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Effects Of Phosphate Solubilizing Bacteria And Arbuscular Mycorrizal Fungi On Contribution Of Inorganic Phosphorus Fractions

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ABSTRACT

Phosphorus is an essential element for plants nutrition. In calcareous soils, concentration of plant available phosphorus is low, while the amount of insoluble phosphate compounds is high. The purpose of this study was to evaluate the effect of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on phosphorus solubility of insoluble compounds and wheat growth. This experiment was a randomized complete block design with four replications. Three levels of phosphate fertilizer (0, 20 and 40 mg kg^{-1}), four levels of inoculums (phosphate solubilizing bacteria, arbuscular mycorrhizal fungi, combination of fungi and bacteria, and control) and four harvest times (25, 50, 75 and 100 days after planting) were used. Measurements were P-Olsen, Ca_{10}-P, O-P, Fe-P, Ca_8-P, Ca_2-P, total P, phosphorus in plant and dry matter weight. Application of 40 $mgkg^{-1}$P increased P-Olsen (153%), Ca_{10}-P (3.6%) and Ca_2-P (108%) compared to the control treatment. .Inoculation reduced the amount of Ca_{10}-P (6-10 mg kg^{-1}) and Ca_8-P (13-18 mg kg^{-1}). Mycorrhizal fungi had no effect on the amount of P-Olsen and Ca_2-P. Amounts of Ca_{10}-P and Ca_8-P after planting decreased over time. Dry matter weight after 25, 50, 75 and 100 days of planting with combination bacteria and fungi were 4.1, 11, 21.3 and 29.4 g pot^{-1}, respectively.

Key words: Dry matter weight, inorganic phosphorus, P forms, mycorrhizal, solubilizing

Introduction

Insoluble inorganic phosphorus (P_i) are not directly uptake by the plant. Calcareous soils contain high concentration of these compounds [16]. Phosphate fertilizers can alter the concentration of inorganic phosphorus in the soil [11]. Mostasahri et al. [16] showed that all forms of Pi (except apatite) increased significantly, with phosphate fertilizer application.

Soil microorganisms also can be effective on P_i fraction. Research on various forms of phosphate compounds and the use of soil fungi on the forest and agriculture, was carried out in Brazil. Results showed that different forms of P_i in the soil are as follow: Fe-P>occluded Al-P>occluded Al-P>Al-P>Ca-P. In this study 14 types of fungi were used and results indicated that only one type can dissolution of total Pi compounds. Nine other species were effective on the Fe-P and Ca-P compounds and four on of Ca-P compounds solubility [1]. Ghaderi et al. [6] investigated influence the effect of three strains of *pseudomonas feteda*, *Pseudomonas fluorescens CHAO* and *Tabriz Pseudomonas fluorescens* on release of phosphorus from iron hydroxides. They reported that the amount of phosphorus released to the strains were, 50.5, 29.2 and 61.6% respectively. *Pseudomonas fluorescens CHAO* strains secreted the highest concentration of the enzyme phosphatase levels. While the other two strains had maximum amount of hydrogen ion secretion. Hydrogen ions are the main factor in the release phosphorus of iron hydroxides. Chul Kang et al. [3] studied the effect of a type of fungi called *Fomitopsis SP* on tri-calcium phosphate, rock phosphate, aluminum phosphate and hydroxyapatite and showed that the maximum solubility was of tri-calcium phosphate, but had no significant effect on hydroxyl apatite concentration. Metin et al. [15] investigated the effect of inoculation agents (fungi and bacteria), inorganic phosphate (tri-calcium phosphate and rock phosphate) and two phosphate concentration on the concentration of soluble phosphorus. All treatments and their interaction effects combination (except the interaction between phosphorus and inorganic phosphorus concentration) had significant impacts on the amount of soluble P.

The purpose of this study was to evaluate the effect of phosphate solubilizing bacteria and arbuscular mycorrizal fungi on solubility of insoluble

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phosphate compounds in soil under wheat cultivated for 100 days period. Also, the evaluated dry matter weight and P uptake by plants were evaluated.

Materials And Methodes

Soil sample was taken from 0-15 cm of was used to grow wheat in green house. Soil subsamples were used to determine chemical properties and soil texture. Electrical conductivity of a saturated extract [19] and pH of a saturated paste were determined. Organic carbon was measured by wet oxidation [17]. Particle size distribution was determined by the pipette method [5]. Calcium carbonate equilibrium was determined by reverse titration [14]. Available P was extracted by Olsen [18].

The experimental treatments were arranged in split plot factorial based on a complete randomized block design including three phosphorus fertilizer levels (0, 20 and 40 mg kg^{-1}P), four levels of phosphate solubilizing microorganisms (PSM). The PSM used in this study were: mixture of three phosphate solubilizing bacteria (PSB) including *Azotobacter chrocooccum* strain 5, *Pseudomonas fluorescens* 187 and *Pseudomonas fluorescens* 36, 2- mixture of arbuscular mycorrizal fungi (AMF) including *Glomus mossea* and *Glomus intraradices*, 3- mixture of PSB and AMF, and 4- control. Sampling time was 25, 50, 75 and 100 days after planting. The experiment was replicated four times; total numbers of treatments were 192.

Pots with a diameter of 10 cm were used. Pots were filled with soils that passed through 2-mm sieve. 20 mg kg^{-1} nitrogen using urea fertilizer was added to each pot. Bacteria were inoculated using seed inoculation method and fungi were inoculated with soil inoculation method. Ten g of fungi were placed 2 cm below the soil surface. Ten wheat seeds (verinak variety) were planted in each pot. Planting time was Nov. 5, 2010.

Sequentially fractionation procedure for inorganic phosphorus (P_i) fractions was performed. Ca_2P by $NaHCO_3$, Ca_8P by NH_4Ac, AL-P by NH_4F, Fe-P by $NaOH$-Na_2CO_3, occluded-P by Na_3Cit-$Na_2S_2O_4$-NaOH, and $Ca_{10}P$ by H_2SO_4 [10].

Five wheat plants were selected from each pot. Dry matter weight and percentage of phosphorus in plant were measured.

Four replicates per treatment were established. Three factor analyses of variance (ANOVA) and Duncan multiple range tests (test at 1 and 5% level of probability) were used to partition the variance into the main effects and the interaction between phosphorus, biological fertilizers and growth times. Statistical analysis was performed using SPSS statistical package 18.

Results And Discussion

The soil contained 42.0, 26.5, and 31.5%, of clay, silt and sand particles, respectively. Soil acidity was 7.6 and the electrical conductivity of soil was 1.4 dS m^{-1}. The organic matter content was 10.2 g kg^{-1}. Soil was calcareous and had 350 g $kg^{-1}CaCO_3$. The concentration of P-Olsen Ca_{10} –P, O-P, Fe-P, Al-P, Ca_8 –P, Ca_2 -P and total P were 421, 25, 21, 23,162, 7.6 and 852 mg kg^{-1} soil, respectively.

The effects of phosphorus (P), biological fertilizers (B) and time (T) on P-Olsen, Ca_{10} –P, Ca_8–P and Ca_2 -P were significant ($P \leq 0.01$) (Table 1). Application of 40 mg kg^{-1} P increased the P-Olsen (153%), Ca_{10}–P (3.6%) and Ca_2–P (107.7%) compared to control. Adding AMF had no effect on P-Olsen and Ca_2 -P while PSB and PSB+AMF treatments increased P-Olsen and Ca_2 -P (Table 2). Inoculation decreased Ca_{10} –P (6-10 mg kg^{-1}) and Ca_8 –P (13-18 mg kg^{-1}). The lowest concentrations of P-Olsen for 50 and 75 day after of planting obtained were 10.6 and 10.16 g kg^{-1}, respectively. The highest concentrations for 25 and 100 days after planting were 8.5 and 8.6 g kg^{-1}, respectively (Table2).

Interactive effects of phosphorus and biological fertilizer, time and biological fertilizer, time and phosphorus on P-Olsen were significant (Table 1). Fig. 1a shows that the highest P-Olsen was in PSB and PSB+AMF inoculation with the combined application of 0 and 20 mg kg^{-1}P and the lowest P-Olsen was in control treatment (4.7 mg kg^{-1}).The lowest P-Olsen of 7 mg kg^{-1} was obtained in soil without phosphorus fertilizers addition after 100 days after planting. In contrast the highest P-Olsen (11.2 mg kg^{-1}) was obtained in soil with addition of P after 50 days after planting (Fig. 1b). The maximum amount of P-Olsen was obtained for inoculated PSB and PSB+AMF treatments at 50, 75 and 100 days after planting (Fig. 1c).

Many researchers have found that soil phosphorus availability increases after fertilizer P application [13,22]. Samadi and Gilkes [20] found that soil Olsen-P increased by 200% in fertilized soil compared with virgin soil in Western Australia [20]. Approximately 10-20% of phosphate fertilizer added to soil can be absorbed by plants and the rest are converted to insoluble phosphorus compounds [4]. A research conducted on calcareous soils showed that P application of 60 mg kg^{-1} increases the concentration of Ca_{10}-P by 18.8% [21]. Enzymes produces many bacteria enhance releasing of phosphate from inorganic P compounds. These bacteria also produce other biological materials such as auxin, gibberlic acid, vitamins and hormones that increase the dissolution of phosphate [9]. Mycorrizal fungi have organ called arbuscular that P directly Into the plant, probably due to low P-Olsen in the plant root environment by direct transfer of phosphate from fungus to root.

The effects of phosphorus (P), biological fertilizers (B) and time (T) on dry matter weight (DMW) and P uptake of wheat were significant

(P≤0.01). The interactive effects between P and T, B and T were also significant (P≤0.01) (Table 2). Application of phosphate fertilizer increased the average DMW and P uptake. The highest DMW (15.4 g pot^{-1}) and P uptake (0.49 %) were at 40 mg kg^{-1} P application. Biological treatments increased the DMW and P uptake compared to control. The maximum amount of DMW (15.5 g pot^{-1}) was obtained of PSB+AMF treatment. Time factor also was effective on dry matter yield and P uptake. DMs of 25, 50, 75 and 100 days after planting were 4.1, 6.9, 19.1 and 26.4 g pot^{-1}, respectively (Table 2). Interactive effects of time and inoculation factors had different effects on dry matter weight. Biological fertilizer treatments during the 25 days after planting had no significant effect on dry matter yield. But at 50, 75 and 100 days after planting had a significant effect. Maximum performance at all sampling times were obtained for the biological treatment of AMF+PSB. So DM at 25, 50, 75 and 100 days after planting were for 4.1, 11, 21.3 and 29.4 g pot^{-1} (Fig 2a). Using of 20 and 40 mg kg^{-1} P at 25 days after planting had no significant effect on dry matter weight. The sampling time of 50 days after planting that received phosphate fertilizers showed 5 to 8% and the time 75 days 8 to 8.5% increased in dry matter weight compared to control treatment (Fig 2b). The P fertilizer addition on treatments receiving biological fertilizers had no significant effect on P uptake. Although addition of 20 mg kg^{-1} P increased P uptake, the application of 40 mg kg^{-1} reduced the amount of phosphorus uptake by the plant (Fig2c).

Table 1: Analysis of variance insoluble phosphorus (P_i) fractions in soil , Plant P and DMW.

Variable	(P-Olsen)	(Ca_{10}-P)	(O-P)	(Fe-P)	(Ca_8-P)	(Ca_2-P)	Total P	P-Plant	DMW
Replication ®	2.2	256.6	10.8	12.5	82.2	10.5	324.1	0.02	3.5
Phosphorus fertilizer (P)	26.9**	306.5*	12.5	14.8	106.8	44.9**	525.4	0.12**	105.5**
Biological fertilizer (B)	79.3**	700.5**	8.8	10.2	456.1**	921.2**	424.2	0.41**	90.9**
P × B	73.1	222.4	14.5	8.9	204.7	22.6	325.4	0.09**	52665.0**
Time (T)	18.5**	224.4	17.4	15.2	78.8	25.9	333.4	0.11**	7.4
P × B	8.2*	225.2	11.2	9.6	72.7	22.5	444.9	0.01	37.5**
T × B	23.8**	215.5	10.8	12.6	221.4	37.4	254.5	0.01	21.5**
P × B × T	2.5	201.5	9.6	8.8	101.1	14.4	125.4	0.02	2.4
Error	5.5	25.8	7.4	7.9	35.1	9.2	82.1	0.01	7.1
CV	8.2	10.2	11.2	12.2	12.1	15.1	17.1	6.6	15.5

** Significant at P ≤ 0.01, ** Significant at P ≤ 0.01, CV: Coefficient of variance P: Phosphorus, DMW: Dry matter weight

Table 2: Mean comparisons of the main effects of insoluble phosphorus (Pi) fractions in soil and P-Plant and DMW.

Treatment	(P-Olsen)	(Ca_{10}-P)	(O-P)	(Fe-P)	(Ca_8-P)	(Ca_2-P)	Total P	P-Plant	DMW
	(mg kg^{-1})							(%)	(g pot^{-1})
P levels									
P_0	4b	420b	22a	27a	125a	5.2c	495a	0.36c	12.8c
P_{20}	8.1ab	423ab	24a	28a	130a	8.8b	520a	0.42b	14.2b
P_{40}	10.1a	435a	25a	30a	133a	10.8a	532a	0.49a	15.4a
Biological fertilizers									
Blank	5.9b	415a	18a	21a	133a	4.9b	502a	0.25b	12.3c
PSB	12.8a	407b	17a	19a	120b	10.6a	495a	0.43a	14.1b
AMF	6.2b	409b	17a	20a	118b	5.5b	480a	0.44a	14.6b
PSB and AMF	13.3a	405b	18a	18a	115b	9.9a	476a	0.45a	15.5a
Time after planting									
T25	8.5b	430a	19a	25a	140a	6.7a	480a	0.43a	4.1d
T50	10.6a	425ab	19a	24a	139a	7.8a	478a	0.39b	6.9c
T75	10.1a	422b	18a	26a	132b	7.6a	465a	0.36c	19.1b
T100	8.6b	410bc	17a	22a	120c	7.7a	458a	0.35c	26.4a

Means with different superscript letter(s) are significantly different at P ≤0.01 according to Duncan test

P_0, P_{20} and P40 = 0, 20 and 40 mg kg^{-1}P, respectively

PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhizal fungi, P: Phosphorus, DMW: Dry matter weight

It is well documented that AMF can increase P uptake by host plants.Some of AMF hyphae can into the plant root system and reduces increase cytokine. Cytokine increases water uptake and expansion of the plant root system. The hyphae exist outside the root system produce organic acids like malic acid. Organic acids can enhance soluble inorganic phosphorus and increase P uptake and biomass of plants [12]. The PSB and AMF treatments led to an increase in DMW due to development of the root length and phosphorus uptake by roots [7,2]. Similar results were reported by Harris and Martin [8].

Conclusion:

P-Olsen increased (102-153%) with increasing P rates. Fertilizer P increased Ca_2-P, Ca_{10}-P, P uptake and dry matter weight contents. The present study demonstrated the benefits of arbuscular *mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) for enhancing P uptake and* dry matter weight of wheat. Application of biological fertilizers reduced Ca_{10}-P and Ca_8-P (2.4 and 13.5%, respectively) and increased P-Olsen and Ca_2-P (126 and 116 % respectively). AMF had no effect on P-Olsen percent.

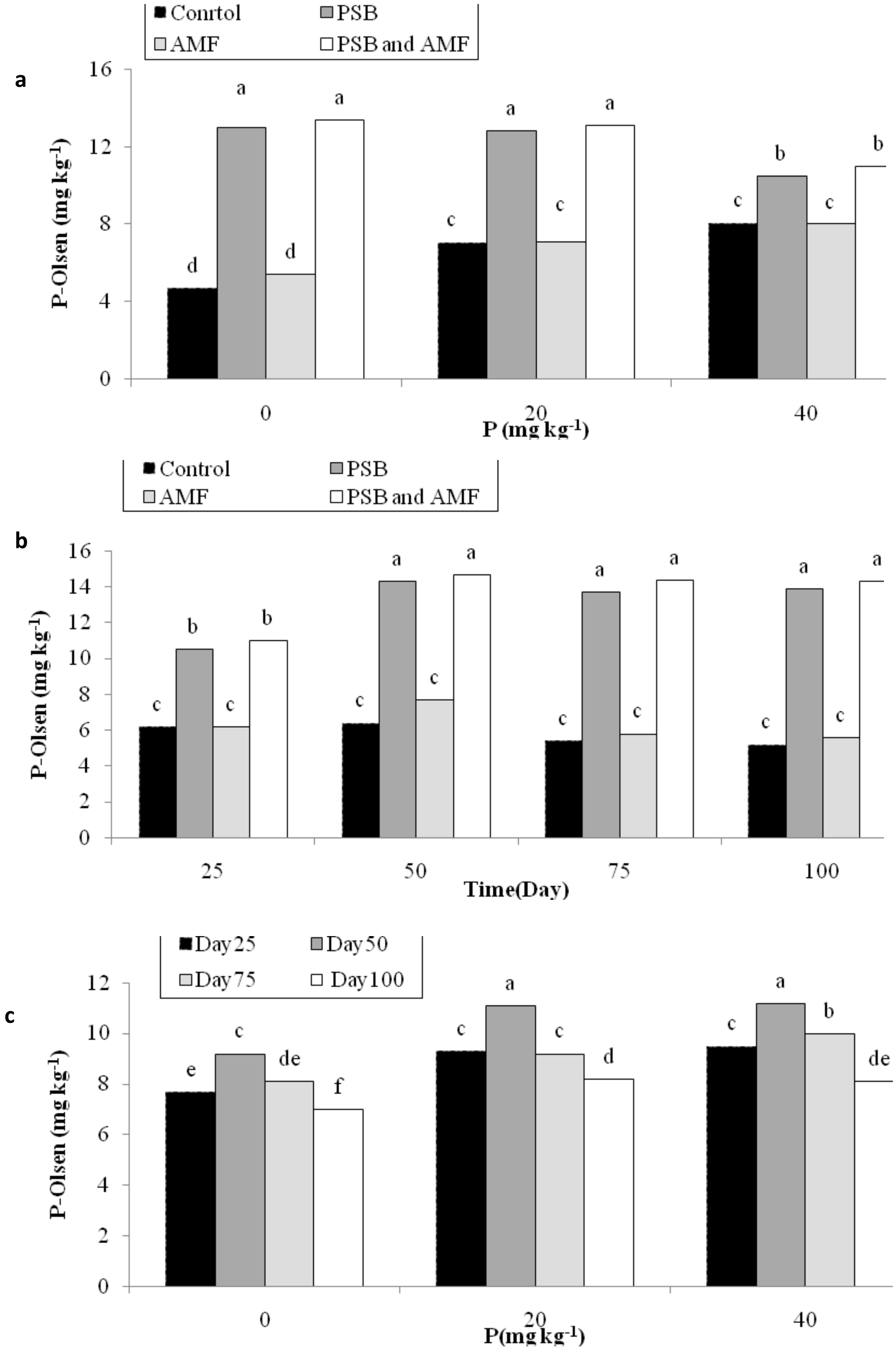

Fig. 1: Interactive effects P fertilizer with biological fertilizer (a), time (b), and time with biological fertilizer (c) on P-Olsen. (PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhizal fungi)

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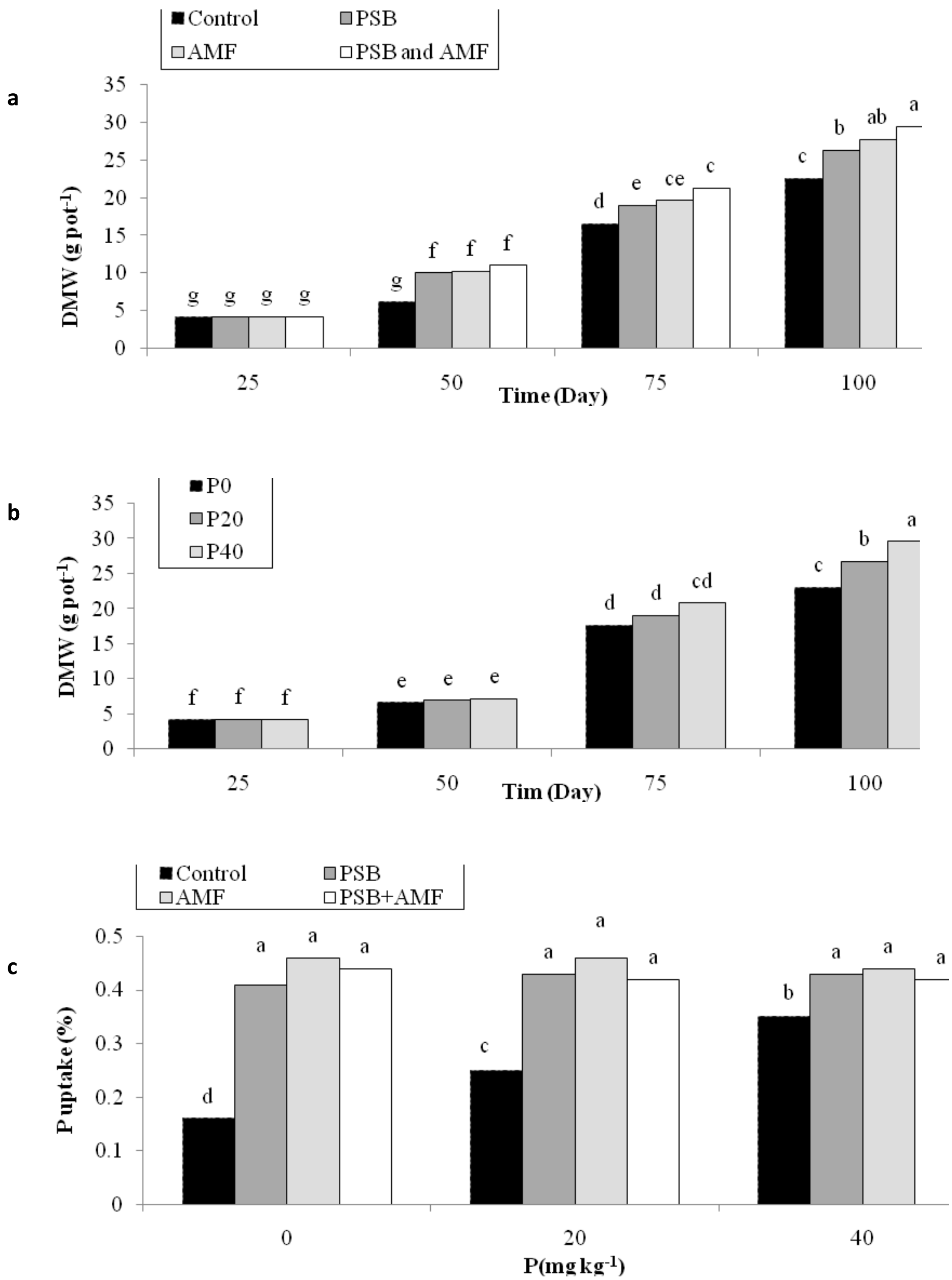

Fig. 2: Interactive affects of time with biological fertilizer (a), fertilizer P (b) on DMW, and biological fertilizer with fertilizer P (c) on P uptake. (PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhizal fungi, DMW: dry matter weight)

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