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### **Original Research Article**

# Integrated Effect of Phosphate Solubilizing Bacteria and Humic Acid on Physiomorphic Attributes of Maize

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#### ABSTRACT

#### Keywords

Phosphate solubilizing bacteria, humic acid, biofertilizer, maize This study aims at elucidating the combined effects of humic compounds and phosphate-solubilizing bacteria to improve the yield characteristics of maize. Use of biofertilizer such as humic acid and phosphate solubilizing bacteria being an environmentally friendly, sustainable and economical approach is attracting the attention of the researchers worldwide and can help to reduce the problems raised by synthetic fertilizer. In order to reduce chemical fertilizer usage, integrated effect of phosphate solubilizing bacteria and humic acid on physiomorphic attributes of maize were tested during the year 2012 at University research farm PMAS, Arid Agriculture University, Rawalpindi. The treatment combinations were the Control, full rate of P fertilizer @ 90 kg P2O5 ha-1, humic acid @ 10 kg ha-1, PSB biofertilizer @ 1 kg ha<sup>-1</sup>, PSB biofertilizer @ 2 kg ha<sup>-1</sup>, PSB biofertilizer @ 1 kg ha<sup>-1</sup> <sup>1</sup> + humic acid @ 10 kg ha<sup>-1</sup>, PSB biofertilizer @ 2 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup>. The experiment was laid out in randomized complete block design (RCBD) with 4 replications. The result showed that the treatment where PSB biofertilizer @ 2 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup> was applied showed an increase in biological yield, grain yield, Stover yield and harvest index by 28, 50, 18 and 22% respectively, as compared to control. It is concluded that the use of chemical herbicide can be minimized by using phosphate solubilizing bacteria and humic acid.

### Introduction

Chemical fertilizers are commonly used to full fill the deficiency of soil nutrients, which results in high environmental contamination and also uneconomical (Dai *et al.*, 2004). Chemical fertilizers have several negative impacts on the environment and sustainable agriculture. Therefore, biofertilizers are recommended in these conditions while PSB biofertilizer is used as a replacement of chemical fertilizers (Wua *et al.*, 2005). Plants use minor concentration of phosphate fertilizers that are applied in the soil and the remaining is quickly changed into insoluble complexes (Vassilev and Vassileva, 2003). This directs to the need of repeated synthetic phosphate fertilizer application, but its use as an even phosphorous source is uneconomical and also unfriendly to the environment (Reddy et al., 2002). It is well known that more than two-third of phosphatic fertilizers is rendered unavailable within a very short period of time. A substantial amount of phosphorus accumulation in soils was after application obtained soon of phosphorous fertilizer usually that is fixed; resulting in low recovery by crops (Alam and Ladha, 2004).

Bolan and Duraisamy (2003) described that phosphate solubilizing bacteria (PSB) play a significant part in phosphorus nutrition by improving phosphorus accessibility to the plants through the release of organic and soil phosphorus by inorganic pools mineralization and solubilization. Application of phosphate solubilizing bacteria as inoculants enhance phosphorous uptake. Humic acid is also an important constituent and an intimate part of the soil organic structure which is highly effective in improving soil condition and plant growth (Pettit, 2004). Humic acid is one of the main components of humic substances. Humic matter is formed through the biological and chemical humification of dead animal and plant parts and through the biological actions of microorganisms, having high molecular weight, resistance to decay and ranging in color from yellow to black. Humic acid, which is used as a commercial product normally contains, 6-8% H, 44-58% C, 0.5-4% N, 42-46% O and many other components (Larcher, 2003). Humic acid may be utilized in agriculture as a fertilizer, plant growth promoter, nutrient carrier and soil conditioner (Bidegain et al., 2000). Small concentrations of humic acid has been reported to enhance shoot length, plant growth, root length, moisture and nutrient

uptake significantly (Ylmaz, 2007). The use of phosphate-solubilizing bacteria as inoculants.

Simultaneously increases P uptake by the plant and crop yield. Strains from the genera Pseudomonas, Bacillus and Rhizobium are among the most powerful phosphate solubilizers (Rodriguez and Fraga, 1999). The principal mechanism for mineral phosphate solubilization is the production of organic acids such as acetic, succinct, prop ionic, butyric, formic, oxalic, and citric acids (Perez et al., 2007). Acid phosphatase plays a major role in the mineralization of organic phosphorous in soil (Gyaneshwar et al., 1998). Phosphate solubilizing bacteria added to media or soil significantly increases acid phosphatase activity at day 7 and maintained this value until the end of 21 days, about 900 µg p-nitrophenyl phosphate day<sup>-1</sup> (Premono 1994). In Pakistan during 2009-2010 maize production was 3,262 thousand tons, which was increased to 3,341 thousand tons in 2010-11, showing an increase of 2.4% (Fahad and Bano, 2012). In Pakistan, maize is the third important crop after wheat and rice (Fahad and Bano, 2012). In the year 2010-2011 total area under maize crop in Pakistan was 974.3 thousand hectares with 3706.9 thousand tons of total production (Fahad and Bano, 2012). The aim and objective of our present study were to investigate the integrated effect of PSB and humic acid on yield attributes of maize.

# Materials and Methods

To investigate the integrated effect of phosphate solubilizing bacteria and humic acid on physiomorphic attributes of maize, an experiment was conducted at the University Research Farm, PMAS Arid Agriculture University Rawalpindi during the spring season, 2012. The soil was silt loam having 0.53 percent organic matter. The seeds of maize (*Zea mays* L.) obtained from National Agricultural research center (NARC) Islamabad, were surface sterilized with 95% ethanol followed by sterilization in 10% chlorox solution for 5min by shaking and cultivated in the field.

The test variety Islamabad gold was sown at the rate of 30 kg ha<sup>-1</sup>. Individual plot size for each treatment was 6m x 8m with row spacing of 75 cm and plant to plant distance of 25 cm. A field experiment was performed by using a RCBD design with four replications. The recommended dose of NPK @ 200-120-125 kg ha-1 was applied. Humic compounds extracted from rice straw compost are added to the soil and are incubated for 30 days. The compost is previously prepared by composting rice straw, naturally in the field (no added chemicals and decomposers). The humic compounds in the compost are extracted by squeezing and diluting the compost in water. The amount of humic compounds obtained from this simple water squeezing and dilution method is 5.63 mg kg<sup>-1</sup> containing 13,000 me COOH 100g<sup>-1</sup> humic compound.

# Statistical Analysis

Data collected for different characteristics were statistically compared at 5% level of probability with a least significance difference (LSD) as described by Montgomery (2001).

# **Results and Discussion**

Data presented in table. 1 revealed that plant height has shown significant differences among different humic acid and PSB treatments. Maximum plant height (159.25 cm) was recorded for treatment where PSB biofertilizer @ 2 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup>were applied. Plant height, i.e. 153.50 cm recorded in the plots where PSB kg ha<sup>-1</sup> were applied. The treatment PSB biofertilizer @ 2 kg ha<sup>-1</sup> was obtained 145.50cm plant height. This increase in plant height is due to the availability of plant nutrients provided by the humic acid and PSB biofertilizers. PSB inoculation and humic acid have the highest efficiency to enhance plant growth by providing nutrients especially phosphorous to the plants. Height of plant is a vital factor and although it is mostly influenced by genetic factors yet it has significantly influenced by nutrient, environmental factor and water stress and weed infestation. More plant height is a symbol of more vegetative growth and less height of the plant indicates under plant growth. Both of them causes poor grain development and results in decrease of final grain yield. An optimal plant height is required for better plant stand and result in high crop yield. Finding of the study in accordance with Sarawgi et al. (1999) who described that inoculation of phosphate solubilizing bacteria increase nodulation, plant growth and grain yield of chickpea crop. David et al. (1994) reported that the addition of humic substances, which act in a similar way to growth hormones, promoted the growth and nutrient uptake of plants. Similarly, the maximum cob length (17 cm) was recorded in the plots where PSB biofertilizer @ 2 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup>were applied. Cobs length (16.4 cm) was recorded in the plots where Full rate of P fertilizer @ 90 kg  $P_2O_5$  ha<sup>-1</sup> was applied. Cobs length was recorded (14.1cm) in the plots where PSB biofertilizer @ 1 kg ha <sup>1</sup>was applied. The increase in cob length was found due to the availability of plant nutrients. The results of the current are also in conformity to the finding of Mohammad pour khan eghah et al. (2012) who testified that cob length of maize enhanced with the application of liquid humic acid.

biofertilizer @1 kg ha<sup>-1</sup> + humic acid @ 10

Treatments	Plant height (cm)	Cob length (cm)	Grains number per cob <sup>-1</sup>	1000-grain weight (g)	Biological yield (kg ha <sup>-1</sup> )	Grain yield (kg ha <sup>-1</sup> )
Control	110.50 d	13.9 c	289 e	190 c	4730.3 c	1238 d
Full rate of P fertilizer @ 90 kg $P_2O_5$ ha <sup>-1</sup>	140.75 abc	16.4 ab	355 c	210 b	5625 ab	1630 bc
Humic acid @ 10 kg	130.25 bcd	15.5 b	323 d	192 c	5288 b	1517 c
PSB biofertilizer @ 1 kg ha <sup>-1</sup>	124.50 cd	14.1 c	299 e	197 c	5185.3 bc	1499 c
PSB biofertilizer @ 2 kg ha <sup>-1</sup>	145.50 ab	16.3 ab	377 b	210 b	5706.3 ab	1689 bc
PSB biofertilizer @ 1 kg ha <sup>-1</sup> + humic acid @ 10 kg ha <sup>-1</sup>	153.50 a	16.4 ab	387 ab	219 b	5875.3 a	1798 ab
PSB biofertilizer @ 2 kg ha <sup>-1</sup> + humic acid @ 10 kg ha <sup>-1</sup>	159.25 a	17.0 a	398 a	232 a	6061.5 a	1957 a
LSD (0.05)	20.802	1.1524	17	9.7	530.43	228.6

**Table.1.** Effect of phosphate solubilizing bacteria (PSB) and humic acid on Plant height, Cob length, Grains number per cob<sup>-1</sup>, 1000-grain weight, Biological yield (kg ha<sup>-1</sup>) and Grain yield of maize

In the meanwhile, highest grains per cob (398) were recorded in the plots where PSB biofertilizer @  $2 \text{ kg ha}^{-1}$  + humic acid @ 10 kg ha<sup>-1</sup> were applied. The least grains numbers per cob was recorded (299) in the treatment where PSB biofertilizer @ 1 kg ha<sup>-1</sup> was applied. Increase in grain number per cob may be attributed to increasing the availability of plant nutrients by the humic acid and PSB biofertilizer. Finding of this study in accordance with Winarso et al. (2011) combined effects of humic acid and PSB (Pseudomonas putida) increase the availability of nutrients and yields of soybean. Whereas, the heavier 1000-grain weight 232 g compared to control was recorded in the plots where PSB biofertilizer @ 2 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup>were applied. The minimum 1000-grain weight that is 192 as compared to control was recorded in the plots where humic acid @ 10 kg ha<sup>-1</sup>was applied. Increase in 1000-grain weight of common millet due to the application of humic acid was informed by (Veysel et al., 2011).

Similar results were described by Yazdani *et al.* (2009) that grain weight of maize increased with the application of phosphate solubilizing microorganisms.

The maximum increase in biological yield 28.14 percent over control was recorded in the plots where PSB biofertilizer @ 2 kg  $ha^{-1}$  + humic acid @ 10 kg  $ha^{-1}$ were applied (table. 1). Twenty four percent increase in biological yield over control was recorded in the plots where PSB biofertilizer @1 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup>. In the plots where humic acid @ 10 kg ha<sup>-1</sup>was applied showed (11.78 percent) increase in biological yield over control. Whereas, full rate of Full rate of P fertilizer @ 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>increase (18.91 percent) biological yield over control. These results agreed with the finding of Ayasand Gulser (2005) who reported that humic acid leads to increased growth and subsequently height and increased biological yield through the increasing nitrogen content of the plant. Tan (2003)

also reported that application of humic acid in nutritional solution led to increased content of nitrogen within aerial parts and growth of shoots and root of maize.

The maximum increase in grain yield (50.04 percent) over control was recorded in the plots where PSB biofertilizer @ 2 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup> was applied, followed by (45.16 percent) increase in grain yield over control was recorded in the plots where PSB biofertilizer @1 kg ha<sup>-1</sup> + humic acid @ 10 kg ha<sup>-1</sup>was applied. The minimum increase in grain yield (21.02 percent) over control was recorded in the plots where PSB biofertilizer @ 1 kg ha<sup>-1</sup>was applied. These results are in conformity with the work of Shahryariand Shamsi (2009) who reported that application of humic acid along with a PSB increase grain yield of wheat. Grain vield is the function of the of interaction various genetic and environmental factors influencing the yield components. Variation in these factors may bring a variation in gain yield. Grain yield of Maize was considerably enhanced by all the treatments over control. These results are also agreeing with Mohamed et al. (2009) who indicated that crop yield in maize increased by application of humic acid based fertilizers. The same results were also reported by Juhi et al. (2011).

Humic substances (HS) are the major fraction of the soil organic matter which represents the final stage of a complex interaction between non-living organic matter and microbial communities. PSB and humic acid have great influence on the production of maize crop. PSB and humic acid improve the ability of soil to solubilize phosphorus for better crop production. Different characteristics of maize were increased in the treatments where different concentration of PSB biofertilizer applied with humic acid. On the basis of this study, it may conclude that integrated effect of PSB and humic acid is a useful, efficient and economical practice for supplying nutrients to the crop plants and enhancing crop yield. However, such studies may be continued for further confirmation of results. This synergy of effects favors the increase in population and activity of selected microbes and the consequent response to plant growth promotion, thus opening opportunities to develop a new generation of biofertilizers for sustainable agricultural systems.

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