Research Article

Foliar Application of Phosphorus to Enhance Phosphorus Utilization and Crop Growth: A Hydroponic Study

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Abstract | A hydroponic study was conducted to investigate the effectiveness of foliar applied phosphorus for enhancing P use efficiency and crop growth at Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar. Wheat (cv. Atta Habib-2010) seeds were first germinated in sand culture and then three weeks old seedlings (7.5 cm) were transferred to water tubs containing Hoagland solution without P. The treatments included 0, 18, 36, 72, 144 and 216 mM as KH_2PO_4 solution foliarly applied three times with one week intervals starting after two weeks of seedlings transplantation. Each treatment unit consisted of 10 seedlings arranged in CR design with three replications. Plant height, shoot and root dry biomass of 6 weeks old seedling were significantly increased with each increment of foliar P suggesting effective absorption through leaf stomata and translocation to other parts of the plant body. The plant height, shoot and root dry biomass yield increased by 55, 40 and 35%, respectively, at 144 mM KH_2PO_4 solution over control. The shoot and root [P] as well as its total uptake by wheat seedlings also showed several times increase over control. Further increase beyond 144 mM KH_2PO_4 though did not show any adverse effect and was no better than lower P levels. It is concluded from the present study that [P] applied as foliar spray up to 144 mM KH_2PO_4 could be an effective way to enhance the plant growth in P deficient condition and application without any detrimental effect on wheat crop.

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Introduction

Foliar application of fertilizers is a widely used practice to correct nutritional deficiencies in plants caused by improper supply of nutrients to roots (Fan and Silberbush, 2002). Foliar fertilization is usually economical and effective under certain conditions that supply nutrients quickly to a target organ (Fageria et al., 2009). This technique is adopted and important for agricultural crops to achieve maximum yield when crop nutrient demand is not fully fulfilled during the crop growth period. The techniques with instant supply of nutrients also help in reducing the exposure of chemicals to the environment and promoting sustainable agriculture. Numerous factors influence the efficacy of foliar applied fertilizers and may elucidate the often-reported discrepancy in plant response to foliar nutrition. Before adopting foliar fertilization some pre-requisites must be met including (i) the nutrient applied must be in an available form for leaf absorption; (ii) the nutrients applied must grasp the target organ (often leaves); (iii) the target organ must have an enough retention area to reduce spread in the environment; (iv) the nutrient chemistry and inter-

Links Researchers

nal leaf structure as well as environmental conditions should be such that nutrients either in ionic or in nonionic form can enter the leaf cuticle and apoplast of the cells of the target organ (Pandey et al., 2013). Moreover, the response to the nutrient of interest is more likely if the plant is deficient in this nutrient prior to application. The main mechanisms by which plants take up the foliar applied nutrients are through leaf stomata (Eichert et al., 1999) and hydrophilic pores within the leaf cuticle. The crop response to foliar P applied depends on soil and climatic conditions, type of crop as well as concentration and source of fertilizers. The main water soluble P fertilizers are single superphosphate [$Ca(H_2PO_4)_2$+$CaSO_4$], triple superphosphate [$Ca(H_2PO_4)_2$], monoammonium phosphate [$NH_4H_2PO_4$], diammonium phosphate [(NH_4) $2H_2PO_4$], monopotassium phosphate (KH_2PO_4) and phosphoric acid [H_3PO_4] which are being used as foliar spray. Phosphorus is absorbed and penetrates to the plant leaves through various pathways when applied to the foliage.

The foliar applied P may be absorbed and assimilated by plants in the same manner as they do in case of micronutrients and sulfur application. The P application through foliar technique may enhance the soil applied P efficacy with potential increase in P use efficiency and reduction of plant dependency on soil P. Resultantly, it helps in reducing the phosphorus recommended levels if applied in combination with foliar applied P. Fan and Silberbush (2002) reported increased in leaf area, fresh and dry weight, and leaf contents of chlorophyll, N, P, and K in response to foliar application of NPK fertilizer. Foliar application of supplementary P and K resulted in increases in dry matter and chlorophyll concentrations of three tomato (*Lycopersicon esculentum*) cultivars (Kaya, 2001). Similarly, Arif et al. (2006) reported that foliar application is a promising technique that can increase the availability of nutrients to crops for obtaining higher yield. According to Mosali et al. (2006) that mid-season P deficiency in winter wheat might be corrected by low rates of foliar applied P and subsequently may result in higher P use efficiencies.

In this study various concentration of KH_2PO_4 solution were foliarly applied to wheat crop grown in P excluded nutrient Hoagland solution to asess the effect of KH_2PO_4 concentration on yield, P uptake and its translocation from leaves to roots.

Materials and Methods

The experiment was conducted at the glass house of Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture; Peshawar during 2013-14, in P excluded nutrient Hoagland solutions. Wheat seeds (cv. Atta Habib-2010) were first germinated in sand and 2 weeks old wheat plants (7.5 cm) were transferred to water tubs (20 L) with foam floating over Hoagland's solution without P. Just after transplanting, P as KH_2PO_4 was applied as foliar spray at the rate of 0, 18, 36, 72, 144 and 216 mM with one week intervals for 4 weeks. The Hoagland solution was prepared (Table 1), according to the recipe developed by Hoagland and Arnon (1950). The experiment continued till 6 weeks and the pH of the solution was monitored after every three days and was adjusted to 7 with either HCl or NaOH. An air pump was installed to provide oxygen to the plant continuously throughout the experiment. Data on plant height, shoot and root biomass (dry), P concentration in roots, shoots and total P uptake was recorded. At the time of termination of the experiment, root and leaves were separated from plants and rinsed with

Table 1: *Hoagland's solution composition (full strength).*

Compound	Final Concentration	Mol. Wt. (g)	g L^{-1}	Stock Concentration (mM)	Volume added to 1 L (mL)
KNO_3	6.5 mM	101.11	82.15	812	8
$Ca(NO_3)_2.4H_2O$	4.0 mM	236.16	118.08	500	8
$MgSO_4.7H_2O$	2.0 mM	246.47	61.62	250	8
H_3BO_3	4.6 µM	61.83	0.284	4.6	1
$MnCl_2.4H_2O$	0.5 µM	197.9	0.099	0.5	1
$ZnSO_4.7H_2O$	0.2 µM	287.54	0.055	0.2	1
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.1 µM	1235.95	0.124	0.1	1
$CuSO_4.5H_2O$	0.2 µM	249.7	0.050	0.2	1
$FeCl_3$	45 µM	162.2	24 ml of 60%	45	1

tape water, roots and leaves were oven dried at 80 ^{0}C for at least 72 hours, ground and subjected to wet digestion for the determination of P concentration in plant leaves and roots. The [P] in the digest was then measured at 880 nm using a spectrophotometer (Perkin Elmer, Lambda-35) and NH_4- molydate color complex (Kuo, 1996). The experiment was repeated for two seasons (2013-14 and 2014-15). Relative plant height was determined using a formula below;

Relative height = (Given value/Maximum value) x 100

Results and Discussion

Plant Height (cm)

The plant height during both years significantly ($p \leq 0.05$) increased with increasing foliar P solution concentration (Table 2) suggesting absorption of P as foliar spray in the form of KH_2PO_4 solution. The mean plant height of both years ranged from 19 cm in control (0 mM) to 43 cm in treatment receiving 144 mM KH_2PO_4 clearly showing increases in plant height with increase in P solution concentration. However, beyond 144 mM KH_2PO_4 application plant height did not increase. The relative plant height showed that with application of 144 mM KH_2PO_4 solution the plant height could be improved by about 60 % over control (Figure 1). The response was more pronounced between control and 18 mM which increased plant height by 55 % over control implying the absorption of P through leaves when foliar applied and its role in plant growth in no P environment. These results suggested no adverse effect of foliar applied P up to 144 mM KH_2PO_4 solution and any level below or equal to this level could be adopted for getting taller plants without experiencing any detrimental effect. The increase in plant growth with foliar P is in line with reports of Soylu et al. (2005), Kenbaev and Sade (2002) and Arif et al. (2006) who observed significant increase in plant height of wheat crop with foliar application of different nutrients individually or in combination. Increase in number of fertile tillers of wheat was also reported with foliar P in early growth stage (Elliot et al., 1997; Grant et al., 2001). The results of the present study confirm that foliar applied P could be absorbed by plants and metabolically take part in plant growth and development as was evident from the increase in plant height. It was observed one week after transplant that the plants grown in P free solution are showing deficiency symptoms and eventually the plants wilted that seem to be due to P starvation. The initial survival seemed to be due to the presence of P in seeds and a fraction of that came from sandy soil initially used for germination as it was not washed.

Table 2: *Plant height, shoot dry weight and root dry weight as influence by different levels of foliar applied phosphorus solution (KH_2PO_4).*

Foliar P		Plant height	Shoot dry weight	Root dry weight
(mM KH_2PO_4)		(cm)	g 10-plants^{-1}	
0	-	19.00 d	18.17 d	15.03 d
18	-	31.33 c	21.00 cd	16.78 c
36	-	36.00 b	22.33 bc	17.84 c
72	-	36.33 b	24.67 ab	20.29 b
144	-	43.00 a	26.50 a	22.45 a
216	-	40.50 a	25.00 ab	23.12 a
LSD at p < 0.05		3.14	2.90	1.11
Average across treatments				
-	2013	33.33 b	23.17 a	18.98 a
-	2014	35.39 a	22.72 a	19.53 a
LSD at p < 0.05		ns	ns	ns

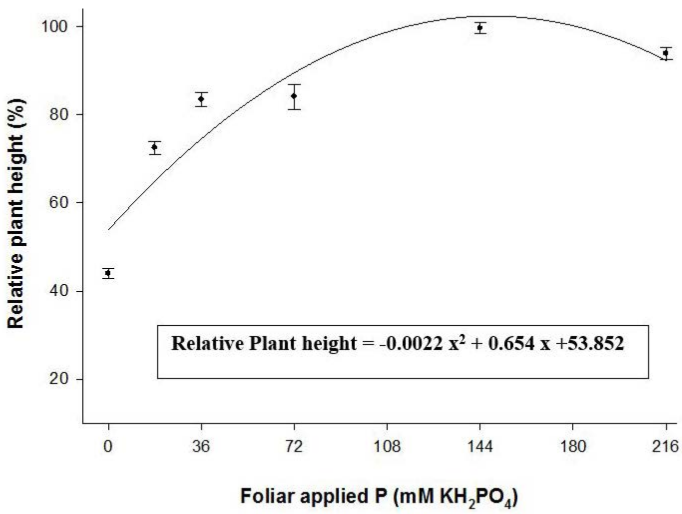

Figure 1: *Relative plant height (%) of 6 weeks old (after transplantation) wheat plants at given foliar KH_2PO_4 application grown in phosphorus excluded Hoagland solution (values are averages of two years and calculated as percent of maximum).*

Shoot dry weight (g 10 plants^{-1})

Mean wheat shoot dry weight also significantly ($p < 0.05$) increased with increase in foliar P solution from 18.2 g in control (0 mM) to 26.5 g 10 plants^{-1} in tubs receiving 144 mM KH_2PO_4 (Table 2). The percent relative shoot dry weight also showed the same trend where it increased with increase in P levels with maximum at 144 mM KH_2PO_4 which was about 40% higher relative yield than control (Figure 2). However, the shoot dry weight at 72, 144 and 216 mM KH_2PO_4 were statistically at par implying that any of three treatments could be applied with no detrimental effect of higher concentration. The level of 144 mM KH_2PO_4 as foliar spray was also better in term of increase in plant height (Table 2) and hence seems more appropriate. By comparing the result of two years it was noted that there were no significant differences in biomass during the two years.

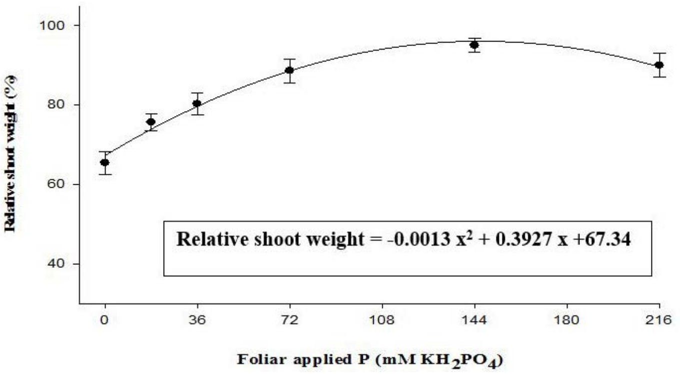

Figure 2: *Relative shoot dry weight (%) of 6 weeks old wheat plants at given foliar KH_2PO_4 application grown in phosphorus excluded Hoagland solution (values are averages of two years).*

The increase in shoot dry weight was associated with increase in [P]. Studies in the past have also reported P adsorption from KH_2PO_4 through leaf stomata when applied in solution form. Pandey et al. (2013) reported that P is absorbed by wheat leaves via stomata through diffusion as well as through biochemical pathways. The results reviewed by Elliot et al. (1997) and Grant et al. (2001) concluded that applying foliar P in early growth stage can increase the number of fertile tillers and crop growth. This response to foliar application will be more in P deficient condition. Usually P is applied to soil at sowing time and as such plants with optimum P supply at early stage may suffer P deficiency at later stage especially in high yield crops (Gray 1997). In such situation, the foliar P application might correct the later stage P deficiency and increase the yield.

Root dry weight (g 10 plants^{-1})

The root dry weight of 6 weeks old plants grown in hydroponic solution excluded P (Table 2) significantly increased with increasing level of foliar P with mean values ranging from 15 at 0 mM to 23.1 g 10 plants^{-1} with 216 mM KH_2PO_4 (when values were averaged across the years). However, the difference between 144 and 216 mM KH_2PO_4 were statistically at par with root weight values of 22.5 and 23.1 g 10 plants^{-1}, respectively revealing that like plant height and shoot dry weight 144 mM KH_2PO_4 solution could the appropriate level. The Figure 3 also showed substantial increase with each increment of foliar applied P with higher percent relative yield at 144 and 216 mM KH_2PO_4 solution (Figure 3).

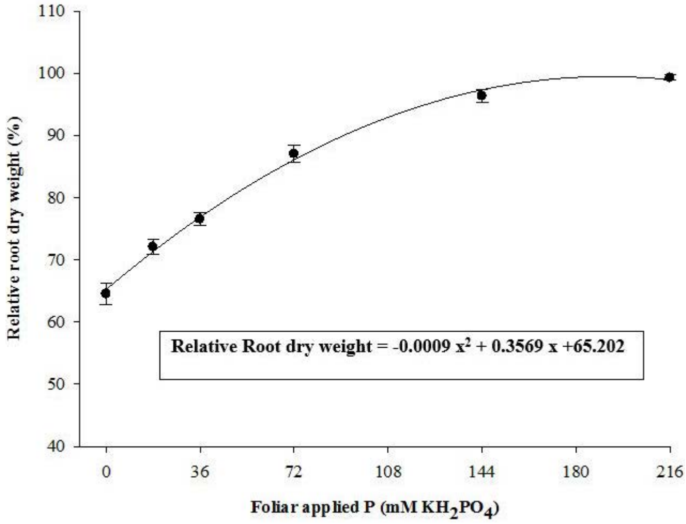

Figure 3: *Relative root dry weight (%) of 6 weeks old wheat plants at given foliar KH_2PO_4 application grown in phosphorus excluded Hoagland solution (values are averages of two years).*

This increase in root growth and yield could be attributed to P absorption through plant leaves as the hydroponic solution did not contain any P. Once the nutrient crosses the plant surface and is absorbed, it can take either apoplastic or symplastic pathway and transported to the other parts of the plant (Pandey et al., 2013). Phosphorus has been established as the second essential nutrient required for plant development, enhanced growth, cell division and protein synthesis which enhances the overall functions of plants (Sharma et al., 2013) and responsible for various physiological growth and biochemical processes of plant (Cozzolino et al., 2013). The most essential function of P is storage and transfer of energy in the form of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and many nucleic a cids, coenzymes, phospholipids, and nucleotides which all together would have increased the growth of plants as well as of roots.

Concentrations of P in wheat shoot

Application of foliar P significantly ($p < 0.05$) increased the shoot P concentration [P] by several-fold over the control (Table 3 and Figure 4). The mean P concentration in leaves of both years was highest (3.9 g kg^{-1}) for 216 mM KH_2PO_4, followed by (3.3 g kg^{-1}) with 144 mM KH_2PO_4 solution. There are various point like stomata, cuticular cracks, ectodesmata, lenticels and aqueous where P can enter to the leaves of the plant (Pandey et al., 2013).

Table 3: *P concentration in plant leaves, roots and total uptake as influenced by different doses of foliar applied phosphorus.*

Foliar P (mM KH_2PO_4)		P concentration in leaves	P concentration in root	Total nutrient uptake
		g kg^{-1}		mg 10 $plants^{-1}$
Average across years				
0	-	0.9 e	1.0 c	30.92 e
18	-	2.1 d	1.7 b	72.22 d
36	-	2.7 c	1.6 b	89.72 c
72	-	3.2 b	2.0 ab	120.27 b
144	-	3.3 b	2.2 a	136.97 a
216	-	3.9 a	1.9 ab	139.49 a
LSD at $p < 0.05$		0.20	0.42	14.8
Average across treatments				
-	2013	2.6	1.8	99.6
-	2014	2.7	1.6	96.9
LSD at $p < 0.05$		ns	ns	ns

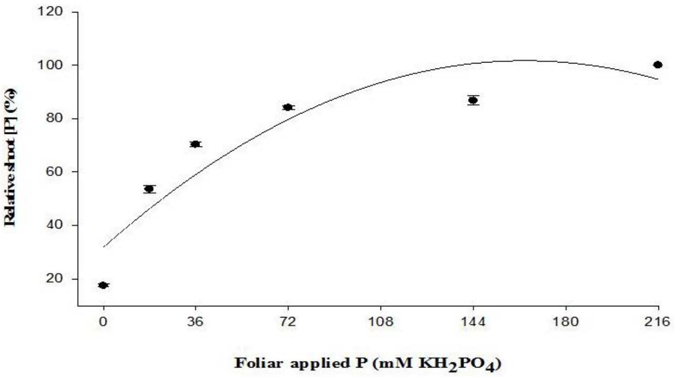

Figure 4: *Relative P concentration in shoots (%) of 6 weeks old wheat plants at given foliar KH_2PO_4 application grown in hydroponics (values are averages of two years and calculate as percent of maximum).*

There was a clear response between control and 18 mM KH_2PO_4 affirming that P was absorbed by the leaves when foliar applied. The shoot [P] increased from 0.9 to 2.1 g kg^{-1} showing 100 % increases with only 18 mM KH_2PO_4 solution. Girma et al. (2007) and Dixon (2003) reported increase in P use efficiency with foliar application over pre- plant applied P and suggested that foliar application of P could be an efficient strategy to enhance P use efficiency. Arif et al. (2006) observed that foliar application enhances availability of nutrients to crops for obtaining higher yield. Furthermore, since no adverse effect of foliar applied P up to 144 or even 216 mM KH_2PO_4 solution (equivalent to 2.0 and 3.0 %, respectively) was observed and thus any level below or equal to this level could be recommended without experiencing any detrimental effect.

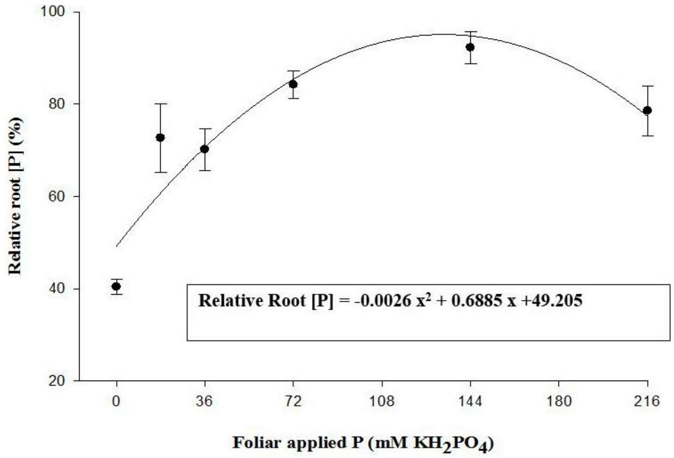

Figure 5: *Relative P concentration in roots (%) of 6 weeks old wheat plants at given foliar KH_2PO_4 application grown in hydroponics (values are averages of two years).*

Concentration of P in wheat roots

In both years, P concentration [P] in roots of 6 weeks old wheat crop was significantly ($p < 0.05$) increased by P foliar application as compared to control. The mean P roots concentration across the two years increased from 1in control to 2.2 g kg^{-1} in treatments receiving 144 mM KH_2PO_4 solution but did not increase beyond this level. Furthermore, the root [P] did not significantly change among 72, 144 and 216 mM KH_2PO_4 solution suggesting that any level between them could be appropriate without harmful effect. The increase in root [P] with foliar application of P suggested its absorption and assimilation as well as translocation within the plant body. The same trend can be seen in Figure 5 where the relative root dry weight increased with increase in foliar KH_2PO_4 solution. Phosphorus absorbs and penetrates to the plant leaves through various pathways when applied to the foliage and increase yield and P concentration in leaves and roots. The absorption and penetration primarily depends on the ionic charge on plant surface and molecular size. Pandey et al. (2013) reported that once the nutrient crosses the plant surface and is absorbed it may take both apoplastic or symplastic pathway and transport to the other parts of the plant. These results suggested no adverse effect of foliar applied P up to 216 mM KH_2PO_4 solution and any level below or equal to this level could be adopted without experiencing any detrimental effect.

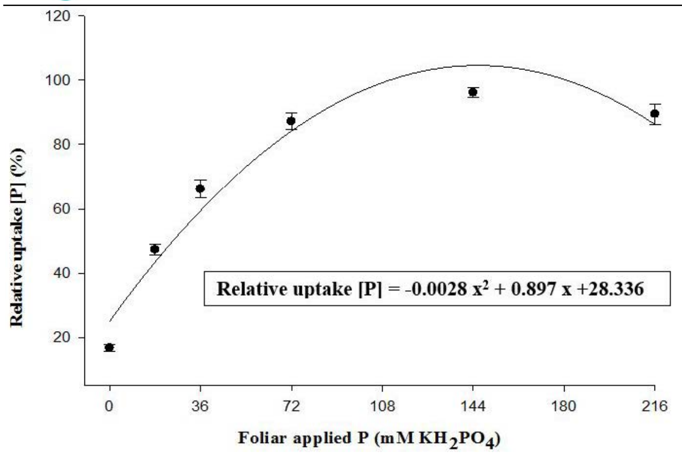

Figure 6: *Total uptake by wheat crop (%) of 6 weeks old wheat plants at given foliar KH_2PO_4 application grown in hydroponics.*

Uptake of P in wheat shoots and roots

The total uptake (sum of root and shoot) of P by 6 weeks old wheat significantly increased with foliar application of KH_2PO_4 at different level (Table 3). The P uptake increased with each increment of P concentration up to 144 mM KH_2PO_4 with values from 30.92 to 139.49 mg 10 plants^{-1} when averaged across the years (Figure 6). The P uptake recorded in treatments recovering 144 and 216 mM KH_2PO_4 were statistically similar with values of 136.97 and 139.49 mg 10 plants^{-1}, respectively. The increase in nutrient uptake in the plants with foliar application of phosphorus were primarily associated with corresponding increase in plant nutrient content as well as overall dry matter production. The non-significant increase in treatments beyond 144 mM implied that any level of 144 or 216 mM could be applied for better P use efficiency and crop growth.

Conclusions

Foliar P application as KH_2PO_4 solution promoted plant height, biomass and P uptake of 6 weeks old wheat plants grown in P free Hoagland solution revealing the effective absorbance of P from solution, assimilation and translocation to other parts of the plant as recorded in case of significantly higher root [P]. The response of relative P uptake clearly indicates that P was absorbed through foliage as the plants were grown in P free Hoagland solutions. No detrimental effect of higher P concentration (216) mM was observed, however, the maximum plant growth or P uptake was recorded with increase in P solution up to 144 mM KH_2PO_4. The non-significant changes among treatment beyond 72 mM further suggested that any level of 72, 144 or 216 mM could be adopted for enhanced P utilization and crop yields without any injurious effect.

Author's Contribution

Rafiullah was the major investigator of the study. He did the laboratory work, collected and analysed the data and wrote the article. Muhammad Jamal Khan supervised the work, provided overall input and concieved the idea of reaserch. Dost Muhammad provided experimental material, helped in data analysis and wrote the article.

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