Effect of Herbicides on Pollen Germination and Pollen Tube Growth in Vitro

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Abstract **- The pollen of weed Sida acuta Burm.f. were germinated in different concentrations of herbicides by adopting 'Hinging drop' technique. The effects of herbicides on pollen germinations and pollen tube growth were observed till the lethal dose was found. The pollen germination and pollen tube growth gradually decreased with the increase in concentration of herbicide.**

The lethal doses for pollen germination were observed at 50, 50 and 50 ppm of 2,4-D, stomp and glyphosate, respectively. At these concentrations the bursting observed. So there was no germination of pollen and no growth of pollen tube found.

The percentage of pollen germination prior to lethal dose found at 40, 40 and 40 ppm were 43.75, 59.09 and 46.00 micron, respectively with pollen tube length counted was 62.50, 62.50 and 79.21 micron, respectively in 2,4-D, stomp and glyphosate, respectively against 84.32 % pollen germination with 287.50-micron pollen tube length in control.

At the time of pollen germination and pollen tube formation, abnormalities like bursting a swelling were the common at all concentrations of herbicides. 2,4-D was more effective than stomp and glyphosate for inhibiting the pollen germination and pollen tube growth.

Index Terms **- Herbicide, Pollen germination, Pollen tube, Sida acuta.**

INTRODUCTION

Weeds are harmful to every living organism directly or indirectly. Weeds have been reported to be quite harmful as they create health hazards both to the animals and human beings. It is very dangerous to field crops. It is one of the factors that reduce the crop yield. Weeds are responsible to cause allergies like hay fever, itching and poison irg. Weeds may poison or seriously slow down the weight gains of the livestock. They are found along the roadside, railway rout, gardens, lawn, play grounds, in irrigation and drainage system and farm. They have tremendous growth; gain more nutrients and water form soil. The cultivated plants compete with that weed around it for soil, sunlight, nutrients, water and space. So it is very necessity to control the weeds. There are various methods of controlling it such as hand pulling, hoeing, spuldding, tillage and mowing. Among them, herbicide is one of the suitable and easiest methods to control the weeds. In order to have proper scientific knowledge of weed control, it is necessary to understand the mechanism of herbicides in relation to weeds. For that, it is necessary to observe their effects on protein. Such studies will be helpful to determine the proper dosages of herbicides to be used for eradication of obnoxious weeds.

Jethro Tull (1731) was the first person who used the word 'weed' in his famous book "Horse Hoeing Husbandry". According to him "A weed is a plant growing where it is not desired". Various learned men have defined weeds in different ways; Branchely (1920) defined it as, "A plant that grows so luxuriantly or plentifully that possesses more valuable nutritive property". Cumming (1977) said, "Weeds are plants but hey are weeds simply when they are growing where they are not wanted". Gupta and Lamba (1978) defined weeds as, "Weeds are the plants growing at places and its times when we wanted either some other plants to grow or no plants to grow at all". Thakur in 1984 said, "An undesirable, injurious, unsightly and troublesome plant which interferes with cultivated crop and affect human affairs".

Although India is facing a serious weed control problem unfortunately, it did not receive the attention it deserved. A number of workers have studied the effects of herbicides on various weeds. Most of them pertain to general weed control methods, morphological changes induced by herbicides and economics of methods, etc. But a very little attention has been paid to pollen germination and pollen tube growth.

Sida acuta Burm.f. is a very common and obnoxious weed in the Vidarbha region of Maharashtra state. Sida acuta Burm.f. belongs to family Malvaceae. It is an erect weed that grows on roadsides, plain ground, garden, barren land, corner of fields, etc. It is perennial herb or subshrub, 30-129 cm in height with stellate hairs on the branches. Leaves are lance3olate, glabrous or sparsely stellate-pilose both dorsally and ventrally, rarely with simple hairs; base is obtuse, margin dentate, apex acute, stipule filiform, petiole 4-6 mm. flowers solitary in the axils of leaves. Calyx shallow cup-shaped, connate in the lower half, lobes 5 with caudate. Corolla yellow in colour, prominent stamina column. Fruit nearly globose with 4-9 mericarps, with 2 awns at apex. Seeds are angular.

MATERIALS AND METHODS

The pollen grains were collected from naturally growing plants Sida acuta at the time of anthesis. This experiment had been conducted at laboratory conditions. Tap water and distilled water were used as a medium for determining the maximum percentage of pollen germination and pollen tube length. The tap water medium gave the maximum percentage of pollen germination and pollen tube length was used for the preparation of solutions of all the herbicides of different concentrations.

The stock solution of 1000 ppm of stomp and glyphosate were prepared using tap water. As 2,4-D did not dissolve directly in tap water, it was first dissolved in absolute alcohol (1 gm. in 5 ml alcohol) and subsequently raising the volume upto 1000 ppm solution with tap water. To study the effect of alcohol (995:5), it was also used as medium. The concentration of each herbicide ranging from 1 ppm to 100 ppm was used. Here, 50 ppm was found to be a higher dose for the pollen germination.

The 'Hanging drop' technique (Vasil, 1960) was adapted for pollen germination and pollen tube growth experiment. The germination percentage of pollen and pollen tube length were calculated with respective concentration of freshly prepared aqueous solution of 2,4-D (1 to 50 ppm), stomp (1 to 50 ppm) and glyphosate (1 to 50 ppm). The germination of pollen and tube growth at different solutions were studied from morning 7 O'clock to onwards. After few hours of sowing of pollen germination, the lengths of pollen tubes were noticed at random. The three replications carried out for each experiment.

Results:

The germination percentage of pollen and pollen tube length of Sida acuta Burm.f. in tap water were 84.32 with 287.50 micron. In absolute alcohol-tap water, there was 76.90 percent of germination and 237.50 micron tube length. In water, the maximum pollen germination percentage (84.32) having maximum pollen tube length (287.50) was observed. And therefore, tap water was considered as a control and stock solutions of the herbicides were prepared by using it as a medium.

2,4-D

In 2,4-D, the germination percentage at 1, 5, 10, 20, 40 and 50 ppm was 78.12, 75.00, 64.00, 62.50, 43.75 and 00.00 micron respectively, and the pollen tube length was 200.00, 184.37, 75.00, 70.50, 62.50 and 00.00 micron, respectively (table-1). The gradual decrease in the percentage of pollen germination and pollen tube length was from 1 to 40 ppm of 2,4-D.

Stomp

In stomp, the different concentration of stomp 1, 5, 10, 20, 40 and 50 ppm pollen germination percentage was 82.75, 80.00, 76.00, 63.15, 59.09 and 00.00 micron respectively, and the pollen tube length was 180.78, 168.75, 112.50, 80.35, 62.50 and 00.00 micron, respectively (table-1). At 1 ppm to onward, the pollen germination and pollen tube length gradually decreased with increase in concentration of herbicide.

Glyphosate

In glyphosate, at 1, 5, 10, 20, 40 and 50 ppm germination percentage was 80.76, 61.90, 60.00, 58.50, 46.00 and 00.00 micron, respectively and pollen tube length was 237.50, 133.33, 133.33, 87.50, 79.21 and 00.00 micron, respectively (table-1). Here also, the pollen germination and pollen tube length gradually decrease with increase in concentration of herbicide. At 5 and 10 ppm, it is little bit constant as compared to other i.e. 61.90 and 60.00 with 133.33 and 133.33 micron, respectively. The lethal doses were observed at 50, 50 and 50 ppm of 2,4-D, stomp and glyphosate, respectively. At these concentrations bursting observed, so there was no germination of pollen and pollen tube growth found.

The abnormalities like bursting and swelling of pollen tube at their tips observed. No pollen germination and pollen tube growth observed in distilled water. More swelling and bursting was observed at and above 10 ppm of 2,4-D, stomp and glyphosate. More swelling and bursting was observed on 2,4-D treated pollen tube than stomp and glyphosate at all concentrations.

Table 1: Effect of herbicide on pollen germination and pollen tube growth in vitro.

Herbicides	Conc. in ppm	Percentage of germination	S.E. (±)	Pollen tube length in micron	S.E. (±)	Abnormalities
Medium	Tap water	84.32	0.50	287.50	0.23	Rarely bursting
2,4-D	1	78.12	1.65	200.00	8.66	Bursting
	5	75.00	0.47	184.37	0.29	-do-
	10	64.00	0.47	75.00	1.24	Swelling and Bursting
	20	62.50	0.23	70.50	0.23	Bursting
	40	43.75	0.11	62.50	0.23	-do-
	50	00.00	0.00	00.00	0.00	-
Stomp	1	82.75	1.31	180.78	0.36	Rarely bursting
	5	80.00	0.47	168.75	0.58	Bursting
	10	76.00	1.24	112.50	1.17	-do-
	20	43.15	1.81	80.35	0.73	Swelling and Bursting
	40	59.09	0.47	62.50	1.18	Bursting
	50	00.00	0.00	00.00	0.00	-do-
Glyphosate	1	80.76	1.44	237.50	0.23	-
	5	61.90	0.89	133.33	0.62	Rarely bursting
	10	60.00	1.41	133.33	1.24	Bursting
	20	58.50	0.23	87.50	0.44	-do-
	40	46.00	1.16	79.21	0.37	Swelling and Bursting
	50	00.00	0.00	0.00	0.00	Bursting

DISCUSSION

In the present study, pollen germination was found more in tap water than in distilled water or in different concentrations of sucrose solution. Hence, tap water was considered as control and medium for further investigation. In the present investigation, alcohol did not affect the rate of germination and tube length for that alcohol solution (995:5). It was observed that drop of alcohol present in tap water did not change the germination percentage of pollen and pollen tube growth. The pollen germination was maximum in control i.e. in tap water. Banergi and Ganguli (1937) reported 94 percentage of pollen germination in tap water in Eichhornia crassipes; Dharurkar (1974) also reported 93 percentage pollen germination in Eichhornia crassipes in tap water. Srinivasu (1986) observed maximum germination percentage in tap water of Parthenium hysterophorus pollen. Similarl observation are made by Kamble (1999) on Hibiscus cannabinus, Dudhe (2002) on Hyptis suaveoluns and Kamble Sanjay (2006) in Hibiscus cannabinus.

2,4-D

In 2,4-D, the percentage of pollen germination decreased gradually and it was 84.32, 78.12, 75.00, 64.00, 62.50, 43.75 and 00.00 percent at control, 1, 5, 10, 20, 40 and 50 ppm, respectively. It was seen that the percentage of pollen germination was zero at 50 ppm. Therefore, this dose was considered as lethal dose. Similar results were observed by Gentle and Gallangher (1972) treated 32 pesticides including 2,4-D in vitro germination in Petunia hybrid and reported majority of them, reduced pollen germination and few of them totally inhibited the pollen germination. Patil (1978) reported inhibitory effect on pollen germination of Catheranthus rosea at 24 ppm of 2,4-D. Indar (1982a,b) observed inhibition of pollen germination in Brunfedsia, Solanum and Datura after 2,5-D treatment. Srinivasu and Bakale (1986b) reported 200 ppm of 2,4-D inhibited the pollen germination in Parthenium hysterophorus. Some researchers like Gopal (1993) in Medicago sativa, Jain (1993) in Chenopodium album, Bobde (1993) in Crotalaria juncea, Kulkarni (1998) in Crotalaria

medicaginea var. laxurians, Tulankar (1998) in Amaranthus lividis, Kamble (1999) in Hibiscus cannabinus, Dudhe (2002) in Hyptis suaveoluns and Kamble Sanjay (2006) in Hibiscus cannabinus reported the reduction of percentage of pollen germination, Reddy and Neelima (2006) reported completely inhibited the pollen germination of Vinca rosea at 20 ppm of 2,4-D and Guo et al. (2008) in Solidago canadensis.

In the present study, pollen tube length also decreased as the concentrations of 2,4-D increased. It was 287.50, 200.00, 184.37, 75.00, 70.50, 62.50 and 00.00 microns at control, 1, 5, 10, 20, 40 and 50 ppm, respectively. At higher concentration of 2,4-D on abnormalities like bursting of pollen and bursting of pollen tube were observed whereas it was rare phenomenon at lower concentrations. The bursting of pollen tube might be due to expanding of cytoplasmic contents which immediately come out with force from tips of the pollen tubes due to higher concentrations of herbicide. Similar results were reported by Rosen (1961) was noticed that bursting in pollen of Lolium longifolium might be due to change in pH, turgor pressure and extensibility of pollen and pollen tubes, Choudhary and George (1962) in brinjal and Dharurkar (1974) in Eichhornia crassipes reported that bursting of pollen was probably due to softening of hardened pollen tube tip by herbicides, Patil and Rahman (1978) in Martyania annua observed bursting might be due to disturbance of herbicides in protein synthesis, Patil (1978) in Catheranthus rosea, Srinivasu and Bakale (1989b) in Parthenium hysterophorus, Jain (1993) in Chenopodium album, Bobde (1993) in Crotalaria juncea, Gopal (1993) in Medicago sativa, Kulkarni (1998) in Crotalaria medicaginea, Tulankar (1998) in Amaranthus lividis, Kamble (1999) on Hibiscus cannabinus, Dudhe (2002) in Hyptis suaveoluns, Kamble Sanjay (2006) in Hibiscus cannabinus, Reddy and Neelima (2006) in Vinca rosea and Guo et al. (2008) in Solidago canadensis.

Stomp

The stomp was found to be efficient herbicide. In the present experiment, by application of stomp, the percentage of pollen germination was decreased gradually. It was 84.32, 82.75, 80.00, 76.00, 63.15, 59.09 and 00.00 percent at control, 1, 5, 10, 20, 40 and 50 ppm, respectively. It was observed that the percentage was zero at 50 ppm. Therefore, this dose was considered as lethal. Jain (1993) in Chenopodium album and Dudhe (2002) in Hyptis suaveoluns reported reduction in percentage in pollen germination.

Similarly, the growth of pollen tube length decreased gradually. It was 287.50, 180.78, 168.75, 112.50, 80.35, 62.50 and 00.00 microns at control, 1, 5, 10, 20, 40 and 50 ppm, respectively. Jain (1993) in Chenopodium album and Dudhe (2002) in Hyptis suaveoluns reported gradual reduction in length of pollen tube. Owing to the treatment of stomp an abnormalities like bursting of pollen and swelling of pollen tubes were observed at higher concentrations. The maximum bursting of pollen tubes at higher concentrations was also observed by Jain (1993) in Chenopodium album and Dudhe (2002) in Hyptis suaveoluns.

Glyphosate

The germination percentage of pollen was decreased gradually due to the application of herbicide. It was 84.32, 80.76, 61.90, 60.0, 58.50, 46.00 and 00.00 percent at control, 1, 5, 10, 20, 40 and 50 ppm, respectively. It was cleared that the percentage of pollen germination was zero at 50 ppm. So this concentration being considered as lethal dose. Workers like Jain (1993) in Chenopodium album, Bobde (1993) in Crotalaria juncea, Gopal (1993) in Medicago sativa, Kulkarni (1998) in Crotalaria medicaginea, Tulankar (1998) in Amaranthus lividis, Kamble (1999) on Hibiscus cannabinus, Dudhe (2002) in Hyptis suaveoluns, Thomas et al. (2004) in Zea mays, Kamble Sanjay (2006) in Hibiscus cannabinus, and Guo et al. (2008) in Solidago canadensis reported reduction of percentage of pollen germination due to application of glyphosate.

In the present study, pollen tube length also decreased gradually as the concentration was increased. It was 287.50, 237.50, 133.33, 133.33, 87.50, 79.21 and 00.00 micron at control, 1, 5, 10, 20, 40 and 50 ppm, respectively. Abnormalities like bursting of pollens, curvature of pollen tubes occurred due to application of glyphosate. Similar results were observed by Jain (1993) in Chenopodium album, Bobde (1993) in Crotalaria juncea, Kulkarni (1998) in Crotalaria medicaginea, Tulankar (1998) in Amaranthus lividis, Kamble (1999) on Hibiscus cannabinus, Dudhe (2002) in Hyptis suaveoluns, Thomas et al. (2004) in Zea

mays, Kamble Sanjay (2005) in Hibiscus cannabinus, and Guo et al. (2008) in Solidago canadensis.

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