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Effectiveness of herbicides against weeds and their residual effect on soil and carrot (*Daucus carota*)

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ABSTRACT

An experiment was conducted to study the efficacy of different herbicides for weed control in carrot (*Daucus carota* L.) and their residual effect on soil as well as on crop, at Research Farm of CCS Haryana Agricultural University, Hisar (2012–13 and 2013–14). Thirteen treatments includes pre-emergence application of three herbicides, viz. oxyfluorfen and oxadiargyl (80, 100, 120 g/ha and 100 g/ha + HW), and pendimethalin (1.0 kg/ha and 1.0 kg/ha + HW), two weedings at 30 and 60 DAS, weed free and weedy check (control) are replicated three times in Randomized Block Design. Residues of these herbicides were estimated in soil and root samples, using GC-MS/MS. Based on two years pooled data it was concluded that pre-emergence application of oxadiargyl 100 g/ha, oxyfluorfen 100 g/ha and pendimethalin 1.0 kg/ha integrated with one hand weeding at 30 DAS resulted in effective weed control (70–72%) in carrot without any phytotoxicity on crop and gave 79.5, 77.2 and 76.4% higher root yield over weedy check, respectively. The residues of these herbicides in soil, carrot root as well as in foliage were found below detectable level at the time of harvest.

Key words: Carrot, GC-MS/MS, Oxyfluorfen, Oxadiargyl, Pendimethalin, Phytotoxicity

Carrot (*Daucus carota* L.) is an important root vegetable cultivated in temperate countries during spring and summer while in tropical and sub-tropical region during winter. It is an annual herb for root production and biennial for seed production. Carrot is an important member of Umbelliferae family, which produce thick fleshy edible tap root of 5–30 cm long (Sulaeman *et al.* 2001). Carrot is grown all over the world, in India the main growing states are Uttar Pradesh, Assam, Karnataka, Andhra Pradesh, Punjab and Haryana. Carrot is used as raw, cooked and preparation of pickles, beverage named *kanji*, etc. It is precious for their taste, good digestibility, high contents of vitamin A, fibers and full with β-carotene that has been used as powerful antioxidant.

There are many reasons for low productivity of carrot in India and among them weeds are one of the most important. The practice of liberal use of FYM and fertilizers, and frequent irrigation encourages the luxuriant growth of weeds in carrot particularly during the initial growth stages. Weed causes up to 57–67% losses in carrot root yield (Farag *et al.* 1994) and reduced the size of their roots through direct competition for nutrients, space and water and, also deform the roots and making them unmarketable (Dittmar and Stall 2012). Manual weed control in carrot is a costly and exhausting operation (Mani *et al.* 1968). Chemical control of weeds in carrot is scanty and very little work has been done on this aspect. While some herbicides recommended for carrot caused phyto-toxic effects in crop.

Persistence of herbicides residues in soil can result in phyto-toxic effects over succeeding crop, contamination of ground and surface water through leaching and surface movements. On the other hand, persistence of residues in edible parts of crop products can result in several severe health hazards in humans and animals. Their bio-magnification at various trophic levels has produced an alarming situation during last decades. Thus, it is essential to know the ultimate fate of the herbicides in soil, crop products and foliage before making any recommendations to farmers and future policies.

MATERIALS AND METHODS

Description of the study area: The experiment were conducted during *rabi*, 2012–13 and 2013–14 at Vegetable Research Farm, CCS Haryana Agricultural University, Hisar, which is situated in the semi-arid, sub-tropical region of north western India in Haryana state. The elevation of this Site is 215.2 m above sea level and lies at 29°10’ N latitude and longitude 75°46’ E. The climatic tract of this region is hot and dry winds during summer and dry, severe cold in winter. The total rainfall as well as its distribution in the region is subjected to large variation. About 80–90% of the total rainfall (400 mm) is received

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through southwest monsoon during July-September. The soil of the experimental field was sandy loam, slightly high in *p*H, low in organic carbon and available nitrogen, medium in available phosphorus and high in available potash. The maximum and minimum temperature as well as the sunshine hours during the crop growth period is shown in Fig 1.

Treatments and experimental design: Thirteen treatments, viz. four levels of oxyfluorfen (80, 100, 120 g/ha and 100 g/ha + one hand weeding at 30 DAS), four levels of oxadiargyl (80, 100, 120 g/ha and 100 g/ha + one hand weeding at 30 DAS) and two levels of pendimethalin (1.0 kg/ha and 1.0 kg/ha + one hand weeding at 30 DAS), two hand weeding at 30 and 60 DAS, weed free and weedy check. All the herbicides were applied as pre-emergence, 4–5 days after sowing of seeds. Each experimental unit consisted of a bed of 3.0 m × 3.0 m, containing 10 rows of plants spaced 30 cm × 6 cm. The eight central rows were harvested as useful plants without the border plants.

The pooled data (Table 1) were the mean values of different parameters. The statistical method described by Panse and Sukhatme (1961) was followed for the analysis of variance and interpretation of experimental results. All the tests of significance were made at 5% level of the significance. Experimental data of different parameters were analyzed in randomized block design with three replications.

*Crop raising and data recording***:** The experimental field was prepared by one deep ploughing and two harrowing each followed by planking to prepare suitable seedbed. The seeds of carrot variety *Hisar Gairic* was sown on last week of September at the rate of 5 kg/ha. Carrot seeds were small in size and sown directly in the field on top of ridges 2 cm deep by hand and covered with soil. The crop was irrigated uniformly on the need basis. Thinning was done to maintain the proper plant population. Weed management in carrot was done as per treatments. Other cultural practices were followed as per package and practices recommended for the crop. The carrot roots were harvested 90 days after sowing and data were recorded on root and shoot (foliage) length, root diameter, root and shoot yield, number of weeds and dry weight of weeds per square meter, etc. The observation of phytotoxicity of herbicides on carrot crop was recorded 15 days after application of herbicides. The weed control efficacy (WCE) was calculated as;

$$\text{WCE} \frac{\text{Dry wt. of weeds in control treatment} - \text{Dry wt. of weeds in treated treatment}}{\text{Dry weight of weeds in control treatment}} \times 100$$

The most dominant weeds recorded during the crop period were *Portulaca oleracea, Cyperus rotundus, Trianthema portulacastrum, Cynodon dactylon, Coronopus didymus, Chenopodium album, Echinochloa crusgalli* and *Polygonum lapathifolium*.

Herbicides residue study: The proposed study for the residue analysis was carried in the Agrochemicals Residues Testing Laboratory, Department of Agronomy, CCS Haryana Agricultural University, Hisar. Samples of soil, root and leaf were collected in triplicate at crop maturity from experimental trial, conducted for screening of different herbicides.

Chemicals and reagents: The technical grade analytical standard of oxyfluorfen, oxadiargyl and pendimethalin were procured from Fluka Sigma Aldrich, Germany. Other chemicals like n-hexane, ethyl acetate, acetone, analytical grade ammonium carbonate, sodium chloride and sodium sulfate (anhydrous) were purchased from Merck. A standard stock solution of pendimethalin was prepared in n-hexane

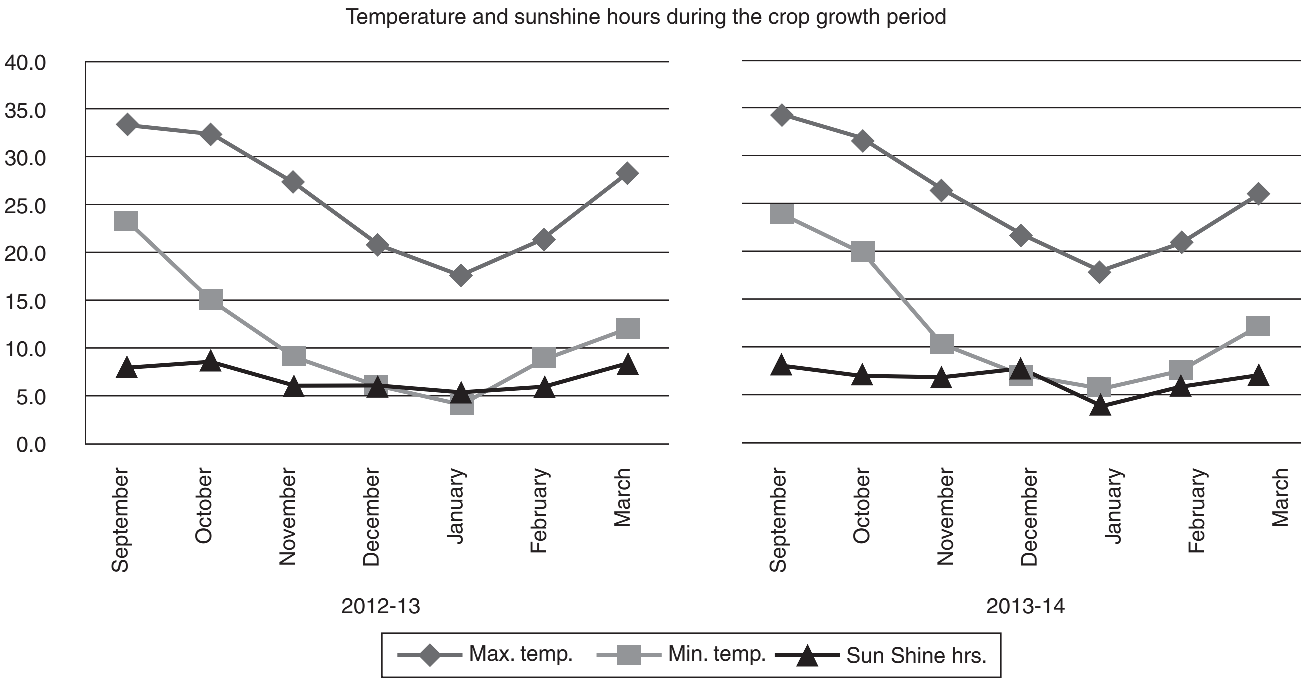

Fig 1 Maximum minimum temperature and sunshine hours during the crop growth period.

Table 1 Effect of different herbicides on growth, root yield, number and dry weight of weed in of carrot cv. Hisar Gairic (Pooled data 2012–13 and 2013–14)

Treatment		Root length (cm)	Shoot length (cm)	Root diameter (mm)	Root yield (q/ha)	Shoot yield (q/ha)	Number of weeds/m2			Dry weight of weed (g/m2)			Weed control efficacy
							30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	
T1	Oxyfluorfen 80 g/ha	22.70F	57.13D	31.23E	214.80F	142.93H	156.00B	125.17B	94.73A	12.93B	37.73B	27.70BC	44.2
T2	Oxyfluorfen 100 g/ha	23.93EF	60.17CD	32.73DE	230.73DEF	176.43F	131.17C	95.50C	90.47A	11.40BCD	30.27CD	23.50CD	52.6
T3	Oxyfluorfen 120 g/ha	25.57BCDE	60.27BCD	33.43DE	248.47CD	186.03DEF	124.60C	95.50C	76.37B	9.93DEF	26.40DE	22.70CD	54.2
T4	Oxyfluorfen 100 g/ha + HW	25.93ABCD	63.47ABC	36.47AB	275.03AB	195.47ABCD	101.40DE	56.00EF	43.17DE	9.03DEFG	11.70F	13.93F	72.0
T5	Oxadiargyl 80 g/ha	24.13DEF	58.90CD	33.20DE	220.63F	157.73G	114.60CD	101.23C	76.67B	12.53BC	34.63BC	32.57B	34.5
T6	Oxadiargyl 100 g/ha	26.23ABC	61.87ABCD	32.97DE	239.93DE	179.50EF	110.90CDE	75.43D	53.97CD	11.30BCD	26.07DE	27.67BC	44.2
T7	Oxadiargyl 120 g/ha	26.40ABC	63.17ABC	33.90CD	247.50CD	190.00CDE	95.70DEF	70.07D	47.50D	10.30CDEF	22.10E	19.90DE	59.9
T8	Oxadiargyl 100 g/ha + HW	27.73A	65.90AB	36.57AB	278.63AB	202.70AB	78.50FG	64.00DEF	35.67E	7.87FG	13.20F	14.43EF	71.0
T9	Pendimethalin 1.0 kg/ha	24.83BCDE	59.33CD	32.90DE	228.73EF	176.07F	95.97DEF	75.43D	62.60C	10.63BCDE	32.47BC	24.50CD	50.6
T10	Pendimethalin 1.0 kg/ha+HW	26.20ABC	66.30A	35.97ABC	273.77AB	197.07ABCD	69.60G	53.00F	35.10E	7.27G	13.47F	14.73EF	70.4
T11	Weeding at 30 & 60 DAS	24.60CDEF	62.70ABCD	34.13BCD	260.70BC	200.63ABC	90.57EF	68.93DE	35.17E	8.53EFG	12.20F	9.23F	81.5
T12	Weed Free	26.63AB	64.07ABC	36.77A	279.47A	208.13A	0.00H	0.00G	0.00F	0.00H	0.00G	0.00G	100.0
T13	Weedy check	20.60G	49.77E	26.83F	155.23G	99.70I	387.87A	192.23A	98.50A	69.07A	75.97A	49.63A	0.0
CD (P=0.05)		2.03	4.90	2.55	18.73	12.70	20.79	13.30	11.62	2.40	5.40	5.69	

NB: Any two means having a common letter, are not significantly different at the 5% level of significance.

and those of oxyfluorfen and oxadiargyl was prepared in methanol. The standard solutions required for constructing a calibration curve (0.001–1.0 µg/ml) were prepared from stock solution by serial dilutions using respective solvents. All standard solutions were stored at -20°C before use.

Extraction and clean-up: For extraction and clean-up of oxyfluorfen, oxadiargyl and pendimethalin, a representative 20 g soil, 20 g meshed carrot roots and 20 g meshed foliage samples were taken in 250 ml conical flask and extracted with 50 ml of acetone over rotary shaker for one h. The contents were decanted in separate conical flask by passing over a bed of anhydrous Na_2SO_4 and concentrated to about 10 ml over rotary evaporator at 35°C. The samples were partitioned with hexane:ethyl acetate (9:1) thrice by taking 50, 30 and 20 ml after adding 50 ml saturated brine solution. The organic phases were collected by passing over Na_2SO_4 in a separate bottle and concentrated over rotary evaporator to 10 ml at 35°C. No further clean-up was required for soil samples as the samples were clear and containing no colour. But the carrot root and foliage samples were further cleaned-up by column containing 10 g alumina and 0.5 g charcoal sandwiched between Na_2SO_4 layer above and below. 100 ml of hexane:ethyl acetate (9:1) was used as eluent for each sample. The extracts were collected by passing over anhydrous Na_2SO_4, concentrated up to dryness over rotary-evaporator and the final volume was made with n-hexane in case of pendimethalin, and methanol in case of oxyfluorfen and oxadiargyl for analysis over GC-MS/MS.

GCMS/MS Parameters: The instrument was tunned properly before injection of standard sample (1 ppm) of pretilachlor and pendimethalin. The tunning parameters of GCMS/MS were found in adequate range. For confirmation and quantification a programming was developed in Multiple Reaction Monitoring (MRM) Mode on the basis of m/z ratio and collision energies used in SCAN and PI monitoring. The operating parameters were, injection port temperature; 280°C. Column, HP-5 (30 m × 0.32 mm i.d. × 0.25 µm film thickness) containing 5% diphenyl and 95% dimethyl polysiloxane. Oven temperature ramping was; 70°C (2 min) → @25°C/min → 150°C (0 min) → @15°C/min → 200°C (0 min) →@8°C/min → 280°C (2 min). Detector: Mass 7000 GCMS/MS; detector parameters were: source temperature, 230°C; emission current, 35 µA; energy, – 70 eV; repeller voltage, 11 V; ion body, 12 V; extractor, –7.2 V; ion focus, –7.4 V; quadrupole one (MS^1) temperature, 150°C; quadrupole two (MS^2) temperature, 150°C. Gas flow rates; helium (carrier gas), 1 ml/min though column and 2.25 ml/min as collision flow/quench flow, nitrogen (collision cell), 1.15 ml/min. Other parameters; split ratio, 1:10; vacuum (high pressure), 2.23×10^{-05} torr; rough vacuum, $1.51\times10^{+02}$ torr; injection volume, 2 µl.

The retention time observed for oxyfluorfen was 20.6, oxadiargyl 22.1 and pendimethalin 19.3 min. The confirmation and quantification of oxyfluorfen, oxadiargyl and pendimethalin was performed by SCAN, product ion programming and finally multiple reactions monitoring (MRM). The ions having relatively high intensity and strong anti-turbulence were monitored and selected as quantitative ions (Table 2).

Table 2 Programming parameters for MRM

Compound	Molecular mass	Precursor ion (m/z)	Collision energies	Monitoring ions (m/z) and relative abundance (in brackets)
Oxyfluorfen	362	252, 361, 300	12, 20, 32	300 (13084), 223 (15326), 196 (19691), 170 (17023), 146 (19696)
Oxadiargyl	341	214, 185	10, 20	152 (275), 187 (217), 150 (22170), 123 (4723)
Pendim ethalin	281	281, 252	2, 4, 10, 20	252 (351508), 208 (686737), 191 (506108), 161 (504652)

GCMS/MS Programming: For confirmation and quantification, a programming was developed in Multiple Reaction Monitoring (MRM) Mode based on m/z ratio and collision energies used in SCAN and PI monitoring as per the programming detail given in Table 2.

Calibration details, linearity check and validation of method: Different known concentrations of respective herbicides were prepared by diluting the stock solution as mentioned above and injected into the instruments for measuring the peak area resulting after elution of compound. A calibration curve was plotted for concentration of the standard injected ver. area observed and the curve was found linear up to the lowest range from 0.001–1.0 µg/ml. The method for estimation of selected herbicides residues in carrot root, foliage and soil using GC-MS/MS was validated by performing recovery experiments. A representative 20 g of meshed carrot root, foliage and 20 g of soil sample was taken in 250 ml Erlenmeyer flasks and fortified at 0.005 and 0.01 µg/g spiking levels with standard solution of the herbicides. These flasks were kept undisturbed overnight. On next day, extraction, clean-up and analysis were done according to the procedures mentioned above.

RESULTS AND DISCUSSION

Crop growth and yield parameters: Growth and yield of carrot roots and foliage was significantly influenced by different weed management practices during both years. The pooled data (Table) indicated that application of different herbicides on carrot crop significantly increased the shoot and root length, root diameter, root and shoot yield over the weedy check. Maximum root length (27.73 cm) was recorded in oxadiargyl 100 g/ha + HW (one hand weeding at 30 DAS) followed by weed free, oxadiargyl 120 g/ha, oxadiargyl 100 g/ha, pendimethalin 1.0 Kg/ha + HW and oxyfluorfen 100 g/ha + HW treatment. Minimum root length (20.60 cm) was reported in weedy check plot where no

Table 3 Percent recovery of different herbicides in carrot and soil

Herbicide	Calibration parameter								Average* Recovery(%) ±SD					
	Linearity check		Limits of analysis						Soil		Carrot root		Carrot foliage	
	Regression equation	R^2	Soil		Carrot root		Carrot foliage		Fortification levels		Fortification levels		Fortification levels	
			LOD (µg/g)	LOQ (µg/g)	LOD (µg/g)	LOQ (µg/g)	LOD (µg/g)	LOQ (µg/g)	0.005 (µg/g)	0.01 (µg/g)	0.005 (µg/g)	0.01 (µg/g)	0.005 (µg/g)	0.01 (µg/g)
Oxyfluorfen	66974x+ 413.9	0.959	0.001	0.005	0.003	0.008	0.003	0.01	83.2±1.6	86.5±1.5	81.3±1.5	85.2±0.2	82.2± 1.8	89.1± 1.0
Oxadiargyl	42432x+ 217.1	0.996	0.003	0.009	0.005	0.01	0.005	0.01	84.6±0.4	89.1±1.3	80.6±1.2	83.6±1.7	83.1± 1.1	90.0± 1.7
Pendimethalin	40245x+ 156.5	0.985	0.001	0.003	0.001	0.005	0.001	0.005	80.2±1.1	90.4±0.6	87.3±1.6	94.2±1.1	89.5± 1.4	95.8± 1.3

*Average of three replicates

weeding was done during crop growth period. Among the treatments, application of pendimethalin 1.0 kg/ha + HW, oxadiargyl 100 g/ha + HW, oxyfluorfen 100 g/ha + HW, weed free and two weeding at 30 and 60 DAS recorded better shoot length and shoot yield. This may be due to positive effect of these treatments in controlling weeds and providing micro climate for growth of the plant which ultimately increases shoot yield.

Maximum root diameter (36.77 cm) and root yield (279.47 q/ha) was observed in weed free treatment, which was statistically at par with oxadiargyl 100 g/ha + HW, oxyfluorfen 100 g/ha + HW, pendimethalin 1.0 Kg/ha + HW. Minimum root yield was recorded in weedy check plot. Pre-emergence application of oxadiargyl 100 g/ha + HW, oxyfluorfen 100 g/ha + HW and pendimethalin 1.0 kg/ha + HW gave 79.5, 77.2 and 76.4% higher root yield, respectively, over weedy check (control). More availability of light, water, nutrients and space with reduction in weed population might have enhanced growth and yield attributes resulting in higher root yield. These results were corroborated with Ojowi *et al.* (2013) in carrot and Yumnam *et al.* (2009) in onion.

Weeds parameters: Number of weeds and dry weight of weeds per square meter area recorded at 30, 60 and 90 days after sowing (DAS) was significantly affected by different weed control treatments (Table 1). There was no weed under weed free treatment at all the stages (30, 60 and 90 DAS). This might be due to removing of weeds as and when appeared in this treatment. Based on pooled mean of two years, minimum number of weeds (69.60) at 30 DAS was recorded in pendimethalin 1.0 kg/ha + HW followed by oxadiargyl 100 g/ha + HW, while at 60 and 90 DAS, pendimethalin 1.0 kg/ha + HW, oxadiargyl 100 g/ha + HW and oxyfluorfen 100 g/ha HW recorded minimum number of weeds. However, maximum number of weeds was recorded under weedy check plot at all the stages of crop growth (30, 60 and 90 DAS).

There was no dry weight of weeds recorded in weed free treatment because weeds were removed as and when it appeared in this treatment. Based on two year pooled data, the treatments oxadiargyl 100 g/ha + HW, oxyfluorfen 100 g/ha + HW, pendimethalin 1.0 kg/ha + HW and two hand weeding at 30 and 60 DAS recorded minimum dry weight of weeds at all stages of crop growth (30, 60 and 90 DAS), which was significantly lower than all other weed control treatments. These treatments also controlled 70.4–81.5% weeds over weedy check on dry weight basis at 90 DAS. However, the maximum dry weight of weed at 30, 60 and 90 DAS was reported under weedy check treatment, where no weeding was done during the crop growth periods. The least dry weight of weeds in weed control treatment may be due to efficiency of herbicides in weed control. Similar findings have been reported by Kumar *et al.* (2001), Singh *et al.* (2010) and Ojowi *et al.* (2013) in carrot and Saini and Walia (2012) in onion.

Phytotoxicity: Phytotoxicity refers to the damage in plants caused by exposure to some toxic stressor. It can be evidenced by acute injury such as obviously damaged, necrotic tissues. The observation of phytotoxicity of herbicides on crop was recorded 15 days after application of herbicides visually and no phytotoxic symptoms on crop was observed with application of oxyfluorfen, oxadiargyl and pendimethalin because these are pre-emergence herbicides and applied at right time on the crop.

Herbicides residue studies: Linearity of calibration curve was studied using standard solutions of oxyfluorfen, oxadiargyl and pendimethalin within the range of 0.001–1 µg/ml. Each run was repeated thrice and the detector response was measured in terms of peak areas. A calibration curve was prepared by plotting concentrations of different herbicides in µg on x-axis against average peak area on y-axis. The response was found to be linear with correlation coefficient (R^2) = 0.959, 0.996 and 0.985 for oxyfluorfen, oxadiargyl and pendimethalin, respectively. The limit of detection (LOD) and limit of quantification (LOQ) for these herbicides in different matrix varied from 0.001–0.01 µg/g (Table 3). Recovery experiments were carried out to check the validity of the method in soil, carrot and foliage samples by fortifying the control samples of each matrix at 0.005 and 0.01 µg/g levels in triplicate. The mean recoveries for different matrix ranged from 80.2–95.8% with relative standard deviation (RSD) below 10% (Table 3). Analysis of soil, carrot and foliage revealed that the residues of oxyfluorfen and oxadiargyl were below detectable limit (BDL) at all applied doses (Table 3). But analysis of carrot and soil samples show persistence of pendimethalin residues in carrot and soil as 0.008 and 0.02 µg/g at applied dose. But the residues were below maximum residue limit (MRL) of 0.05 µg/g (US EPA 2012). The pendimethalin residues were not detected in foliage.

Based on two years pooled data it may be concluded that pre-emergence application of oxyfluorfen 100 g/ha, oxadiargyl 100 g/ha, pendimethalin 1.0 kg/ha integrated with one hand weeding at 30 DAS in carrot root crop provided 70–72% control of weeds without any phytotoxic effect on crop and gave 79.5%, 77.2% and 76.4% higher yield, respectively, over weedy check. At harvest, the residues of oxyflurofen and oxadiargyl were not detected. However, pendimethalin residues were found in carrot roots and soil but below its maximum residues limit of 0.05 µg/g.

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