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In vitro evaluation of low dosages of 2,4-D on germination and seedling growth of wheat and associated weeds

Avneet Kaur* and Navjyot Kaur

Department of Agronomy, Punjab Agricultural University, Ludhiana, Punjab 141 004, India

**Email: avneetatwal1995@gmail.com*

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ABSTRACT

Hormesis is a biphasic dose response to an environmental agent characterized by low dose stimulation and high dose inhibitory or toxic effect. In order to develop an effective weed management system, the stimulatory and/or inhibitory response of low doses of herbicides on germination and growth of weeds should be considered. Thus, a laboratory study was conducted to test the effect of low dosages of 2,4-D on wheat (*Triticum aestivum* L.) and associated dicotyledonous weeds. Low dosages of 2,4-D (0, 5, 10, 20, 40 and 50 g/ha) failed to accelerate either seed germination or seedling growth of *T. aestivum* and tested four weed species –*Phalaris minor* Retz., *Avena ludoviciana* Durieu., *Medicago denticulata* Willd. and *Rumex dentatus* L. under *in vitro* conditions in Petri-dishes. The application of 2,4-D inhibited the seed germination and seedling growth of these weeds in a dose dependent manner with higher concentrations being more inhibitory. The sodium formulation of 2,4-D more adversely affected the germination of *P. minor* and *A. ludoviciana* as compared to its ethyl ester formulation. The results indicated that 2,4-D when used as a post-emergence herbicide in wheat fields can delay the emergence of new flushes of weeds like *P. minor*, *A. ludoviciana*, *M. denticulata* and *R. dentatus* due to its inhibitory effect on their germination. It could be concluded that low doses of 2,4-D did not enhance germination/ seedling growth of wheat and associated weeds.

INTRODUCTION

In the North Western states of India, wheat production is under a severe threat due to the increasing infestation of weeds in the crop. Herbicides are the most effective, practical and economical means for weed management. But improper use of herbicides can do harm rather than benefitting the productivity. The selection of best herbicide, proper time and proper dose of herbicide application are important factors for achieving good herbicide efficacy. Wheat fields are infested with many monocotyledonous and dicotyledonous weeds which may cause the yield loss of 7 to 50% based on the kind of weed flora and their intensity (Chhokar *et al*. 2012). The major weeds prevalent in the wheat fields of Punjab are *Phalaris minor*, *Medicago denticulata*, *Rumex dentatus*, *Avena ludoviciana*, *Chenopodium album*, *Fumaria parviflora*, *Cirsium arvensis*, *Anagallis arvensis*, *Melilotus indica* and *Lathyrus aphaca*. Among these, *Medicago denticulata* and *Rumex dentatus* are the major dicotyledonous weeds and *Phalaris minor* and *A. ludoviciana* are the major monocotyledonous weeds in irrigated wheat under the rice-wheat cropping system in India (Chhokar *et al*. 2006).

Hormesis refers to biphasic dose response to an environmental agent characterized by low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect (Mattson 2008). Some patches of crop and weeds may receive very low doses of herbicides due to herbicide drift, incorrect methods or improper time of herbicide application or herbicide dilution in the soil due to the degradation and immobilization. Herbicides can also be applied at sub-lethal rates due to lateral and vertical movements of the nozzle boom, weed coverage by the crop plants or mulches, leaf contact of treated and untreated plants and herbicide resistance. Rainfall and dew drops may also dilute the herbicide concentration (Souza *et al*. 2007). The low doses of herbicides may exert stimulatory effect on crops and weeds boosting their growth and seed production potential. Hormetic effect of herbicides depends on many factors such as type of herbicide, herbicide dose, time of application, type of crop or weeds species, light, nutrients, temperature, carbon dioxide, and many other management practices (Belz *et al*. 2008). The growth stimulation due to herbicides under uncontrolled field conditions is generally unpredictable (Belz *et* *al*.2011). Hormetic responses have been demonstrated in response to many herbicides like glyphosate in *Cicer arietinum* L. (chickpea) and *Vicia faba* (faba bean) (Abbas *et al*. 2015, El- Shahawy and Sharara 2011); fenoxaprop-p-ethyl in *Phalaris minor* (littleseed canarygrass) and *Avena fatua* (wild oats) (Abbas *et al*. 2016). 2,4-D at very low concentration (0.1 kg/ha) has been reported to enhance seed production ability of *Chenopodium album* (Hume and Shirriff 1989).

The stimulatory response of plants at very low doses of herbicides have been reported for different physiological and biochemical parameters such as gene expression, enzyme activity, growth, biomass, protein content and chlorophyll content (Duke *et al*. 2006). Low doses of herbicides have been reported to stimulate growth in various crop species under field as well as controlled conditions (Cedergreen *et al*. 2007, Velini *et al*. 2008). Growth stimulation due to low herbicide doses to the extent of 20-30% and 10-15% has been reported under controlled and field conditions, respectively (Cedergreen *et al*. 2005, 2007, 2009). Nadeem *et al.* (2016) reported that sub-lethal doses of glyphosate solution in the range of 65–250 g/ha promoted the seed germination and seedling growth of four broad-leaved weeds–*C. didymus*, *C. album*, *R. dentatus* and *L. aphaca*. However, at high concentration (500 g/ha) inhibition of germination and seedling growth was reported. When crops are influenced by the hormetic effects, farmers may occasionally benefit from this phenomenon. But, it may intervene in the weed management or decline the crop production if hormetic phenomenon is produced in weeds (Velini *et al*. 2010).

2,4-D (2,4-dichlorophenoxyacetic acid) is an effective herbicide against dicotyledonous weeds, which is a synthetic auxin and effective plant growth regulator but limited studies have been undertaken on hormetic effect of 2,4-D in wheat crop and weed flora associated with this crop. It was hypothesized that sub-toxic concentrations of 2,4-D may affect germination and seedling growth of weeds that can influence the crop-weed competition. So, the present study was conducted to study the hormetic effect of low doses of 2,4-D on germination and seedling growth of wheat and four major weeds associated with wheat- *P. minor*, *A. ludoviciana*, *M. denticulata* and *R. dentatus.*

MATERIALS AND METHODS

Four annual winter season weeds (*P. minor*, *A. ludoviciana*, *M. denticulata* and *R. dentatus*) were selected to test the hormetic effects of 2,4-D. The seeds of these species were collected from wheat fields of Punjab Agricultural University, Ludhiana. 2,4-D ethyl ester 38% EC and 2,4-D sodium salt 80% WP were used as source of 2,4-D. The seeds of wheat cv. '*PBW 677*' were procured from Punjab Agricultural University, Ludhiana.

Germination protocol

Uniform sized seeds of *T. aestivum*, *M. denticulata*, *R. dentatus*, *P. minor* and *A. ludoviciana* were surface sterilized with 0.1% mercuric chloride for two minutes.Seeds were then washed several times with distilled water to avoid any fungal infection. The seeds of each species were kept for germination separately in Petri-dishes lined with Whatman No. 1 filter paper. The germination ability of seeds under different concentrations of 2,4-D (5, 10, 20, 40, 50 and 500 g/ha) was examined. At the beginning of the experiment, 5 ml of treatment solution was added in Petri-dishes. Petri-dishes were then kept at 20°C in an environmental chamber (Model MAC MSW-127, Delhi, India). The seeds germinated using distilled water served as control. Each treatment was replicated thrice and experiment was repeated three times.

Observations recorded

Germination counts were daily made for 15 days after initiation of the experiment. The visible protrusion of the radicle was regarded as the germination criteria. Germination percentage was calculated as:

Germination (%) = (no. of seeds germinated / total no.of seeds) x 100

Speed of germination (germination index) was computed as described by the Association of Official Seed Analysts (1983) using the following formula.

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + - - + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Mean germination time (MGT) was calculated according to the following equation of Ellis and Roberts (1981):

$$MGT = \Sigma\,(Dn) / \Sigma\,n$$

Where *n* is the number of seeds that had germinated on day *D* and *D* is the number of days counted from the initiation of germination.

Seedling vigour index (SVI) was calculated using the following formula (Abdul-Baki and Anderson 1973):

Seedling vigour Index I = seedling length (cm) x germination (%)

Statistical analysis

All the experiments were repeated three times in a completely randomized design (CRD) using three replicates, and data were analyzed by analysis of variance (ANOVA) using statistical analysis software version 9.2 (SAS 2009). Means were separated at $\sqrt{x+0.5}$ using Fisher's Protected Least Significant Difference (LSD) test (Cochran and Cox 1957).

RESULTS AND DISCUSSION

Effect of 2,4-D on germination and seedling growth of *P. minor*

When ethyl ester formulation was used, 2,4-D concentrations higher than 10 g/ha significantly reduced the germination (%) and germination speed with concomitant increase in mean germination time. Abnormal germination was observed at 40 and 50 g/ha 2,4-D with emergence of only plumule and radicle failed to emerge at these concentrations **(Table 1)**. *P. minor* seeds possessed the ability to germinate upto 50 g/ha 2,4-D with complete inhibition at 500 g/ha 2,4-D. Increasing concentration of 2,4-D from 0 to 50 g/ha caused significant decrease in seedling growth and vigour **(Table 2)**. Significant reduction in germination of seeds was observed at and above 5 g/ha2,4-D sodium salt formulation and no germination was recorded at 500 g/ha2,4-D. Mean germination time was increased with concomitant decrease in germination speed with increase in 2,4-D concentration. Radicle emergence of *P. minor* was completely inhibited in the presence of 2,4-D sodium salt concentrations higher than 10 g/ha in the germination medium.

Effect of 2,4-D on germination and seedling growth of *Avena ludoviciana*

The increase in 2,4-D concentration (ethyl ester formulation) from 5 to 500 g/ha significantly decreased seed germination of *A. ludoviciana*; and germination was found to be abnormal at highest dose (500 g/ha 2,4-D) where radicle failed to emerge and only shoot emergence was observed **(Table 1)**. Seeds germinated at 2,4-D concentration of 500 g/ha took maximum time to start germination along with reduced speed of germination and increased mean germination time. Increasing concentration of 2,4-D ethyl ester from 5 to 500 g/ha significantly decreased the growth of *A. ludoviciana* seedlings with a more pronounced effect on root length. There was 64.9% reduction in vigour index I at 2,4-D concentration of 5 g/ha in comparison to control. Sodium formulation of 2,4-D more adversely affected seed germination and abnormal germination was observed when 2,4-D concentration was increased above 5 g/ha in the germination media. Germination was completely inhibited in the presence of 500 g/ha concentration of 2,4-D sodium. Even the lowest dose of 2,4-D sodium (5 g/ha) caused significant decline in growth and vigour of *A. ludoviciana* seedlings **(Table 2).**

Effect of 2,4-D on germination and seedling growth of *Medicago denticulata* Willd

Ethyl ester and sodium formulations of 2,4-D exhibited similar effects on germination and seedling growth of *M. denticulata* at similar concentrations. Either ethyl ester or sodium formulation at concentration 5 g/ha 2,4-D caused no significant effect on germination of *M. denticulata* seeds but germination observed was abnormal as there was only emergence of plumule and radicle failed to emerge. Germination of *M. denticulata* was completely inhibited at and above 10 g/ha 2,4-D concentrations**.** Application of 5 g/ha 2,4-D (ethyl ester formulation) recorded 2.1 fold reduction in germination index and 2.3 times increase in mean germination time in comparison to control **(Tables 1 and 2)**.

Effect of 2,4-D on germination and seedling growth of *R. dentatus*

The seeds exhibited significant reduction in germination at and above 5 g/ha 2,4-D concentrations (ethyl ester formulation). However, germination observed even in the presence of sub-lethal doses of 2,4-D ranging from 5-50 g/ha was abnormal with only emergence of plumule and radicle failed to emerge at these herbicide concentrations. Germination was completely inhibited at 500 g/ha concentration of 2,4-D **(Table 1)**. At 50 g/ha2,4-D concentration, germination speed was decreased by about 2.6 times and mean germination time was enhanced by about 1.5 times than control. Seedling vigour index was reduced by about 93.4% as compared to control when seedlings were grown in the presence of 50 g/ha 2,4-D **(Table 2)**. Sodium formulation of 2,4-D exhibited effects similar to its ethyl ester formulation at different concentrations.

Effect of 2,4-D on germination and seedling growth of *Triticum aestivum*

2,4-D ethyl ester at 5 g/ha caused no significant effect on percent germination, speed of germination and mean germination time. However, significant reduction in germination of *T. aestivum* seeds was observed with 2,4-D concentrations higher than 5 g/ha while the germination of seeds was completely inhibited at 500 g/ha 2,4-D concentration. Increasing 2,4-D sodium salt concentration from 0 to 40 g/ha caused no significant effect on germination of *T. aestivum* seeds. 2,4-D concentrations higher than 40 g/ha caused significant decline in germination of *T. aestivum* seeds. Abnormal germination was observed in the presence of 40 and 50 g/ha concentrations of 2,4-D (ethyl ester and sodium salt) where only plumule was able to emerge and radicle exhibited complete failure to emerge **(Table 1)**. At 50 g/ha

Table 1. Effect of 2,4-D on germination and speed of germination of *P. minor*, *A. ludoviciana*, *M. denticulata*, *R. dentatus* and *T. aestivum*

2,4-D concentration (g/ha)	Germination (%)					Speed of germination				
	P. minor	*A. ludoviciana*	*M. denticulata*	*R. dentatus*	*T. aestivum*	*P. minor*	*A. ludoviciana*	*M. denticulata*	*R. dentatus*	*T. aestivum*
2,4-D ethyl ester formulation										
0 (Control)	100.0±0.00	96.1±2.00	91.0±4.04	88.33±1.67	100.0±0.00	6.45±0.01	3.15±0.14	3.98±0.25	2.85±0.12	9.61±0.24
5	95.0±1.44	83.8±2.32	86.3*±6.17	80.00*±2.89	96.7±1.67	5.96±0.13	1.49±0.02	1.85±0.07	1.98±0.15	9.12±0.15
10	94.2±1.67	81.3±1.53	NG	75.00*±2.89	93.3±3.33	5.21±0.00	1.59±0.07	-	1.87±0.08	8.93±0.29
20	72.5±1.44	78.3±2.54	NG	71.67*±1.67	88.3±1.67	2.94±0.05	1.31±0.07	-	1.41±0.04	8.19±0.28
40	60.0*±4.33	78.9±1.67	NG	63.33*±1.67	85.0*±2.89	2.38±0.10	1.22±0.01	-	1.36±0.04	7.56±0.25
50	45.8*±0.83	83.9±1.67	NG	46.67*±1.67	78.3*±1.67	1.31±0.03	1.28±0.06	-	1.09±0.06	6.51±0.16
500	NG	42.2*±4.84	NG	NG	NG	-	0.57±0.05	-	-	-
LSD (p=0.05)	5.89	7.88	8.46	6.04	6.04	0.21	0.22	0.29	0.26	0.66
2,4-D sodium salt formulation										
0 (Control)	100.0±0.00	90.0±0.00	88.66±4.67	90.0±2.89	100.0±0.00	6.28±0.10	2.16±0.07	3.65±0.20	2.64±0.05	9.47±0.27
5	86.7±1.67	63.3±3.33	86.33*±6.17	73.3*±7.27	93.3±4.41	5.67±0.13	0.94±0.07	1.81±0.15	1.56±0.18	8.74±0.46
10	88.3±1.67	66.7*±6.67	NG	73.3*±4.41	90.0±5.00	5.64±0.06	0.91±0.12	-	1.52±0.09	8.65±0.40
20	71.7*±1.67	43.3*±3.33	NG	71.7*±1.67	90.0±2.89	4.48±0.12	0.61±0.02	-	1.36±0.06	8.22±0.23
40	65.0*±5.77	50.0*±5.77	NG	58.3*±4.41	90.0*±5.00	3.81±0.16	0.60±0.07	-	1.27±0.06	8.22±0.66
50	71.7*±1.67	46.7*±3.33	NG	45.0*±2.89	83.3*±3.33	3.75±0.31	0.52±0.06	-	1.00±0.02	6.82±0.43
500	NG	NG	NG	NG	NG	-	-	-	-	-
LSD (p=0.05)	7.64	12.08	8.87	12.08	10.81	0.46	0.21	0.28	0.25	1.21

Data are mean ± standard error; *abnormal germination with only emergence of plumule; NG: No germination

concentration of 2,4-D sodium salt, speed of germination was decreased by about 1.4 times and mean germination time was increased by about 1.4 times than control **(Table 2)**.

Unlike many studies which indicate hormetic effect of herbicides namely glyphosate and fenoxaprop-p-ethyl at low doses (Velini *et al.* 2008, Abbas *et al.* 2016, Nadeem *et al.* 2016), the results of present investigation demonstrate that low concentrations of 2,4-D have failed to stimulate either germination or seedling growth of *T. aestivum* and tested four weed species – *P. minor*, *A. ludoviciana*, *M. denticulata* and *R. dentatus*. The application of 2,4-D herbicide inhibited the seed germination and seedling growth of these weeds in a dose dependent manner with higher concentrations being more inhibitory. The sodium formulation of 2,4-D adversely affected the germination of *P. minor* and *A. ludoviciana*as compared to its ethyl ester formulation. 2,4-D sodium salt at 500 g/ha concentration completely inhibited the germination of *A. ludoviciana* while germination was drastically reduced (56.1%) by 500 g/ha concentration of ethyl ester formulation of 2,4-D. Radicle emergence of *A. ludoviciana* was observed in the presence of 5-50 g/ha concentrations of 2,4-D ethyl ester. However, radicle emergence was completely inhibited in the presence of 10-50 g/ha 2,4-D sodium salt in the germination medium. 2,4-D herbicide possessed the ability to reduce seed germination and seedling growth of *R. dentatus* even at very low doses ranging from 5-50 g/ha. In case of *M. denticulata*, the inhibition in germination was more pronounced as there was no emergence of radicle even at the lowest concentration of 2,4-D (5 g/ha). Low doses of 2,4-D in the range of 5-20 g/ha did not significantly reduce germination of *T. aestivum*. Radicle emergence of *T. aestivum* was completely inhibited in the presence of 2,4-D concentrations equal to 40 g/ha 2,4-D sodium or higher concentrations. Even at lowest dose of 2,4-D *i.e.* 5 g/ha, root and shoot length were reduced by 80.6 and 43.8% as compared to control. Similar to our study, Khan and Aslam (2006) also reported the inhibitory effect of 2,4-D sodium at different concentrations (0.01% , 0.1% , 1.0%) on germination and seedling growth of *Triticum aestivum* and *Phalaris minor* with higher suppression of germination in *P. minor* than *T. aestivum*. Shoot and root length were reduced with the increase in dose of herbicide and radicle was more severely affected than coleoptile. In wheat, the swelling of hypocotyl was observed due to endogenous production of ethylene.

The results of present study reflected that different concentrations of 2,4-D used were toxic to all the five species as germination and seedling growth were declined as compared to control. The results indicate the possibility that 2,4-D when used as a post-emergence herbicide in wheat fields can delay the emergence of new flushes of weeds like *P. minor*, *A. ludoviciana*, *M. denticulata* and *R. dentatus* due to its inhibitory effect on their germination. However, unlike herbicides glyphosate and fenoxaprop-P-ethyl (Abbas *et al.* 2015, 2016), sub-lethal concentrations of 2,4-D failed to exhibit any hormetic effect in terms of enhanced germination/seedling growth of *P. minor*, *A. ludoviciana*, *M. denticulata* and *R. dentatus*.

The results indicated inhibitory effect of low doses of 2,4-D under *in vitro* conditions in the

Table 2. Effect of 2,4-D on mean germination time (MGT) and seedling vigour index I *P. minor*, *A. ludoviciana*, *M. denticulata*, *R. dentatus* and *T. aestivum*

2,4-D concentration (g/ha)	MGT(days)					Seedling vigour index I				
	P. minor	*A. ludoviciana*	*M. denticulata*	*R. dentatus*	*T. aestivum*	*P. minor*	*A. ludoviciana*	*M. denticulata*	*R. dentatus*	*T. aestivum*
2,4-D ethyl ester formulation										
0 (Control)	3.22±0.02	4.43±0.33	3.53±0.25	7.03±0.38	2.13±0.09	1133±55.62	1389±69.74	409±16.93	334±16.88	1584±9.40
5	3.37±0.17	7.87±0.30	8.03±0.58	8.64±0.27	2.24±0.07	443±10.29	487±94.93	74±7.37	113±5.20	661 ±20.48
10	4.19±0.09	6.93±0.22	-	8.66±0.11	2.18±0.03	321±28.38	503±18.80	-	79±4.35	520±46.76
20	6.15±0.14	8.19±0.28	-	10.09±0.38	2.36±0.10	148±8.62	448±32.67	-	59±4.75	339±23.99
40	6.20±0.61	7.85±0.22	-	10.74±0.10	2.47±0.10	90±8.34	387±20.49	-	38±3.00	197±6.76
50	7.65±0.90	8.25±0.46	-	10.85±0.31	2.82±0.12	94±3.36	370±37.85	-	22±1.63	158±17.41
500	-	9.51±0.51	-	-	-	-	143±8.88	-	-	-
LSD (p=0.05)	1.28	1.06	0.73	0.79	0.25	73.93	150.47	21.00	21.91	68.97
2,4-D sodium salt formulation										
0 (Control)	3.30±0.08	4.96±0.26	3.76±0.10	8.19±0.46	2.17±0.09	1478±38.89	667±83.85	451±45.41	285±34.35	1480±129.91
5	3.08±0.02	7.07±0.38	7.84±0.13	9.52±0.16	2.27±0.06	440±17.61	109±48.76	73±11.02	71±7.74	545±22.97
10	3.20±0.04	8.29±0.44	-	9.74±0.12	2.16±0.04	319±12.61	203±38.97	-	53±1.91	388±40.98
20	3.30±0.08	8.46±0.03	-	9.25±0.50	2.40±0.12	271±41.42	184±47.84	-	48±3.07	281±10.03
40	3.62±0.28	8.95±0.33	-	10.44±0.44	2.44±0.10	173±45.36	237±18.95	-	39±3.05	181±25.40
50	4.27±0.51	9.67±0.44	-	11.26±0.54	2.94±0.36	172±18.76	99±30.69	-	21±2.26	113±18.71
500	-	-	-	-	-	-	-	-	-	-
LSD (p=0.05)	0.68	0.96	0.19	1.13	0.46	89.59	138.12	53.57	40.81	162.85

Data are mean ± standard error

laboratory on germination and seedling growth of *T. aestivum* and tested four weed species – *P. minor*, *A. ludoviciana*, *M. denticulata* and *R. dentatus*. The low doses of 2,4-D did not enhance germination/seedling growth of wheat and associated weeds.

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