

# **Plant Archives**

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# GROWTH AND PRODUCTIVITYOF INDIAN MUSTARD (*BRASSICA JUNCEA* L.) AS INFLUENCED BYDIFFERENT PRE AND POST EMERGENT APPLICATION OF HERBICIDES UNDER FOOTHILLS OF SHIVALIK HIMALAYAN, INDIA

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Field experiment was conducted during Rabi season of 2019-2020 at G.B. Pant University of Agriculture & Technology, Pantnagar (Uttarakhand) for exploration of weed management module for Indian mustard (Brassica juncea L.). The experiment was laid out in Randomized Block Design taking three replications of thirteen treatments. The treatments undertaken were T<sub>1</sub>: Pendimethalin @ 1kg a.i./ha (PE), T<sub>2</sub>: Pendimethalin  $@ 0.5 \text{kg } a.i./\text{ha} (P E), T_3$ : Pendimethalin  $@ 1 \text{kg } a.i./\text{ha} (P E) + HW 30 DAS, T_4$ : Pendimethalin @ 0.5 kg a.i./ha**ABSTRACT** (P E)+ HW 30 DAS,  $T_5$ : Oxadiargyl @ 0.09kg *a.i.*/ha (P E),  $T_6$ : Oxadiargyl @ 0.045kg *a.i.*/ha (P E),  $T_7$ : Oxadiargyl @ 0.09kg a.i./ha (P E) + HW 30 DAS, T<sub>s</sub>: Oxadiargyl @ 0.045kg a.i./ha (P E) + HW 30 DAS, T<sub>s</sub>: Clodinafop @ 0.06kg a.i./ha (Po E), T<sub>10</sub>: Clodinafop @ 0.03kg a.i./ha (Po E), T<sub>11</sub>: Clodinafop @ 0.06kg a.i./ha (Po E) + HW 60 DAS, T<sub>12</sub>: Clodinafop @0.03kg *a.i.*/ha (Po E) + HW 60 DAS and T<sub>13</sub>: Control (Weedy Check). Indian mustard variety NRCHB-101 was sown in a geometry of 30cm × 10cm on 01 November 2019 and harvested on 21 March 2020. Growth attributes like plant height (149.1 cm), dry matter accumulation (37.8 g) and yield attributes like primary branches (4.1), secondary branches (10.7), number of siliquae/plant (186), length of siliquae (4.4 cm), number of seeds/siliquae (14.3), seed weight/plant (12.7 g) and 1000-seed weight (4.77 g) were obtained highest with application of Pendimethalin @1kg a.i./ha (PE) + HW 30 DAS.Seed yield (3373 kg/ha) and harvest index (34.3%) were also influenced by the application of Pendimethalin @ lkg a.i./ ha (PE) + HW 30 DAS compared to other treatments.

Key words: Brassica juncea, Clodinafop, Net return, Oxadiargyl, Pendimethalin, Yield

# Introduction

Indian mustard, a key oilseed crop in India from the Brassicaceae family ranks third most important oilseed crop after soybean and groundnut in India in terms of oil production (Singh *et al.*, 2023). It ranks second in terms of area (25%) and third in production (24%) after Canada and China among all oilseeds and contributes about 6-7% of the world's oilseed production (Rathore *et al.*, 2022). The share of India in rapeseed-mustard growing area of the world is 17.2% but its contribution in

production is just 8.54% (Dash and Shukla, 2023). This gap in area and production is due to various constraints like environmental, technological, economic and organisational (Baghel *et al.*, 2024; Tamboli *et al.*, 2024). Among all these, environmental constraint has a big role to play. It includes both, biotic as well as abiotic factors. In abiotic factors there is temperature, soil, rainfall pattern, frost, wind and nutrient availability. Biotic factors include insects, weeds and disease-causing organisms. Among biotic factors maximum damage is caused by weeds causing 37% damage followed by insects with 29% damage, next to it are insects causing 22% harm, and finally rodents and other pests causing 12% injury to crops. In mustard itself weeds cause a yield loss of about 40% reducing the crop productivity and quality by competition with the crop for available resources like nutrients, sunlight, water and space (Dash and Shukla, 2023). Some of the weeds occurring in mustard fields as reported by DRMR, 2019 are Ageratum conyzoides, Amaranthus spinosus, Anagallis arvensis, Argemone mexicana, Asphodelus tenuifolius, Avenafatua etc. Other than causing yield loss, weeds also have many detrimental effects like, the allelo-chemicals cinnamic and benzoic acid of Argemone mexicana inhibit the germination and seedling vigour of mustard. Another weed, Convolvulus arvensis, binds with the mustard plant and makes harvesting extremely cumbersome. Parasitic weed Orobancheaegyptica causes a loss of about 80-100% in affected field as it is a total root parasite. Apart from direct damage weeds like Chenopodium album, Convolvulus arvensis and Anagallis arvensis also cause indirect damage by acting as alternate host for pathogen Alternaria solani, which causes leaf blight and further reduce the yield (Bal et al., 2014). These evidences show that, weed management is very important to obtain a good yield. The critical period of crop weed competition for mustard is 20-40 DAS (DRMR, 2012). In the later stages as the crop grows its branches, it smothers the weeds. Thus, at later stages the crop itself becomes competitive, and the weed management during critical period can give us the best results. There are various methods available to effectively control the weed population. Among them, the mechanical and manual methods are quite laborious, time consuming and costly. The information available on weed control by integration of pre and post herbicides along with manual hand weeding in the tarai region of Uttarakhand is sparse and inadequate. In view of the above considerations, the present investigation with the objectives of to determine the impact of the herbicidal molecules on yield, oil content in seeds and on economics of mustard was carried out.

#### **Materials and Method**

#### Site specifications

The experiment was conducted during the *rabi* season of 2019-20 in the E-5 (Oilseed Agronomy Block) at Norman E. Borlaug Crop Research Centre of the G. B. Pant University of Agriculture and Technology, Pantnagar, District: Udham Singh Nagar, Uttarakhand (India). Geographical location of Pantnagar is 29°01'20" N latitude and 79°29'20 "E longitude with an altitude of 243.8 m above mean sea level in the foothills of the Shivalik range of the Himalayas, known as *tarai* region. The region lies under warm, humid and subtropical climate.

#### **Experimental details**

The experiment was laid out in Randomized block design (RBD) consisting of thirteen treatments, with three replications. The treatments were, Pendimethalin @ 1kg a.i./ha as PE (T<sub>1</sub>), Pendimethalin @0.5kga.i./ha as PE  $(T_2)$ , Pendimethalin @ 1kg a.i./ha as PE + HW @ 30 DAS (T<sub>2</sub>), Pendimethalin @ 0.5kg a.i./ha as PE + HW @ 30 DAS (T<sub>4</sub>), Oxadiargyl @ 0.090kg a.i./ha as PE  $(T_{\epsilon})$ , Oxadiargyl @ 0.045kg *a.i.*/ha as PE  $(T_{\epsilon})$ , Oxadiargyl @ 0.090kg a.i./ha as PE + HW @ 30 DAS  $(T_{7})$ , Oxadiargyl @ 0.045kg a.i./ha as PE + HW @ 30 DAS (T<sub>o</sub>), Clodinafop @ 0.060kg a.i./ha as PoE at 35 DAS  $(T_o)$ , Clodinafop @ 0.030kg *a.i.*/ha as PoE at 35 DAS  $(T_{10})$ , Clodinafop @0.060kg a.i./ha as PoE at 35 DAS + HW @ 60 DAS ( $T_{11}$ ), Clodinafop @0.030kg *a.i.*/ha as PoE at 35 DAS + HW @ 60 DAS  $(T_{12})$ , Control  $(T_{133})$ . The experimental crop was fertilized uniformly with 120:40:20 kg/ha of N, P and K, respectively. Seeds of mustard variety NRCHB 101 were sown at the rate of 5 kg/ha with gross plot area  $4.2m \times 4.0m = 16.8m^2$  and spacing of 30cm×10cm. The other cultural operations followed the standard package of practices of mustard

## **Herbicide Application**

Water requirement for pre-emergence herbicide application was 750 l/ha, whereas for post-emergence herbicide application it was 450 l/ha. The herbicide solutions were sprayed uniformly in the experimental plots as per treatment with the help of knapsack sprayer. Pendimethalin and Oxadiargyl were applied a day after sowing as pre-emergence herbicide. Clodinafop was applied at 35 DAS as post-emergence herbicide.

# **Observations recorded**

**Growth attributes:** The plant height was measured with the help of a scale at 25, 50, 75 days and maturity from five randomly selected plants from each plot and the values were averaged and expressed in cm. The same five plants were subjected to oven dry at  $65 \pm 5^{\circ}$ C temperature till the constant weight was obtained.

**Yield attributes:** The total numbers of primary, secondary and tertiary branches and Numbers of siliquae were counted by selecting five plants from the tagged plants of the observational rows of each plot at maturity. Length of ten siliquae of the main shoot and the branches was measured with the help of meter scale. Number of seeds in ten siliquae of the main shoot and three different types of branches was counted and their average was worked out. The seeds obtained after threshing the

The second se	Treatments	Plant height (cm)				Plant dry weight (g)			
Ir. No.		25	50	75		25	50	75	
			DAS	DAS	Maturity	DAS	DAS	DAS	Maturity
T <sub>1</sub>	Pendimethalin 1kg a.i./ha (P.E.)	14.0	41.4	118.0	146.9	1.57	13.3	29.6	32.4
<b>T</b> <sub>2</sub>	Pendimethalin 0.5kg a.i./ha (P.E.)	13.1	42.1	116.3	143.5	1.00	10.4	22.1	27.3
T <sub>3</sub>	Pendimethalin 1kg a.i./ha (P.E.) + HW 30 DAS	14.5	47.9	130.4	149.1	1.77	18.8	35.2	37.8
T <sub>4</sub>	Pendimethalin 0.5kg a.i./ha (P.E.) +HW 30 DAS	13.5	44.5	121.7	147.4	1.63	13.3	22.4	29.5
T <sub>5</sub>	Oxadiargyl 0.09kg a.i./ha (P.E.)	13.7	46.3	120.3	143.6	1.33	8.3	23.3	26.1
T <sub>6</sub>	Oxadiargyl 0.045kg a.i./ha (P.E.)	12.7	37.7	116.7	141.6	0.80	6.1	22.4	24.3
T <sub>7</sub>	Oxadiargyl 0.09kg a.i./ha (P.E) + HW 30 DAS	13.8	47.5	123.5	145.0	1.37	16.1	25.9	28.4
T <sub>8</sub>	Oxadiargyl 0.045 kg a.i./ha (P.E.) + HW 30 DAS	13.1	40.3	119.2	144.2	0.87	14.0	24.6	27.7
T <sub>9</sub>	Clodinafop 0.06kg a.i./ha (30 DAS)	11.9	40.5	124.1	137.4	1.40	8.4	21.1	27.8
T <sub>10</sub>	Clodinafop 0.03kg a.i./ha (30 DAS)	12.3	44.4	117.8	133.3	0.77	6.0	20.4	25.4
T <sub>11</sub>	Clodinafop 0.06 kg <i>a.i.</i> /ha (30 DAS) + HW 60 DAS	12.1	47.5	125.2	138	0.80	8.0	27.3	29.8
T <sub>12</sub>	Clodinafop 0.03 kg <i>a.i.</i> /ha (30 DAS) + HW 60 DAS	12.1	44.5	120.2	137	1.10	5.1	26.3	28.1
	Control (Weedy check)	9.3	37.1	111.00	130.7	0.37	2.5	10.3	16.9
	SEm±	0.6	4.1	4.4	5.4	0.11	0.8	2.3	0.4
	CD(P=0.05)	1.9	NS	NS	NS	0.31	2.2	6.8	1.1

Table 1: Plant height (cm) and dry weight (g) as influenced by weed management practices in Indian mustard.

siliquae of tagged plants were separated and total seed weight was recorded in gram. One thousand seeds were counted using electronic seed counter.

**Yield:** Seed yield and stover yield was recorded in kg per plot and finally converted into kg/ha by using the conversion factor.

Harvestindex(%) = 
$$\frac{\text{Economicyield}}{\text{Biologicalyield}} \times 100$$

#### **Statistical Analysis**

The experimental data obtained from various observations on growth parameters, yield attributes, yield and quality were analyzed using standard procedure for Randomized block design with the help of computer having analysis programmer for RBD, programmed and developed by the Department of Mathematics and Statistics, College of Basic Science and Humanities (CBSH). The treatment comparision are made at 5% level of Significance.

# **Results and Discussion**

# **Growth Attributes**

**Plant height:** The plant height increased gradually with the progression of the crop stages and reached its maximum at maturity (Table 1). At 25 DAS, significant differences were observed among the various treatments. The tallest plants were seen in Pendimethalin 1kg *a.i.*/ha + HW at 30 DAS applied plots, which were statistically on a par with all the other Pendimethalin and Oxadiargyl applied plots. The shortest plants were recorded in case of control where no herbicide was applied. At 50 DAS, 75 DAS and maturity, though no significant differences

were found among the treatments, the tallest plants were recorded in Pendimethalin 1kg *a.i.*/ha+HW at 30 DAS compare to control. At 25 DAS due to application of the pre-emergence herbicide Pendimethalin (1kg *a.i.*/ha and 0.5kg *a.i*/ha) and Oxadiargyl (0.09kg *a.i.*/ha and 0.045kg *a.i.*/ha) on the second day after sowing, weeds were controlled effectively due to which the crop did not face much competition in the early stages, and was able to use all the resources efficiently for its growth and development. Similar result was reported by Yadav *et al.*, 2017 and Yernaidu *et al.*, 2022.

Dry matter accumulation: Plant dry weight was affected significantly by various weed management practices (Table 1). The rate of accumulation was maximum from 50 to 75 days stages. At 25 DAS, the significantly maximum dry matter accumulation was recorded with application of Pendimethalin 1kg a.i./ha (P.E) + HW 30 DAS, which remained statistically on a par with that with the application of Pendimethalin 0.5 kg a.i/ha (P.E.) + HW 30 DAS. The same trend followed at 50 DAS and it continued to remain highest subsequently in 75 days stage and maturity. Although at 75 DAS, the lone application of Pendimethalin 1kg a.i./ha (P.E.) was statistically on a par with it. The total dry matter of the plant is credited to the decreased weed population and lesser dry weight of the weeds which resulted in more availability of moisture, light, space and nutrients for the crop. Similar result was reported by researchers like Singh, 2017.

# **Yield attributes**

Number of branches: The number of primary branches per plant recorded at the time of harvest did

 Table 2:
 Branches at harvest, No. of siliquae/plant, Length of siliqua (cm), No. of seeds/ siliqua, Seed weight/plant (g) and 1000 seed weight (g) as influenced by different weed management practices.

Treatments	Primary branches	Secondary branches	No. of siliquae/ plant	Length of siliqua (cm)	No. of seeds/ siliqua	Seed weight/plant(g)	1000-seed weight (g)
T <sub>1</sub>	4.0	8.8	165	4.1	12	9.3	4.70
T <sub>2</sub>	3.7	7.8	144	3.9	11	7.4	4.67
T <sub>3</sub>	4.1	10.7	186	4.4	14.3	12.7	4.77
T <sub>4</sub>	4.0	9.5	177	4.2	12.7	10.6	4.73
T <sub>5</sub>	3.6	5.7	132	3.8	10.0	6.2	4.67
T <sub>6</sub>	3.4	5.2	101	3.5	9.0	4.1	4.63
T <sub>7</sub>	3.8	7.5	152	4.0	11.3	8.1	4.68
T <sub>8</sub>	3.7	7.2	122	3.8	10.7	5.9	4.60
T <sub>9</sub>	3.0	5.0	113	3.2	9.0	4.6	4.53
T <sub>10</sub>	3.0	4.7	97	3.0	7.7	3.4	4.43
T <sub>11</sub>	3.6	5.7	120	3.4	9.3	4.9	4.47
T <sub>12</sub>	3.4	5.0	115	3.2	8.3	4.3	4.46
T <sub>1</sub>	2.9	4.6	84	3.0	7.0	2.5	4.40
SEm±	0.3	0.4	11	0.2	0.6	0.6	0.03
CD(P=0.05)	NS	1.1	31	0.5	1.8	1.6	0.10

not show any significant difference among the various weed management practices (Table 2). However, Pendimethalin 1kg a.i./ha (P.E.) + HW 30 DAS had the highest number of primary and secondary branches. The secondary branches were more than primary branches in numbers in case of all the treatments. Branching in mustard is affected by temperature as well as the resources availability. As weed management helped the crop to effectively absorb and assimilate all the supplied nutrients and water, those plants had copious branching. The lowest number of branches in both the cases was noted in case of Control or the Weedy Check (Yernaidu *et al.*, 2022).

Number of siliquae per plant: Number of siliquae per plant as influenced by weed management practices (Table 2). Most number of siliquae per plant was recorded with application of Pendimethalin 1kg a.i./ha (P.E.) + HW 30 DAS. The lone application of Pendimethalin 1kg a.i./ha (P.E.) and Pendimethalin 0.5kg a.i./ha (P.E.) + HW 30 DAS were statistically on par with it. The lowest number of siliquae per plant, as can be understood, was noted in case of Control or the Weedy Check (Yernaidu *et al.*, 2022). Further, flowering in mustard is temperature dependent but the number of flowers is also affected by branching. Thus, higher the branches more will be the flowering. These results are in line with that obtained by Patel (2013) and Yadav *et al.*, 2017.

**Length of siliqua:** The length of siliqua as affected by various treatments (Table 2). Pendimethalin 1 kg a.i./ha (P.E.) + HW 30 DAS produced the longest siliquae which, however, remained statistically on a par with that produced with the lone application of Pendimethalin 1kg a.i./ha (P.E.) and Pendimethalin 0.5kg a.i./ha (P.E.) + HW 30 DAS. All these treatments were significantly superior the others. On the other side, the shortest siliquae were formed in case of Control (Weedy Check) (Yadav *et al.*, 2017).

**Number of seeds per siliqua:** The number of seeds per siliqua was affected significantly by the treatments (Table 2). The highest number of seeds per siliqua was computed in case of Pendimethalin 1kg a.i./ha (P.E.)+ HW at 30 DAS which is also reported by Bazaya *et al.*, (2017) and Yadav *et al.*, 2017. Application of Pendimethalin 0.5kg a.i./ha (P.E.) + HW at 30 DAS and the lone pre-emergence application of Pendimethalin 1kg a.i./ha remained statistically on a par with it compared to control. An effective translocation of photosynthates along with the length of siliquae per plant affects the number of seeds per siliqua (Yernaidu *et al.*, 2022).

**1000-seed weight:** The recorded values of 1000seed weight showed that it was significantly influenced by different weed management practices (Table 2). The maximum 1000-seed weight was produced with application of Pendimethalin 1kg *a.i.*/ha (P.E.)+ HW at 30 DAS which is in conformity with that documented by Bazaya *et al.*, (2017) and Yadav *et al.*, 2017. Some of the other treatments which were statistically on a par with Pendimethalin 1kg *a.i.*/ha (P.E.)+ HW at 30 DAS are Pendimethalin 1kg *a.i.*/ha (P.E.), Pendimethalin 0.5kg *a.i.*/ha(P.E.)+HW 30 DAS, Pendimethalin 0.5kg *a.i.*/ha (P.E.), Oxadiargyl 0.09kg *a.i.*/ha(P.E.) and Oxadiargyl 0.09kg *a.i.*/ha(P.E)+ HW 30 DAS. Usually the 1000seed weight is generally influenced by genetics. But the variation here could also be due to proper translocation and accumulation of photosynthates in the seeds in case of the effectively weed managed plots.

**Seed weight per plant:** Seed weight per plant showed significant variations due to the various weed management (Table 2). The crop applied with Pendimethalin 1kg *a.i.*/ha (P.E.)+ HW 30 DAS, bore significantly highest seed weight per plant. The lone application of Clodinafop 0.030kg/ha at 30 DAS and the untreated Weeded Check, which did not differ statistically, had the least seed weight per plant. Due to proper weed management in case of Pendimethalin 1kg *a.i.*/ha (P.E.)+ HW 30 DAS, the crop faced low competition for nutrients, light and space resulting in a good amount of economic yield per plant.

# Yield

Seed yield: The seed yield (kg/ha) was significantly influenced by weed management practices (Table 3). Application of Pendimethalin @ 1kg a.i./ha (P.E.) + HW 30 DAS recorded the highest seed yield being significantly superior over all the other weed management practices. Similar results have been reported by Bazaya et al., (2017). The yield recorded with application of Pendimethalin was much higher than what was harvested with the pre as well as post emergence application of other herbicides, due to weed free conditions, which was in accordance with the report by AICRP-RM (2008) and Singh, 2017 compared to control which remained statistically on a par with that of the post-emergence application of Clodinafop. As in case of mustard slow growth during the initial stages leads to dominance of the weeds which later affects the yield, which is also confirmed by Chauhan et al., (2005). Even the hand weeding 60 DAS along with the post-emergence application of Clodinafop 0.03 kg a.i./ ha could not change the prospects of the crop ending up with the Weedy Check statistically.

**Stover yield:** The stover yield (kg/ha) varied significantly due to different weed management practices (Table 3). Comparison among all the treatments showed that Pendimethalin 1kg a.i./ha (P.E.) + HW at 30 DAS showed the highest stover yield. All the other Pendimethalin applied treatments along with Oxadiargyl 0.09kg a.i./ha (P.E.) and Oxadiargyl 0.09kg a.i./ha (P.E.) + HW 30 DAS were statistically on a par with Pendimethalin 1kg a.i./ha (P.E.) + HW at 30 DAS in terms of the stover yield. The lowest stover yield was recorded when no weed management was done (Singh, 2017). Just like in case of the seed yield, it remained statistically on a par with what was produced with the

Table 3:	Seed yield (kg/ha), stover yield (kg/ha), biological
	yield (kg/ha) and harvest index (%) as influenced
	by different weed management practices.

	Seed	Stover	Biological	Harvest	
Treatments	Yield	Yield	Yield	Index	
	(kg/ha)	(kg/ha)	(kg/ha)	(%)	
T <sub>1</sub>	2550	6139	8689	29.9	
T <sub>2</sub>	2306	6124	8430	27.6	
T <sub>3</sub>	3373	6464	9837	34.3	
T <sub>4</sub>	2820	6373	9193	31.6	
T <sub>5</sub>	2055	5765	7820	26.3	
T <sub>6</sub>	1376	4722	6097	23.1	
T <sub>7</sub>	2044	6165	8209	24.9	
T <sub>8</sub>	1932	5186	7118	27.3	
T <sub>9</sub>	1445	4383	5829	24.8	
T <sub>10</sub>	1148	3757	4905	23.4	
T <sub>11</sub>	1659	4495	6154	27.0	
T <sub>12</sub>	1346	3749	5096	26.4	
T <sub>1</sub>	968	2982	3951	24.7	
SEm±	177	407	338	2.0	
CD(P=0.05)	515	1189	986	5.7	

post-emergence application of Clodinafop. Even the hand weeding 60 DAS along with the post-emergence application of Clodinafop 0.03 kg a.i./ ha ended up with the Weedy Check statistically (Meena *et al.*, 2023).

**Biological yield:** Biological yield (kg/ha) is the sum total of the stover and the seed yields (Table 3). It was significantly influenced by different weed management techniques. Application of Pendimethalin 1kg *a.i.*/ha (P.E.) + HW 30 DAS produced the highest biological yield as it also had the highest stover and seed yields. Pendimethalin 0.5kg *a.i.*/ha (P.E.) +HW 30 DAS was also statistically on a par with it. The lowest biological yield was noticed in case of control which was statistically on a par with the post-emergence application of Clodinafop 0.030kg *a.i.*/ha at 30 DAS (Meena *et al.*, 2023; Singh, 2017).

**Harvest index:** Application of Pendimethalin 1kg a.i./ha (P.E.) + HW 30 at DAS had the highest harvest index (%) being statistically on a par with that obtained by the combo of Pendimethalin 0.5kg a.i./ha (P.E.) + HW 30 DAS and the lone pre-emergence application of Pendimethalin 1kg a.i./ha (Table 3). Due to weed free conditions during the critical period of weed competition resulted in good vegetative and reproductive stages leading to a good economic yield (Meena *et al.*, 2023). All the remaining treatments were statistically on a par with each other along with the Weedy Check, which had the least harvest index.

# Conclusion

Based on the findings of the present study, it can be concluded that application of Pendimethalin 1kg a.i./ha(P E) + HW 30 DAS performed well for all the parameters undertaken in the study. Pendimethalin can be recommended as the herbicide for weed management in mustard which is highly economical and manages the weeds properly at early stage during the critical period of weed control. Effective weed management helped the crop for better vegetative and reproductive growth, and the quality of the economic part also enhanced as compared to other treatments. No phytotoxic effect of the herbicide was noticed in germination or the earlier stages or later in the maturity stages.

**Conflict of Interest:** Authors declares no conflict of interests.

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