Pak. J. Bot., 12 (1): 97–106, 1980.

HERBICIDE INFLUENCE ON GERMINATION AND SEEDLING GROWTH OF *VIGNA MUNGO* (L.) HEPPER AND *V. RADIATA* (L.) WILCZEK.

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Abstract

Tolerance of *Vigna mungo* and *V. radiata* to Chloro-phenoxyacetic acid, Chloro-phenoxy-butyric acid and triazine herbicides was evaluated. 2, 4-D and 2, 4, 5-T were found to be highly inhibitory to germination and early seedling growth of the legumes. MCPB suppressed the germination of *V. radiata* at ⩾ 50 ppm and that of *V. mungo* at 800 ppm, but inhibited root as well as shoot growth at ⩾ 25 ppm. The triazines viz. prometryne and terbutryne, did not inhibit germination upto 400 ppm. Triazines stimulated root growth of *V. mungo* but that of *V. radiata* was slightly retarded by terbutryne. Shoot growth of the legumes remained uninfluenced by triazines upto 50 ppm. Value of 50% tolerance level (TL_{50}) was highest in prometryne, > 800 ppm for *V. mungo* and 717.42 ppm for *V. radiata*, suggesting that prometryne is the safest herbicide among those tested for weed control in leguminous crops.

Introduction

The use of certain categories of herbicides, especially the triazines, chlorophenoxy acids and phenoxybutyric acids is recommended for the efficient weed control in leguminous crops (Muzik, 1973). The application of these herbicides and the residual herbicide remaining in the soil may also influence the growth of legume species and have a direct impact on the crop yield.

The effect of triazine herbicides on germination has been a controversial issue for the last two decades. Gast *et al.* (1955, 1956) reported that chloro-amino-triazines do not influence seed germination. Wakonig & Arnason (1958), however, noted that germination of barley was almost completely suppressed beyond 200 ppm concentration of triazine herbicides. Similar results are reported by Shaukat & Soni (1974) and Shaukat *et al.* (1976).

In contrast, the inhibitory effect of chlorophenoxyacetic and chlorophenoxy-butryric herbicides of moderate concentrations is well estabilished (Rojas – Garciduenas *et al*, 1962; Kozlowski & Sasaki, 1968; Shaukat & Soni 1974; Smith, 1975) However, all the aforesaid group of herbicides are known to be invariably toxic to recently germinated seedlings, particularly in case of susceptible species or varieties (Kozlowski & Kuntz, 1963; Shaukat *et al,* 1975; Smith, 1975) consequently trials for the determination of suitable herbicides and their dosages for a specific crop are essential.

The purpose of this investigation was to assess the influence of certain chlorophenoxyacetic, chlorophenoxybutyric and triazine herbicides on germination and seedling development of *Vigna mungo* (L.) Hepper and *V. radiata* (L.) Wilczek and to determine the relative susceptibility of the two legume species to these herbicides.

Material and Methods

Lots of 20 surface-sterilized (with 0.1% mercuric chloride) of either *V mungo* var. Pak-22 or *V. radiata* (local variety of Sind) were placed on Whatman No. 1 filter paper in 9 cm Petri plates containing 5 ml of an aqueous solution of prometryne (6 methylmercapto-2, 4 bis (isopropylamino)s-triazine), terbutryne (2-ethylamino-4-methylthio-6-butylamino-1 3.5-triazine), MCPB (Methyl-chlorophenoxybutyric acid), 2, 4-D (2, 4-dichlorophenoxyacetic acid) or 2, 4, 5-T (2, 4, 5-trichlorophenoxyacetic acid) at 25, 50, 100, 200, 400 and 800 ppm concentrations. All concentrations were based exclusively on the proportion of active ingredients. Distilled water was used for controls. The Petri plates were maintained at 25±2°C in a growth chamber. Light intensity at the top of dishes was 4 K Lux (12 h day length). Small amounts of water were added periodically when it was obvious that Petri dishes were beginning to dry out. Seed germination counts were made daily for 5 days. A seed was considered germinated when the radicle had attained a length of not less than 1.5 mm (Taylor, 1942). At the end of 5th day, roots and shoots of all the germinated seedlings were measured. The treatments were replicated three times and the collected data was subjected to appropriate statistical analysis following Steel & Torrie (1976). A 50% tolerance level (TL_{50}) the concentration at which shoot growth was reduced to 50% was computed using the formula adduced by Davis *et al.* (1972) as follows:

$$TL_{50} = C_1 + [(C_2 - C_1)(50 - P_1)]/(P_2 - P_1) \ ;$$

Where

C_1 = highest concentration giving less than 50% growth reduction

C_2 = lowest concentration giving more than 50% growth reduction;

P_1 = percentage growth at C_1; and P_2 = percentage growth at C_2.

Results

a) *Effect of herbicides on germination*

The phenoxyacetic herbicides 2, 4-D and 2, 4, 5-T strongly inhibited germiation of both *V. mungo* and *V. radiata* (Fig. 1; Table 1). The inhibitory effect of these herbicides was more pronounced at higher concentrations and the germination was completely

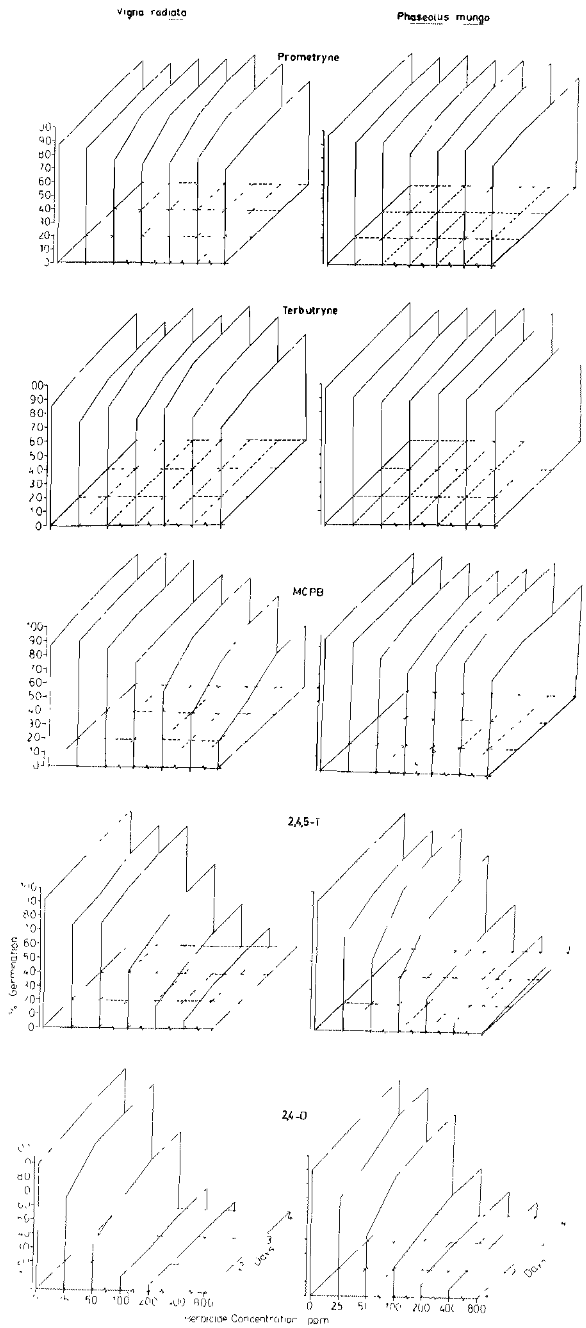

Fig. 1. Effect of five herbicides on germination of *Vigna mungo* and *V. radiata*.

Table I. ANOVA table for the germination data (percentage germination was transformed into arcsin values).

Source of variance	SS	df	MS	F
Species (S)	14006.88	1	14006.88	475.77***
Herbicides (H)	469671.52	4	117167.88	3979.88***
Time (T)	16568.88	4	4142.22	140.70**
Concentration (C)	3132166.57	6	52027.76	1767.24**
First order interactions				
S x H	4753.25	4	1188.31	40.36**
H x T	482.68	16	30.167	1.02
H x C	245880.82	24	10245.03	347.99***
C x S	2290.29	6	381.71	12.96***
C x T	1753.46	24	73.06	2.48***
S x T	49.70	4	12.42	0.42
Second order interactions				
S x H x T	870.64	16	54.41	1.84*
S x C x T	776.99	24	32.37	1.09
H x C x S	12842.08	24	535.09	18.17***
H x C x T	5493.31	96	57.22	1.99***
Interaction of all factors				
S x H x C x T	622.67	96	6.48	0.22
Residual	20613.34	700	29.44	—
Total	1107663.08	1049	—	—

$LSD_{0.05} = 8.68$ $LSD_{0.01} = 11.42$ $LSD_{.001} = 14.57$.

suppressed by 2, 4-D at 400 and 800 ppm in *V. radiata* and *V. mungo* respectively. Germination of *V. radiata* was affected to a greater degree than that of *V. mungo* by both the phenoxyacetic herbicides (SXH, $p < 0.001$). Phenoxybutyric herbicide MCPB delayed germination upto 100 ppm in case of *V. radiata* and reduced the germination upto 100 ppm in case of *V. radiata* and reduced the germiantion percentage at $\geqslant 50$ ppm. On the other hand, the germination of *V. mungo* was inhibited by MCPB only at 800 ppm though the rate of germination remained slightly suppressed at all the dosages (HXTXC $p < 0.001$). Likewise, the triazines, i.e., prometryne and terbutryne delayed the germination of both the species at < 100 ppm leaving the final percentage much the same as con-

Table 2. Effects of herbicides on root and shoot growth of *Vigna mungo* and *V. radiata*.

Herbicide	Concentration ppm	*Vigna mungo* Root length cm	*Vigna mungo* Shoot length cm	*Vigna radiata* Root length cm	*Vigna radiata* Shoot length cm
Prometryne	0	2.88±0.28	5.34±0.36	3.68±0.24	5.80±0.33
	25	4.20±0.41	4.64±0.20	3.17±0.23	4.77±0.25
	50	4.81±0.52	5.16±0.38	3.54±0.30	5.01±0.18
	100	5.05±0.51	5.14±0.27	3.64±0.31	4.48±0.23
	200	6.32±0.36	4.61±0.43	3.29±0.33	3.92±0.40
	400	8.33±0.70	4.12±0.52	4.06±0.35	3.02±0.20
	800	7.40±0.76	3.04±0.27	3.44±0.24	2.80±0.14
Terbutryne	0	3.11±0.31	5.26±0.49	3.45±0.40	5.51±0.28
	25	4.18±0.40	5.06±0.35	3.40±0.29	4.07±0.31
	50	4.76±0.39	4.22±0.28	2.42±0.11	4.52±0.19
	100	4.91±0.36	5.50±0.26	2.59±0.42	3.70±0.22
	200	4.54±0.23	3.88±0.40	3.73±0.28	3.58±0.24
	400	8.04±0.55	3.07±0.46	3.10±0.20	3.34±0.27
	800	7.84±0.46	1.56±0.38	2.64±0.12	2.06±0.19
MCPB	0	3.05±0.44	5.21±0.38	3.54±0.29	5.72±0.60
	25	1.15±0.17	2.12±0.40	1.00±0.15	4.06±0.35
	50	0.80±0.09	1.13±0.13	0.42±0.12	1.56±0.19
	100	0.67±0.03	0.42±0.04	0.09±0.02	1.13±0.06
	200	0.25±0.07	1.12±0.09	0.20±0.017	1.10±0.15
	400	0.11±0.08	0.46±0.15	0.17±0.07	0.84±0.16
	800	0.07±0.01	0.29±0.18	0.13±0.05	0.25±0.13
2, 4-D	0	2.76±0.32	5.38±0.43	3.52±0.36	5.87±0.46
	25	0.52±0.08	2.24±0.12	0.47±0.16	2.84±0.36
	50	0.24±0.05	0.82±0.08	0.32±0.09	2.15±0.29
	100	0.21±0.05	1.12±0.14	0.26±0.07	1.16±–.18
	200	0.18±0.03	0.54±0.056	0.11±0.01	1.09±0.12
	400	0	0	0.07±0.008	0.72±0.09
	800	0	0	0	0
2,4, 5-T	0	3.05±0.36	5.14±0.32	3.46±0.31	5.48±0.37
	25	0.89±0.17	3.02±0.22	0.73±0.21	3.56±0.33
	50	0.28±0.12	2.70±0.153	0.48±0.09	2.48±0.27
	100	0.35±0.08	2.26±0.11	0.27±0.05	2.54±0.31
	200	0.16±0.004	1.25±0.06	0.05±0.007	1.68±0.17
	400	0.12±0.02	1.20±0.05	0.08±0.01	1.15±0.12
	800	0	0	0	0

± S.E are given against the means.

trols but the germination percentage was significantly reduced at 800 ppm (HXTXC, $p < 0.001$). The adverse effect on the rate of germination was relatively greater for terbutryne in comparison to that of prometryne and *V. radiata* was comparatively more susceptible to triazines than was *V. mungo* (SXHXT, $p < 0.05$).

b) Effect of herbicides on seedling growth

i) *Root growth:* The root growth of *Vigna mungo* was significantly inhibited by 2, 4-D, 2 4, 5-T and MCPB but was remarkably promoted by the triazines (i.e. prometryne and terbutryne) (Table 2). The relative order of increasing deleterious effect on root growth of *V. mungo* by the herbicides were 2, 4-D > 2, 4, 5-T > MCPB. Stimulation of root growth of *V. mungo* by the two triazines was more or less of equal magnitude. On the othr hand, root growth of *Vigna radiata* was inhibited by all the herbicides except prometryne which had no significant influence on root development (Table 2.). Root growth of *V. radiata* was retarded by the herbicides in the order 2. 4-D > 2, 4, 5-T > MCPB > Terbutryne. Whereas 2, 4-D, 2, 4, 5-T and MCPB retarded the root growth at all the concentrations, terbutryne exhibited the detrimental effect only at 400 and 800 ppm.

ii) *Shoot growth:* The shoot growth of *V. mungo* was suppressed by all the herbicides though at lower dosages the triazines did not induce significant inhibition of shoot elongation (Table 2). Shoot growth of *V. mungo* was suppressed by the herbicides in the order: 2, 4-D> MCPB > 2, 4. 5-T > terbutryne > prometryne. Likewise, shoot growth of *V. radiata* was also retarded by all the herbicides· the relative order of deleterious effect being MCPB > 2, 4-D > 2, 4, 5-T > terbutryne > prometryne.

c) Comparison of 50% tolerance levels (TL_{50}). to the herbicides

The tolerance of both the species to the five herbicides under study varied in the order: prometryne > terbutryne > 2.4, 5-T ⩾ MCPB > 2. 4-D (Table 3). *Vigna mungo* exhibited a very high degree of tolerance to prometryne, though *V. radiata* was also fairly resistant to this herbicide. Tolerance to terbutryne was slightly more for *V radiata* in comparison to that of *V. mungo* and both crops were fairly resistant to this herbicide.

Table 3. 50% tolerance level (TL_{50}) based on hypocotyl lengths of herbicide treated *Vigna mungo* and *V. radiata* seedlings.

	HERBICIDES				
Species	Prometryne	Terbutryne	MCPB	2, 4-D	2.4, 5-T
V. mungo	>800	517.42	<25	<25	64.72
V. radiata	717.42	582.7	37	<25	43.98

MCPB – tolerance was substantially more for *V. mongo* in comparison to that of *V. radiata;* the reverse being true for 2, 4, 5-T, but both crops were extremely susceptible to, 2, 4-D.

Discussion

The impediment of germination rate by prometryne and terbutryne and the suppression of germination at the highest dosage (800 ppm), which seems to be previously unreported, can be attributed to the inhibition of respiration and its associated oxidative phosphorylation by these herbicides (Truelove & Davis, 1969; Thomson *et al.* 1970; Kirkwood, 1976). It has been demonstrated by Shaukat *et al.* (1976) that prometryne, at higher concentration, suppresses the amylase activity of germinating seeds; this would restrict the mobilization of sugars and consequently hinder the rate of germination.

MCPB, which is a recommended chemical for weed control in leguminous crops (Wain, 1965; Brian, 1965), also not only delayed germination of the legumes but at higher dosages, reduced the final germination percentage over the controls. Wain (1965) ascribed the inherent tolerance of certain legumes to phenoxybutyric acids to their inability to beta-oxidize the relatively less toxic phenoxybutyric compounds to more toxic phenoxyacetic analogues. But recently it has been shown that both susceptible as well as resistant species to phenoxybutyric acids are equally effective in metabolizing (beta-oxidizing) these chemicals (Hawf & Behrens, 1974; Naylor, 1976). However, the results of the present study suggest the prevalance of the former mechanism of MCPB tolerance, as the detrimental influence of MCPB was of comparatively lesser order than that of phenoxyacetic acid herbicides.

Critical concentrations of the phenoxyacetic herbicides for inhibition of seed germination were relatively low in comparison to that of MCPB and triazines. 2, 4-D remarkably lowered seed germination at concentrations of $\geqslant 50$ ppm and 2, 4, 5-T at $\geqslant 100$ ppm but the rate of germination was impeded at all the dosages. This is in accordance with the earlier findings (Rojas – Garciduenas *et al.,* 1962; Kozlowski & Sasaki, 1968; Shaukat & Soni, 1974). The actual mechanism whereby phenoxyacetic herbicides cause the inhibitioon of germination has not been brought to light, but it is well known that these chemicals disturb a number of metabolic processes e.g., (a) production of abnormal quantities of RNA (Robertson & Kirkwood, 1970), (b) accumulation of coumarin in the tissue (Van Overbeek *et al.,* 1951), (c) increased respiration (Williams & Dun, 1961) and the consequent breakdown of starches (Tomizawa & Koike, 1954) and sucrose (Flood *et al.*, 1970).

The inhibition of shoot growth by the triazines can be explained on the grounds that they inhibit non-cyclic photophosphorylation and consequently the rate of phtosynthesis (Exer, 1961; Good, 1961; Van Orschot, 1964; Shaukat *et al.*, 1975; cf. Ebert & Dumford, 1976). Further, it is well known that factors which affect photosynthesis also consequently influence plant growth (Sweet & Wareing, 1966). The inhibition of photosynthesis led to reduction in shoot growth due to scarcity, and increased downward translocation of assimilates, as indicated by increased root growth. Enhancement of the root growth of *V. mungo* by the two triazines seems to be a previously unreported phenomenon. Reasons for such an effect are not known, but certain hypothetical possibilities can be raised. The enhancement of root elongation could be due to the phytohormonal activity of the triazines (Jordan *et al.*, 1966); alternatively, it could be the result of the disruption of the balance of source and sink where roots became more active as a sink. The marked reduction in shoot growth caused by 2, 4-D, 2, 4, 5-T and MCPB is presumably the result of (a) accumulation of coumarin (Van Overbeek *et al.*, 1951) and (b) reduction in photosynthetic rate (Robertson & Kirkwood, 1970; Shaukat, 1973). Root growth was also inhibited to a much greater extent by the phenoxyacid herbicides in comparison to triazines which is certainly due to phytohormonal activity of the phenoxyacids. Audus (1959) indicated that the optimal auxin concentration for root elongation is 1/100,000 the optimum concentration for the shoot. He found that 2, 4-D is 450 times more active than IAA in the inhibition of root growth. Keeping this in view the lethal action of phenoxyacids (also called hormone herbicides) can easily be visualised. Inhibition of root elongation corroborates the earlier findings with the other plant species (Eliasson & Palen, 1972; Shaukat, 1973; Shaukat & Soni, 1974; Smith, 1975).

The present investigation emphasizes the necessity for clearly distinguishing between absolute phytoxicity of herbicides from the apparent toxicity as determined in soil culture. The degree of herbicide toxicity often varies greatly with experimental conditions. Herbicide toxicity is generally well marked when seeds or seedlings are maintained cotinuously in direct contact with the herbicides (as in the present investigation) but only mild or negligible toxicity result when herbicides are applied in soil culture or in the field (Kozlowski & Torrie, 1965; Shaukat, 1973). Soil-applied herbicides often are lost to plants by microbial or chemical degradation (Allexander & Aleem, 1961; Day & Clerx, 1964), leaching (Rodgers, & Wilcox, 1963) and irreversible adsorption on the soil (Shaw *et al.*, 1960). The 50% toxic levels TL_{50} are, therefore, calculated on the basis of absoltue toxicity as a preliminary step towards evaluating the approved chemicals for weeding in the leguminous crops. Comparison of TL_{50} values of the five test herbicides suggests that prometryne would probably be the safest herbicide for the control of weeds in the fields of *V. mungo* and *V. radiata.*

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