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RESEARCH ARTICLE

Cumulative Effect of Biochar, Microbes and Herbicide on the Growth and Yield of Wheat (*Triticum aestivum* L.)

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ABSTRACT
In Pakistan, per acre yield of wheat (*Triticum aestivum* L.) suffers greatly due to low organic matter and immobilized form of nutrients in soils. Various strategies have been adopted by agronomists to cope these issues. In order to investigate the effect of an integrated strategy of application of biochar (Green waste), microbes (*Pseudomonas, Agrobacterium, Azospirillum, Enterobacter*) and herbicides on the growth and yield of wheat, an experiment was conducted at the farm area of the Islamia University of Bahawalpur, Pakistan during 2012-13. The experiment was laid out in randomized complete block design with split plot arrangements containing three replications. The main plots were with and without the application of herbicide, while subplots carried four treatments viz. microbial inoculation, biochar application, microbial inoculation in combination with biochar, and an untreated control. Maximum grain yield (5.04 t ha^{-1}) and yield components were recorded in herbicide treated plants with the combined application of biochar and microbes, while minimum (2.14 t ha^{-1}) was measured in untreated control where herbicide was not applied. Biological yield and leaf area showed that both biochar and microbes in combination with herbicide application were most effective than all other treatments. It is concluded that biochar and plant growth promoting rhizobacteria (PGPR) along with herbicides was considered to be a best combination that enhanced the productivity of wheat.

INTRODUCTION

Organic matter is regarded as a very important parameter for soil productivity (Hussain et al., 2001). Pakistani soils are deficient in organic matter contents which are less than 2 % and it can be replenished by the application of organic matters and composts (Sarwar, 2005). Pakistani soils are low in iron, aluminum and organic matter contents, which resulting in poor aggregate stability and (Nizami & Khan 1989). Higher soil organic matter concentrations have been proved to enhance the yield and yield components of cereals (Sarwar, 2005) as well as soil aeration, density and water holding capacity (Zia et al., 1998).

Biochar is an efficient alternative supplement for increasing the soil organic matter content (Ngo et al., 2014). However in Pakistan, this alternative strategy has not yet been benefited to great extent. Biochar which is one of the waste materials can improve the fertility status of the soil and environmental reimbursement. At present, the results about the effect of biochar adding on crop production are indecisive. They illustrate either negative, zero, or positive response depending on the kind and properties of biochar as well as experimental circumstances such as soil type, crop species, and environmental conditions. This makes it essential to further examine biochar effects on crop production under precise site conditions (Jeffery et al., 2011).

In addition to biochar as soil amendment, use of microorganisms specifically bacteria have also been isolated and characterized to improve soil health and mobilize macro and micro nutrients to plants (Glick, 2012; Shahid et al., 2012; Tahir et al., 2013). Application of biochar in combination with microbes not only improves soil organic matter content but also positively affects its physical and chemical properties which ultimately lead to enhanced crop yield (Ngo et al., 2014). Microbial inoculants also help to overcome the ecological problems and enhance availability and efficiency of nutrients (Jeffery et al., 2011). Strains of *Agrobacterium*, *Pseudomonas*, *Azospirrillum* and *Enterobacter* have been reported as potent plant growth promoting rhizobacteria (PGPR) by researchers around the globe (Egamberdiyeva, 2004; Mehnaz et al., 2001; Shahid et al., 2012; Tahir et al., 2013)

The activity of the micro-organism is increased under the shelter of weeds that compete with the inoculants to eliminate them from the vicinity (Dawar et al., 2014). Thus, the application of herbicide becomes crucial to eradicate weeds and provide semi open play-ground for microbes to exert their effects. Conversely, many studies also documented the lethal effects of herbicides application on microbial inoculants (Cullimore, 1971). We adopted an integrated strategy with the hypothesis that biochar and microbes improve the soil physio-chemical properties not only by enhancing the organic matter status but also by restricting leaching and mobilization of the nutrients to plant roots. The sole objective was to test the application of this integrated strategy for augmentation of wheat growth and yield.

Keeping the importance of biochar and microbes in view, the present study was designed to know the cumulative effect of biochar, microbes and herbicide on the growth and yield of wheat under the agro-climatic conditions of Bahawalpur during 2012-13.

MATERIALS AND METHODS

A field experiment was conducted at the experimental farm area of The Islamia University of Bahawalpur, Pakistan to examine the cumulative effect of biochar, microbes and herbicides on the growth and yield of wheat (*Triticum aestivum* L.) during 2012-13. The treatments were replicated four times using randomized complete block design (RCBD) with split spot arrangement. The main plots were either applied with herbicides Fenoxaprop-P-Ethyl and Bromoxynil (both 500 ml acre^{-1}) to control broad and narrow leave weeds or kept untreated with the herbicide. Herbicides were applied at tillering stage. Sub-plots were subjected to four separate treatments viz. application of biochar, microbial inoculation, a combination of biochar and microbes and untreated control. Inoculated bacteria were *Pseudomonas, Agrobacterium, Azospirillum, Enterobacter*. The source of biochar was green waste containing 0.25 % Nitrogen, 0.049 % Phosphorus and 0.0072 % Potassium. Biochar was applied at the rate of 500 kg ha^{-1}. Biochar, microbes and two levels of herbicides were evaluated comparatively. The meteorological data of the crop growth period during 2012-13 was collected from arid zone research institute Bahawalpur, Pakistan (Fig. 1).

Seed bed was prepared by cultivating the soil 2-3 times with tractor mounted cultivator to a depth of 10-12 cm each followed by planking. Wheat variety PUNJAB-

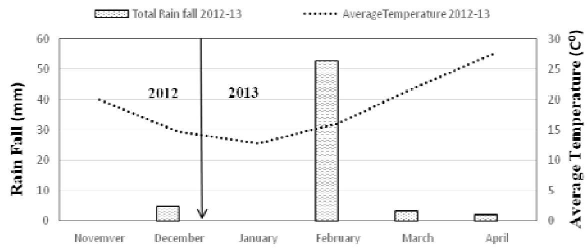

Fig. 1: Data of total rain fall and average temperature in Bahawalpur during crop period 2012-2013 of wheat

2011 (Ayub Agricultural Research Institute) was shown on November 22, 2012 after cotton. Sowing was done with hand drill using 120 kg ha^{-1} seed rate in 25 cm apart rows in plots measuring 5m×1.5m. The NPK fertilizer was applied at rate of 130-115-62 kg ha^{-1} respectively. Entire dose of the phosphorus, potassium and 1/3 nitrogen were applied at the time of sowing. Remaining nitrogen was applied in two splits along with first and second irrigations. First irrigation was applied at 18 days after sowing the crop while the remaining irrigations were given according to the crop requirement. All other agronomic and management practices were kept constant.

Data collection

The data representing the growth and yield parameters like plant height at maturity, number of spikelets per spike, biological yield and economic yield were calculated by using standard principles and procedures (CIMMYT, 1988). The threshing was done by small thresher (Murshid Farm Industries) available at Agronomy farm of The Islamia University Bahawalpur. Leaf area index was calculated with leaf area meter (CL-202) fortnightly.

The data collected were statistically analyzed to check the significance of treatment means by means of ANOVA and the treatment means were compared by least significant difference (LSD) test at 5% level of probability (Steel et al., 1997).

RESULTS AND DISCUSSION

Biochar and microbes application either alone or in combination with herbicide has incredible effects on soil conditions. Optimum amount and suitable source of microbes and biochar improve the crop growth and yield attributes of the crop (Dawar et al., 2014). When biochar and microbes are applied with or without herbicide improved the considerable yield and components. Leaf area index (LAI) of wheat is central photosynthetic equipment. It is used to predict the photosynthesis capacity of a crop and as a reference tool for crop growth. Higher the LAI more energy will be trapped that will increase the photosynthetic rate and ultimately increased final yield of crop. Numbers of productive tillers per unit area contribute positively to the economic yield. Vegetative growth including plant height, number of fertile tillers and leaf area index were greatly influenced by the application of different treatments. Significantly ($P<0.05$) higher number of fertile tillers (49.8 tillers m^{-2}), greater leaf area index (3.10) and plant height (87.3 cm) were obtained by the application of biochar (source of organic matter) and PGPR in combination with herbicide as compared with control and all other treatments (Table 1). Biochar is a source of organic matter and has been reported to improve soil fertility, performance of PGPR and availability of essential nutrients to the plants which in turn increases the vegetative growth of wheat plant. As biochar contains micro and macro nutrients, so provision of these nutrients directly to the plants also exerts beneficial effects on the growth components of wheat. Similarly bacterial strains belonging to *Azospirillum, Bacillus, Enterobacter* and *Pseudomonas*have also have been reported to improve growth attributes of wheat by providing fixed atmospheric nitrogen, solubilized phosphorus and producing phytohormone like indol-3-acetic acid. Herbicides provide efficient weed control which in turn reduces the competition of weeds with main crop plants for nutrients, light and space. Efficient weed control also reduces the competition of beneficial bacteria with pathogenic bacteria. This ultimately results in provision of proper amount of nutrients to the plants and improved plant growth in terms of higher plant height, greater number of fertile tillers and leaf area index. Our results are in line with the previous studies on biochar (Asai et al., 2009; Bot and Benites, 2005), inoculation of PGPR like *Azospirillum, Bacillus, Enterobacter* and *Pseudomonas* (Ahmad et al., 2014; Bhardwaj et al., 2014; Bhattacharyya and Jha, 2012; James and Baldani, 2012; Mathivanan et al., 2014; Mehnaz, 2015; Mehnaz et al., 2001; Tahir et al., 2013; Vessey, 2003) and application of herbicides for efficient weed control (Malik and Yadav, 2008; Larid 2008) have increased the growth and yield components of crop.

Spike length is a prime contributor among different components for the determination of yield. Production potential of wheat is estimated by the length of its spike. Spike length of wheat contributes great share in final economical yield by influencing number of spikelets, grain size and number of grains per spike. The more the length of spike, the more number of spikelets will be and ultimately higher number of grains. Maximum spike length (10.0 cm), number of grains per spike (33.5), 1000-grain weight (37.1 g), and significantly higher grain and biological yield (5.04 t ha^{-1}, 21.0 t ha^{-1} respectively) was recorded where biochar and PGPR were applied in combination with herbicide as compared to control and all other treatments (Table 2, 3). As discussed earlier leaf is photosynthetic equipment and higher the leaf area, the higher would be the photosynthetic rate which positively contributes towards better accumulation of photosynthates in grain. This has resulted in increased spike length, number of grains per spike, 1000 grain weight and grain yield of wheat. Application of biochar improved the soil nutrient status through its function as organic matter, PGPR inoculation provided available P supply to plants by phosphate solubilization, fixed atmospheric nitrogen for the plants and supplied growth hormones to the plants. Application of herbicide efficiently controlled the weed competition which resulted in efficient use of nutrients and other resources. All these factors contributed towards increased spike length, number of grains per spike, 1000 grain weight and finally the higher grain yield. Our results confirmed the findings of previous studies that application of biochar increased the growth and yield of various crops (Asai et al., 2009; Bot and Benites, 2005).

Our findings also coincided with the investigation of (Ebrahimian and Bybordi, 2011) who reported that growth and grain yield attributes of wheat crop increased with biochar application. Inoculation of wheat with PGPR has been reported to improve nutrient uptake, growth and yield of wheat, rice, sugarcane and maize (Asai, et al., 2009; Ali et al., 2008; Ahmad et al., 2014; Bhardwaj et al., 2014; Bhattacharyya and Jha, 2012; James and Baldani, 2012; Mathivanan et al., 2014; Mehnaz, 2015; Mehnaz et al., 2001; Tahir et al., 2013; Vessey, 2003). Our results confirm the findings that herbicide application in wheat has significant effect on number of grains (Malik and Yadav, 2008). The increase in grains perhaps was mainly because of the improvement in the cation exchange capacity (CEC) of the soil, a decrease in the hydraulic conductivity of the soil and an increase in the carbon, nitrogen and phosphorus content of the soil (Chan and Xu, 2009). The application of herbicides provided more space, nutrients, light and water availability to the plants which ultimately increased the seed yield (Laird, 2008). Herbicides mitigated the harmful effects of any stress and biochar and microbes provided the balanced nutrients.

The data about economic analysis presented in the table 4 reveals that there was an overall increase in the net benefit in different treatments when compared with that of control. Among different treatments the highest benefits were obtained from the plots treated with biochar, microbes and herbicide giving a net benefit (of PKR 79646 ha^{-1}). Control treatment with herbicide application led to a net benefit (of PKR 11346 ha^{-1}) and control treatment without herbicide application resulted in a net benefit (of PKR 11346 ha^{-1}). Experimental treatment with biochar and herbicide application gave a net benefit (of PKR 55246 ha^{-1}). Application of microbes and herbicides were as experimental treatment

Table 1: Influence of various treatments on agronomic attributes of wheat

Treatments	Leaf Area Index			Number of tillers m^{-2}			Plant height (cm)		
	Herbicide	Control	Means	Herbicide	Control	Means	Herbicide	Control	Means
Control	2.68 bc	2.42 c	2.55 B	325.3 d	297.0 h	311.1 D	77.5 de	75.1 e	76.3 C
Microbes	2.50 bc	2.62 ab	2.56 B	333.3 c	308.6 g	321.0 C	81.3 cd	79.2 d	80.2 B
Biochar	2.77 bc	2.55 bc	2.66 B	338.6 b	316.0 f	327.3 B	85.0 b	83.7 bc	84.3 A
Microbes + Biochar	3.10 a	2.98 ab	3.04 A	346.6 a	320.6 e	333.6 A	87.3 a	86.2 ab	86.8 A
Means	2.76	2.64		336.0 A	310.5 B		82.7	81.1	
LSD ≤0.05	Treatment = 0.35, Herbicide = NS, Interaction = 0.66			Treatment = 2.35, Herbicide = 3.21, Interaction = 4.44			Treatment = 3.36, Herbicide = NS, Interaction = 7.12		

Table 2: Influence of various treatments on yield components of wheat

Treatments	Spike length (cm)			Number of spikelets per spike			Number of grains per spike		
	Herbicide	Control	Means	Herbicide	Control	Means	Herbicide	Control	Means
Control	7.86 de	7.30 e	7.58 C	14.2 de	13.6 e	13.9 C	28.8 cd	27.6 d	28.2 C
Microbes	8.03 de	7.30 e	7.66 C	15.1 bc	14.7 cd	14.9 B	29.9 bc	28.6 cd	29.3 BC
Biochar	8.86 bc	8.13 cd	8.50 B	15.7 b	15.4 bc	15.5 B	31.3 b	29.8 bc	30.5 B
Microbes + Biochar	10.0 a	9.36 ab	9.68 A	17.9 a	17.4 a	17.6 A	33.5 a	31.3 ab	32.4 A
Means	8.69 A	8.02 B		15.7 A	15.2 B		30.9 A	29.3 B	
LSD ≤0.05	Treatment = 0.53, Herbicide = 0.63, Interaction = 0.86			Treatment = 0.76, Herbicide = 0.37, Interaction = 0.99			Treatment = 1.52, Herbicide = 1.37, Interaction = 2.22		

Table 3: Influence of various treatments on yield and yield components of wheat

Treatments	1000- grain weight (g)			Biological yield (t ha^{-1})			Grain yield (t ha^{-1})		
	Herbicide	Control	Means	Herbicide	Control	Means	Herbicide	Control	Means
Control	33.6 de	32.4 e	33.0 C	13.3 f	12.0 g	12.7 D	2.25 ef	2.14 f	2.19 D
Microbes	35.0 b	33.5 de	34.3 B	16.0 d	14.7 e	15.3 C	3.24 cd	2.56 e	2.90 C
Biochar	36.5 a	34.0 bc	35.2 A	18.7 b	17.3 c	18.0 B	4.12 b	3.12 d	3.62 B
Microbes + Biochar	37.1 a	34.9 bc	36.0 A	21.0 a	18.7 b	19.8 A	5.04 a	3.69 bc	4.36 A
Means	35.5 A	33.7 B		17.2 A	15.7 B		3.66 A	2.87 B	
LSD ≤0.05	Treatment = 0.88, Herbicide = 0.95, Interaction = 1.38			Treatment = 0.94, Herbicide = 0.72, Interaction = 1.31			Treatment = 0.48, Herbicide = 0.15, Interaction = 0.49		

Table 4: Variable cost for economic analysis

	T_1H_1	T_2H_1	T_3H_1	T_4H_1	T_1H_2	T_2H_2	T_3H_2	T_4H_2	Remarks
Grain yield of wheat	2.25	3.24	4.12	5.04	2.14	2.56	3.12	3.69	t ha^{-1}
Adjusted grain yield	2.02	2.91	3.70	4.53	1.92	2.30	2.80	3.32	10% less than actual to bring at farmer's level
Gross benefit	60600	87300	111000	135900	57600	69000	84000	99600	PKR 120040 kg^{-1}
Cost of biochar	-	-	6500	6500	-	-	6500	6500	PKR 2600 $acre^{-1}$
Cost of microbes	-	500	-	500	-	500	-	500	PKR 200 $acre^{-1}$
Cost of Herbicide	3000	3000	3000	3000	-	-	-	-	PKR 600 $acre^{-1}$
*Fixed cost	46254	46254	46254	46254	46254	46254	46254	46254	PKR
Cost that vary	3000	3500	9500	10000	-	500	6500	7000	PKR
Total cost	49254	49754	55754	56254	46254	46754	52754	53254	PKR
**Net benefit	11346	37546	55246	79646	11346	22246	31246	46346	PKR

*Fixed cost: fixed cost includes the cost on land preparation, sowing, seed, irrigation, fertilizer (SSP, Urea), harvesting and threshing; **Net benefits = Gross benefits – Total cost; H_1 = with herbicide, H_2 = without herbicide, T_1 = Control, T_2 = Microbes, T_3 = Biochar, T_4 = Biochar & Microbes

provided a net benefit (of PKR 37546 ha^{-1}). Treatment application of where biochar, microbes and combination of biochar and microbes led to a net benefit (of PKR 31246 ha^{-1}, 22246 $ha^{-1}$and 46346 ha^{-1}, respectively).

Conclusions

The present study led to the conclusion that a combined application of PGPR and biochar increases the growth and yield of wheat under agro-climatic conditions of Bahawalpur. Efficacy of herbicides also improves due to application of biochar. It is concluded that biochar and PGPR along with herbicides was considered to be a best combination that enhanced the productivity of wheat. This strategy may help to increase the grain yield of wheat and is recommended for commercial application.

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