JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

NUCLEIC ACIDS AND PROTEIN CONTENT CHANGES IN SEEDLINGS OF *PSORALEA CORYLIFOLIA* INDUCED BY 2,4-DICHLOROPHENOXY ACETIC ACID

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Abstract: *The present investigation deals with the influence of 2,4-dichlorophenoxy acetic acid (2,4-D)on macromolecular synthesis, i.e. DNA, RNA and protein content of weed Psoralea corylifolia L. belonging to family Fabaceae. It is commonly known as 'Bawachi' growing in a wild and cultivated field in Vidarbha region of Maharashtra State, India. The macromolecular content of seeds was treated with different concentrations of 2,4-D and grown to get seedlings. Six days old seedlings were tested for DNA, RNA and protein content. As the concentrations of 2,4-D increased the percentage of DNA, RNA and protein content gradually decreased.*

The percentage of DNA decreased from 6.96×10^{-3} to 4.02×10^{-3} at 100 to 1000 ppm respectively. The percentage of RNA in treated seedling decreased from 9.18×10^{-2} to 3.20×10^{-2} at 100 to 1000 ppm respectively. The percentage of protein gradually decreased from 5.24 to 2.32 at 100 to 1000 ppm respectively. The percentage of DNA, RNA and protein content of control seedlings were 7.20×10^{-3}, 9.80×10^{-2} and 6.35 percent respectively.

Keywords: Psoralea corylifolia, Seedlings, 2,4-dichlorophenoxy acetic acid, DNA, RNA, Protein.

I. INTRODUCTION

The loss of crop due to competition from weeds is one of the most pernicious and troublesome one. It adversely affects agricultural productivity and the losses are perhaps more than those caused by pests and diseases. Various methods such as mechanical, cultural and biological have been employed from time to time for weed control. None of them, however, has proved to be satisfactory. In recent years considerable progress has been made in chemical weed control. The use of certain growth regulating substances as herbicides has recently attracted much attention and has led to important practical applications.

The primary objective of introducing herbicides in any country has been to replace torturous and back-breaking manual weeding and to give farmer the much needed leisure, of which he has been deprived since ages. The spared labour could be utilized more effectively for bringing additional land under cultivation and improving irrigation, fertilizer and other pesticide applicationpractices, enhancing thus the overall production in the country. The present investigation was undertaken to study the effect of 2,4-dichlorophenoxy acetic acid (2,4-d) on weed *Psoralea corylifolia* Linn, belonging to the family Papilionaceae (Fabaceae) which is the noisome weed in fields, fallows and waste lands in Vidarbha and Marathwada regions of Maharashtra State.

1.1. Details about Psoralea corylifolia L.

Psoralea corylifolia Linn.(Vern. Bawachi) belonging to family Fabaceae, is an erect annual herb and grow to a maximum height of 1.5m . The stems and branches are covered with conspicuous glands and white hairs. Leaves are round, dotted with black glands on both surfaces. Flowers appear from the axils of leaves in bunches. Individual flowers are small, bluish purple in colour. Each fruit bear one smooth seed. The species is native to India and Arab. In India, it is distributed in M.P., U.P. Rajasthan, Bihar, Gujarat, A.P. and Maharashtra and found as weed in
waste places.

1.2. Particulars about 2,4-dichlorophenoxy acetic acid

2,4-D belongs to aromatic compound with phenyl ring attached to oxygen which in turn is attached to a carboxylic groups. 2,4-D was discovered in USA independently by Zimmerman and Hitchcock (1942) at Boyce Thompson Institute.The unique properties of 2,4-D and some of its analogues in chemical weed control are extremely high toxicity, wide selectivity and ability to translocate through plants and kill the underground root system (Crafts, 1946).The chlorophenoxy compounds are growth regulator with hormone like activities at relatively low doses.

II. RESEARCH METHODOLOGY

The seeds of *Psoralea corylifolia* were treated with different concentrations 100, 200, 400, 600 and 800 ppm of 2,4-D for 24 hours. After treatment, seeds were washed thoroughly with distilled water and kept for germination in petridishes, lined with double layers of moistened filter paper under laboratory condition. 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 nucleic acids and total proteins. The number of seedlings per gram were counted and noted every time. For extraction of nucleic acids, the method suggested by Ogur and Rosen (1950) and Schneider (1945) were adopted and for protein extraction the Kjeldahl's method was followed.The four replicates were used for each sample at each concentration of herbicide

The DNA content of sample was calculated from standard graph of calf-thymus DNA. The DNA per seedling in sample was calculated by using formula

$$\text{DNA per seedling} = \frac{\text{Total DNA}}{\text{Total number of seedlings per sample}} \times 100$$

The RNA content in sample was calculated by using standard graph of yeast RNA. The RNA per seedling in a sample was calculated by the formula

$$\text{RNA per seedling} = \frac{\text{Total RNA}}{\text{Total number of seedlings per sample}} \times 100$$

The percentage of nitrogen in the seedling was calculated by using formula

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

From the obtained nitrogen percentage, the total protein of sample was calculated as followed

Total protein = Nitrogen percentage x 6.25

Similarly the percentage of protein per seedling was calculated as follows

$$\text{Protein per seedling} = \frac{\text{Total protein}}{\text{Total number of seedlings per sample}} \times 100$$

III. RESULTS AND DISCUSSION

The results obtained after the treatment of 2,4-D, it shows that the percentage of DNA per seedling was $6.96x10^{-3}$, $6.60x10^{-3}$, $5.58x10^{-3}$, $5.14x10^{-3}$, $5.04x10^{-3}$ and $4.02x10^{-3}$ at 100, 200, 400, 600, 800 and 1000 ppm, respectively, where as in control it was $7.20x10^{-3}$. This shows that the DNA content per seedling was gradually decreased with an increasing concentration of 2,4-D (Table 3.1).

Table 3.1: Showing effect of 2,4-dichlorophenoxy acetic acid (2,4-D) on DNA of seedling of *Psoralea corylifolia* L.

Herbicide	Concentration in ppm	Percentage of DNA seedling^{-1}	Standard error (±)
	Control	7.20×10^{-3}	0.01
2,4-D	100	6.96×10^{-3}	0.02
	200	6.60×10^{-3}	0.01
	400	5.58×10^{-3}	0.02
	600	5.14×10^{-3}	0.01
	800	5.04×10^{-3}	0.02
	1000	4.02×10^{-3}	0.02
	1200	4.02×10^{-3}	0.02

In the present study 2,4-D treated seedlings showed gradual decrease in DNA percentage with increase in concentration of herbicide. Many investigators like West *et al.* (1960), Key and Hanson (1961), Basler and Nakazawa (1961) and Fites (1965) reported increase in DNA content at lower concentration of 2,4-D treatment. Key *et al.* (1966) critically analysed the 2,4-D induced changes in DNA, RNA and protein at different zones of hypocotyl of soybean and found that DNA synthesis was stopped in hypocotyl apical zone. In the present study the decrease in DNA percentage was due to arrest of DNA synthesis at interphase in meristematic cells as evident from the cytological studies where most of the cells were found to be in an interphase.

The percentage of RNA per seedling was $9.18x10^{-2}$, $7.10x10^{-2}$, $5.65x10^{-2}$, $5.40x10^{-2}$, $4.78x10^{-2}$ and $3.20x10^{-2}$ at 100, 200, 400, 600, 800 and 1000 ppm, respectively where as in control it was $9.80x10^{-2}$. This results shows that the sudden decrease in percentage of RNA was observed at 200 and 400 ppm and thereafter, it decreases gradually (Table 3.2).

In 2,4-D treated seedling the RNA content decreased gradually with an increasing concentration of 2,4-D in present study. Many workers have noticed decrease in RNA content of seedling after 2,4-D treatment. Key (1963) in corn and Shannon *et al.* (1964) in mesocotyl tissue of *Zea mays* found inhibition of RNA synthesis with 2,4-D treatment. Fites (1965) reported decrease in DNA and RNA content in soybean tissue following 2,4-D treatment. Moreland and Malhotra (1969) noticed effect of 22 different herbicides including 2,4-D on RNA synthesis in maize mesocotyl and soybean hypocotyl and found that 14 of herbicides inhibited RNA synthesis. Chen *et al.* (1972) observed progressive decrease in RNA levels in wheat by treatment with 2,4-D and 2,4,5-T, dicamba and picloram.

In the present investigation, the reduction in DNA and RNA contents after treatment of 2,4-D may be proved by growth of seedlings. The elongation of hypocotyls and radicles were almost inhibited apparently due to inhibition of cell division and cell enlargement. From the previous study, it was evident that as concentrations increased the inhibition of mitotic activity also increased with increasing percentage of abnormalities. Badr and Eklington (1982) concluded that reduction in amount of DNA and RNA has closely associated with the inhibition of mitotic activity in *Allum cepa*. They found that metobromuron and isopromuron herbicides might inhibit DNA synthesis, which resulted in lower mitotic value. Similar results were also noticed by Mohandas and Grant (1972), Cartwright (1976), Chand and Roy (1981) and Badr *et al*. (1983) reported that herbicides inhibits DNA and RNA synthesis by reducing the oxidative phosphorylation in various plants resulting in lower levels ATP. This supports the hypothesis of Ashton and Crafts (1973) that herbicides reduce ATP, which are

Table 3.2: Showing effect of 2,4-dichlorophenoxy acetic acid (2,4-D) on RNA of seedling of *Psoralea corylifolia* L.

Herbicide	Concentration in ppm	Percentage of RNA seedling^{-1}	Standard error (±)
	Control	9.80×10^{-2}	0.02
2,4-D	100	9.18×10^{-2}	0.02
	200	7.10×10^{-2}	0.01
	400	5.65×10^{-2}	0.02
	600	5.40×10^{-2}	0.01
	800	4.78×10^{-2}	0.02
	1000	3.20×10^{-2}	0.02
	1200	3.20×10^{-2}	0.02

strong inhibitor of RNA and protein synthesis. Fedtke (1982) reported inhibition of RNA synthesis in corn plant roots by 2,4-D in dark and light conditions. Kolhe (1979) in three farm weeds, Srinivasu (1986) in *Parthenium hysterophorus*, Bobde (1993) in *Crotalaria juncea*, Jain (1993) in *Chenopodium album*, Gopal (1993) in *Medicago sativa*, Tulankar (1998) in *Amaranthus lividus* and Taduwadi (2004) in *Cleome viscosa* reported decrease and increase in DNA, RNA and protein contents after treatment of 2, 4-D. Mhaiskar (2022) studied that the RNA contents of seedlings decreased gradually with the increased in concentration of herbicides. Gopal and Mahakhode (2022) reported that the decreased in percentage of DNA and RNA with increase in the concentrations due to mitotic inhibition by the herbicide attributed to blocking of mitotic cycle during interphase which may results from the inhibition of nucleic acid synthesis.

The protein percentage per seedling was 5.24, 4.45, 3.70, 3.20, 2.85 and 2.32 at 100, 200, 400, 600, 800 and 1000 ppm, respectively against 6.35 percent in control. There was gradual decrease in protein content was observed with increased in concentration of 2,4-D (Table 3.3).

In the present study, the total protein percentage of seedling showed progressive decrease as the concentrations of 2,4-D increased. Many papers have been published on the effect of 2,4-D and other phenoxy alkonic herbicides on the aspects of protein synthesis in many plants. Ezerzha and Kulikov (1973) subsequently examined the interaction of 2,4-D with nucleotides from twelve crop species and postulated that interaction may occur between 2,4-D and DNA spiral with the formation of supermolecular complexes between 2,4-D and adenine.

In the present study, treatment with 2,4-D have caused changes in protein content and treatment of *Psoralea corylifolia* seedlings with 2,4-D results in inhibition of ribonuclease, deterioration on certain protein fractions and decline of simple proteins. Grzesiuk *et al.* (1973) also reported that 2,4-D may have a retarding effect on the process of transcription in differentiating nuclei and an accelerating effect inhistone synthesis. Gruzdev *et al.* (1973) found that plants of spring wheat treated with 2,4-D amine showed an initial increase in the rate of protein synthesis which, however, gradually declines as the growing season progresses and free amino acid content of wheat foliage and roots was increased. Gruzdev *et al.* (1979) reported that treatment of 2,4-D on barley showed decline in percentage of proteins and decline at the initiation of grain formation. Mahakhode and Jachak (2015) reported progressive reduction in DNA, RNA and protein content as the concentrations of paraquat increased.

Table 3.3: Showing effect of 2,4-dichlorophenoxy acetic acid (2,4-D) on protein content of seedling of *Psoralea corylifolia* L.

Herbicide	Concentration in ppm	Percentage of protein seedling^{-1}	Standard error (±)
	Control	6.35	0.04
2,4-D	100	5.24	0.02
	200	4.45	0.02
	400	3.70	0.01
	600	3.20	0.03
	800	2.85	0.04
	1000	2.32	0.02
	1200	2.32	0.02

In the present investigation, the decrease in protein percentage might be due to the formation of supermolecular complexes between 2,4-D and proteins and might be the morphological characteristic symptoms of phenoxy acid damage causes changes accompanied by changes in nitrogen content of seedlings. Key *et al.* (1966) studied the protein synthesis in apical zone of hypocotyl following 2,4-D treatment and they reported that 2,4-D blocked the protein synthesis in soybean. Moreland *et al.* (1969) reported inhibition of protein synthesis in maize and soyben hypocotyls following various herbicides treatment. Srinivasu (1986) observed gradual decrease in protein content of *Parthenium hysterophorus* seedlings as concentrations of 2,4-D increased. Bobde (1993) in *Crotalaria juncea*, Jain (1993) in *Chenopodium album*, Gopal (1993) in *Medicago sativa*, Tulankar (1998) in *Amaranthus lividus*, Dudhe (2002) in *Hyptis suaveolens* and Taduwadi (2004) in *Cleome viscosa* reported decrease in protein content in the respective plants as the concentration of 2,4-D increased.

IV. CONCLUSION

In the above discussion, it was cleared that 2,4-D blocked DNA synthesis in the seedlings as compared to control in the present investigation. With respect to decrease in DNA synthesis, RNA and protein contents of seedlings also decreased. Owing to decrease in synthesis of nuleic acids (DNA and RNA) and protein contents, the rate of cell division decreased and reduction in growth or length of the seedlings also noticed. Therefore, it may be concluded that 2,4-D reduced DNA, RNA and protein content at all concentrations.

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