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RESEARCH ARTICLE

INFLUENCE OF SPRAY APPLICATION OF N-(PHOSPHONOMETHYL) GLICINE ON MORPHOANATOMICAL CHARACTERS OF *Psoralea corylifolia* L.

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ABSTRACT

The plant of *Psoralea corylifolia* sprayed with glyphosate (N-(phosphonomethyl)glycine') showed some morphological changes such as scorching, wilting of leaves and stems, crumpling and yellowing of leaves and drying of all the parts of the plant at all concentrations (100,200,400,600 ,800, 1000 and 1200ppm). On fourth day of spraying plant showed yellow spots on lower leaves at all concentrations. When glyphosate sprayed at concentration of >= 400ppm lamina showed burning effects and they dried on completely. Stem became pale green on eight day after spraying. At 600ppm and above concentration, apical and lateral growth was found inhibited, whereas at 1200ppm the leaf dried completely, thus this dose acts as a lethal dose to this plant. Glyphosate induced anatomical changes in stem, root, petiole and leaf. At 400ppm phloem showed abnormal meristematic activity owing to periclinal and anticlinal divisions of phloem, the cortex was pushed outward and crushed. The xylem and pith cells disintegrated at many places and formed lacunae at 800ppm. Similarly at 800ppm root showed ruptured epidermis and disorganization of cortex forming lacunae. At 1000ppm, the leaf epidermis showed cellular disorganization of the spongy and palisade tissue.

INTRODUCTION

Psoralea corylifolia L. is belonging to family Papilionaceae is an erect annual herb and grow to maximum height of 1.4 m. The stem and branches are covered with conspicuous glands and white hairs. Leaves are round, dotted with black glands on both surface. Flower appears form the axil of leaves in bunches. It is commonly known as 'Bawachi'. This species is native to India and Arab (Brenchley, 1920). This plant has harmful effects on growing crops, interference in land uses, and rank among the most important enemies to agricultural production (Crafts and Robbins, 1973). 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 labour and equipment, render harvesting difficult, reduce the quality and marketability of agricultural commodities, harbor insects, fungal, viral and bacterial micro-organisms and some are poisonous to human being and cattle. These losses are far greater than usually realized (Mahakhode and Somkuwar, 2012). Phytotoxic influence of herbicides is quite various. It is expressed in morphological, anatomical, physiological and biochemical modifications, which occur in sensitive plants and cause deterioration in plants that even lead to death. The biochemical, physiological or anatomical modifications precede the morphological ones. Herbicides reduce the chloroplast content in assimilation parenchyma cells of leaf (Jung *et al*., 2008), distrurb plant biochemical and physiological processes (Warabi *et al*., 2001; Ha *et al*., 2003; Jung *et al*., 2004; Yang *et al*., 2006), causing anatomical and morphological modifications (Guh and Kuk, 1997; Kamble, 2007a, b), lead to growth inhibition and death of plants (Martin and Fletcher, 1972; Gorske and Hopen, 1978; Muniyappa *et al*., 1980; Bakale, 1989; Ferrel *et al*., 1989; Tripathi *et al*., 1992; Mukharji,1994). The objective of the study was to determine the effect of herbicide glyphosate on some morphoanatomical indices of the plant organs such as leaves, stem, and root of *Psoralia corylifolia* plants.

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MATERIALS AND METHODS

Seeds of *Psoralea corylifolia* were randomly collected from naturally growing population at different places of Nagpur and were grown in earthen pots for two months till they attained the height of 8 to 10 inches. (i.e. vegetative growth). Different concentrations of herbicide glyphosate (viz., 100, 200, 400, 600, 800, 1000 and 1200 ppm) were prepared in distilled water. Six pots, each containing 2 plants were given through foliage spray of each concentration. The herbicide solution contained a pinch of sodium lauryl sulphate as surfactant. The field and pot culture experiments were conducted in the month of October and November. Spraying was done in the evening when the wind was slow and the temperature was comparatively lower than rest of the hours of the day to reduce evaporation and to help absorption of herbicide solution by the leaves. To avoid contamination from surrounding, the pots were kept in high walled card boardbox at the time of spraying and the pots to be sprayed were taken away to a considerable distance. Control pots were sprayed with water. After 48 hours of spray, pots were watered daily. All pots were kept preferably in shade in order to get the maximum effects on them. Similarly field trial was conducted on naturally growing plants in plots of the size of approximately 4 x 4 square feet. Fresh and dry weight of shoots and roots of control and treated plants were taken after 8-10 days of spraying to determine the biomass of plants. The morphological changes were noted daily till the death of the plants.

In order to study the anatomical changes induced by the herbicides, the plant parts mainly root, stem, leaf and petiole of the treated as well as the control plants were fixed in F.A.A. (Formalin-Acetic acid-Alcohol) solution for 24 hours, and then washed and stored in 70% alcohol. The materials were embedded in paraffin wax following customary method (Sass, 1951). Sections were cut as 15-20 microns using spencer rotary microtome, stained according to the crystal violet-erythrosine schedule and mounted in DPX medium. Microphotographs of various sections of both control and treated plants were taken using research microscope (Metzer Optical Instruments Pvt. Ltd. India).

RESULTS AND DISCUSSION

A) Morphological responses

The plants of *Psoralea corylifolia* sprayed with glyphosate showed morphological changes such as scorching, wilting of leaves and stems, crumpling and yellowing of leaves and ultimately it was followed by drying of all the parts of the plant. The first two days, treated plants did not show any marked effects but, on fourth day of spraying showed yellow spots on lower leaves at all concentrations i.e. 100 to 1200 ppm. It was gradually proceeded towards upper leaves and ultimately leaves became yellow in colour. Along with chlorosis, rolling of leaves towards inner side was also observed. Later on at and above 400 ppm lamina showed burning effects and leaves dried off completely (Fig. 3). On seventh day the young leaves crumpled upwards and later on they dried off completely at 600 ppm and above concentrations. Stem became pale green on eighth days after spraying. After spray application yellow spots on lower leaves, this gradually proceeded towards upper leaves as the concentration increases; it may probably due to photodistruction of chlorophyll and chloroplast disruption. Many workers noticed the yellowing of leaves in various plants. Jaworski (1972) in *Lemna gibba*, Suwunnamek and Parker (1975) in *Cyperus rotundus* and Campbell *et al*. (1976) in *Agropyron repense* reported disruption of chloroplast envelope in mesophyll cells after glyphosate treatment. The activities of apical and lateral meristematic tissues of plants were suppressed at the concentration of 600 ppm and above. On the thirteenth day terminal part of stem showed desiccation and dried off at all concentrations. The plants at 1200 ppm growing in pots dried completely on the sixteenth day, thus this dose acts as lethal to this weed (Fig. 1 and 2). The root system became stunted in appearance and reductions in growth of lateral roots were observed (Fig. 4). Field trials showed similar results (Fig. 5). The fresh and dry weight of shoots and roots of plants were found to be decreased gradually as the doses of herbicide increased (Table 1). Jain (1993) noticed the complete death of *Chenopodium album* at 1500 ppm of glyphosate on 9th day. Bobde (1993) observed the death of *Crotalaria juncea* on 16th day after glyphosate treatment and Tulankar (1998) also observed in *Amranthus lividus* complete death on 16th day at 2200 ppm. Carlson and Donald (1988) in *Cirsium arvense*, Ali and Fletcher (1977) in corn reported drastic inhibition of root growth and stated that roots lost their turgidity and were discoloured.Time of herbicides application is important in determining the effectiveness and length of weed control duration (Carter *et al*., 2007; James *et al*., 2007). In case of *Psoralea corylifolia*, due to progressive desiccation of plants, there was gradual decrease in fresh and dry weights of plants as the concentration of herbicide increased. The rate of desiccation of plants was directly proportional to the concentration of glyphosate increased. Villanueva *et al.* (1985) in *Cyperus rotundus* and Richard (1991) in sugarcane reported that glyphosate decreased fresh and dry weights of shoot and root due to inhibition of respiration.

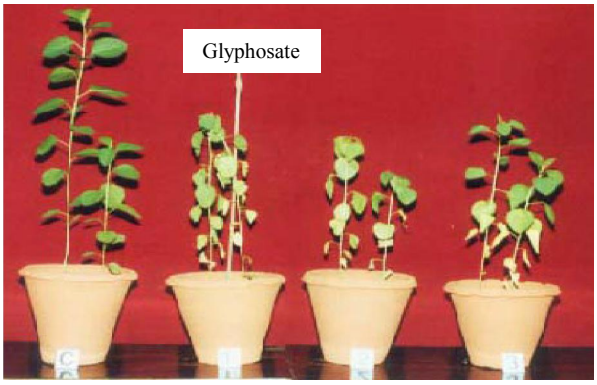

Fig. 1. C-Control.1,2 and 3-plant after spray application at 100,200 and 400 ppm of glyphosate, respectively showing retardation of vegetative growth and drying of shoot.

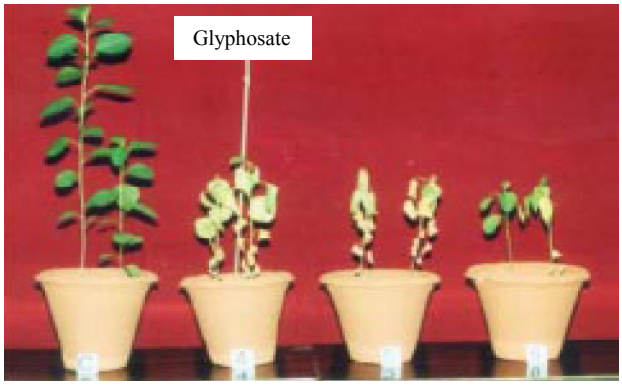

Fig. 2. C-Control. 4,5 and 6-plant sprayed with 600, 800 and 1000 ppm of glyphosate, respectively showing retardation of vegetative growth and drying of shoot.

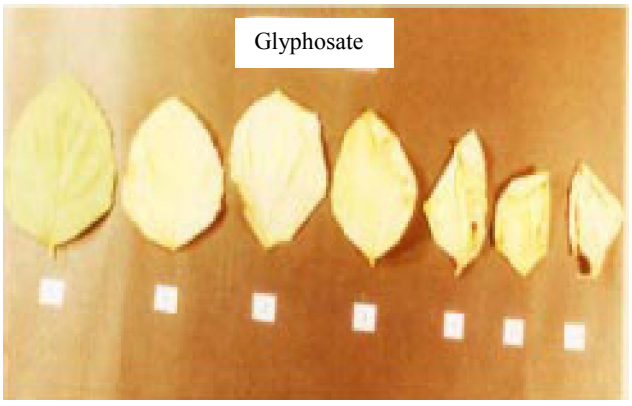

Fig. 3. C-Control. 1,2,3,4,5 and 6- leaves after spray application of glyphosate at 100,200,400,600,800 and 1000 ppm, respectively showing chlorosis and progressive

Table 1. Effect of glyphosate on fresh and dry weight (g) of shoots and roots * of *Psoralea corylifolia* L.

Herbicides	Concentration in ppm	Shoot		Root	
		Fresh weight(g)	Dry weight	Fresh weight(g)	Dry weight(g)
Glyphosate	Control	35.20	14.70	10.35	4.28
	100	22.85	10.30	6.80	2.45
	200	15.20	5.54	4.85	1.60
	400	11.45	3.25	2.70	0.96
	600	9.62	2.28	2.10	0.58
	800	7.70	1.80	1.90	0.42
	1000	6.68	1.20	1.65	0.35
	1200	6.68	1.20	1.65	0.35

* Average of four replicates.

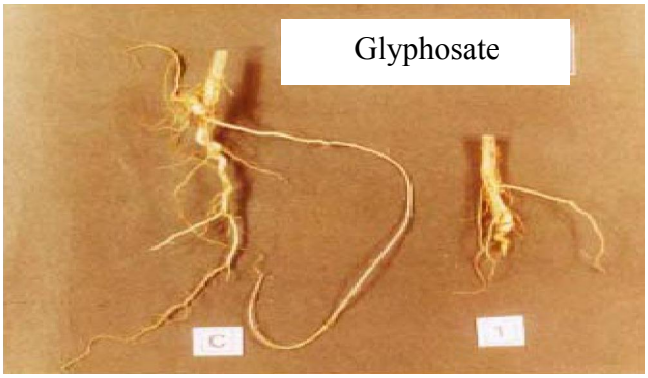

Fig. 4. C-Con trol. 1-after application of glyphosate at 1000 ppm showing decrease of lateral roots

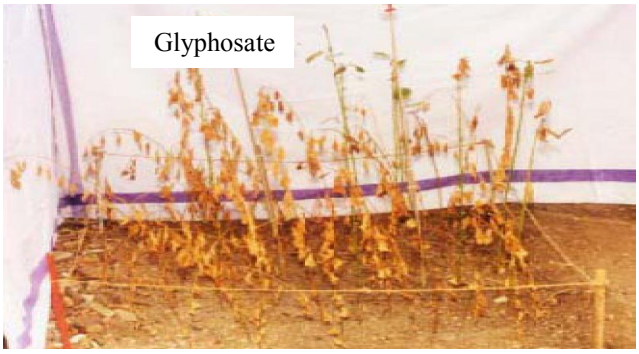

Fig. 5. Field photograph of plant after spraying at 1200 ppm (lethal) of glyphosate

(B) Anatomical responses

Control

The nontreated stem of *Psoralea corylifolia* showed a typical dicotyledonous structure with ridges and furrows bounded by the epidermis. The outer region of cortex, bordering the epidermis includes collenchymas below the ridges followed by 4-5 layers of chlorenchyma. The pericycle was in the form of intermittent ring of fibers. The Vascular bundles are arranged in a ring and encloses large parenchymatous pith (Fig. 6). Anatomically petiole in untreated plants showed five large vascular bundles, one below each ridge alternating with 1-2 small vascular bundles below the furrows. Collenchyma in the ridges was prominently seen, and it was a single layered below the furrow followed by two layers of chlorenchymatous ground tissue (Fig. 7).

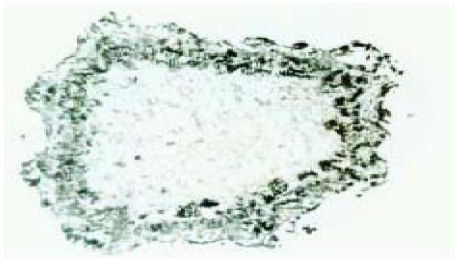

Fig.6. T.S. stem, control

Fig. 7. T.S.petiole, control

Roots of untreated plants showed secondary growth and consisted of an outer epidermis and parenchymatous cortex, followed by a band of secondary phloem. The xylem forms a cylinder in the center of the root (Fig. 8). The leaf lamina has upper and lower epidermis and mesophyll in between them. The mesophyll comprises upper palisade and lower spongy tissue. The midrib was formed of parenchymatous tissue, embedding vascular bundles (Fig. 9).

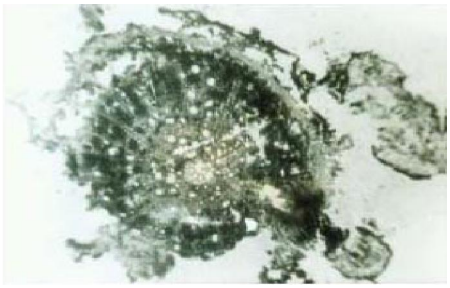

Fig. 8. T.S. root, control

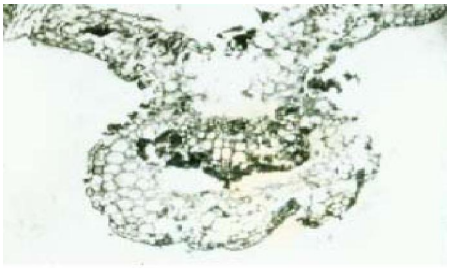

Fig. 9. T.S. leaf, control

Glyphosate induced anatomical changes in stem, root, petiole and leaf. The stem of *Psoralea corylifolia* at 800 ppm showed disintegration in the outer part of pith cells and forming lacunae. The epidermal, hypodermal and cortical cells were observed to be distorted and formed lacunae (Fig. 10). In petiole, due to glyphosate spray, the phloem tissue proliferated and form mass of meristematic cells at 800 ppm which exerted the pressure on the cortical cells and thereby forming cavity in petiole. The xylem and pith cells disintegrated at many places and formed intercellular spaces.(Fig. 11).

Fig. 10. T.S. stem at 800 ppm

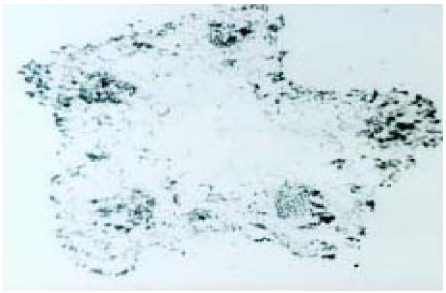

Fig. 11. T.S. petiole at 800 ppm

After spray application, glyphosate induced some anatomical nomalies in plant part of *Psoralea corylifolia*. The stem showed phloem proliferation to form meristematic masses. These masses penetrate in cotex and ruptured the cortical cells. This might be due to

that glyphosate accumulated in plant meristems and induced abnormal division to produce masses of meristematic cells which rupture the cortical region. Claus and Behrens (1976) reported that accumulation of glyphosate following foliar application to *Elytrigia repens* was greater in nodes near the rhizome tip and least in nodes near the mother shoot. Wilson (1998), Cole *et al.* (1980) and Smid and Hiller (1981) reported that glyphosate was accumulated in their respective plant meristems. Petioles of plants sprayed with glyphosate showed proliferation and disintegration of phloem cells. This might be due to the glyphosate subjected to export and accumulation in the living cells during migration across the mesophyll along the sieve tubes. The root showed ruptured epidermis and disorganization of cortex forming lacunae at 800 ppm. There was disintegration and distortion of xylem and phloem tissues. The cortex and epidermis sloughed off at certain places (Fig. 12). The lamina of the leaf at 1000 ppm showed some structural changes which induced disturbance in cellular organization at spongy and palisade cells. There was complete disorganization of vascular tissues of lateral veins. Phloem present in the midrib region proliferated and pressure exerted on the parenchymatous cells caused distortion of the same. The depletion of chloroplast was observed. The part of the leaf away from midrib also crumpled resulting thereby in crumpling the mesophyll cells and epidermal cells and lost their identity (Fig.13).

Fig. 12. T.S. root at 800 ppm

Fig. 13. T.S. leaf at 1000 ppm

The root showed proliferation of phloem and cambium cells and damaged xylem vessels in the present study. This may be due to the uptake of soil applied herbicide and the glyphosate accumulated within the root apical meristems which results into the proliferation damaging tissues. Similar findings were reported by Cole *et al*. (1983) in some dicotyledonous weeds, Camacho and Moshier (1991) in *Sorghum halepense* and Taduwadi (2004) in *Cleome viscosa*. The leaves of this weed showed disorganization and desiccation of mesophyll cells in the present study. This might be due to the inhibition of photosynthesis and photosynthetic electron transport by the herbicide which lead to destruction of the existing chlorophyll pigment and ultimately destruction of mesophyll tissue. Similar results were noticed by Canal *et al*. (1990) in yellow nutsedge, Stock and Davies (1994) in some dicotyledonous plants.

Conclusion

There was complete inhibition of vegetative growth of plant by using glyphosate herbicide. This was confirmed on the basis of the fresh and dry weights of shoot and root. Glyphosate kills the plant by inducing some anatomical changes which might be resulting in reducing metabolic activities of plants. Weeds accumulate and convert larger amount within a relatively short period and thus affected more rapidly than crop plants that escape injuries because of slow conversion process and the non-lethal level accumulated in their tissues. Therefore, herbicide application is a skill that allows farmers take an advantage from morphological, physiological or biochemical differences between weeds and crop plants in response to herbicides and thus may be considered as an opportunistic operation should be implemented on time and when conditions permit.

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