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IMPACT OF MEGAFOL (BIO-STIMULATOR) IN COMBINATION WITH HERBICIDES TO OVERCOME THE HERBICIDAL STRESS ON WHEAT

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ABSTRACT

Field experiments were conducted at the farm of Chaudhary Khair Din & Sons at Mandiala Warriach, Gujranwala Pakistan, for three years 2010-11 to 2012-13. The experiments were designed in RCBD with three replications having 5x50 m plot size. The efficacy and economics of four herbicides namely Atlantis 3.6% WG (mesosulfuron-methyl + ideosulfuron-methyl sodium), Sencor 70WP (metribuzin), Affinity 50WP (isoproturon + carfentrazone-ethyl) and Pallas 45OD (pyroxsulam) alone and in combination with Megafol (bio-stimulator) were tested for reducing herbicide phytotoxicity against wheat. All the herbicides alone and in combination with Megafol provided good control of narrow and broad leaved weeds. Highest mortality of narrow leaved weeds (98.58 and 98.16%) and broad leaved weeds (98.17 and 95.77%) was obtained when Atlantis and Pallas were mixed with Megafol, respectively. All tested herbicides mixed with Megafol produced taller plants of wheat resulting in overcoming the herbicide induced oxidative stress in wheat. Similarly, number of tillers m^{-2}, grains $spike^{-1}$ and 1000-grain weight were significantly higher when herbicides were mixed with Megafol as compared to their alone application. Yield was also significantly increased by the addition of Megafol in herbicides as a result of better recovery from stress caused by herbicide phytotoxicity. Therefore, higher grain yield was obtained from the plots treated with Atlantis + Megafol (4.99 t ha^{-1}), Pallas + Megafol (4.86 t ha^{-1}), and Affinity + Megafol (4.65 t ha^{-1}) with higher net return (Rs. 44812, 40600 and 34925 ha^{-1}), respectively. While Sencor alone provided minimum net return (Rs. 17225 ha^{-1}) as compared to other herbicides.

Key words: *Triticum aestivum* L., herbicidal, Megafol, weed, economics, yield.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal in the world, giving about one-third of the total production followed by rice. It is the major grain used for human consumption in the country. Weeds have inhibitory effect on crops and can result in yield reduction by 57% and even complete failure of crop can occur in extreme cases (Singh *et al*., 1997; Marwat and Khan, 2007). Various environmental conditions (cold, heat, drought, salt) and man-made secondary stress factors (wear, traffic, herbicides, irrigation scheduling or other cultural practices) constantly interact and either enhance or alleviate a wide range of primary abiotic and edaphic stresses on crop plants which ultimately decreases their growth and production (Lee *et al*., 2007). Varshney *et al*. (2012) witnessed the potential use of certain phytohormones in augmenting abiotic stress to get rid of yield gap. Khan *et al*. (2003) and Yin *et al*. (2008) reported that isoproturon 75WP has a phytotoxic effect on wheat crop. Significant decrease in chlorophyll content at low concentration of isoproturon was observed. Song *et al.* (2007) conducted to know the response of wheat to chlorotoluron a phenyl urea herbicide and it induced oxidative stress in wheat. Herbicides drastically influence all aspects of primary and secondary metabolisms in crops. Herbicide applications in fields limit harmful effects of weeds. However, they can have certain effects on changes in physiological process of crop (Zarzecka and Gugala, 2003). Ali *et al*. (2004) showed that herbicidal treatments of metribuzin, isoproturon + diflufenican and isoproturon + carfentrazone produced smaller plants which can be due to their phytotoxic effect on wheat crop. Similarly, metribuzin significantly reduced shoot biomass of plants. These findings indicate an induced oxidative stress in wheat following metribuzin treatment. Under the conditions employed, the increased activities of antioxidant enzymes could be suggested to play a role in wheat tolerance to metribuzin (Nemat *et al.,* 2008; Rajabi *et al.,* 2012). Isoproturon, metribuzin + fenoxaprop-p-ethyl and chlorsulfuron was reported to be phytotoxic to crop plants and hence decreased wheat yield (Shehzad *et al.,* 2012).

The addition of amino acid based foliar fertilizer such as Megafol may be able to improve the efficacy of herbicides and overcome the herbicidal induced stress on crop. The concept of using foliar applications with a nutritive aim other than a phytoiatric one is common practice in modern agriculture. However, its use in Pakistan is relatively new and minimal research data has been published. There are many reports on stress-protective effects of bio-stimulators, followed by growth, yield and quality enhancement in different species (Muralidharan *et al.,* 2000; Vernieri *et al*., 2002). Megafol contains many free amino acids as well as highly mobile and soluble potassium and nitrogen. Poincelot, (1993) and Nelson (2003) stated that microbiological fertilizers, different bio-stimulators and bio-regulators affects plant development. Megafol treatment resulted in the highest leaf biomass plant^{-1} and stimulated plant metabolism (Marija *et al.,* 2010). Field trials confirmed positive effect of Megafol on yield and economic result in pepper production (Loncaric *et al*., 2011). Megafol gave significantly higher fruit yield in comparison with control plants and those treated with Viva (Aleksandar *et al*., 2010). Jelacic *et al*. (2007) stated that application of Megafol and Viva made a significant effect on the quality of rosemary seedlings. When Megafol was applied, a production of 104.36 t ha^{-1} of Mangel-Wurzel-Bet was achieved, which is significantly superior to the untreated sample. Application of Megafol also determines the highest profit rate (66.66%) and they recommend the utilization of the Megafol (Cojocariu *et al*., 2009). The purpose of present study was to determine the impact of Megafol (bio-stimulator) in addition with different herbicides for weed control, wheat productivity and to overcome the herbicidal induced oxidative stress in wheat.

MATERIALS AND METHODS

Field experiment was conducted at the farm of Chaudhary Khair Din & Sons (CKD) at Mandiala Warriach Gujranwala, Pakistan during the year 2010-11 and repeated in 2012-13. Wheat variety Faisalabad-2008 was drilled at a seed rate of 125 kg ha^{-1} in 22.5 cm apart rows in the 2nd week of November in each year. The experiment was laid-out in RCBD with three replications having 5x15 m plot size. The herbicides alone and in combination with Megafol (bio-stimulator) were sprayed at 4-6 leaf stage of weeds in moist fields after first irrigation with hand operated knapsack sprayer in a spray volume of 300 l ha^{-1}. A check plot was kept un-weeded for comparison. Fertilizers NPK @136-114-62 kg ha^{-1} were applied. All P, K and 1/3rd N was applied at sowing, 1/3rd N at first irrigation and 1/3rd at 2nd irrigation. The experiment comprised the treatments were listed in Table-1.

The weeds under study were naturally accruing in the field. The common weeds observed in wheat field were, *Avena fatua* L. (wild oat), *Phalaris minor* Retiz (bird's seed grass), *Chenopodium album* L. (common lamb's quarters), *Vicia sativa* L. (common vetch), *Medicago polymorpha* L. (burclover/ medic) and *Rumex dentatus* L. (broad leaf dock).The addition of amino acid based foliar fertilizers such as Megafol may be able to improve the efficacy of herbicides. It is also very useful to overcome stress conditions of herbicides. Application of Megafol assures a balanced development of the plant. Bio-stimulators are commercial liquid mixtures of different natural compounds extracted from plants such as amino acids, glycosides, vitamins, micro-elements, growth hormones and humic acids. Chemical composition and physical characteristics of Megafol are presented in Table 2 & 3.

Table-1: Herbicidal treatments in combination with Megafol (bio-stimulator) used in the experiment.

Sr. No.	Name of herbicides		Recommended dose ha^{-1}
	Trade name of herbicides	Chemical name of herbicides	
1.	Atlantis 3.6% WG	mesosulfuron-methyl + ideosulfuron-methyl sodium	400 g
2.	Atlantis 3.6% WG+Megafol	mesosulfuron-methyl + ideosulfuron-methyl sodium + Megafol	400 g + 1.00 lit
3.	Sencor 70 WP	Metribuzin	250g
4.	Sencor 70 WP + Megafol	metribuzin + Megafol	250g + 1.00 lit
5.	Affinity 50 WP	isoproturon + carfentrazone – ethyl	2.00 kg
6.	Affinity 50 WP + Megafol	isoproturon + carfentrazone – ethyl + Megafol	2.00 kg + 1.00 lit
7.	Pallas 45 OD	Pyroxsulam	310 ml
8.	Pallas 45 OD + Megafol	pyroxsulam + Megafol	310 ml + 1.00 lit
9.	Control (un-weeded)	------	------

Table-2: Chemical composition of Megafol (bio-stimulator)

Total amino acid	Nitrogen (N)		Potassium Oxide (K2O)	Organic Carbon
	Total Nitrogen	Organic Nitrogen		
28%	4.5%	4.5%	2.9%	15.0%

Table-3: Physical characteristics of Megafol (bio-stimulator)

S. No.	Characteristics	Observations
1	Appearance	Liquid
2	Colour	Brown
3	Conductivity (g cm^{-3})	1.26
4	pH (1% aquatic solution)	7.6
5	Solubility 1% (mS/cm)	0.380
6	Freezing point	$-5C^0$

Weed count was recorded before and 04 weeks after spray of herbicides and Megafol from one meter square area randomly selected from each experimental plot. The data on tiller count, number of grains spike^{-1}, 1000-grain weight, plant height and grain yield were recorded at harvesting. The data were statistically analyzed by Fisher's analysis of variance method and the treatment means were compared with LSD test at 5% level of significance (Steel *et al.,*1997). Economic analysis was carried on the basis of extra income obtained from the enhanced yield and by extra cost incurred for each treatment at prevailing market prices.

RESULTS AND DISCUSSION

Weed density (m^{-2})

All tested herbicides alone and in combination with Megafol provided good control of narrow and broad leaved weeds, causing significant reduction in their density as compared to control (Table-4). The highest mortality of narrow leaved weeds i.e. 98.58% and 98.16% was obtained when Atlantis and Pallas were mixed with Megafol, respectively. These treatments were followed by Atlantis alone (97.09%), Pallas alone (96.47%), Affinity + Megafol (94.86%) and Affinity alone (92.84%). All these treatments were statistically at par with each other. Amongst the herbicides, application of Sencor alone and in combination with Megafol provided significantly less mortality of narrow leaved weeds (87.02 and 90.79%), respectively. Similarly, when Megafol was mixed with Atlantis, Pallas and Affinity also provided higher weed kill percentage of broad leaved weeds of 98.17, 95.77 and 93.91%, respectively. However, Atlantis and Pallas alone gave statistically similar results regarding the control of broad leaved weeds (95.68 & 93.79%), respectively. Affinity alone, Sencor alone and Sencor + Megafol were less effective against broad leaved weeds (89.75, 84.53 & 89.44%), respectively. Mixture of all herbicides with Megafol slightly increased the mortality of weeds than individual application of herbicide. Varshney *et al*. (2012) also concluded that natural growth regulator (Megafol) overcome the herbicidal stress (Varshney *et al*.*,* 2012). Herbicides application in fields limits harmful effects of weeds (Zarzecka and Gugala*,* 2003; Afridi and Khan, 2014; 2015). Similar results were also recorded by Khan *et al*. (2003) and Shehzad *et al*. (2012).

Table-4: Effect of different herbicides in combination with Megafol for weed control in wheat (Pooled data of Rabi 2010-11 to 2012-13)

S. No.	Treatments	Narrow leaved weeds density (m^{-2})			Broad leaved weeds density (m^{-2})		
		Before spray	Four weeks after spray	Mortality of weeds (%)	Before spray	Four weeks after spray	Mortality of weeds (%)
1.	Atlantis 3.6% WG	103.11	3.00 cd	97.09	32.17	1.39 cd	95.68
2.	Atlantis 3.6% WG + Megafol	101.17	1.44 d	98.58	36.67	0.67 d	98.17
3.	Sencor 70 WP	98.00	12.72 b	87.02	38.58	5.94 b	84.53
4.	Sencor 70 WP + Megafol	99.56	9.17 bc	90.79	35.22	3.72 bc	89.44
5.	Affinity 50 WP	98.44	7.05bcd	92.84	35.50	3.64 bc	89.75
6.	Affinity 50 WP + Megafol	101.53	5.22 cd	94.86	38.28	2.33 cd	93.91
7.	Pallas 45 OD	102.20	3.61 cd	96.47	36.25	2.25 cd	93.79
8.	Pallas 45 OD + Megafol	96.67	1.78 d	98.16	35.45	1.50 cd	95.77
9.	Control (un-weeded plot)	101.72	103.08a	---	37.19	38.00 a	---
	LSD at 0.05	NS	7.091	NS	NS	2.914	NS

Means sharing the same letter in a column do not differ significantly (P = 0.05), using LSD, test.

Number of tillers (m^{-2})

Application of herbicides either alone or in addition with Megafol significantly increased the number of tillers m^{-2} over untreated plot (Table-5). Atlantis, Pallas, Affinity and Sencor when mixed with Megafol produced significantly higher number of tillers i.e. 348.31, 345.92. 343.84 and 338.83 m^{-2}, respectively and all these treatments were statistically at par with each other. Herbicides alone produced less number of tillers as compared to their combinations with Megafol. Amongst the herbicides, application of Sencor alone provided less number of tillers (319.00 m^{-2}) as compared with other herbicides. Whereas, minimum numbers of tillers (295.83 m^{-2}) were recorded in control plot. Similar results have been reported by Ali *et al*. (2004) that tillers m^{-2} of wheat were maximum in clodenafop and fenoxaprop treated plots. However, isoproturon + carfentrazone and metribuzin proved significantly to be inferior to other herbicides. Sanaullah *et al*. (2011) and Shehzad *et al*. (2012) also reported similar results that isoproturon, metribuzin and chlorsulfuron seemed someway phytotoxic to wheat plants which decreased the number of tillers m^{-2}.

Plant height (cm)

The results presented in table-5 revealed that plant height of wheat was significantly influenced with the application of herbicides alone and in combination with Megafol. Taller plants having values of 103.96, 103.42, 102.85 and 102.63 cm was obtained by the application of Pallas, Atlantis, Affinity and Sencor when mixed with Megafol, respectively and these treatments were at par with each other. Whereas, when Pallas, Atlantis, Affinity and Sencor were applied alone they produced smaller plants with height of 100.19, 99.87, 99.17 and 98.69 cm, respectively. This can be attributed to induced oxidative stress in wheat by these herbicides. Whereas, the lowest plant height (96.45 cm) was observed in control plot which was primarily due to the weed competition. Nemat *et al*. (2008) and Rajabi *et al*. (2012) reported that metribuzin significantly reduced plant height due to induced oxidative stress in wheat. They also stated that under the conditions employed, the increased activities of antioxidant enzymes could be suggested to play a role in wheat tolerance to metribuzin. Similarly Ali et al. (2004) showed that herbicidal treatments of metribuzin, isoproturon + diflufenican and isoproturon + carfentrazone produced smaller plants which can be due to their phytotoxic effect on wheat crop. Zarzecka and Gugala (2003) and Song *et al*. (2007) also reported that environmental contamination and herbicide application may cause a variety of physiological or oxidative stress in plants. The general symptom of plants under such stress is the inhibition of growth.

Number of grains spike^{-1}

Various herbicidal treatments produced significant effect on the number of grains spike^{-1} as compared to untreated plot (Table-5). Maximum number of grains spike^{-1} (35.91) was obtained by the application of Atlantis + Megafol followed by Pallas + Megafol (35.37) and these treatments were significantly at par with each other. Affinity + Megafol and Sencor + Megafol provided the intermediate number of grains spike^{-1} (34.76 and 34.18), respectively and were not statistically differed with each other. Amongst the herbicides Sencor alone and Affinity alone produced less number of grains spike^{-1} (32.35 and 32.64), respectively. However, when Megafol was added in the herbicides, they produced higher number of grains spike^{-1} as compared to their alone application. Statistically minimum number of grains spike^{-1} (30.01) was recorded in weedy check plot. Sanaullah *et al*. (2011) reported that significantly more number of grains spike^{-1} was recorded in all the treated plots as compared with control and it may be attributed to availability of more nutrients because weed population reduced. They also stated that grain production spike^{-1} was the lowest in case of Sencor and Partner treated plot which ultimately resulted in poor yield. Ali *et al*. (2004) also stated that the increase in number of grains spike^{-1} was due to the use of different herbicide and lowest grains spike^{-1} (32.87) was noted in metribuzin treated plot. Marija *et al*. (2010) reported that Megafol might stimulate plant metabolism and growth.

Table-5: Effect of different herbicides in combination with Megafol on yield and yield components of wheat (Pooled data of Rabi 2010-11 to 2012-13)

S. No	Treatments	No. of tillers (m^{-2})	Plant height (cm)	No. of grains s pike^{-1}	1000-grain weight (g)	Yield (t ha^{-1})
1.	Atlantis 3.6% WG	330.33 bcd	99.87 b	33.46 de	36.37 d	4.38 cd
2.	Atlantis 3.6% WG + Megafol	348.31 a	103.42 a	35.91 a	38.88 a	4.99 a
3.	Sencor 70 WP	319.00 d	98.69 b	32.35 g	35.02 f	3.94 f
4.	Sencor 70 WP + Megafol	338.83 abc	102.63 a	34.18 cd	37.29 c	4.43cd
5.	Affinity 50 WP	325.50 cd	99.17 b	32.64 fg	35.60 ef	4.01 ef
6.	Affinity 50 WP + Megafol	343.84 ab	102.85 a	34.76 bc	37.71 bc	4.65 bc
7.	Pallas 45 OD	326.17 cd	100.19 b	33.25 ef	35.93 de	4.28 de
8.	Pallas 45 OD + Megafol	345.92 a	103.96 a	35.37 ab	38.35 ab	4.86 ab
9.	Control (un-weeded plot)	295.83 e	96.45 c	30.01 h	33.16 g	3.32 g
	LSD at 0.05	14.786	2.060	0.788	0.701	0.314

Means sharing the same letter in a column do not differ significantly (P=0.05), using LSD test.

1000-Grain weight (g)

Grain weight is an important yield component of wheat. All treatments showed significant effect on 1000-grain weight as compared to untreated plot (Table-5). Atlantis + Megafol gave the highest 1000-grain weight (38.88 g) followed by Pallas + Megafol (38.35 g) and these two treatments were statistically similar with each other. Combination of Affinity and Sencor with Megafol provided the intermediate 1000-grain weight (37.71 and 37.29 g), respectively and these treatments were also at par with each other. Amongst the herbicides, Sencor and Affinity alone produced lower grain weight (35.02 and 35.60 g), respectively. The minimum 1000-grain weight (33.16 g) was recorded in untreated plots. Similar results were reported by Sanaullah *et at.* (2011) that the application of Sencor and

Partner produced lowest grain weight and this reduction in grain weight may be due to the induced oxidative stress in wheat by herbicides. Ali *et al*. (2004) also reported that metribuzin and isoproturon + carfentrazone treated plots produced less grain weight. This reduction might be due to more weeds and their phytotoxic effect.

Grain yield (t ha^{-1})

Wheat grain yield in treated plots was significantly higher than untreated one (Table-5). The highest grain yield (4.99 t ha^{-1}) was obtained by the application of Atlantis + Megafol followed by Pallas + Megafol (4.86 t ha^{-1}) and these treatments had non-significant differences. However, application of Affinity + Megafol, Sencor + Megafol and Atlantis alone provided intermediate yield i.e. 4.65, 4.43 and 4.38 t ha^{-1} , respectively. These treatments were statistically at par with each other. Megafol applied with post-emergence herbicide increased yield and yield components. Amongst the herbicides, application of Affinity alone and Sencor alone gave significantly lower yield (4.01 and 3.94 t ha^{-1}), respectively as compared with other herbicides. However, the lowest yield (3.32 t ha^{-1}) was recorded in untreated plot (un-weeded plot). Subsequently grain yield was increased by the application of Megafol as a result of better recovery from stress caused by herbicide phytotoxicity. Earlier scientists reported that untreated weed infestation can result in dramatic reduction in wheat yield by 57% and complete failure of crop can occur in extreme cases (Singh *et al.,* 1997). Varshney *et al*. (2012) reported that potential use of certain phytoharmones in augmenting abiotic stress to get rid of yield gap and productivity constraints. Megafol treated plot gave significantly higher yield in comparison with control plot and those treated with Viva (Aleksandar *et al.,* 2010). When the Megafol was applied, a production of 104.35 t ha^{-1} of Mangel–Wurzel-Bet was achieved, which is significantly superior to the untreated plot (Cojocariu *et al.,* 2009).

Economic analysis

The economic analysis showed that all tested herbicides alone and in combination with Megafol proved economical as compared to control (Table-6). The analysis of the profit rate clearly reflects that the application of Atlantis + Megafol, Pallas + Megafol and Affinity + Megafol gave higher net return (Rs 44812, 40600 and 34925 ha^{-1}) with CBR of 1:8.47, 1:7.25 and 1:7.02, respectively. Sencor + Megafol and Atlantis alone gave the lower income (Rs 29325 and 29112 ha^{-1}), respectively. Amongst the herbicides Sencor alone provided minimum net return (Rs. 17225 ha^{-1}) with highest CBR 1:12.53. CBR given by Sencor was higher than that of other herbicides, which was due to its lower cost. The data in Table-6 clearly depicted that when Megafol mixed with herbicides made pronounced impact on yield and resultantly it provided reasonable higher profit than the herbicides applied alone. Similar result was reported by Loncarvic *et al*. (2011) that field trials confirmed positive effect of Megafol on yield and economic result in pepper production. Cojocariu *et al*. (2009) reported that the application of Megafol determines the highest profit rate (66.66%) and they recommended the utilization of the Megafol.

Table-6. Economic analysis of different herbicides+Megafol applied to wheat (Pooled data of 2010-11 to 2012-2013)

S. No.	Treatments	Yield (t ha^{-1})	Increase in yield over check(t ha^{-1})	Additional income (Rs ha^{-1})	Additional expenditure (Rs ha^{-1})	Net income over check (Rs ha^{-1})	(CBR)
1.	Atlantis 3.6%WP	4.38	1.06	31800	2688	29112	1:10.83
2.	Atlantis 3.6%WP + Megafol	4.99	1.67	50100	5288	44812	1:8.47
3.	Sencor 70 WP	3.94	0.62	18600	1375	17225	1:12.53
4.	Sencor 70 WP + Megafol	4.43	1.11	33300	3975	29325	1:7.38
5.	Affinity 50 WP	4.01	0.69	20700	2375	18325	1:7.72
6.	Affinity 50 WP + Megafol	4.65	1.33	39900	4975	34925	1:7.02
7.	Pallas 45 OD	4.28	0.96	28800	3000	25800	1:8.60
8.	Pallas 45 OD + Megafol	4.86	1.54	46200	5600	40600	1:7.25
9.	Control (un-weeded plot)	3.32	---	---	---	---	---

CONCLUSION

Application of natural bio-stimulator (Megafol) in addition with herbicides made pronounced impact for controlling weeds to increase the yield and yield components of wheat, as a result of better recovery from stress caused by herbicides. Moreover, Magafol in combination with herbicides is more efficient and economical as compared to the herbicides alone.

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