Indian Journal of Weed Science **51**(4): 352–357, 2019

Print ISSN 0253-8040 Online ISSN 0974-8164

Effect of sub-lethal doses of 2,4-D sodium salt on physiology and seed production potential of wheat and associated dicotyledonous weeds

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Article information

DOI: 10.5958/0974-8164.2019.00074.1

Type of article: Research article

Received : 4 July 2019

Revised : 29 November 2019

Accepted : 3 December 2019

Key words

Chlorophyll content index

Chlorophyll fluorescence

Hormesis

Wheat

ABSTRACT

A field experiment was conducted in *Rabi* 2016-17 and 2017-18 to assess the effect of 2,4-D sodium salt at 5, 10, 20, 40 and 50 g/ha along with the recommended dose of 500 g/ha on wheat and associated dicotyledonous weeds (*Medicago denticulata* and *Rumex dentatus*). Foliar applied 2,4-D in the range of 5-20 g/ha increased the chlorophyll content index (CCI) and chlorophyll fluorescence (Fv/Fm) values in wheat as compared to control, demonstrating biphasic dose response (hormetic effect) of this auxinic herbicide. But the stimulatory effect of 2,4-D at low doses was observed only upto flag leaf stage of crop. Grain and straw yield of wheat was not boosted significantly by the low doses of 2,4-D, indicating that growth stimulation by sub-toxic doses of 2,4-D was not sustained over time. The foliar application of 2,4-D at ultra-low doses of 5-50 g/ha did not exert hormetic effect in *R. dentatus* and *M. denticulata* as application of different doses of 2,4-D led to a reduction in seed production potential of these weeds.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely cultivated winter cereal crop of North-Western Plain Zone and Central Zone of India, occupying an area of about 3.50 million hectares with total production of 17.64 million tonnes and average productivity of 5.05 t/ha in 2016-17 (Anon. 2018). In wheat, weed infestation is the major but less recognized constraint, causing 37-50% yield reduction. Burclover (*Medicago denticulata* Willd.) and toothed dock (*Rumex dentatus* L.) are two major dicotyledonous weeds infesting irrigated wheat under the rice-wheat cropping system in the country. Generally, herbicides are used as the cost-effective tool of weed management for reducing early crop-weed competition and achieving higher yields in wheat (Balyan and Malik 2000).

Many chemicals including herbicides are reported to exhibit stimulatory or beneficial effect at ultra-low doses, but they are toxic at higher doses. Ultra-low or sub-toxic doses of many herbicides stimulate the growth and yield of many crop species under field as well as controlled conditions. Such growth stimulating potential (herbicide hormesis) may change the use of herbicides from crop protection to crop enhancement (Cedergreen *et al.* 2007, Velini *et al.* 2008). Due to herbicide drift, some patches of crop and weeds growing at field edges may receive low doses of herbicides (1-10% of recommended field rate). This low or sub-lethal dose of herbicides equalizes to stimulatory dose, resulting in hormesis in crop plants with respect to different physiological and biochemical parameters such as gene expression, enzyme activity, growth, biomass, protein and chlorophyll content (Nadeem *et al.* 2016). Herbicides are also applied at sub-lethal rates due to lateral and vertical movements of nozzle boom and coverage by taller crop plants and/or mulches which cut down the number of spray drops that can reach weeds (Souza *et al.* 2007). Hormetic effect of herbicides depends mainly on the type of herbicide, its dose and time of application, type of crop or weed species, plant development stage and environmental parameters (light, nutrients, temperature, carbon dioxide) and many other management practices. Low doses of herbicides are reported to cause growth stimulation to the extent of 10-15 and 20-30% under field and controlled conditions, respectively (Cedergreen *et al.* 2007, 2009). Use of herbicides to stimulate crop growth may also promote weed growth and may alter intra-weed and crop-weed competition. If crop plants are affected by hormetic effects, the phenomenon of hormesis sometimes becomes beneficial. However, it may interfere in the management of weeds if hormetic effects are produced in weeds. Therefore, growth stimulation due to herbicides under uncontrolled condition often becomes unpredictable (Belz and Leberle 2012).

Hormetic responses have been demonstrated with respect of certain herbicides like glyphosate in maize, soybean, eucalyptus, coffee, chickpea and faba bean (Velini *et al.* 2008, Abbas *et al.* 2015, El-Shahawy and Sharara 2011); fenoxaprop-P-ethyl in littleseed canarygrass (*Phalaris minor*) and wild oats (*Avena fatua*) (Abbas *et al.* 2016). 2,4-D and metribuzin when used at low dose are effective hormetization herbicides for enhancing growth of cotton and corn plants (Allender *et al.* 1997).

Limited research studies are available regarding the effect of herbicide hormesis on crop and weed growth, crop yield and seed production potential of weeds. Most of the studies documented so far are related to the hormetic responses of herbicides on crop growth (Cedergreen *et al.* 2007). It is equally important to examine the effect of ultra-low doses of herbicides on the growth of weeds so far as practical application of hormesis to stimulate crop growth and yield is concerned. 2,4-Dichlorophenoxyacetic acid (2,4-D) is the first commercial herbicide used to control dicotyledonous weeds, and it is also a synthetic auxin and effective plant growth regulator. Studies on the hormetic effect of 2,4-D in wheat and associated weed flora are meager. Hence, the present study was taken up.

MATERIALS AND METHODS

Field experiments were conducted during *Rabi* season of 2016-17 and 2017-18 at the Research Farm, Punjab Agricultural University (PAU), Ludhiana, Punjab. The seedbed was prepared by ploughing the field with a disc harrow, followed by two passes with a field cultivator and two plankings. The seeds of *R. dentatus* and *M. denticulata* were broadcasted uniformly in the field prior to wheat sowing. The wheat variety "*PBW 677*" was sown by hand on 8 November, 2016 and 13 November, 2017 using a seed rate of 100 kg/ha, in 22.5 cm spaced rows. Uniform density of *M. denticulata* and *R. dentatus* was maintained by keeping fifteen plants of each weed species in individual experimental plot of 4.0 x 2.5 m. Other weeds emerging in the field were regularly uprooted from the field. Six treatments including different doses of 2,4-D sodium salt 80% WP (5, 10, 20, 40, 50 and 500 g/ha) were assigned in a randomized complete block design with three replicates. Irrespective of herbicide dosage, it was sprayed as post-emergence at 35 days after sowing of wheat using knapsack sprayer fitted with a flat fan nozzle. Simple water spray as the control treatment was included for comparison.

Visual observations on crop and weed injuries were recorded at 1, 3, 7, 15, 21 and 30 days after treatment (DAT) by comparing with water sprayed control plots. Toxicity was rated on visual scale of 0-10 (0 = no phytotoxicity and 10 = complete mortality). For recording weed biomass, five plants of *M. denticulata* and *R. dentatus* from each plot were uprooted, dried in sunlight and then placed in paper bags for oven-drying at 60°C for 48 hours. Dry weight was taken till constant weight was achieved. Weed control efficiency (WCE) was calculated by using the following formula suggested by Das (2008) and expressed in percentage:

$$\mathrm{WCE} = \frac{\mathrm{DMC} - \mathrm{DMT}}{\mathrm{DMC}} \times 100$$

Where, DMC is the dry matter of weeds in control (unweeded) plot and DMT is the dry matter of weeds in treated plot.

Chlorophyll fluorescence and chlorophyll content index (CCI) were recorded at 1, 3, 7, 15 and 30 DAT, and also at flag leaf stage of wheat from fully expanded apical leaves, using a portable Chlorophyll Fluorometer (Model-OS-30p, Opti-Sciences, Inc.) and portable Chlorophyll Content Meter (Model-CCM-200, Opti-Sciences, Inc.), respectively. The data on chlorophyll fluorescence and CCI were recorded from ten tagged plants of *R. dentatus* and *M. denticulata* at 7, 15, 21 and 30 DAT. For recording the observations on chlorophyll fluorescence, the middle portion of the leaf from which data was recorded was dark-adapted with plastic clips before exposing to the light emitted by the fluorometer. The readings were expressed as Fv/Fm (variable fluorescence/maximum fluorescence).

Spike length, number of grains/spike, grain weight/spike and 1000-grain weight were recorded from ten randomly selected spikes from each plot at three days before crop harvest. Data on grain and straw yield of wheat were also determined at harvest. Five plants of *R. dentatus* and *M. denticulata* were uprooted from each plot at maturity, and the number of fruits and seeds/plant were counted, averaged and expressed as fruit and seed number/plant. Seeds collected from each plot were bulked, cleaned, properly dried and stored at room temperature. A sample of 1,000 seeds of each treatment were counted and weighed.

The results of both the years were pooled before subjecting to ANOVA in randomized block design using statistical analysis software version 9.2 (SAS

2009). Means were separated at α ≤ 0.05 using Fisher's Protected Least Significant Difference (LSD) test (Cochran and Cox 1957).

RESULTS AND DISCUSSION

Effect on phytotoxicity and weed control efficiency

Post-emergence application of 2,4-D sodium salt at different concentrations (5-500 g/ha) did not show any phytotoxic effect on wheat at any stage, indicating that this herbicide formulation is safe to wheat. Application of 2,4-D sodium salt at sub-lethal doses (5-50 g/ha) did not show any phytotoxicity symptoms on *R. dentatus* and *M. denticulata*, whereas its highest dose (500 g/ha) adversely affected growth of both the weed species at 21 DAT, but failed to cause their complete mortality. 2,4-D sodium salt at 500 g/ha provided weed control efficiency (WCE) of about 60 and >75% against *R. dentatus* and *M. denticulata*, respectively, indicating poor herbicidal efficacy against these dicotyledonous weeds (**Figure 1**). The results of 2,4-D sodium salt on crop phytotoxicity were in consonance with the findings of Biswas *et al.* (2016), who reported non-phytotoxic effect due to application of 2,4-D ethyl ester at different doses (225, 450, 675 and 900 g/ha) in wheat. There was a good control of *R. dentatus* with the use of 2,4-D at 500 g/ha (Chhokar *et al.* 2007) and poor control with 2,4-D amine salt at 750 g/ha (Chhokar *et al.* 2015). Likewise, application of 2,4-D ethyl ester at 600 g/ha provided only 60% control of *R. spinosus* in wheat (Singh *et al.* 2011).

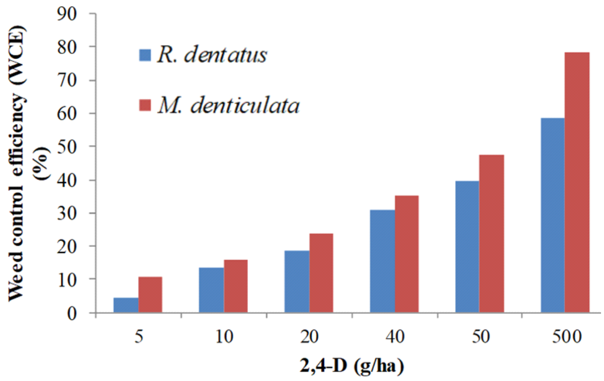

Figure 1. Effect of different doses of 2,4-D sodium salt on weed control efficiency against toothed dock and burclover weeds at 20 days after spray (pooled data of 2016-17 and 2017-18).

According to Grossmann (2010), stimulation of ethylene production due to 2,4-D application played an important role in eliciting herbicidal symptoms in the sensitive plants.

Effect on chlorophyll content index and chlorophyll fluorescence

Low doses of 2,4-D sodium salt (5-20 g/ha) increased the values of chlorophyll content index (CCI) and chlorophyll fluorescence (Fv/Fm) in comparison to control, exhibiting the highest values at the dose of 20 g/ha and hormetic effect of the herbicide at the sub-lethal doses till flag leaf stage (70 DAT) of wheat. 2,4-D sodium salt at 20 g/ha increased the CCI (**Table 1**) and Fv/Fm (**Table 2**) at 30 DAT by about 5.6 and 1.9% respectively as compared to control. There was a

Table 1. Effect of different doses of 2,4-D sodium salt on chlorophyll content index at different stages of wheat (pooled data of 2016-17 and 2017-18)

2,4-D (g/ha)	Chlorophyll content index					
	1 DAT	3 DAT	7 DAT	15 DAT	30 DAT	70 DAT (flag leaf stage)
5	17.3	24.6	26.9	31.3	47.9	49.9
10	19.2	25.2	28.5	33.0	48.3	50.8
20	24.2	28.2	29.8	33.7	49.9	53.6
40	15.5	22.6	24.8	28.9	45.8	47.8
50	14.4	21.7	23.7	28.0	45.0	47.8
500	13.7	13.1	12.6	26.8	43.9	45.5
Control (water spray)	15.9	24.3	26.6	29.8	47.1	49.1
LSD (p=0.05)	2.14	2.46	3.90	1.82	1.72	2.11

Table 2. Effect of different doses of 2,4-D sodium salt on chlorophyll fluorescence (Fv/Fm) at different stages of wheat crop (pooled data of 2016-17 and 2017-18)

2,4-D (g/ha)	Chlorophyll fluorescence					
	1 DAT	3 DAT	7 DAT	15 DAT	30 DAT	70 DAT (flag leaf stage)
5	0.583	0.590	0.661	0.706	0.764	0.685
10	0.588	0.597	0.671	0.709	0.767	0.688
20	0.590	0.605	0.681	0.715	0.771	0.700
40	0.569	0.580	0.642	0.687	0.736	0.673
50	0.562	0.572	0.606	0.663	0.725	0.661
500	0.554	0.532	0.520	0.648	0.720	0.655
Control (water spray)	0.574	0.584	0.632	0.695	0.756	0.683
LSD (p=0.05)	0.012	0.011	0.042	0.029	0.021	0.015

DAT- Days after treatment

constant increase in CCI from 1 to 70 DAT in the unsprayed control as well as with 2,4-D application at the doses of 5-50 g/ha. The lowest value of Fv/Fm and CCI was recorded under the highest dose of herbicide (500 g/ha) at all the dates of observation.

Application of different doses of 2,4-D sodium salt caused a decrease in CCI and Fv/Fm of *R. dentatus* (**Table 3**) and *M. denticulata* (**Table 4**) relative to the water sprayed check. The CCI and Fv/Fm values were decreased up to 15 DAT with the herbicide doses of 5-50 g /ha. Such decline was followed by a constant increase up to 30 DAT with the exception of dose level at 500 g /ha, which caused the CCI and Fv/Fm values to further decrease up to the final observation.

The CCI and Fv/Fm values decreased with the increase in herbicide dose, indicating the stress and lack of hormesis imposed by low doses of 2,4-D sodium salt in *M. denticulata* and *R. dentatus* plants. The decrease in chlorophyll content could be due to increased degradation of chlorophyll or by reduction of chlorophyll synthesis (Santos 2004). Herbicide stress might induce decline in chloroplast number (Cakmak *et al.* 2009). Arunrangsi *et al.* (2013) found that application of 2,4-D amine salt to detached basil leaves in the range of 2.16-10.80 g/L resulted in reduction of chlorophyll content, indicating the ability of this herbicide to degrade chlorophyll. In contrast to the results of present study, hormetic effect of metamitron and glyphosate was reported on growth and chlorophyll content of lamb's-quarters (*Chenopodium album*) at sub-lethal doses of these herbicides (Ketel 1996).

Effect on yield attributes and yield of wheat

Being the most important economic component, grain yield of a crop reflects the resultant impact of all

Table 3. Effect of different doses of 2,4-D sodium salt on chlorophyll content index (CCI) and chlorophyll fluorescence (Fv/Fm) at different stages of toothed dock weed (pooled data of 2016-17 and 2017-18)

2,4-D (g/ha)	Chlorophyll content index				Chlorophyll fluorescence			
	7 DAT	15 DAT	21 DAT	30 DAT	7 DAT	15 DAT	21 DAT	30 DAT
5	13.7	12.4	23.4	37.4	0.649	0.643	0.676	0.793
10	13.0	11.9	21.8	36.6	0.636	0.629	0.650	0.787
20	11.5	10.6	20.4	34.5	0.626	0.619	0.636	0.778
40	11.2	10.5	20.2	33.1	0.617	0.597	0.624	0.775
50	10.5	9.9	19.6	22.8	0.604	0.587	0.610	0.770
500	10.2	9.2	8.9	8.6	0.570	0.563	0.462	0.412
Control (water spray)	16.1	21.3	27.4	43.1	0.658	0.659	0.695	0.796
LSD (p=0.05)	1.46	1.72	4.61	5.00	0.027	0.041	0.028	0.039

DAT-Days after treatment

Table 4. Effect of different doses of 2,4-D sodium salt on chlorophyll content index (CCI) and chlorophyll fluorescence (Fv/Fm) at different stages of burclover weed (pooled data of 2016-17 and 2017-18)

2,4-D (g/ha)	Chlorophyll content index				Chlorophyll fluorescence			
	7 DAT	15 DAT	21 DAT	30 DAT	7 DAT	15 DAT	21 DAT	30 DAT
5	17.1	15.3	26.5	30.4	0.672	0.661	0.676	0.720
10	15.8	14.6	24.1	26.9	0.671	0.655	0.669	0.708
20	15.7	14.0	22.4	24.5	0.652	0.646	0.665	0.703
40	15.1	13.3	21.1	23.4	0.632	0.622	0.652	0.686
50	14.5	12.7	19.2	21.8	0.617	0.615	0.646	0.677
500	14.2	12.3	11.7	9.9	0.605	0.566	0.464	0.391
Control (water spray)	18.0	23.9	28.3	34.2	0.677	0.685	0.692	0.731
LSD (p=0.05)	NS	1.90	1.57	2.67	0.046	0.026	0.059	0.017

Table 5. Effect of different doses of 2,4-D sodium salt on yield attributes and yield of wheat at harvest (pooled data of 2016-17 and 2017-18)

2,4-D (g/ha)	Spike length (cm)	Grains/spike	Grain weight/spike (g)	1000-grain weight (g)	Grain yield (t/ha)	Straw yield (t/ha)
5	10.75	52	2.40	45.84	4.26	8.43
10	10.63	53	2.45	45.14	4.19	8.35
20	10.82	53	2.53	48.82	4.23	8.29
40	11.02	58	2.60	46.87	4.32	8.49
50	11.13	55	2.50	46.74	4.34	8.59
500	11.37	58	2.63	50.17	4.52	8.63
Control (water spray)	10.60	51	2.32	44.73	4.41	8.37
LSD (p=0.05)	0.50	3.58	NS	NS	NS	NS

DAT-Days after treatment, NS- Non-significant crop growth parameters as well as yield attributes as influenced by various treatments. It was evident from the results that the treatments didn't show any significant difference in terms of influencing the grain yield of wheat (**Table 5**). 2,4-D sodium salt at 500 g/ha provided 2.4% higher grain yield as compared to control. Although no significant differences were noted, the crop plants in the plots treated with 2,4-D sodium salt at 500 g/ha produced longer spikes and bold grains having higher 1000-grain weight and number of grains/spike, while minimum spike length and 1000-grain weight was produced by the plants in water sprayed control.

The results of present study clearly indicated that 2,4-D sodium salt did not exhibit any hormetic effect in influencing grain or straw yield of wheat. Similar to the present findings, Cedergreen *et al*. (2009) observed that glyphosate application in the range of 4-63 g/ha did not affect grain production by influencing the grain number and grain weight in barley. Glyphosate and metsulfuron were reported to increase the biomass by about 25% when used at 5-10% of their recommended field rates, whereas the herbicides *viz*. acifluorfen-sodium, diquat, haloxyfop-P-methyl, MCPA, pendimethalin and terbuthylazine did not promote hormesis, thereby under-estimating the theory of hormesis as a general stress response (Calabrese and Baldwin 2001). Hormesis with glyphosate was not found in the growth of coffee plant at the doses of 57.6-460.8 g/ha. However, a significant decrease in plant height, leaf area, stem and leaf dry mass, and root density was observed as well as decline in the nutrient content of leaf were verified (Franca *et al*. 2010). In contrast to the results of present study, many studies showed that some herbicides at low doses promoted crop growth. Low doses of glyphosate application were reported to increase yield of many crops like faba bean, soybean and chickpea (Abbas *et al*. 2015, Silva *et al*. 2015, El-Shahawy and Sharara 2011). Although some studies revealed herbicide-induced hormetic effect in different crop species by improving their growth and yield (Cedergreen *et al*. 2009) but the unpredictability of hormetic response might be a question for its practical implementation in the field to increase growth and yield of different crop species.

Effect on seed production potential of weeds

The seeds of *R. dentatus* were enclosed in acutely trigonous brown nut (fruit) with one seed/fruit, and the seeds of *M. denticulata*, were enclosed in coiled pods called burs (fruit) with 3-5 seeds/pod. The results of fruit and seed production ability of *R. dentatus* and *M. denticulata* showed that the application of 2,4-D sodium salt at different doses led to a numerical reduction in fruits and seeds/plant relative to the unsprayed control (**Table 6**). The number of fruits/plant decreased with the increase in herbicide dose from 5 to 500 g /ha. Application of 2,4-D sodium salt at and above 10 and 20 g/ha caused a significant decrease in fruit number/ plant of *M. denticulata* and *R. dentatus*, respectively. In response to 2,4-D application at the highest dose of 500 g/ha, the plants of *R. dentatus* and *M. denticulata* produced 76.3 and 39.6% lesser number of seeds/plant as compared to their respective controls. Even though the treatments didn't show any significant difference from each other in terms of influencing the 1000-seed weight of *R. dentatus* and *M. denticulata*, the highest 1000-seed weight was observed for the seeds collected from water sprayed control plots while 2,4-D application at 500 g/ha recorded the lowest 1000-seed weight.

2,4-D sodium salt at ultra-low doses of 5-50 g/ha failed to exhibit any hormetic effect in *R. dentatus* and *M. denticulata*. The seed production potential of *R. dentatus* and *M. denticulata* decreased consistently with the increase in 2,4-D concentration from 0 to 500 g/ha. In contrast to the results of

Table 6. Effect of different doses of 2,4-D sodium salt on seed production potential and ancillary attribute of toothed dock and burclover weeds at harvest (pooled data of 2016-17 and 2017-18)

2,4-D (g/ha)	Weed species			1000-seed weight (g)	
	R. dentatus	*M. denticulata*		*R. dentatus*	*M. denticulata*
	Seeds/plant	Fruits/ plant	Seeds/plant		
5	2627	154	617	2.27	3.43
10	2548	139	557	2.15	3.30
20	2381	128	511	2.23	3.40
40	2422	130	521	2.22	3.37
50	1750	112	448	2.25	3.35
500	655	101	403	2.13	3.31
Control (water spray)	2766	167	667	2.35	3.50
LSD (p=0.05)	357	18	71	NS	NS

NS- Non-significant

present study, Nadeem *et al*. (2016) reported that foliar application of glyphosate at low doses in range of 4-32 g/ha resulted in stimulation of root and shoot growth, dry biomass and increase in seed number/ plant of different weed species (*Coronopus didymus*, *Chenopodium album*, *Rumex dentatus* and *Lathyrus aphaca*). On the other hand, glyphosate application at high dose of 64 g/ha significantly declined seed number/ plant in all the weed species. Abbas *et al*. (2016) reported that low dose of fenoxaprop-p-ethyl in the range of 1-6 g/ha produced hormetic effect in *Phalaris minor* and *Avena fatua*. They reported 28 and 17% increase in seed number/ plant in *P. minor* and *A. fatua*, respectively in response to fenoxaprop-P-ethyl application at ultra-low dose of 6 g/ha.

Low doses of 2,4-D sodium salt (5-20 g/ha) increased the CCI and Fv/Fm values in comparison to control, exhibiting the hormetic effect of this herbicide at sub-lethal doses upto flag leaf stage of wheat. But no significant difference was observed in grain and straw yield of control and treated plots at harvest, indicating that growth stimulation by sub-toxic doses of 2,4-D was not sustained over time. Foliar application of 2,4-D at low doses of 5-50 g/ha did not exert hormetic effect in *R. dentatus* and *M. denticulata* in terms of seed production potential of these weeds.

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