



## COMPARATIVE ASSESSMENT OF THE EFFECT OF DIFFERENT HERBICIDES ON WEEDS REPORTED IN CULTIVATION OF NATURAL SWEETENER PLANT: *STEVIA REBAUDIANA* BERTONI

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### Abstract

Weeds are uninvited guests which pose a biotic threat to agriculture, that compete for space, nutrients and out-grow crop plants. *Stevia rebaudiana* is one among the commercially important crops which has attained global attention due to its “zero calories sweetening power” (300-400 times sweeter than sucrose). Despite numerous benefits, Stevia has weaker ability to compete with weeds, especially at early stage of growth. This study examines the effectiveness of four commercial herbicides: pendimethalin, atrazine, paraquat, and 2, 4-D, for successful control of weeds that affect stevia cultivation. Three major weeds: *Erigeron sumatrensis*, *Parthenium hysterophorus*, and *Solanum nigrum* were selected, all of which grow in stevia fields. Each weed was treated with three different concentrations of single herbicide and each experiment was conducted in triplicate. To determine the effect of each herbicide on each weed, chlorophyll content, leaf area and weed height was studied at first, second and third week after treatment. All the three concentrations of each herbicide have shown significant effect on all three weeds but maximum effect observed was under highest concentration of each herbicide: pendimethalin at 0.45mg/ml, atrazine at 0.40mg/ml, paraquat at 0.34mg/ml and 2, 4-D at 0.25mg/ml. In all the three parameters, effectiveness shown by all herbicides was expressed as; 2, 4-D > paraquat > atrazine > pendimethalin. Based on these findings, 2, 4-D can be recommended as the best herbicide for controlling the aforementioned weeds in Stevia fields.

**Keywords:** Weeds, Herbicides, 2,4-D, *Erigeron sumatrensis*, Natural sweetener

### Introduction

Despite the global dependence on agriculture to feed the rapidly growing population, crop production is faced with different *biotic* and *abiotic* constraints globally. Among the biotic constraints, weeds are considered as most harmful to agriculture production (Gharde *et al.*, 2018; Kumar and Dwivedi, 2018a; Kumar *et al.*, 2018b; Kumar *et al.*, 2018c). Weed plants can be explained as “any type of plant located at a place where it is not required or needed” (Flamini, 2012; Rana and Rana, 2000). However, this definition is sometimes context-dependent; to one person a particular plant may be considered a weed, whereas to another person it may be desirable plant (Zimdahl, 2018; Kumar and Dwivedi, 2018d; Kumar Purnima *et al.*, 2018e). Weeds have special characteristics that make them unwanted: they out-grow crop plants, compete for space and nutrients with crop plants, act as secondary hosts for pests, and increase labor and other agricultural expenditures, all of which leads to reduction in the quantity and quality of agricultural produce (Rana and Rana, 2000; Zimdahl, 2018). Weeds block water flow in canals and drainage, also deoxygenate water and prove to be detrimental for aquatic organisms when grow densely (Abouziena and Haggag, 2016). Weeds are more tolerant than normal crops, and some species have the ability to reproduce both by seed and by vegetative means, as reported in Canada thistle, field bindweed, leafy spurge, quack grass and *Cyperus rotundus*, that can propagate through tubers as well as seeds (Kraehmer *et al.*, 2014; Kumar and Pathak, 2019f; Kumar *et al.*, 2019g; Siddique and Kumar, 2018h; Siddique *et al.*, 2018i; Pathak *et al.*, 2017j). Weeds have been the major universal threat to agriculture as weed management practices significantly cost about 40 billion of dollars annually (Abouziena and Haggag, 2016; Girdhar *et al.*, 2014a; Girdhar *et al.*, 2014b; Prakash and Kumar, 2017k;

Kumar and Mandal, 2014L; Kumar *et al.*, 2014m; Kumar *et al.*, 2014n). In a study on agricultural yield losses, it has been reported that among all the losses, 45% is through weeds, 30% is through insects, 20% is through diseases and 5% is through other pests. This brings about an estimate of nearly 10-15% annual losses in agricultural produce worldwide. In India, approximately 31.5% of the agricultural yields are lost due to weeds (Abouziena and Haggag, 2016). For two decades now, nearly twenty to twenty-eight billion Indian rupees have been lost due to weeds (Gharde *et al.*, 2018). Thus, the problem of weeds is ubiquitous and for almost every crop (Meena *et al.*, 2017; Singh and Aulakh, 2018; Kumar, 2013o; Kumar and Dwivedi, 2015p; Gogia *et al.*, 2014q; Kumar, 2014r; Kumar *et al.*, 2012s; Mishra *et al.*, 2012t).

Different practices for weed management have been adopted by farmers worldwide, some of which includes: (a) preventive measures, (b) cultural practices (c) mechanical methods, (d) plant breeding, (e) biotechnological strategies: use of transgenic approach, (f) biological control, (g) mulching, (h) soil solarization, (i) chemical method etc. (Duke, 2006; Meena *et al.*, 2017). However, the use of herbicides (chemical method) is the most adopted means of weeds control (Derksen *et al.*, 2002; Meena *et al.*, 2017; Jaswal *et al.*, 2017; Singh *et al.*, 2018). It has been reported that the trend of herbicides for weed control was initiated over a century ago with the introduction of few inorganic compounds, such as sulfuric acid, copper salts, sodium chlorate, and 2,4-dichlorophenoxyacetic acid (Kraehmer *et al.*, 2014). The use of herbicides is largely practiced by both local small-scale and large-scale crop producers (Harrington *et al.*, 2011). Some herbicides are used /sprayed alone or as an aid to tillage, before sowing or prior to plant maturity, or

as recommended by the manufacturer (Kraehmer *et al.*, 2014).

However, issues regarding environmental pollution together with fear of herbicide(s) resistance by weeds are also reported. Foxtail (*Setaria viridis*) is reported to be resistant against trifluralin and ethalfluralin in Canada due to excessive use of herbicides (Harrington *et al.*, 2011). It shows that, no single strategy is fully efficient alone, and that use of herbicides should be part of integrated weed management coupled with other practices for sustainable weed management (Gnanavel, 2015; Bahadur *et al.*, 2015). Eco-friendly approaches such as use of “bio-herbicides” should be given much consideration for successful weed control, so as to avoid ecological imbalance, pollution and destruction of beneficial microorganism (such as fungi, bacteria and protozoa) that combat disease causing pathogens (Bahadur *et al.*, 2015). The present study focuses on the identification and collection of different weeds reported in *Stevia rebaudiana* cultivation. I also aim at comparative evaluation of the effect of four known herbicides on different weeds to report the most efficient herbicide for successful weed-control.

## Materials and Methods

### Plant materials

Three different weeds, *E. sumatrensis*, *P. hysterophorus* and *S. nigrum*, (Fig. 1) that out-grow in *S. rebaudiana* cultivation and affect its production, were identified and their seedlings were collected. All the weeds were kept in plastic pots and maintained in green house facility provided by Lovely Professional University, Punjab, India. After a month, each weed plant was treated with three different concentrations of four commercially selected herbicides: (a) atrazine at 0.30, 0.35 and 0.40 mg/ml, (b) pendimethalin at 0.35, 0.40 and 0.45 mg/ml, (c) paraquat at 0.24, 0.29 and 0.34mg/ml, (d) 2, 4-D at 0.15, 0.20 and 0.25 mg/ml, and the controls were treated with distilled water. The chemicals (herbicides) were procured from Sigma-Aldrich, USA. The experiment was conducted in triplicate and the effect of each concentration of all the herbicides on each weed species was recorded after one week, two weeks and three weeks of spray (treatment). The parameters studied were, total chlorophyll contents (mg/g), plant heights (cm) and total leaf area (cm<sup>2</sup>).



**Fig. 1:** Weed plants, (A) *Erigeron sumatrensis*, (B) *Parthenium hysterophorus*, and (C) *Solanum nigrum*.

### Methodology

#### Chlorophyll estimation

The total chlorophyll contents were determined using the method of Arnon (1949) (Prasann *et al.*, 2018; Kumar *et al.*, 2018; Kumar *et al.*, 2019). Approximately 100 mg of leaves of each weed under test and the control plant was grinded separately in 10 ml of 80% acetone, using mortar and pestle. After incubation for 45 min at room temperature, the

sample was centrifuged for 10 min at 4000 rpm. The supernatant was transferred into 100 ml flask and the volume was made to 50 ml with 80% acetone. The absorbance of the diluted leaf extract was recorded at 663 and 645nm using a spectrophotometer (80% acetone as blank). The calculation of chlorophyll a, b as well as total chlorophyll was done using following formula:

Chl. a (mg/g) = [(12.7 × A<sub>663</sub>) - (2.6 × A<sub>645</sub>)] × ml acetone / mg leaf tissue.

Chl. b (mg/g) = [(22.9 × A<sub>645</sub>) - (4.68 × A<sub>663</sub>)] × ml acetone / mg leaf tissue.

Total Chlorophyll = Chl. a + Chl. b

#### Leaf area estimation

For determining the leaf area, leaf lamina was traced on the Whatman 1 MM paper, cut and weighed. Leaf area was calculated by dividing the weight of paper leaf by the weight of one cm<sup>2</sup> Whatman paper. This exercise was done with randomly selected large and small leaves from every weed-plant and the individual leaf area was multiplied to the total leaf count of that particular plant to get the total leaf area of the plant.

#### Plant height estimation

The height of all the weed plants was recorded (in cm) manually, using a scale. Readings for all the parameters were thrice i.e., after one week, two weeks and three weeks of herbicides spraying.

#### Statistical analysis

Each experiment was performed in triplicates. The results of these experiments were presented as a mean ± standard deviation. Experimental data was subjected to ANOVA test at 5% level of probability, using IBM SPSS 22 (statistic software).

## Results and Discussion

### Effect of herbicides on chlorophyll content

Under this experiment, effectiveness of three different concentrations of four selected herbicides was studied. A reduction in chlorophyll contents was observed in the treated weeds while the control plants (water sprayed weed plants) remained unaffected (Fig. 2). This reduction in chlorophyll contents was observed at all concentrations of each herbicide, with maximum effect at the highest concentrations (Fig. 4). The pattern of herbicides effectiveness on each weed was: *E. sumatrensis*- 2, 4-D > paraquat > atrazine > pendimethalin; *P. hysterophorus*- 2, 4-D > paraquat > atrazine > pendimethalin; and *S. nigrum* - 2, 4-D > paraquat > atrazine > pendimethalin. Among all the three herbicides used, maximum reduction of total chlorophyll contents in all three weeds was recoded with 2, 4-D treatment, followed by atrazine and paraquat, and least reduction was observed using pendimethalin treatment (Kumar *et al.*, 2011u; Kumar *et al.*, 2011v; Kumar and Pathak, 2016w; Pathak *et al.*, 2016x; Kumar *et al.*, 2018y; Kumar *et al.*, 2018z; Kumar *et al.*, 2018aa; Kumar *et al.*, 2018bb; Kumar *et al.*, 2018cc).

### Effect of herbicides on leaf area

Total leaf area of each of the three weeds was affected at all concentrations of each herbicide, compared to the control-weeds (water sprayed weed plants). The reduction in total leaf area of each weed was significantly increased with

the increase in herbicide(s) concentration, and the major reduction was observed at the highest concentration of each herbicide (Fig. 3). Each weed responded differently to each herbicide and the pattern of herbicide effectiveness for each weed, is as follows: *E. sumatrensis* - paraquat > 2, 4-D > atrazine > pendimethalin; *P. hysterophorus* - 2, 4-D > atrazine > paraquat > pendimethalin; and *S. nigrum* - 2, 4-D > paraquat > pendimethalin > atrazine. Out of these three weeds, *P. hysterophorus* and *S. nigrum* were more severely affected by 2, 4-D, whereas *E. sumatrensis* was affected more by paraquat (Singh *et al.*, 2020a; Singh *et al.*, 2020b; Sood *et al.*, 2020; Bhadreacha *et al.*, 2020; Singh *et al.*, 2020c; Sharma *et al.*, 2020; Singh *et al.*, 2020d; Bhati *et al.*, 2020; Singh *et al.*, 2019; Sharma *et al.*, 2019).

### Effect of herbicides on weed height

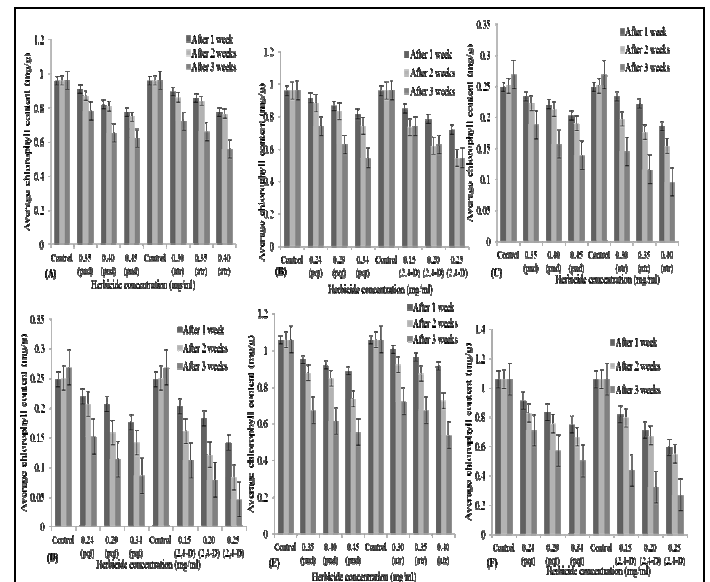
The average plant height(s) of the weeds were also timely studied after each herbicide spray treatment. After the treatment with three different concentrations of four different herbicides, plants heights were severely affected under all concentration of herbicides. But, each herbicide has shown maximum effect at its highest concentration (Fig. 4). The response of each weed to each herbicide differs from the other as follows: *E. sumatrensis* - pattern obtained by the effect of herbicides on plant height was - 2, 4-D > paraquat > atrazine > pendimethalin; *P. hysterophorus* - 2, 4-D > paraquat > atrazine > pendimethalin, whereas, for *S. nigrum*, it differs slightly- 2, 4-D > atrazine > paraquat > pendimethalin. It was observed that in all three weeds, 2, 4-D has the highest effect, under which highest reduction of average plant height was observed. However, all control plants (water sprayed weeds) remained unaffected, and some increase in height was recorded.

The chlorophyll is an important molecule present in certain algae and green plants, located in greenish parts such as leaves, flowers and stem (Pareek *et al.*, 2018). Being a coloured pigment, its physiological role is to capture sunlight and convert it into food through a process known as photosynthesis (Rajalakshmi and Banu, 2015). Generally, chlorophyll is classified into six types (a, b, c, d, e and f), with two main types (chl. a and b) reported in angiosperms. Chl. a is mostly dominant over chl. b, with an approximate ratio of 3:1. As the main source of energy production in plants, chlorophyll synthesis is determined by the availability of sunlight (Kamble *et al.*, 2015). With these known functions of chlorophyll, growth and productivity, as well as health status of plants depends on the availability of chlorophyll contents. The higher the chlorophyll contents, the more productive plant is and vice-versa. On the other hand, insufficiency of chlorophyll may lead to death of the plant.

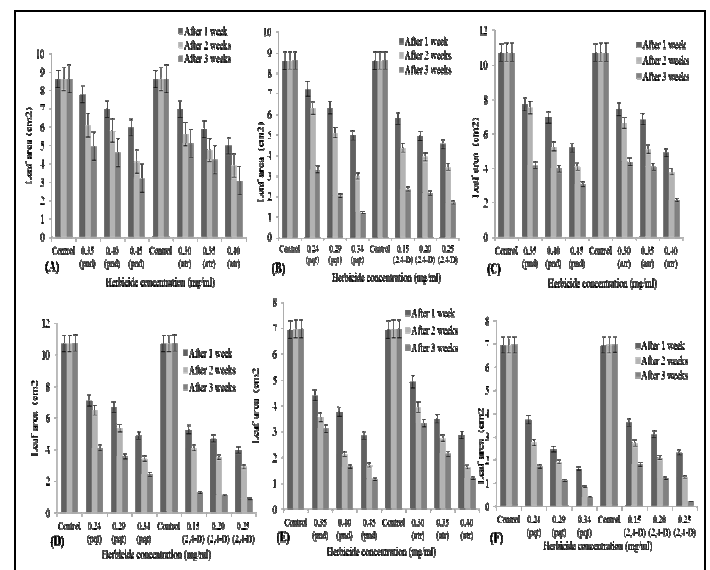
This study described that all three concentrations of pendimethalin, atrazine, paraquat and 2, 4-D affected the chlorophyll contents of *E. sumatrensis*, *P. hysterophorus* and *S. nigrum*, after at one week, two weeks and three weeks of spray. But maximum effect was recorded under highest concentration of each herbicide. However, compared to the other herbicides, 2, 4-D significantly reduced the chlorophyll contents of all the weeds (Gill *et al.*, 2017).

Height is one of the ecological parameters of plants to ensure survival, throughout their life span. Plants attain maturity and yield seeds to ensure continuity of their kinds. In every species of plants, height correlates with plants strength, maturity and ability to compete for resources

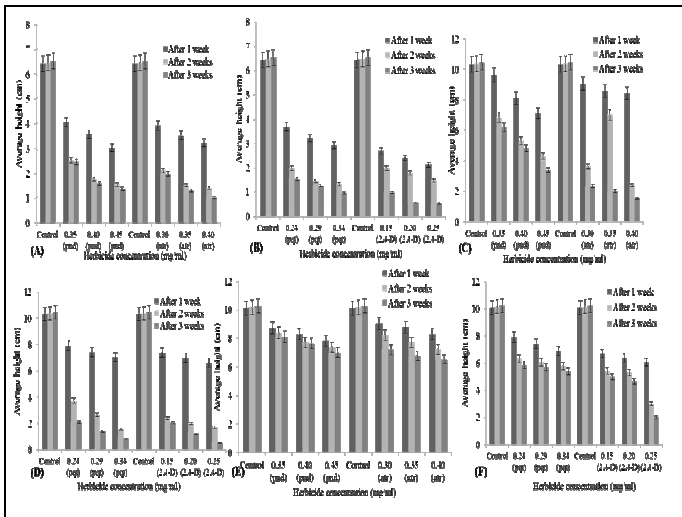
(Moles *et al.*, 2004). Plant heights can be used to study health status of a plant as well as its growth, both at normal environmental conditions and under various stresses such as salinity, drought, and herbicides (Shrestha *et al.*, 2002). Results of average weeds heights described that all three concentrations of each herbicides have shown effect on all weeds heights with highest concentration of each herbicides. More than other three herbicides, 2, 4-D significantly affected the heights of all the weeds (*E. sumatrensis*, *P. hysterophorus* and *S. nigrum*).



**Fig. 2:** Graphical representation of the effect of different herbicides on chlorophyll content (mg/g) of weeds, after first, second and third week of treatment. (A) Effect of pendimethaline and atrazine on *E. sumatrensis*, (B) Effect of paraquat and 2,4-D on *E. sumatrensis*, (C) Effect of pendimethaline and atrazine on *P. hysterophorus*, (D) Effect of paraquat and 2,4-D on *P. hysterophorus*, (E) Effect of pendimethaline and atrazine on *S. nigrum*, and (F) Effect of paraquat and 2,4-D on *S. nigrum*.



**Fig. 3:** Graphical representation of the effect of different herbicides on leaf area (cm<sup>2</sup>) of weeds after first, second and third week of treatment. (A) Effect of pendimethaline and atrazine on *E. sumatrensis*, (B) Effect of paraquat and 2,4-D on *E. sumatrensis*, (C) Effect of pendimethaline and atrazine on *P. hysterophorus*, (D) Effect of paraquat and 2,4-D on *P. hysterophorus*, (E) Effect of pendimethaline and atrazine on *S. nigrum*, and (F) Effect of paraquat and 2,4-D on *S. nigrum*.



**Fig. 4 :** Graphical representation of the effect of different herbicides on height (cm) of weeds after first, second and third week of treatment. (A) Effect of pendimethaline and atrazine on *E. sumatrensis*, (B) Effect of paraquat and 2,4-D on *E. sumatrensis*, (C) Effect of pendimethaline and atrazine on *P. hysterophorus*, (D) Effect of paraquat and 2,4-D on *P. hysterophorus*, (E) Effect of pendimethaline and atrazine on *S. nigrum*, and (F) Effect of paraquat and 2,4-D on *S. nigrum*.

### Conclusions

Weeds are global threat to agriculture as they are good competitors for space and nutrients. Weeds affect every commercial plantation, by reducing quality and quantity of crops. Among the commercially important crops, *Stevia rebaudiana* has received global attention due to “zero calories-sweetening power”. However, despite numerous benefits of this plant, *Stevia* has weaker ability to compete with weeds, especially at early stage of growth. In present study maximum weed control efficacy was observed with maximum concentration of each herbicide: pendimethalin (0.45 mg/ml), atrazine (0.40 mg/ml), paraquat (0.34 mg/ml) and 2,4-D (0.25 mg/ml). Level of effectiveness shown by all herbicides for three parameters can be expressed as: 2, 4-D > paraquat > atrazine > pendimethalin. Based on these findings, this study recommends 2, 4-D herbicide for best weed management in stevia fields as compared to other herbicides. Besides this further field trials should be conducted on a large scale, in order to check the efficacy of these herbicides for the same.

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### Conflict of interest

The authors declare no conflict of interest.

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