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RESEARCH ARTICLE

Herbicides for weed management in onion and analysis of herbicide residues using liquid chromatography tandem mass spectrometry

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ABSTRACT

Management of weeds is the major challenge to the success of onion. Chemical weed control has been intensively used to reinforce crop yield; however, even selective herbicides can potentially interfere with biochemical and physiological changes in onion. A field experiment was conducted to evaluate the efficacy of different herbicides pendimethalin, oxyfluorfen and quizalofop-ethyl individually and in combinations on weed control efficiency, yield attributes, changes in photosynthetic pigments, membrane injury and persistence of herbicides in onion. The combined application of oxyfluorfen and quizalofop-ethyl efficiently influenced the weed density and biomass causing higher yield and net returns. The application of oxyfluorfen recorded highest weed control efficiency, while a decline in photosynthetic pigments caused lesser yield. The results of QuEChER method of liquid chromatography tandem mass spectrometry (LC-MS/MS) revealed that herbicide residue did not find in both leaves and bulb in any treatment.

Key words: Chlorophyll, Herbicide residue, Liquid chromatography, Onion, QuEChER, Tandem mass spectrometry, Weeds

INTRODUCTION

Onion (*Allium cepa* L.) is an important commercial vegetable crop worldwide and India is the second largest producer of onion after China. In India, Maharashtra state is the leading onion producing state with an area of 4.81 mha, production 37.34 mt and productivity of 14.0 t/ha (National Horticultural Research and Development Foundation 2016-17). In Maharashtra, Nashik is the leading district in area and production of onion (Indian Horticulture Database 2017). Low productivity of onion in the country might be the resultant of a number of factors like poor yielding genotypes, non-availability of quality seeds and poor agronomic practices. Among the agronomic factors, proper weed management may be a serious issue. Onion is a slow growing plant with narrow upright leaves and non-branching habit due to which crop cannot compete well with weeds. In onion crop, weeds not only compete vertically and horizontally for space, but also consume essential nutrients, much needed water and acts as a reservoir for several pathogenic pests and insects due to which yield loss have estimated to the 40-58% (Channapagoudar and Biradar 2007) or even ranging from 40-80% depending upon the type of weed flora and their competitiveness (Prakash *et al.* 2000). There are a number of methods available by which weeds can be controlled effectively like manual methods and chemical weed control. Unlike, the horticultural developed countries, use manual weeding techniques which are time consuming and costly. At the earlier stages of crop weed infestation significantly reduces the bulb yield, the pre-emergent herbicides application may not control the weed population long enough. To optimize the bulb yield, post emergence herbicides control weed population effectively. Therefore, proper weed control is the prime need and essential to obtain maximum productivity and under such circumstances chemical method of weed control has shown good promise.

Improper herbicide application causes phytotoxicity and high dosages causes alterations in biochemical and physiological changes that leads to the formation of reactive oxygen species causing oxidative stress, which has been identified as a consequence of different abiotic stresses including herbicides usage in crops for weed management (Song *et al.* 2007). Although, the reactive oxygen species are inevitable products of plant metabolism under normal circumstances, yet coordinated

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antioxidant defence mechanisms inside plant cells maintain a balance between their synthesis and scavenging (Mittler 2002). While, in severe conditions the damage due to reactive oxidative stress cannot overcome (Langaro *et al.* 2017). Under such circumstances, one of the protection mechanisms is the enzymatic antioxidant defence mechanism, which operates with sequential and simultaneous actions of several anti-oxidative enzymes including superoxide dismutase, ascorbate peroxidase and catalase. In the non-enzymatic antioxidant system includes phenolic compounds like ascorbic acid, glutathione, chlorophylls, carotenoids, proteins and amino acids. Carotenoids pigments are responsible for the photo protection of the photosynthetic membranes, acting as auxiliary pigments. The carotenoids also act in the dissipation of the excited state of chlorophyll and neutralization of reactive oxygen species (Kreslavski *et al.* 2009).

Farmers are using wide range of herbicides in onion growing pockets, among these pendimethalin, oxyfluorfen and quizalofop-ethyl are very new chemical substances in Nashik region of India. Due to improper application of these herbicides, the chemical residue may persistent in bulb may become an important issue in major onion growing and export zone of India. Different herbicides detected in some food products and estimated its concentration by Gas chromatography (GC) due to its high selectivity and high sensitivity for thermo-stable and volatile molecules (Xin *et al.* 2009). However, it is limited because nowadays commonly used pesticides are polar, less volatile and /or thermo-labile compounds, which are not directly traceable by GC (Fernandez *et al.* 2001). Most of these polar pesticides like fungicides, carbamates and herbicides in vegetables can be efficiently separated by liquid chromatography (LC) without a preceding laborious risk. Recent developments in the detection and separation by LC have extended its application in pesticide residue analysis (Choi *et al.* 2001). High performance liquid chromatography and Tandem mass spectrometry (LC-MS/MS) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample extraction method was used to determinate herbicides for fruits and vegetables (Wilkowska and Biziuk 2011). It was found that it is the best method for determination of herbicides in some food products in terms of high recovery, short time of analysis, low cost and safety (Renata 2014). Therefore, in this study, we evaluated optimised and validated by the QuEChERS procedure for the determination of three herbicides residues in onion. The study was supposed to provide scientific evidence and implement recommendations with chemicals for management of weeds in onion with minimum toxic effects and environmental safety.

MATERIALS AND METHODS

The present investigation was carried out at the research farm of Regional Research Station, National Horticultural Research and Development Foundation (NHRDF), Nashik, Maharashtra, India, during *Kharif* 2015, 2016 and 2017. The experimental site is located at an altitude of about 492 m above sea level, latitude of 20' N and has longitude of 73º 57' E. The agro-meteorological data of experimental site during the cropping period has given in **Figure 1**. The experiment was laid out in randomized block design with three replications. The fifty days old seedlings of onion variety '*Agrifound Dark Red*' developed and released by NHRDF were transplanted in mid of August during three seasons under drip irrigation system. Three herbicides were used in this experiment, which includes pre-emergence herbicide pendimethalin [N-(1-ethylpropyl)-3, 4-dimethyl 1-2,6 dinitrobenzenamine], pre- and post-emergence herbicide oxyfluorfen [2-chloro-4 (trifloromethyl) phenyl-3-oxy-4- nitrophenol ether] and post-emergence herbicide quizalofop-ethyl [(R)-2-[4-(6-chloroquinoxaline -2- phenoxy]-ethyl propionate] with different combinations as tank mixture and applied to the onion crop two times, the first application was done at pre-transplanting (pre-emergence of weed and second application was done 30 days after transplanting (DAT). The treatments includes oxyfluorfen 23.5% EC 1.5ml/l of water at pre-transplanting and second at 30 DAT, oxyfluorfen 23.5% EC1.5 ml/l of water at pre-transplanting + quizalofop-ethyl 5% EC 3.0 ml/l of water at 30 DAT, combined spray of oxyfluorfen23.5% EC 1.0 ml + quizalofop-ethyl 5% EC 2.0ml/l of water at pre-transplanting and second at 30 DAT, pendimethalin 30% EC 5.0 ml/l of water at transplanting and second at 30 DAT, pendimethalin 30% EC 5.0 ml/l of water at pre-transplanting + quizalofop-ethyl 5% EC 3.0 ml/l of water 30 DAT, combined spray of pendimethalin 30% EC 3.0 ml + quizalofop-ethyl 5% EC 2.0 ml/l of water at pre-transplanting and second at 30 DAT, weed free check (three times manual weeding was done at 25, 40 and 55 DAT) and weedy check (No manual weeding and no herbicide application throughout cropping period - kept as control). The required quantity of herbicides was dissolved in water and sprayed with the help of a knapsack sprayer fitted with flat fan nozzle. Soil of the experimental area was deep heavy clay with pH- (7.6), organic carbon (0.75%), available N (374 kg/ha), available P (49.05 kg/ha), available K (414.4 kg/ha), water holding capacity (62.8%), field capacity (38.9%) and permanent wilting point (24.6%).

Data collection and analysis

During the course of the study, data was recorded on various parameters such as weed density (number of weeds/m²) counted based on quadrate of size 1.0 x 1.0 m placed randomly at three sites per plot and weeds growing within this quadrate were counted, fresh weeds biomass (fresh weight of weeds collected from one m² area), for dry weeds biomass (dry weight of weeds collected from one m² area), when fresh weeds were kept in electric oven at 66˚C for 72 ± hr then weighed. Weed control efficiency (WCE) denotes the magnitude of weed reduction due to weed control treatment was calculated by using formula suggested by Mani *et al.* (1973) and expressed in percentage *i.e.* $WCE = \frac{DW1 - DW}{DW1} \times 100$ where; DW_1 is dry weight of un-weeded control and DW is dry weight of treatments. Weed index (WI) was determined by the formula given by Gill and Vijayakumar (1969), *i.e.* $WI = \frac{X-Y}{X} \times 100$, where; X = Total yield from the weed free check, Y = Total yield from the treatment.

Biochemical parameters

The photosynthetic pigments were extracted at 35 DAT (after 5 days of post-emergence herbicide application) and 60 DAT (bulb developing stage) by the method described by Gunes *et al.* (2007) using dimethyl sulphoxide (DMSO). Twenty-five mg of leaf tissue was placed in a vial containing 3 ml DMSO at room temperature till the tissue became chlorophyll free (12-16 h). The extract was transferred to a graduated tube and absorbance was read at 665, 645 and 454 nm as described by Kaloyereas (1958) on a computer aided spectrophotometer (CHEMITO Spectro Scan UV 2700 – Double Beam UV VIS Spectrophotometer) running a multiple wave length programme. Calculations for different pigments were made according to the formulae given by Lichtenthaler (1987). Quantities of these pigments were calculated in mg/g fresh weight (FW) of tissue. Membrane stability was assessed by the method of Vanstone and Stobbe (1977). Leaf samples were collected from control as well as herbicide sprayed plants, from these 100 mg of leaf tissue was taken separately in 20 ml test tubes containing 10 ml of de-ionized water. These samples were incubated for 24 hr at 4˚C. The conductance of decanted liquid containing efflux electrolytes was determined at 25˚C with a conductivity meter and designated as Ec a (before boiling). Then the samples were subjected to heating at 100 ˚C in a water bath for 10 min. After cooling, the electrical conductivity of the solutions was measured and designated as Ec b (after boiling). The electrolyte leakage was expressed as membrane stability was assessed by the following formula; Membrane stability $= \frac{Ec\ a}{Ec\ b} \times 100$.

Data were analysed using randomized block design and all the parameters were compared using critical difference (CD) at 5% level of significance. Data were analysed analyses of variance (ANOVA).

Liquid chromatography and mass spectroscopy (LC MS/MS) analysis

The ≥96% purity certified pesticide reference materials and HPLC acetonitrile solvent were obtained from Dr. Ehrenstorfer GmbH, Augsburg, Germany and J T Baker, USA, respectively. The analytical grade with ≥97% purity of Ammonium formate, acetic acid,

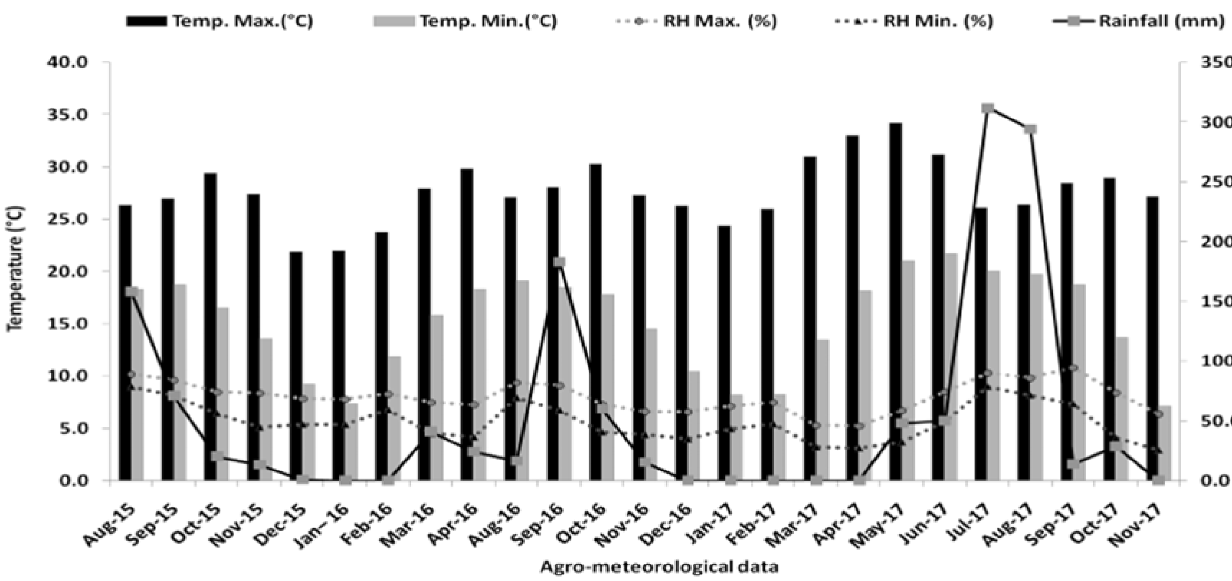


Figure 1. Agro-meteorological data during crop growing *Kharif* season of 2015, 2016 and 2017

diethylene glycol, magnesium sulphate and sodium sulphate anhydrous were from Merck India Ltd. The stock solutions of each analytes prepared with the concentration of 1000 mg/kg by dissolving standard in acetonitrile. The HPLC, Shimadzu, Japan equipped with DGU-20 degasser, 20-AD pumps A and B, SIL-20 AC HT auto sampler, and CTO-20 AC oven. The separation was performed by 10 µl sample injecting on reverse phase zorbaxelipse C-18 column (4.6 id × 100 mm, 5 µm, Agilent) maintained at 30˚C. The triple quadrupole system an API 4000 Q TRAP (ABSCIEX, CA, USA) LC-MS/MS spectrometer fitted with an electro spray ionization interface and operated in positive polarity mode. The analytical method validation parameters have given in **Table 1** as standardized by Yadav *et al.* (2017).

Modified QuChERS method for extraction of onion bulbs for LC MS/MS analysis Anastassiades *et al.* (2003). Onion bulbs separated at neck region very close to the bulb from the leaves and were separately blended and homogenized at speed of 3000 rpm for 1 min, for bulbs 1:1w/v ratio and 1:5 w/v for leaves were prepared by adding of water. The 10 g sample from these, centrifuged and extracted 10 ml of acetonitrile, after added magnesium sulphate anhydrous and sodium acetate. Finally, the sample was homogenized and centrifuged for phase separation. The 50 g of primary secondary amine added to 4 ml of acetonitrile and centrifuged at 10000 rpm. The supernatant layer of 2 ml quantity was evaporated with 200 µl 10% diethylene glycol in methanol. The 2 ml of residues were dissolved and reconstituted with methanol and 0.1% acetic acid (1:1 v/v) and filtered with 0.22 µm polyvinyl idene fluoride filter and injected 10 µl quantity in to LC-MS/MS (Yadav *et al.* 2017).

RESULTS AND DISCUSSION

Effects on weeds population

In the present investigation important monocot weeds and dicot weeds are described in **Table 2**. The *Cyperus Rotundus* and *Cynodon dactylon* from monocot and *Portulaca oleracea* and *Scoparia dulcis* form dicot were major weeds. All the treatments for weed control were effective in reducing both monocot and dicot weed population as compared to weedy check. The lowest monocot weed density and dicot weed density were recorded in combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT followed by combined spray of pendimethalin 3.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT and pendimethalin 5.0 ml/l at transplanting + quizalofop-ethyl 3.0 ml/lat30 DAT. Among the herbicide treatments, the lowest dicot weed density was recorded in oxyfluorfen 1.5ml/l at transplanting and second at 30 DAT. The reduction in weed population in these treatments could be attributed due to the effect pre-emergence and post emergence herbicide applications. Oxyfluorfen acts as a contact herbicide and kills weeds by destroying cell membranes by inhibiting the enzyme protopor-phyrinogen oxidase within leaves and shoots. At lower rates act as a contact herbicide, though it has good pre-emergence activity at higher rates and also has post emergence activity at lower rate. The combine application of pre and post emergence herbicide is one of the options to the farmers to eliminate monocot and dicot weed population at the early and later stages of the crop and to achieve higher weed control efficiency. The herbicide treatments caused significant reduction in weed

Table 1. Analytical method validation parameters of herbicides recovery, precision, accuracy, LOD, LOQ, linearity and uncertainty

Herbicide	Recovery (10 µg/kg)								
	1	2	3	4	5	6	Average	Standard deviation	Relative standard deviation
Pendimethalin	8.81	9.42	8.80	9.93	9.48	9.85	9.38	0.45	4.75
Oxyfluorfen	10.36	11.69	9.89	10.95	10.99	10.99	10.81	0.56	5.21
Quizalofop-ethyl	9.55	10.08	8.90	10.03	9.18	9.27	9.50	0.44	4.58
	Precision (10 µg/kg)								
Pendimethalin	10.15	10.30	9.57	10.04	9.77	9.69	9.92	0.26	2.64
Oxyfluorfen	8.70	11.74	11.78	10.25	8.37	11.44	10.38	1.40	13.51
Quizalofop-ethyl	9.36	9.60	9.24	9.44	9.52	8.87	9.34	0.24	2.55
	Accuracy (10 µg/kg)								
Pendimethalin	9.02	10.07	9.73	10.04	9.51	10.13	9.75	0.39	4.03
Oxyfluorfen	8.51	9.76	8.73	9.55	9.44	9.18	9.20	0.45	4.85
Quizalofop-ethyl	49.72	56.86	56.28	54.02	51.54	57.59	54.33	2.88	5.31
	LOD		LOQ		Linearity		Uncertainty		
Pendimethalin	10.0 µg/kg		5.0 µg/kg		0.9985		± 4.509 at 20.0 µg/kg		
Oxyfluorfen	10.0 µg/kg		5.0 µg/kg		0.9988		± 2.330 at 20.0 µg/kg		
Quizalofop-ethyl	10.0 µg/kg		5.0 µg/kg		0.9958		± 3.336 at 20.0 µg/kg		

population compared with the weedy check, in weedy check highest weed plants competition impact on plant growth. The magnitude of reduction in density varied with different treatments can be attributed to the fact that the herbicides which could kill most of the weed population more effective. The results are similar with the findings of Chattopadhyay *et al.* (2016) and Singh *et al.* 2017. Application of herbicides is sufficient to reduce the weed population at early stage of crop growth, but at later stages there might be degradation of herbicide in the root or by volatilization or leaching effect. Hence, there was increase in weed population at bulb development stage. In manual weeding also some weed population was recorded was due to weekly manual weeding allowed sufficient light, space and received adequate nutrients for remaining buried weed seeds to geminate. Application of quizalofop-ethyl at 30 DAT is the most effective selective herbicides for controlling *Chenopodium album*, *Parthenium hysterophorus* and *Cyperus rotundus* on the basis of weed relative density, while *Cyperus rotundus* could not be controlled completely by any of these herbicides. Quizalofop-ethyl inhibits the acetyl CoA carboxylase (ACCase) activity which is inhibiting the lipid biosynthesis could be possible due to better weed control. The results are in similar line with the results of Dhawan *et al.* (2010). Effectiveness of various herbicides against different weed species in onion crop has been previously reported by Tripathi *et al.* (2013), Vishnu *et al.* (2015) and Singh *et al.* (2017).

Effects on weed biomass, weed control efficiency and weed index

The biomass of monocot and dicot weed population is the index to determine the efficiency of herbicides to control the weeds in onion. The biomass of both monocot and dicot were significantly affected by different treatments had the marked effect on fresh and dry biomass. Among the herbicide treatments, lower monocot fresh and dry biomass were recorded in combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT followed by combined spray of pendimethalin 3.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT and pendimethalin 5.0 ml/l at transplanting + quizalofop-ethyl 3.0 ml/L at 30 DAT, while in manual weeding treatment, the lowest monocot fresh and dry biomass were recorded. These results are in line with those reported by Kalhapure and Shete (2013), Vishnu *et al.* (2015) and Chattopadhyay *et al.* (2016). Recording of WCE under particular treatment can be useful to understand the competition stress of weeds on crop. The maximum WCE was recorded in manual weeding, however among herbicide treatments, the highest WCE was recorded in oxyfluorfen 1.5ml/l at transplanting and second at 30 DAT followed by combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT and pendimethalin 5.0 ml/l of water at transplanting + quizalofop-ethyl 3.0 ml/l at 30 DAT (**Table 3**). It is obvious from the results that those treatments which inhibits weed population growth and had lesser weed dry matter resulted in higher WCE. At 60 DAT, weed density and dry matter were maximum as compared to that at harvesting stage. But, in case of WCE it was found higher at 60 DAT than at harvesting. It might be due to various fate of herbicides like leaching, volatile movement and decomposition which ultimately decrease their efficiency with passage of the time. The results are in close conformity with the findings of Vishnu *et al.* (2015) and Chattopadhyay *et al.* (2016). Weed index also indicates that the yield reduction caused would be due to competition of

Table 2. Common monocot and dicot weed population found in experimental area of onion field

	Dominant weeds details				
Sr. No.	Common name	Botanical name	Family	Habit	Reproduction
		Sedge			
1	Purple nut sedge	*Cyperus rotundus*	Cyperaceae	Perennial	Vegetative
		Monocot			
2	Bermuda grass	*Cynodon dactylon*	Poaceae	Perennial	Seed
3	Viper grass	*Dinebra retroflexa*	Poaceae	Annual	Seed
4	Tropical spiderwort	*Commelina banghalensis*	Commelinaceae	Annual	Seed
6	Jungle rice	*Echinochloa colona*	Poaceae	Annual	Seed
		Dicot			
7	Asthma herb	*Euphorbia hirta*	Compositae	Annual	Seed
8	Slender amaranth	*Amaranths viridis*	Amaranthaceae	Annual	Seed
9	Parthenium	*Parthenium hysterophorus*	Compositae	Annual	Seed
10	Common purslane	*Portulaca oleracea*	Portulacaceae	Annual	Seed
11	Sunberry	*Physalis minima*	Solanaceae	Annual	Seed
12	Licorice weed	*Scoparia dulcis*	Scrophulariaceae	Perennial	Seed
13	Lambs quarter	*Chenopodium album* L.	Chenopodiaceae	Annual	Seed
14	Field bindweed	*Convolvulus arvensis*	Convolvulaceae	Perennial	Seed

major weeds under weedy check. The maximum reduction in weeds and increase in yield was recorded in combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT. This could be described that under these treatments, there was a lower impact of weeds on yield. The findings are in close proximity to that of Chattopadhyay *et al.* (2016).

Effects on morphological characters

The growth parameters such as plant height, number of leaves and neck thickness were influenced significantly by all weed control treatments. Plant height and number of leaves are one of the most important phenological characters of the plant growth and development, maximum plant height and number of leaves were recorded in combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT which was followed by manual weeding and combined spray of pendimethalin 3.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT. While, the poor growth and development was recorded in weedy check followed by oxyfluorfen 1.5ml/l at transplanting and second at 30 DAT. All the herbicide treatments significantly increased the plant growth and development over weedy check. The superior plant growth and development could be due to lower weed population count, moisture and nutrient competition and higher exposure to sunlight. The inferior plant growth and development in treatment of weed check was due to prolonged competition of weeds caused by poor exposure to sunlight and emulation for nutrient and water. The findings are in agreement with earlier results reported by Ghadage *et al.* (2006), Channappagoudar and Biradar (2007), Vishnu *et al.* (2015) and Chattopadhyay *et al.* (2016).

Effects on yield and yield attributes

The bulb yield is the final index of the experiment indicates the success or failure of any herbicide treatments. It is evident from results that highest gross yield as well as marketable yield were recorded in treatment of manual weeding and the yield was found at par with treatment of combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT and in the same treatment highest benefit ratio (3.07) recorded (**Table 4**). This is due to the strenuous growth of the crop by control of weeds resulting into poor weed competition from the transplanting stage to maturity stage and thus enhanced availability of moisture, nutrient, light and space which hastening the photosynthetic rate thereby quacking the supply of carbohydrates and overall improvement in vegetative growth, which favourably influenced the bulb development and ultimately resulted into increased bulb yield. Results are supported by the earlier findings of Warade *et al.* (2008). While in weedy check reverse happened and weeds seriously affected average bulb weight and drastically reduced yield to the tune of 62.2% and these variabilities were due to effectiveness of weed control. The results clearly indicated the adverse impact of weed infestations in onion crop, which in term affected the bulb yield. The results are in agreement with Tripathi *et al.* (2013), Vishnu *et al.* (2015), Chattopadhyay *et al.* (2016) and Singh *et al.* (2017).

Table 3. Efficacy of different herbicides on various monocot and dicot weed population in onion

Treatment	Monocot population m^2	Dicot /population / m^2	Monocot		Dicot		Weed control efficiency (%)	Weed index
			Fresh weight (g/m^2)	Dry weight (g/m^2)	Fresh weight (g/m^2)	Dry weight (g/m^2)		
Oxyfluorfen 1.5ml/L of water at transplanting and second at 30 DAT	15.21	1.97	84.81	40.66	39.92	11.39	86.41	20.96
Oxyfluorfen 1.5 ml/L of water at transplanting + quizalofop-ethyl 3.0 ml/L of water at 30 DAT	13.16	3.04	93.86	48.95	120.9	63.31	41.57	17.33
Combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/L of water at transplanting and second at 30 DAT	11.79	2.89	58.79	20.66	85.62	25.95	73.65	9.78
Pendimethalin 5.0 ml/L of water at transplanting and second at 30 DAT	15.67	5.83	95.87	67.04	184.14	69.78	32.38	14.25
Pendimethalin 5.0 ml/L of water at transplanting + quizalofop-ethyl 3.0 ml/L of water 30 DAT	14.17	3.44	72.03	56.65	90.87	30.75	70.72	20.83
Combined spray of pendimethalin 3.0 ml + quizalofop-ethyl 2.0 ml/L of water at transplanting and second at 30 DAT	12.08	3.52	71.57	51.5	147.5	42.68	57.75	14.09
Weed free check (three hand weeding at 25, 45 and 60 DAT)	6.33	0.99	28.28	14.03	26.65	8.25	91	0
Weedy check	29.65	8.76	659.09	236.4	325.48	94.43	0	62.18
LSD (p=0.05)	3.26	0.69	12.59	16.63	39.12	11.53	7.38	2.80
Year								
2014	18.25	3.92	126.46	88.33	167.13	32.42	58.49	18.92
2015	15.25	4.25	163.51	50.68	169.13	48.7	68.84	16.94
2016	15.15	3.25	163.51	50.68	159.13	48.7	68.84	20.94
LSD (p=0.05)	8.02	0.42	7.71	10.19	23.96	7.06	4.52	1.72

Photosynthetic pigments and membrane permeability

The effect of herbicidal treatments on photosynthetic pigments content in foliage of onion at 35 DAT and 60 DAT showed variable results. Amongst the early physiological responses after herbicide applications in crop as well as weed plants included stunted growth, leaf chlorosis and increase in cell membrane were highest chlorophyll-a content recorded. Due to herbicide treatments a small decline was observed, however minute difference (3.37%) was observed in combined spray of oxyfluorfen and quizalofop-ethyl (**Figure 2a**), while individual spray of oxyfluorfen caused highest decline (14.98%) at 35 DAT and the same treatment efficiently controlled both monocot and dicot weed population with higher WCE, however due to reduction in photosynthetic pigments a small decline in yield. Similar trend was observed in chlorophyll-b content (**Figure 2b**). The small decline was observed in total chlorophyll content in all the treatments including weed check at 35 DAT and 60 DAT over weed free check in the range of 3.80 (%) to 16.79 (%) and 7.97 (%) to 27.70 (%) at 35 DAT and 60 DAT, respectively (**Figure 2c**). The similar results were observed in decline of photosynthetic pigments after herbicide treatments by Vanstone and Stobbe (1977), Bhasker *et al.* (2015) and Langaro *et al.* (2017). The oxyfluorfen is a potential inhibitor of protoporphyrinogen oxidase and has a direct effect on chlorophyll synthesis route, it may interfere with photosynthesis. Therefore, the decline of these compounds may compromise photosynthetic pigments. Due to less toxic effect on onion by various herbicide treatments has checked most of the weeds and thus allowed the crop to grow more vigorously, resulting in higher yield. A small decline in carotenoid content was observed in all herbicidal treatments (**Figure 2d**). The ratio of chlorophyll-a and chlorophyll-b showed mixed results (**Figure 3a**) and the ratio of chlorophyll- a and carotenoid showed declining trend, whereas chlorophyll-b and carotenoid showed increasing trend over weed check. The increase in ratio of chlorophyll-b and carotenoid indicates that carotenoid involved in oxidative defence mechanism (**Figure 3b**), as carotenoids plays an important role in plants protect the photosynthetic apparatus from oxidative stress. The results are in line with the results of Wahid and Ghazanfar (2006) under abiotic stress condition.

Lipid peroxidation is one of the most investigated consequences of the actions of reactive oxygen species on membrane structures, being one of the first responses to damage induced by stress in plant tissues (Amri and Shahsavar 2010). Due to herbicide injury on leaf membrane ion efflux increased in all treatments sprayed with herbicide,

Table 4. Morphological and yield attributing characters under various herbicidal treatments in onion (pooled data)

Treatment	Plant height (cm)	No. of leaves/ plant	Neck thickness (cm)	Gross yield (t/ha)	Marketable yield (t/ha)	Cost: Benefit ratio
Oxyfluorfen 1.5ml/L of water at transplanting and second at 30 DAT	47.0	6.91	1.22	15.93	14.66	2.68
Oxyfluorfen 1.5 ml/L of water at transplanting + quizalofop-ethyl 3.0 ml/L of water at 30 DAT	49.49	7.29	1.22	16.69	15.33	2.78
Combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/L of water at transplanting and second at 30 DAT	53.27	7.58	1.23	18.11	16.73	3.07
Pendimethalin 5.0 ml/L of water at transplanting and second at 30 DAT	48.47	7.07	1.18	17.51	15.90	2.89
Pendimethalin 5.0 ml/L of water at transplanting + quizalofop-ethyl 3.0 ml/L of water 30 DAT	50.0	6.96	1.23	15.75	14.68	2.66
Combined spray of pendimethalin 3.0 ml + quizalofop-ethyl 2.0 ml/L of water at transplanting and second at 30 DAT	50.07	7.18	1.22	17.08	15.93	2.92
Weed free check (three hand weeding at 25, 45 and 60 DAT)	51.96	7.40	1.17	19.58	18.55	2.40
Weedy check	41.64	5.58	0.98	8.86	7.01	1.32
LSD (p=0.05)	1.22	0.33	0.07	1.36	1.43	-
Year						
2014	51.31	7.90	1.31	16.07	14.81	2.60
2015	47.83	6.54	1.12	16.25	14.87	2.59
2016	47.83	6.54	1.12	16.25	14.87	2.87
LSD (p=0.05)	0.75	0.20	0.04	0.83	0.88	-

Table 5. List of herbicides and MRM parameters in liquid chromatography tandem mass spectrometer

Name of herbicide	Class of chemical	RT (min)	Q	Q1[a]	DP(V)[a]	CE(V)[a]	CXP(V)[a]	Q2[b]	DP(V)[b]	CE(V)[b]	CXP(V)[b]
Pendimethalin	Triazine	8.9	216	174	54	26	14	104	54	45	6
Oxyfluorfen	Nicotinoid	5.8	223	126	60	27	7	56	60	35	3
Quizalofop-ethyl	Organophosphorus	2.5	184	143	48	14	5	125	48	29	4

RT, retention time; Q, protonated parent ion; Q1, quantifier ion; Q2, qualifier ion; DP, de-clustering potential; CE, collision energy; CXP, collision cell exit potential; [a] quantifier (1st transition) mass parameter; [b] qualifier (2nd transition) mass parameter

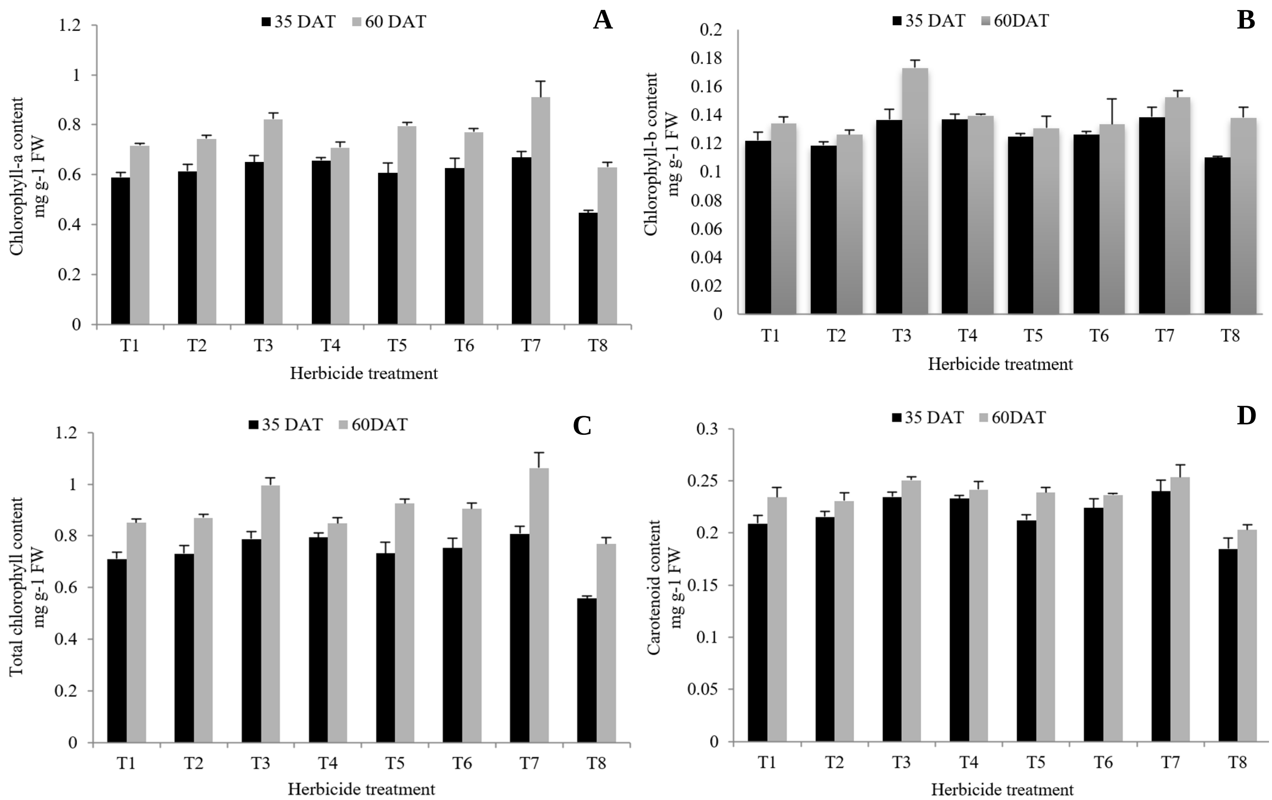
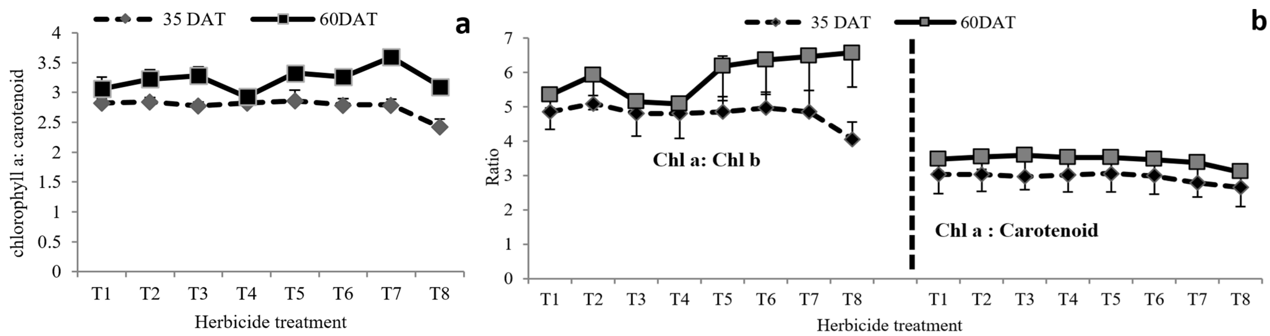


Figure 2. Changes in chlorophyll-a (a), chlorophyll-b (b), total chlorophyll (c) and carotenoid (d) content in onion leaves as affected by different herbicide treatments



Note for figure 2, 3 and 4:- T1: Oxyfluorfen 1.5ml/L of water at transplanting and second at 30 DAT; T2: Oxyfluorfen 1.5 ml/L of water at transplanting + quizalofop-ethyl 3.0 ml/L of water at 30 DAT; T3: Combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/L of water at transplanting and second at 30 DAT; T4: Pendimethalin 5.0 ml/L of water at transplanting and second at 30 DAT; T5: Pendimethalin 5.0 ml/L of water at transplanting + quizalofop-ethyl 3.0 ml/L of water 30 DAT; T6: Combined spray of pendimethalin 3.0 ml + quizalofop-ethyl 2.0 ml/L of water at transplanting and second at 30 DAT; T7: Weed free check (three hand weeding at 25, 45 and 60 DAT); T8: Weedy check

Figure 3. Changes in chlorophyll a: chlorophyll b (a) and chlorophyll a: carotenoid and chlorophyll b: carotenoid (b) in onion leaves affected by different herbicide treatments

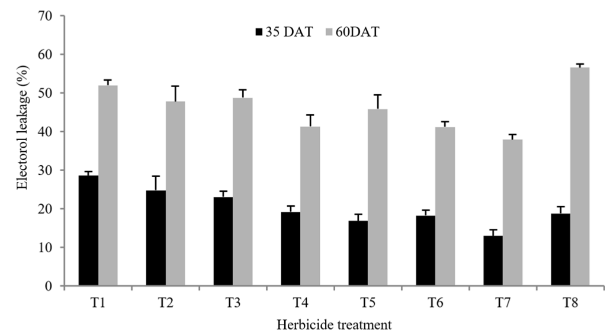


Figure 4. Changes in membrane injury of leaves in onion affected by different herbicide treatments

highest leakage was recorded at 35 DAT in individual spray of oxyfluorfen at 30 DAT, while in weed check at 60 DAT maximum leakages was recorded (**Figure 4**) due to high weed infestation causes poor development of leaf and competes for space, nutrients and water results highest ion leakage.

LC-MS/MS method optimization

The method is designed to detect and quantify the herbicides in a single run. The herbicides chosen in this experiment were applied to the onion crop to control the weed population. The Liquid chromatography (LC), gradient elution program was run in binary gradient solvent of LC i.e. methanol - water (20:80, v/v) with 5 mM ammonium formate and methanol-water (90:10, v/v) with 5 mM ammonium formate with the following programme of 0-1 min, 20% B, 1-8 min 20% - 100% B, 8-16 min. 100% B, 16-17 min 100% - 20% B, 17-20 min 20% B as reported by Yadav *et al.* (2017). The rate of flow kept at 0.6 $mlmin^{-1}$ and all the solvents were on-line degassed with a degasser. The targeted three herbicides i.e. pendimethalin, oxyfluorfen and quizalofop-ethyl chromatographically well separated with good retention time (**Figure 5**). The herbicide residue detection and quantification were performed in schedule multi reaction mode. The mass dependent specific parameters of target herbicides were mentioned in **Table 5**, revealed that the three herbicides presenting different retention, *viz*. pendimethalin 8.9 min, oxyfluorfen 5.8 min and quizalofop-ethyl 2.5 min, in theses ranges there was no detection was recorded in both the leaves and bulb. The precursor and herbicide quantification and confirmation ion pairs, the de-clustering potential and collision energies are summarised in **Table 5**. The optimized method was applied for the analysis onion bulbs and leaves and limit of quantification for three herbicides was 0.01 mg/kg and standard and sample chromatograms summarized in chromatograms. This was conducted for all trails and from results, it was concluded that no any plant part contains residue of these herbicides. Islam *et al.* (2017) reported that by using the similar QuEChERs method followed by LC-MS/MS detected herbicide residues in fruits and vegetables.

Conclusion

Field experiment conducted during *Kharif*, 2015, 2016 and 2017 on onion variety '*Agrifound Dark Red*' revealed that manual weeding practice throughout growing season of the crop controlled all monocot and dicot weed population, which resulted in highest yield, but it is the most laborious and un-economical method to control all weeds may be replaced with chemical weed management practises. Applications of herbicides are effective in the control of monocot and dicot weeds. The combined spray of oxyfluorfen 1.0 ml + quizalofop-ethyl 2.0 ml/l at transplanting and second at 30 DAT with different weed control spectrum were beneficial in reducing weed population and improving the onion growth and also these herbicides had no harmful effect on crop and it was free of any residue.

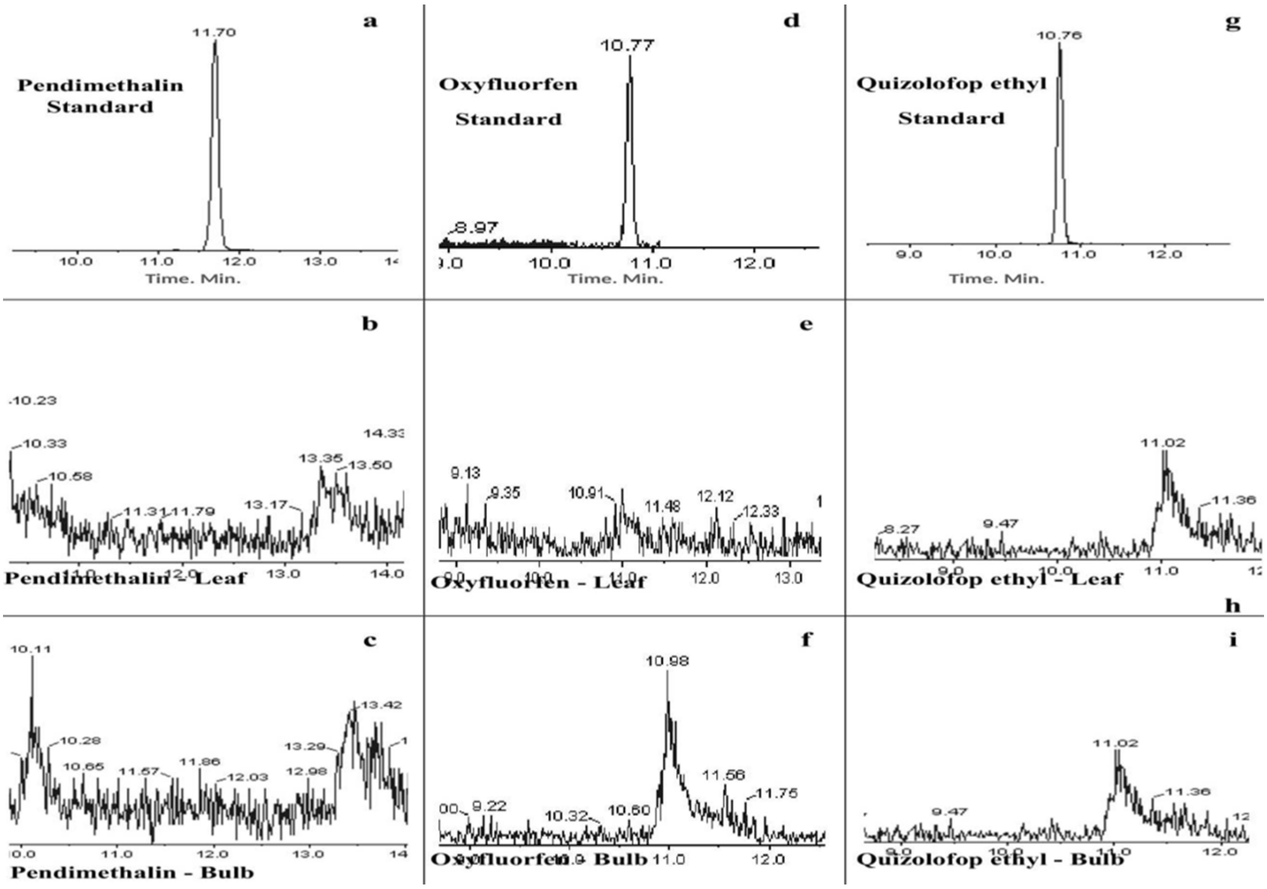


Figure 5. Comparison of pendimethalin (a, b and c), oxyfluorfen (d, e and f) and quizalofop-ethyl (g, h and i) herbicides residue in onion leaf and bulb with standard chromatogram

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