Research Inventy: International Journal of Engineering And Science
Vol.12, Issue 2 (February 2022), PP 10-14
Issn (e): 2278-4721, Issn (p):2319-6483, www.researchinventy.com

Effect of Piriformospora Indica- symbiotic fungus formulation on the growth of wheat varieties

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ABSTRACT
Field experiment was conducted during the rabi season of 2012 -2013 at Ambala college of engineering (ACE) and applied research campus and Tepla, Ambala to study the efficacy of biofertilizer (Piriformospora Indica) formulation along with different doses of chemical fertilizers on growth and yield of wheat (Triticum aestivum). Two varieties named as HD-2989, PBW-343 were selected for this experiment. Gur was used as a media which assist the adherence of biofertilizer on wheat during overnight incubation. After approximately 20-30 days of sowing of wheat seed into the equally divided plots of one acre of land, different phenotypic parameters were start to noted like no. of seed germination per sq. feet, no. of tillers per plant, height of plant, no. of spikelets/spike, maturity in no. of days, yield etc. The result showed significant response of bio-fertilizer on growth and productivity of wheat.

KEY WORDS: *Biofertilizer, Piriformospora Indica, Wheat*

Date of Submission: 05-02-2022 Date of Acceptance: 18-02-2022

I. INTRODUCTION

Wheat (*Triticum aestivum*, L.) is one of the main cereal crops, cultivated to demands of the population for human feeding (Mesbah *et al.*, 2009). Wheat (*Triticum aestivum* L.) grain and its products are one of vital components of our daily diet. Wheat is number one food grain crop consumed directly by the humans and its production leads other food grains such as rice, maize, barley sorghum, oat and millet in the world. On global basis wheat covers maximum area as well as production as compared to other food grain crops. Wheat alone can meet all the daily requirements of thiamine and niacin whereas it can provide more than half of the total daily requirements for iron and riboflavin (Khan 1984). It is also the cheapest and principle source of carbohydrates and proteins in our daily diet. In our country, about 70% of the total wheat produced is utilized in the form of unleavened flat bread known as chapatti while in NWFP it is mainly used in the form of leavened bread and locally known as (Khamiri roti). The rest 30% is used for other bakery products such as bread, cookies, cakes, pastries etc. It has been an elusive task for many years to provide a precise definition as to wheat contributes quality in wheat. *Piriformospora indica*, a root colonizing and growth promoting basidiomycete fungus, was recognized in the Indian Thar desert. *P. indica* has been found to be a potent new candidate symbiont for providing enormous growth-promoting activity to a broad spectrum of plants, including agricultural and medicinal crops (Tsimilli-Michael and Strasser, 2013). In this perspective, *P. indica* has become a paramount tool in improving the productivity of several crops such as Brassica campestris sp. chinensis, Lycopersicon esculentum, Hordeum vulgare, Piper nigrum, Glycine max, Cicer arietinum Arabidopsis sp., Oryza sati va and Nicotiana tabacum under natural and/or stress conditions (Ansari et al.,2013; Trivedi et al., 2014a; Trivedi et al., 2014b). *P. indica* colonized Triticum aestivum plants showed relatively better survival under high salinity with respect to non-colonized plants (Zarea et al., 2012). Hence, the study was planned to evaluate the effect of *P. indica* formulation on wheat growth.

II. MATERIALS AND METHODS

Fungal formulation

The symbiotic plant growth promoting root endophytic fungus (*P. indica*) formulation was procured from the Amity Institute of Microbial Technology (AIMT), Amity University, Noida, and Uttar Pradesh for determining the effect on the performance of field grown wheat varieties.

Experiment Details

The wheat seed varieties namely PBW-343 and HD-2989 were purchased from local market of Ambala Cantt, Haryana. Forty kilogram of different seeds of Wheat varieties was mixed with 1.0 kg fungal powder and Jaggery

(gur) suspension (4% w/v) manually and then overnight incubation for adherence. The field preparation was done by watering followed by ploughing and leveling by using tractor. One acre of land is divided into four equal plots at ACE Campus, Ambala and 3 equal plots at Tepla field, Ambala. 10 Kg of seed sowed in each plot (0.25 Acre). Forty five kilogram of seed sowed in each plot (one acre). Details of the treatment are mentioned in Table 1.

Table 1: Details of the treatments

Treatment	Details	Urea and DAP (Di-amonium phosphate)	Insecticide
Treatment 1	Control Chemical fertilizers with no fungus formulation	14kg	Torpid insecticide100ml/acre was used at ACE Campus, Ambala. Insecticide of Confidor-Bayer and Fungicide of Hexaconazole - Hexcon Plus was used at Tepla field, Ambala with a dose of 100ml/acre.
Treatment 2	50% Chemical fertilizers with fungus formulation	11kg	
Treatment 3	75% Chemical fertilizers with fungus formulation	7.0kg	
Treatment 4	100% Chemical fertilizers with fungus formulation	14kg	

Evaluation of wheat growth

Seed Germination was recorded by counting the number of seeds was observed per sq ft in each field and then compared. The number of tillers and spikelets was observed. The maturity of wheat was observed in different plots in days. Harvesting was done by manually and then thrashing by machine. The thrashing machine was arranged on rent (Rs.-2000/Acre). After thrashing, the weight of wheat seeds from each plot was weighed taken observed and compared the weight of wheat between control and treated plots.

III. RESULTS AND DISCUSSION

The wheat field trial along with fungus (*P. indica*) was done at ACE campus and Tepla field, Ambala were same. The date of sowing was 25th November 2012, date of harvesting was 25th April 2013, total period was 5 months, area of each plot was 0.25 acre and weight of seed sowing in the field was 40 kg/ acre. The wheat seeds were sowed at ACE campus in the four plot such as plot-1, plot-2, plot-3 and plot-4 and plot-1, plot-2, plot-3 at Tepla field, Ambala. The different stages of wheat growth at ACE campus and Tepla Field, Ambala were measured such as no. of seed germination, no. of tillers per plant, height of plant, no. of spikelet per spike formation, maturity period and yield estimation, Estimated cost/acre w.r.t Govt. rate of Rs.1350/Quintal in each plot are shown in Table 2 .

Table 2: Performance of wheat treated with *P.indica* at different crop growth stages at ACE Campus and Tepla Field, Ambala.

	T1		T2		T3		T4	
	L 1	**L 2**	**L 1**	**L 2**	**L 1**	**L 2**	**L 1**	**L 2**
No of seed germination/ sq. ft	27	26	30	29	32	31	33	-
No. of tillers/plant	13	10	18	12	15	13	19	-
Height of plant (in feet)	2.5	2.5	2.8	2.6	2.6	2.7	1.6	-
No. of Spikelets	54	21	55	24	51	25	52	-
Maturity (no. of days)	130	130	120	120	120	120	120	-
Yield (quintal/Acre)	11.24	12.50	15.20	15.26	13.84	15.45	15.48	-
Estimated cost/Acre w.r.t. Govt. rate of Rs.1350/Quintal	15174	16875	20520	20601	18684	20857	20898	-

L1: Location 1, ACE Campus, Ambala; L2: Location 2, Tepla Field, Ambala

The no. of Seed germination was observed of 27(per sq. feet) in plot-1, 30(Per sq. ft.) in Plot-2, 32(Per sq. ft.) in Plot-3, 33 (Per sq. ft.) in Plot-4 at ACE Campus, Ambala. Seed germination stage was observed of 26(per sq. feet) in plot-1, 29(Per sq. ft.) in Plot-2, 31(Per sq. ft.) in Plot-3 at Tepla field, Ambala. The total number of tillers per plant was observed of 13 in the plot-1followed by 18 in plot-2, 15 in plot-3 and minimum 19 in plot-4 at ACE Campus, Ambala. Height of plant was observed of 2.5 feet in plot-1, 2.8 feet in Plot-2, 2.6 feet in Plot-3 and 1.6 feet in Plot-4 at ACE Campus, Ambala. Height of plant was observed of 2.5 feet in plot-1, 2.6 feet in Plot-2, 2.7 feet in Plot-3 at Tepla field, Ambala. The no. of Spikelets per spike formation was observed of 54 in plot-1, 55 in plot-2, 51 in Plot-3, 52 in Plot-4 at ACE Campus, Ambala. The no. of spikelets/Spike formation was observed of 21 in plot-1, 24 in plot-2 and 25 in Plot-3 at Tepla field, Ambala . Maturity was observed of 130 days in plot-1, 120 days in plot-2, plot-3 and plot-4 at ACE Campus, Ambala. Maturity was observed of 95 days in plot-1, 90 days in plot-2 and in plot-3 at Tepla field, Ambala. Yield (Quintal/Acre) was observed of 11.24 in plot-1, 15.20 in plot-2, 13.84 in plot-3, 15.48 in plot-4 at ACE Campus,

Ambala. Yield estimation (Quintal/Acre) was observed of 12.50Q/Acre in plot-1, 15.26Q/Acre in plot-2 and 15.45Q/Acre in plot-3 at Tepla field, Ambla. The estimated cost/Acre with respect to Govt. rate of Rs.1350/Quintal was found Rs. 15174 in plot-1, Rs.20520 in plot-2, Rs.18684 in plot-3, Rs. 20898 in plot-4 at ACE Campus, Ambala. The Estimated cost/Acre with respect to Govt. rate of Rs.1350/Quintal was found Rs.16875.00/Acre in plot-1, Rs.20601.00/Acre in plot-2 and Rs.20857.50 in plot-3 at Tepla field, Ambala. Plant growth promoting fungi are a heterogeneous group of fungi that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. The direct promotion by plant growth promoting fungi entails either providing the plant with plant growth promoting substances that are synthesized by the fungi or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when plant growth promoting fungi prevent deleterious effects of one or more phytopathogenic microorganisms. The exact mechanisms by which plant growth promoting fungi promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene (Arshad *et al*.,1993), (Glick, 1995) (ii) asymbiotic N_2 fixation (Boddey *et al*.,1995) (iii) antagonism against phytopathogenic microorganisms by production of siderophores (Scher FM *et al.,* 1982) antibiotics (Shahnahan *et al.*,1992) and cyanide (Flaishman *et al.,* 1996)(iv) solubilization of mineral phosphates and other nutrients (De Freitas *et al.*,1990; Gaur,1990). The endophyte *P. indica* colonizes roots of a range of host plants and increases biomass production and resistance to fungal pathogens and, thus has been considered a biocontrol fungus. Traditionally, fungi have been regarded as pathogens by agronomists. However, in recent years, symbiotic fungi providing benefits to crop plants have become an additional focus of research. In addition to the arbuscular mycorrhizal fungi (AMF) that constitute a distinct fungal phylum, the *Glomeromycota* (Schussler *et al*., 2001), endophytes that mainly belonging to the *Ascomycota* or *Basidiomycota*, have been shown to improve the vigour of their hosts (Ernst *et al.*, 2003; Hashiba *et al.*, 2005; Schardi *et al*., 2004; Varma *et al*.,1999). One particular endophyte, *P. indica*, a member of the *Sebacinales* order of the *Basidiomycota*, recently has received some attention. Originally, this fungus was recovered from the rhizosphere of shrubs growing in the Thar desert of Rajasthan, India (Singh *et al*., 2000; Verma *et al*., 1998). In contrast to the obligate biotrophic arbuscular mycorrhizal fungi, *P. indica* can be cultivated easily on synthetic media, where it forms typical pear-shaped chlamydospores. Earlier work established that *P. indica* increased the biomass of several host plants belonging to a wide range of taxa (Singh *et al*., 2000; Varma *et al.*, 1999; Waller *et al*., 2005). This fungus even colonized mustard, cabbage, and spinach that belong to the *Brassicaceae*, which do not form a symbiosis with arbuscular mycorrhizal fungi (Kumari *et al.*, 2003). The enhanced biomass of the host apparently results from improved nutrient supply. In the current system, the results support reduced fertilizer rates down to 75% if plant growth promoting rhizobacteria was added because that is the minimum at which results were consistent. This is different from the observations of Canoblat *et al*. (2006), Elkoca *et al*. (2008) who reported no significant difference in root and shoot biomass of barley or seed yield and biomass of roots and shoots of chickpea, respectively, when inoculant alone or fertilizer alone was used. Based on those results, it was suggested that inoculants could be an alternative to fertilizer for chickpea (Elkoca *et al*., 2008). Results indicate that the time of sampling tissue for nutrient analysis could be an essential factor to consider when making conclusions about the impact of inoculants on plant nutrient uptake. The plant growth promoting rhizobacteria promote the growth of the plant and increase the root surface area or the general root architecture (Biswas *et al*., 2000; Lucy *et al*., 2004). Plants growing better in turn release higher amounts of C in root exudates. The release of more C prompts increase in microbial activity, and this process continues in a cycle. In the present investigation the maturity was observed of 120-130 days in the control (130 days) and 120 day in the fungus treated wheat field. In addition, the fungal community structure at 120 days growth (maturity stage) differed most from the other growth stages. These community shifts during plant growth probably resulted from a modification in the root exudation pattern, which is different at maturity. In fact, after flowering most of the assimilated carbon is transported to the grain, the amount of rhizodeposit would therefore diminish at a later stage of wheat growth (Kuzyakov and Domanski, 2000). Plant age had a lesser impact in the RS (rhizophere soil) than in the RE (rhizoplane/endorhizosphere) and explained the same proportion of variance in the DGGE (Denaturing Gradient Gel Electrophoresis) profiles as the type of field. In the RS, the fungal community structure of the HH (High input high yield) field was very different from those of the LL (low input low yield) and LM (low input moderate yield) fields. The main difference in agricultural practice between High input high yield and the other two fields was a higher level of fertilization. High input high yield received 60 kg/ha of urea and 20 kg/ha of diammonium phosphate (DAP), compared to 50 kg/ha of urea and no diammonium phosphate in low input low yield and low input moderate yield. This higher level of fertilization is likely to have affected the rhizofungal community, as previously reported (Liljeroth *et al*., 1990; Marschner *et al*., 2004; Sturz *et al*., 2004). This observation indicates that the fungal community in the rhizosphere soil is likely to be more sensitive to modifications in the agricultural practices than in the rhizoplane. While reviewing the applications of plant growth promoting rhizobacteria in agronomy, Lucy *et al*. (2004) stressed the inconsistency of results between

the laboratory, greenhouse and field studies due to differences in soil type or climatic variability and the fact that plants responded better if the plant growth promoting rhizobacteria strains were isolated from the native rhizosphere. The positive response of wheat to plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi inoculation, as well as the higher mycorrhizal root colonization of arbuscular mycorrhizal fungi treated plants, might be explained by the fact that these micro-organisms were adapted to their environment in terms of soil characteristics, plant genotype and climate. Indeed, they had been selected in the wheat rhizosphere, from the same species and agricultural area. This approach might have limited the discrepancies that could have occurred between greenhouse and field trials, in the plant response to bio-inoculations.

IV. CONCLUSION

The present investigation has special significance as the fungus is being exploited for biotechnological applications in the area of agriculture particularly in cereal grain (wheat). *P. indica* has growth-promoting effects on a broad range of plants, as do the Arbuscular mycorrhizal fungi, but has the added trait of being able to be grown in axenic cultures. The above findings suggested that *P. indica* formulation is a good candidate to improve commercial plant production and might be especially useful in agroforestry and flori-horticulture applications. The future genetic research with *P. indica* would focus on clarifying mycorrhizal symbiosis through genetic manipulation of the fungal component by introducing genes of desirable attributes, such as those coding for fluorescent markers, for tracing their distribution and dispersal in nature, as well as for studying their colonization patterns and demographic factors during the symbiotic status. It would be necessary to obtain fundamental information on relevant genes and their expression by establishing and screening the complementary DNA and genomic libraries. Molecular genetics mechanism(s) underlying nutrient uptake, especially phosphate, and its transmembrane translocation are to be investigated.

ACKNOWLEDGEMENTS

The authors are thankful to Director and Management of Ambala College of Engineering and Applied Research, Ambala for providing research Lab facilities and encourage us to complete this research work.

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Gaurav Kumar, et. al. "Effect of Piriformospora Indica- symbiotic fungus formulation on the growth of wheat varieties." *International Journal of Engineering and Science*, vol. 12, no. 2, 2022, pp. 10-14.