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EFFECT OF HERBICIDES 2,4-D, GLYPHOSATE AND STOMP ON THE PROTEIN IN WEED SEEDLINGS

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ABSTRACT:
The effect of herbicides on macromolecular seedlings was studied at the concentrations form 10 to 100 ppm of 2,4-D and 100 to 40,000 ppm of glyphosate and stomp. The protein contents of seedlings decreased gradually with the increased concentration of herbicides. The protein content of control seedlings was observed 2.10×10^{-4}. In 2,4-D treated seedlings protein contents were 1.60×10^{-4}, 1.58×10^{-4}, 1.46×10^{-4}, 1.31×10^{-4}, 1.18×10^{-4} and 1.60×10^{-4} at 10, 20, 40, 60, 80 and 100 ppm, respectively. Glyphosate treated seedlings, the percentage of protein content per seedlings at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm were 1.95×10^{-4}, 1.90×10^{-4}, 1.83×10^{-4}, 1.75×10^{-4}, 1.50×10^{-4} and 1.20×10^{-4} , respectively. Following stomp treatment proteins content per seedling at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm were 2×10^{-4}, 1.98×10^{-4}, 1.60×10^{-4}, 1.60×10^{-4}, 1.16×10^{-4} and 1.13×10^{-4} respectively. Thus, in the present study, it is concluded that 2,4-D was more effective in inhibiting macromolecular synthesis while glyphosate and stomp have the less inhibitory effect on protein as compared to 2,4-D.
Key words: 2,4-D; glyphosate; stomp; herbicide; *Sida acuta*;.

Introduction:

Weeds are harmful to every living organism directly or indirectly. Weeds have been reported to be quite harmful as they create health hazards both to the animals and human beings. It is very dangerous to field crops. It is one of the factors that reduce the crop yield. Weeds are responsible to cause allergies like hay fever, itching and poison irg. Weeds may poison or seriously slow down the weight gains of the livestock. They are found along the road side, railway rout, gardens, lawn, play grounds, in irrigation and drainage system and farm. They have tremendous growth; gain more nutrients and water form soil. The cultivated plants compete with that weed around it for soil, sunlight, nutrients, water and space. So it is very necessity to control the weeds. There are various methods of controlling it such as hand pulling, hoeing, spuldding, tillage and mowing. Among them, herbicide is one of the suitable and easiest methods to control the weeds. In order to have proper scientific knowledge of weed control, it is necessary to understand the mechanism of herbicides in relation to weeds. For that, it is necessary to observe their effects on protein. Such studies will be helpful to determine the proper dosages of herbicides to be used for eradication of obnoxious weeds.

Jethro Tull (1731) was the first person who used the word 'weed' in his famous book "Horse Hoeing Husbandry". According to him "A weed is a plant growing where it is not desired". Various learned men have defined weeds in different ways, Branchely (1920) defined it as, " A plant that grows so luxuriantly or plentifully that possesses more valuable nutritive property". Cumming (1977) said, "Weeds are plants but hey are weeds simply when they are growing where they are not wanted". Gupta and Lamba (1978) defined weeds as, " Weeds are the plants growing at places and its times when we wanted either some other plants to

grow or no plants to grow at all". Thakur in 1984 said, " An undesirable, injurious, unsightly and troublesome plant which interferes with cultivated crop and affect human affairs".

Sida acuta is a common weed, belonging to family Malvaceae. It is a branched shrubby plant, growing up to the height of one foot. Its leaves are simple lanceolate. The flowers are yellow, solitary or occasionally in pairs. It is a pantropical weed and is found in agricultural fields, causing heavy damage to the crop plants. In the present study the effect of Stomp, (pendimethalin) - which is a broad-spectrum systemic herbicide, was studied on this weed.

Materials and Methods:

The seeds of Sida acuta Burm.f. were treated with different concentration of 2,4-D, glyphosate and stomp herbicides for 24 hours in test tube. After treatment, seeds were washed thoroughly with distilled water and kept for germination in petridishes with double layered moistened filter paper in laboratory conditions. Seeds soaked in distilled water for 24 hours were used as control. The treated and untreated seeds were allowed to grow for six days.

Each sample containing one-gram fresh weight of six days old seedlings were taken for extraction and estimation of protein. The number of seedlings per gram was counted and noted every time. For protein extraction, the Kjeldahl's method was followed. The three replicates were used for each sample at each concentration of herbicides.

Extraction and Estimation of total proteins:

The treated and untreated (control) seedlings of each concentration were dried in oven at 40-60^{0}C for 24 hours. The weighed dried samples (500mg) of each concentration were taken in Kjeldahl's flask. About 30 ml of concentrated sulphuric acid together with potassium sulphate and copper sulphate (6:1) were added. The flask was then heated gently in an inclined position. The heating was continued till the brown colour of liquid was produced, and then it disappeared and left behind clear contents. The Kjeldahl's flask was then left for cooling and contents were diluted with some distilled water and carefully transferred into one litre round bottom flask. An excess of 40 % sodium hydroxide solution was poured down the sides of flask and it was fitted with Kjeldahl trap and a water condenser. The lower end of condenser dipped in 25 ml of 0.1 N sulphuric acid solution containing 2 drops of phenolphthalein indicator. The liquid in round bottom flask was then heated and liberated ammonia got distilled into sulphuric acid contained in a beaker. When no more ammonia passes over (tested the distillate with the litmus paper), the receiver was removed. The excess of acid was then determined by titration with N/10 sodium hydroxide solution using phenolphthalein as indicator and noticed the buriet reading. The standardization of normality of alkali and acid was determined by titration of PHT (potassium hydrogen thallate). The content of nitrogen in the seedling was calculated by using formula.

$$N_2\% = \left[\begin{matrix}\text{Normality}\\ \text{of standard acid}\end{matrix}\right] \times \left[\begin{matrix}\text{Volume}\\ \text{of acid}\end{matrix}\right] - \left[\begin{matrix}\text{Normality}\\ \text{of alkali}\end{matrix}\right] \times \left[\begin{matrix}\text{Volume}\\ \text{of alkali}\end{matrix}\right] \times \frac{14}{1000} \times \frac{100}{\begin{matrix}\text{Weight of}\\ \text{Sample (500 mg)}\end{matrix}}$$

From the obtained nitrogen content, the total protein of sample was calculated as follows.

$$Total \text{ Protein} = \text{Nitrogen content} \times 6.25$$

Similarly, the content of protein per seedling was calculated as follows.

$$Protein\ per\ seedling = \frac{Total\ Protein}{Total\ no.of\ seedlings\ per\ sample}$$

Results:

After treatment with 2,4-D, glyphosate and stomp protein contents were found to be decreased as the concentration of herbicides increased.

Table 1: Protein contents of the seedlings of *Sida acuta* Burm.f. following treatment of herbicides.

Herbicides	Conc. in ppm	Protein per dry seedling (mg)	Standard error (±)
	Control	2.10×10^{-4}	0.04×10^{-4}
2,4-D	10	1.16×10^{-4}	0.10×10^{-4}
	20	1.58×10^{-4}	0.03×10^{-4}
	40	1.46×10^{-4}	0.06×10^{-4}
	60	1.31×10^{-4}	0.05×10^{-4}
	80	1.18×10^{-4}	0.09×10^{-4}
	100	0.50×10^{-4}	0.03×10^{-4}
Glyphosate	100	1.95×10^{-4}	0.02×10^{-4}
	1000	1.90×10^{-4}	0.06×10^{-4}
	5000	1.83×10^{-4}	0.02×10^{-4}
	10,000	1.75×10^{-4}	0.02×10^{-4}
	20,000	1.50×10^{-4}	0.02×10^{-4}
	40,000	1.12×10^{-4}	0.004×10^{-4}
Stomp	100	2.00×10^{-4}	0.23×10^{-4}
	1000	1.98×10^{-4}	0.009×10^{-4}
	5000	1.60×10^{-4}	0.04×10^{-4}
	10,000	1.60×10^{-4}	0.05×10^{-4}
	20,000	1.16×10^{-4}	0.01×10^{-4}
	40,000	1.13×10^{-4}	0.009×10^{-4}

2,4-D

Protein per seedling decreased with an increase in concentration of this herbicide. It was 1.60×10^{-4}, 1.58×10^{-4}, 1.46×10^{-4}, 1.31×10^{-4}, 1.18×10^{-4} and 1.60×10^{-4} at 10, 20, 40, 60, 80 and 100 ppm, respectively as against in control content of protein was 2.10×10^{-4} (table. 1).

Glyphosate

The gradual decrease of protein content was observed as the concentration of herbicides increases. At 100, 1000, 5000, 10000, 20000 and 40000 ppm the content of protein per seedling was 1.95×10^{-4}, 1.90×10^{-4}, 1.83×10^{-4}, 1.73×10^{-4}, 1.50×10^{-4} and 1.12×10^{-4}, respectively, whereas it was 2.10×10^{-4} (table. 1).

Stomp

The amount of protein content per seedling also found to be decreased with the increase in concentration of herbicide stomp. It was 2.00×10^{-4}, 1.98×10^{-4}, 1.60×10^{-4}, 1.60×10^{-4}, 1.16×10^{-4} and 1.13×10^{-4} at 100, 1000, 5000, 10000, 20000 and 40000 ppm, respectively as against 2.10×10^{-4} (table. 1).

Above results of protein of 2,4-D, glyphosate and stomp decrease with increase concentration as compared to standard graph was observed.

Discussion:

These herbicides affected protein contents of seedlings. Protein content decreases per seedling with increase in concentration of herbicides. Thus, it may be concluded that herbicides were effective to reduce protein content in *Sida acuta* Burm.f. with gradual increase in concentration.

2,4-D

In the present study, protein contents of the 2,4-D treated seedlings decreased with the increase in the concentration. Similar results were stated by Stahler and Whitehad (1950) in sugarbeet, Freiberg and Clark (1952) and Key *et al*. (1966) in soybean. They studied the protein synthesis in different apicals zone of hypocotyl of soybean following 2,4-D treatment reported that 2,5-D blocked protein synthesis in apical zone of hypocotyls. Kolhe (1979) noted decrease in the protein content of *Solanum surattense*, following 2,4-D treatment, Chand and Roy (1981) in Nigella sativa, Sairam *et al*. (1986) reported that accumulation of 2,4-D in oat crop resulted in an increase protein contents at initial stage, however, at later it reduced drastically. Srinivasu and Bakale (1989a) also noticed gradual decrease in the protein content of *Parthenium hysterophorus* seedlings with an increase concentration of 2,4-D. Sen *et al*. (1987) reported that protein

content in *Cyperus rotundus* increased initially but decreased after application of 2,4-D. Jain (1993) in *Chenopodium album*, Gopal (1993) in Medicago sativa, Bobde (1993) in Crotalaria juncea, Kulkarni (1998) in *Amaranthus lividis*, Kamble (1999) in *Hibiscus cannabinus*, Dudhe (2002) in *Hyptis suaveoluns*, Taduwadi (2002) in Cleome viscosa and recently Kamble Sanjay (2006) in *Hibiscus cannabinus* also found gradual decrease in the protein contents per seedling with the increase in concentration of 2,4-D.

Glyphosate

In the present study, the total protein content of seedling decreased as concentration of herbicide increased. Pillai *et al*. (1978) on Glycine max reported decrease in protein content by glyphosate treatment. Tymonko and Foy (1978) in soybean, Cole *et al*. (1980), Dutee and Hoagland (1981) in Glycine max observed percentage of protein decreased by this herbicide. Foley et al. (1983) in *Xanthium pensylvanicum* found that inhibition of protein synthesis of root tissues. Jain 91993) in *Chenopodium album*, Amenda and Matschke (1995) in *Picea pungens*, Kulkarni (1998) in *Crotalaria medicaginea* var. luxurians, Dudhe (2002) in *Hyptis suaveoluns* and recently Kamble Sanjay (2006) in *Hibiscus cannabinu*s reported decrease in protein content due to application of glyphosate.

Stomp

Since, DNA and RNA is concerned in protein synthesis, the decrease in amount of nucleic acid will also affect the protein content of seedling. The gradual decrease in protein content of seedling was observed in the present study. In general, protein as a major storage protein is hydrolysed during seed germination by proteolytic enzyme that is essential for seedling growth. Ashton *et al*. (1968) reported trifluralin at 1.5×10^{-6} M inhibited dipeptidase activity of squash cotyledon 15 % compared to control. Ashton *et al*. (1977) also reported 80 % inhibition of protein synthesis at 5×10^{-5} treatment of trifluralin in *Phaseolus vulgaris*. It is clear that, dinitroaniline herbicides have direct effects on enzymes, which are responsible for protein synthesis. Similar effect may occur in the present study and resulted into decrease in amount of protein content of the seedling after stomp treatment. It suggests that the herbicide action of degradation of reserve materials on the control during germination may be one of the factors responsible for greater susceptibility of seedlings to herbicides. Jain (1993) and Dudhe (2002) in *Chenopodium album* and *Hyptis suaveoluns*, respectively, observed gradual decrease in protein content of seedlings.

The foregoing discussion indicates that 2,4-D, glyphosate and stomp decrease protein content of seedlings of *Sida acuta* Burm.f.

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