72	0.37	0.26	0.23	0.09	1.15	0.95	0.46
T4	0.78	0.63	0.28	0.22	0.21	0.16	1.00	0.84	0.44
T5	0.90	0.23	0.19	0.26	0.11	0.06	1.16	0.34	0.25
T6	0.65	0.21	0.19	0.17	0.09	0.05	0.82	0.30	0.24
T7	0.98	0.89	0.78	0.31	0.29	0.26	1.29	1.18	1.04
S.E(m)	0.01	0.06	0.01	0.01	0.03	0.01	0.02	0.03	0.007

DAS – Days after spraying the herbicide

Table 5: Effect of post emergence herbicides on carotene content in weeds

	Carotene content (mg/g fresh weight)					
S. NO	Broad leaved weeds			Grassy weeds		
	3 DAS	7 DAS	10 DAS	3 DAS	7 DAS	10 DAS
T1	0.72	0.42	0.14	0.44	0.32	0.24
T2	0.62	0.34	0.12	0.40	0.28	0.15
T3	0.64	0.46	0.15	0.45	0.40	0.40
T4	0.58	0.45	0.08	0.42	0.39	0.22
T5	0.67	0.28	0.15	0.47	0.23	0.19
T6	0.57	0.27	0.14	0.38	0.28	0.17
T7	0.71	0.53	0.42	0.55	0.47	0.44
S.E(m)	NS	0.02	0.004	0.05	0.02	0.01

DAS – Days after spraying the herbicide

Table 6: Effect of post emergence herbicides on protein content in weeds

	Protein content (mg/g fresh weight)					
S. NO	Broad leaved weeds			Grassy weeds		
	3 das	7 das	10 das	3 das	7 das	10 das
T1	2.82	2.19	2.03	11.59	10.57	7.69
T2	2.18	2.00	1.85	11.16	10.05	6.31
T3	3.41	2. 54	2.13	11.33	9.28	7.83
T4	3.18	2.39	2.03	10.02	7.35	6.29
T5	2.39	2.00	1.87	9.52	4.79	3.61
T6	2.30	1.89	1.78	8.49	4.65	4.36
T7	3.57	3.27	3.24	12.38	12.51	12.01
S.E(m)	0.02	0.15	0.14	0.35	0.57	0.09

DAS – Days after spraying the herbicide

Glyphosate application on 10th day

Propaquizafop application on 10th day

Effect of herbicides on broad leaved weed/dicot: *Indigofera hirsuta* at 10th day after application

Control

Effect of herbicides on narrow leaved weed/monocot- *Pennisetum pedicellatum* at 10th day after application

Control

Glyphosate application on 10th day

Conclusion

From the experiment it can be concluded that application of broad spectrum herbicide glyphosate is very effective in controlling all weeds very quickly.

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