

## Research Article



## Morphoanatomical and Structural Alterations in *Psoralea corylifolia* (L.) Induced by Glyphosate

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### ABSTRACT

The present study deals with the effect of glyphosate on the morpho-anatomical structures of *Psoralea corylifolia* seedling growing in cultivated fields of Vidarbha regions of Maharashtra State. The seeds were treated with different concentrations (100, 200, 400, 600 and 800 ppm) of the herbicide glyphosate. There was a gradual decrease in growth of radicle and hypocotyl with an increase in concentrations of glyphosate. The length of radicle at control and 100 ppm was 18.2 mm and 9.6 mm, respectively. The decrease in length of hypocotyl at 100 ppm was 16.2 mm as against 32.4 mm in control, respectively. In the present study inhibition of lateral roots and change in colour of cotyledons from green to creamy white was observed in early seedling growth. It also induced some remarkable anatomical changes in the hypocotyl, radicle and cotyledon of *Psoralea corylifolia* seedling.

**Keywords:** *Psoralea corylifolia*, glyphosate, morphology, anatomy, seedlings.

### INTRODUCTION

*Psoralea corylifolia* Linn. belongs to family Fabaceae. The species is native to India and Arab. It grows throughout the plains of central and east India in the cultivated land or in nearby areas. Battle with weeds is as old as agriculture. It started during the prehistoric period when man decided to raise certain plants in preference to others for his sustenance. Weeds have harmful effects on growing crops and their interference in land uses, rank among the most important enemies of agricultural production. Weeds reduce crop yields on account of their competition with crops for water, soil nutrients and light. Certain weeds may further reduce crop growth and subsequently field by releasing inhibitors or poisonous substances into the soil. They increase the cost of labor and equipment, render harvesting difficult, reduce the quality and marketability of agricultural commodities, harbor insects, fungal, viral and bacterial microorganisms and some are poisonous to human being and cattle. These losses are far greater than usually realised.

The leaf anatomy study of the weeds is distinguished as an important technique to identify and describe the structures which may influence on the herbicide absorption besides to assist in the differentiation of the species. The thick cuticle, the high content of epicuticular wax, the high density of trichomes, the low stomatal density and the high epidermis thickness in the adaxial side as the major barriers to herbicide penetration in weed leaves<sup>1</sup>. Hess<sup>2</sup> highlighted the importance of morphological diversity of leaf epidermis in several species, and denote the topography, the trichomes and stomata types influence on the deposition of the herbicides on the leaf surface. The leaf anatomic study may improve the understanding of the barriers each

species determine to the herbicides penetration and so, promote subsidies to search the control strategies. Hence, the leaf anatomic study may contribute in selecting the herbicides for weedy species<sup>2</sup>.

The first detectable symptom after glyphosate treatment is growth inhibition, followed by a noticeable yellowing (chlorosis) of treated tissue. Five to 10 days after the treatment, the chlorosis turns into necrosis and the plants begin to die<sup>3</sup>. Glyphosate disrupts chloroplasts, cell membranes, cell walls, alters protein and nucleic acid synthesis, inhibit photosynthesis<sup>4</sup> and reduces chlorophyll content<sup>5</sup>. Glyphosate binds to 5-enol pyruvyl shikimate-3-phosphate synthase (EPSPS) enzyme (EC 2.5.1.19), which consequently inhibits the biosynthesis of the aromatic amino acids, tryptophan, tyrosine and phenylalanine<sup>6</sup>. With the inhibition of the EPSPS, shikimate-3 phosphate accumulates and is cleaved to shikimate in the tonoplast or vacuole<sup>7</sup>. An assay measuring shikimate provides evidence of glyphosate flow in addition to visual or morpho-anatomical symptoms.

Estimates have been made in our country of appreciable losses is grain production and substantial depletion of nutrients, both native in and added to the soil, by unwanted weed growth in arable land. Mani<sup>8</sup> compiled exhaustive data on losses in crop yields due to weeds from several experiments conducted in different parts of India. It can be noted from the data collected by him that weeds incurred a loss of 31.5% in the yields of food grain crops, 22.7% in the Rabi and 36.5% in the Kharif seasons. When calculated in terms of losses in rupees, it amounts to Rs. 4200 million annually<sup>9</sup>. At Weed Research Organisation, Oxford, it is estimated that food lost to weeds nearly about 300,000,000 metric tones per year or 11.5% of the present food production<sup>10</sup>. The F.A.O. has recently estimated that world agriculture is losing up to



75 billion dollars per year due to pests, including insect pests, diseases and weeds<sup>11</sup>.

These facts and figures about the economic losses and production setbacks caused by weeds have now become a major consideration in the implementation of programs of agricultural self-sufficiency.

Considering the above problem the experiment was undertaken to see the morphoanatomical structural alterations in *psoralea corylifolia* (L) induced by glyphosate.

## MATERIALS AND METHODS

The selected taxa were collected during their flowering and fruiting seasons from the Nagpur region for the correct botanical identification.

The seeds of *Psoralea corylifolia* L. were collected randomly from naturally growing plants of different fields in the study area (Fig. 3).

Mechanically injured seeds were treated with different concentrations (100, 200, 400, 600 & 800 ppm) of the herbicide glyphosate, which is freely dissolved in distilled water for 24 hours.

The treated seeds were washed thoroughly with distilled water and allowed to grow for 72 hrs. in 15cm petridishes containing double layered filter paper.

Four replicates of this treated and control seeds were carried out.

The lethal doses were assessed on the basis of growth of radical and hypocotyls at this stage. The pH value of control and solutions of herbicides was also determined by pH meter.

Morphological abnormalities were observed and recorded. The actual increase in lengths of hypocotyls and radical has been graphically presented on the basis of mean values of four replicates.

To study the anatomical changes in the seedlings induced by the herbicides the radicles, hypocotyls and cotyledons were fixed from control and the treated seedlings in F.A.A. (Formalin-Acetic acid-Alcohol) solution.

After 24 hours they were washed thoroughly with distilled water and preserved in 70% alcohol. The materials were embedded in paraffin wax following customary methods<sup>12</sup>.

Sections were cut at 10 to 15 microns and stained with crystal violet and erythrosine schedule and mounted in D.P.X.

The series of various sections of different concentrations of herbicides were observed and recorded the anatomical abnormalities.

Microphotographs of various sections of both control and treated plants were taken using research microscope (Metzer Optical Instruments Pvt. Ltd. India).

## RESULTS AND DISCUSSION

### Morphological Responses



**Figure 1:** C-Control. 1, 2, 3 and 4 - seedlings treated with glyphosate at 100, 200, 300 and 400 ppm respectively.

The herbicide glyphosate was found to be inhibited the growth of hypocotyls and radicle. There was a progressive decrease in growth of radicle and hypocotyl.

The length of radicle was 9.6, 6.4, 2.2 and 2.2 mm at 100, 200, 400 and 500 ppm as against 18.2 mm in control, respectively (Table 1). Similarly decrease in length of hypocotyl was 16.2, 12.4, 8.7, 5.2 and 5.2 mm at 100, 200, 400, 500 and 600 ppm respectively (Table 1). The lethal doses for the linear growth of radicle and hypocotyl were 500 and 600 ppm respectively. The hypocotyl was, however, found to be more resistant than radicle and symptoms of injury were visible from 200 ppm onwards and it turned brownish. The pH value of control, 500 and 600 ppm of herbicide was 7.0, 5.8 and 4.3, respectively. Glyphosate induced very few morphological peculiarities in early seedling growth, such as inhibition of lateral roots and change of colour of cotyledons from green to yellow white (Fig. 1).

In the present study, the abrupt decreased in length of radicle was observed in 100 ppm. It may probably due to rapid inhibition of radicle and further lost their turgidity. Baird and Sprankle<sup>13-14</sup> noticed that glyphosate inhibited corn root and shoot growth relatively more than root growth.

In the present study, the growth of radicle was more inhibited than hypocotyl. It may due to rapid decline rate of respiration in radicle. Similar results were also found by Ali and Fletcher<sup>15</sup> in corn and Tanphiphat and Appleby<sup>16</sup> in *Arrhenatherum elatius* var. *bulbosum* seedlings treated with glyphosate. Similar observations were noted by Bobde<sup>17</sup> in *Crotalaria juncea* and Tulankar<sup>18</sup> in *Amranthus lividus*.

Inhibition of lateral roots at and above 100 ppm was noticed in the present investigation. It may due to preventing the emergence of young primordia already initiated or inhibiting the formation of new primordia by disrupting the organization of vascular cylinder. This is in accordance with the finding of Bingham<sup>19</sup> in several grasses to glyphosate.

In the present investigation, the colour of cotyledon has changed from green to creamy white. Many workers reported about the change in colour of cotyledons like Hoagland<sup>20</sup> in eleven species of crops and weeds, Stecko<sup>21</sup> in nine species of crops and weeds. This was probably due to the depletion of chlorophyll pigments in the cotyledons.

The range of pH from control, 500 and 600 ppm was 7.0, 5.8 and 4.3 respectively i.e. solution became more acidic. Hence it may be concluded that as pH value of glyphosate solution decreases, the linear growth of radicle and hypocotyl of seedlings also decreased proportionately. This is according to the finding of Audus, Hanson and Behrens.<sup>22-24</sup>

## Anatomical responses

### Control

The hypocotyl of *Psoralea corylifolia* in control has single layered epidermis, 12 to 15 layers of parenchymatous cortex, which was limited on the inside by single layer of endodermis. Immediately within the endodermis lies the pericycle.

Vascular bundles were not organized yet (Fig. 2.1). The radicles of *Psoralea corylifolia* seeds showed uniseriate epidermis with unicellular hairs.

The parenchymatous cortex was 8-10 cells thick. Immediately within the endodermis lies the pericycle. The primary phloem consists of four strands of tissues alternating with tetra-arched xylem (Fig. 2.2).

Longitudinal section of the same is shown in (Fig. 2.3). Cotyledon in control consists of palisade tissue, spongy parenchyma with large number of intercellular spaces and vein bundles bounded by upper and lower epidermis (Fig.2.4). Glyphosate induced some remarkable anatomical changes in hypocotyl, radicle and cotyledon.

In the hypocotyl at 200 ppm slight dividing activities was seen in the region of pericycle and endodermis forming a small mass of cells growing in the cortical region.

The cortical cells showed slight enlargement resulting in swelling. At higher concentration (400 ppm) the cortical cells became irregular in shape and disorganised at some places resulting in the formation of lacunae. The epidermal cells were crushed and lost their identity (Fig. 2.5). In the hypocotyl, enlargement of pith cells and meristematic zone developed around the vascular tissues at lower concentrations observed in the present study.

The proliferation of cells due to increased meristematic activity which may be due to the stimulatory effect of glyphosate in phloem cells. Jain<sup>25</sup> in *Chenopodium album* reported more strands of xylem and phloem in the seedlings due to application of glyphosate. Bobde<sup>17</sup> in *Crotalaria juncea* reported ruptured epidermis in hypocotyl due to enlargement of cortical cells in glyphosate treated seedlings.

In radicle at 400 ppm from the immature endodermal and pericycle zone masses of meristematic cells developed opposite to each other however, only two of them grew considerably and penetrated in the cortex.

The cortical cells were pressed by these meristematic masses and became elongated and compact. The pith cells were disorganised (Fig. 2.6).

The longitudinal section showed meristematic mass and rupturing of cortical and epidermal cells (Fig. 2.7). In radicle, lateral root primordia arose from the vascular cylinder.

This primordial growth inhibited by glyphosate at higher concentrations. It also showed enlargement of cortical cells, crushed pith cells and ruptured epidermis. Similar results were reported by Canal<sup>26</sup> in *Cyperus rotundus*.

The cotyledon showed dehydration of mesophyll cells and developed lacunae at 200 ppm. The mesophyll cells lost their identity and palisade cells deformed from spongy cells. At 400 ppm layer of palisade cells increases their number due to which some crackings were formed. Epidermis lost their shape and deformed and distorted and several lacunae were formed in palisade cells.

As a result of enlargement in the spongy cells, the cotyledons curved to ventral side. Formation of the notch at the deformed region was observed (Fig. 2.8).

Cotyledon treated with glyphosate showed disorganization of mesophyll cells and ultimately lacunae were formed.

This was probably due to dehydration of mesophyll cells. The lower and upper epidermis was also ruptured in several places. Kamble<sup>27</sup> in *Hibiscus cannabinus* and Dudhe<sup>28</sup> in *Hyptis suaveolens* observed similar results.

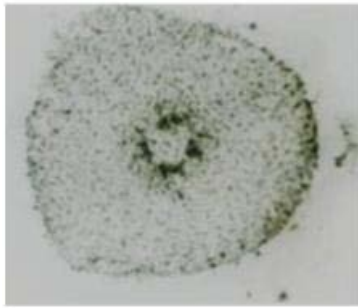
## CONCLUSION

The inhibition of the linear growth of the seedlings was directly proportional to the concentration of glyphosate. For the present study, it was also observed that the radical of the seedlings was more susceptible to the action of herbicide than the hypocotyl.

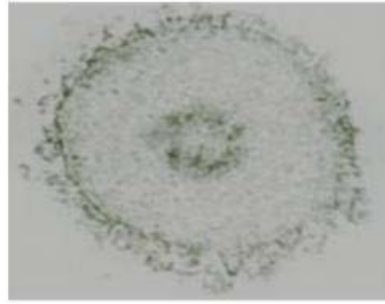
It was also revealed that pH plays an important role in the effect of glyphosate on the growth of seedlings. The pH values of the concentrations decreased towards activity, there was suppression in the length of the radical & hypocotyls with the result that started seedlings of the weed were produced. Treated seedlings were studied for anatomical changes by the glyphosate. These were correlated with the death of seedlings. The treated seedlings exhibited some anatomical abnormalities.

Glyphosate induced proliferation from pericycle and phloem in hypocotyls and radical at 200 & 400 ppm respectively. Owing to increased pressure on these shoulders, the cortical cells were disorganized and epidermis ruptured in several places.





**Figure 2.1:** T.S. of hypocotyl, control.



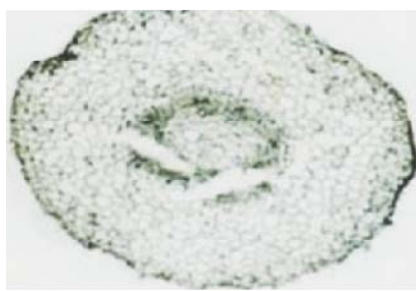
**Figure 2.2:** T.S. of radicle, control.



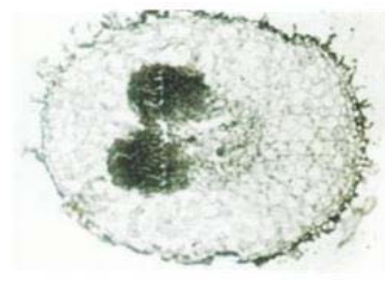
**Figure 2.3:** L.S. of radicle, control.



**Figure 2.4:** T.S. of cotyledon, control.



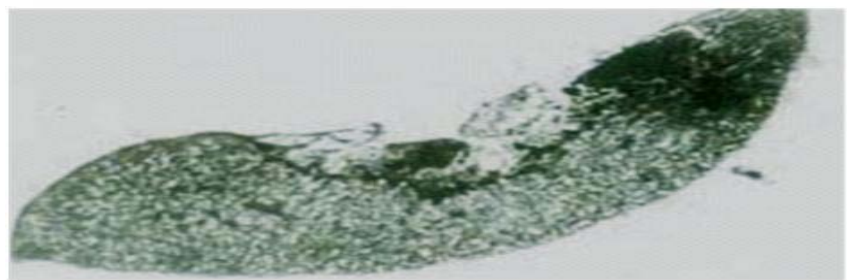
**Figure 2.5:** T.S. of hypocotyl at 400 ppm.



**Figure 2.6:** T.S. of radicle at 400 ppm.



**Figure 2.7:** L.S. of radicle at 400 ppm.



**Figure 2.8:** T.S. of cotyledon at 400 ppm.

**Figure 2:** The photographs 2.1 to 2.8 shows anatomical abnormalities induced by glyphosate in *Psoralea corylifolia* L.



**Figure 3:** The photographs of *Psoralea corylifolia* L.

**Table 1:** Effect of different concentrations of herbicide glyphosate on linear growth (in mm) of hypocotyl and radicle after 72 hours.

Herbicide	Concentration in ppm	Increase in hypocotyl length (mm)	Standard error ( $\pm$ )	Increase in radicle length (mm)	Standard error ( $\pm$ )
	Control	32.4	3.20	18.2	1.10
Glyphosate	100	16.2	1.36	9.6	0.52
	200	12.4	0.85	6.4	0.84
	400	8.7	0.80	2.2	0.86
	500	5.2	0.42	2.2	0.86
	600	5.2	0.42	---	---

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