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Activated Carbon and Phosphorus Application Influence the Growth of Continuously Replanted Asparagus (*Asparagus Officinalis* L.)

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Abstract - The influences of activated carbon (AC) and phosphorus (P) to asparagus growth were assessed under laboratory conditions. The asparagus cultivar; Gijnlim (G) of the European origin, were cultivated in different continuous replanting system. AC was incorporated into sandy soil, was amended with 15 years old root residue (RR) before the sowing of asparagus for the first, second (first replanting), third (second replanting) and fourth (third replanting) time of continuous planting. In all the planting, P was applied weekly basis at P0 (0), P1 (7.5), P2 (15.5) and P3 (22.5) $mg \cdot l^{-1}$. After third replanting, in without AC, P0 and with RR treatment, root biomass inhibited ($P<0.05$) by 55% and total P uptake by 52 %. Controversially, when AC was incorporated into the soil as phosphorus (P3) increased level and without RR, asparagus root biomass inhibited ($P<0.05$) up to 18%, total P uptake by 20 %. AC with applied P alters soil P availability and plant growth even in the presence of the RR allelopathic agent.

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ACTIVATED CARBON AND PHOSPHORUS APPLICATION INFLUENCE THE GROWTH OF CONTINUOUSLY REPLANTED ASPARAGUS ASPARAGUS OFFICINALIS L

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Activated Carbon and Phosphorus Application Influence the Growth of Continuously Replanted Asparagus (*Asparagus Officinalis* L.)

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Abstract - The influences of activated carbon (AC) and phosphorus (P) to asparagus growth were assessed under laboratory conditions. The asparagus cultivar; Gijnlim (G) of the European origin, were cultivated in different continuous replanting system. AC was incorporated into sandy soil, was amended with 15 years old root residue (RR) before the sowing of asparagus for the first, second (first replanting), third (second replanting) and fourth (third replanting) time of continuous planting. In all the planting, P was applied weekly basis at P0 (0), P1 (7.5), P2 (15.5) and P3 (22.5) $mg \cdot l^{-1}$. After third replanting, in without AC, P0 and with RR treatment, root biomass inhibited ($P<0.05$) by 55% and total P uptake by 52 %. Controversially, when AC was incorporated into the soil as phosphorus (P3) increased level and without RR, asparagus root biomass inhibited ($P<0.05$) up to 18%, total P uptake by 20 %. AC with applied P alters soil P availability and plant growth even in the presence of the RR allelopathic agent. This study provided the evidence of AC and P application will be required to improve growth and nutrient uptake by overcoming nutrient immobilization under continuous replanting.

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I. Introduction

Asparagus (*Asparagus officinalis* L.) is a high-value perennial vegetable crop cultivated worldwide. Successive culture of the same crop on the same land for years cause soil sickness or replanting injuries (Tsuchiya, 1990). There are many reasons for the replanting problems such as disease, insect, pathogen, microorganism, soil physical and chemical properties, reduced number and diameter of stems, crowns and roots, and collapse of storage roots (Blok and Bollen, 1993). Moreover, the continuous cropping for asparagus production caused serious replanting problem, which inhibited mainly phosphorus (P) uptake in mineral nutrients (Yeasmin et al., 2013). Therefore, in addition to being scarce, P is also poorly available to plants that are not adapted to these conditions. The involvement of autotoxins from root residues (RR) of former asparagus crops was evaluated for the reduction of P (Yeasmin et al., 2013). In general, under low P availability plant biomass accumulation decreases and root morphology is modified (Lambers et al., 2006). P is a critical macronutrient required for numerous functions in plants and is one of the limiting factors for plant growth due to its rapid immobilization by soil organic and inorganic components (Richardson et al., 2009). The evolutionary adaptations of plants to P deficiency include responses that help them enhance the soil P availability, increase its uptake and improve the use efficiency of P within a plant (Lambers et al., 2006).

Activated carbon (AC), with its large surface area and pore volume, as well as its polarity, has tremendous adsorptive capacity (Lau et al., 2007). AC adsorbs the putative allelochemical due to its great affinity for organic molecules and has very little affiliation for inorganic molecules (Batish et al., 2007). AC additions would also be expected to increase the growth of plants (Kulmatiski and Beard, 2006). Young and Chou (1985) have confirmed the allelopathic activity of chemical substances released by asparagus and allelochemicals such as the substances are considered to be one of the potentially important causes of the asparagus replanting problem (Putnam, 1985). One of the establishment of seedling technique was reported the utilization of AC for absorbing the allelopathic compounds (Lau et al., 2007), but there have been no other detailed studies on the effect of AC in replanting cultivation. Therefore to recover the growth and P uptake after the replanting, the purpose of the present investigation was to evaluate the growth of asparagus in subsequent cropping while investigating the impact of AC and P in mitigating the continuous replanting problem.

II. Materials and Methods

a) *Planting Materials*

A replant culture system was employed to identify the growth inhibitory activity of asparagus. Seeds of asparagus cultivar, Gijnlim of the European origin, were obtained from a local commercial seed company Pioneer Ecoscience Co., Ltd, Tokyo, Japan

b) *Characteristics of the Sandy Soil*

Physico-chemical properties of the soil are illustrated in Table 1. Sandy soil used in this experiment

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was first sterilized at 121°C for 15 min in an automatic high pressure steam sterilized autoclave (MLS-2420; Sanyo, Tokyo, Japan). Prior to showing, the soil was characterized for EC and pH of the soil: water suspension (1:5 w/v). EC and pH were measured with EC and pH meters (Horiba DS-14 and Accumut M-10, TOA electronics Ltd., Tokyo, Japan, respectively). Exchangeable cations (K^+, Ca^{2+}, Mg^{2+}, and Na^+) were measured using an atomic absorption spectro-photometer (Model Z-2300; Hitachi Co., Tokyo, Japan) after extraction with neutral ammonium acetate.

Table 1 : Soil physico-chemical properties

Soil Parameter	Value
EC (1:5) water	0.03 ds· m^{-1}
pH	6.36
Total N	0.02%
Available-P	1.5 mg P2O2 100 g^{-1}
Exchangeable K^+	0.06 cmol·kg^{-1}
Exchangeable Ca^{2+}	0.34 cmol·kg^{-1}
Exchangeable Mg^{2+}	0.45 cmol·kg^{-1}
Exchangeable Na^+	0.10 cmol·kg^{-1}
CEC (Cation exchange capacity	2.40 cmol·kg^{-1}
Bulk density	1.47 g·cm^{-1}
Infiltration rate	30 mm·min^{-1}
Hydraulic conductivity	0.05 cm·sec^{-1}
Texture	Sand

c) *Treatment Conditions*

The goals of this experiment were to investigate the effects of AC and P on asparagus growth. Physico-chemical properties of the AC are illustrated in Table 2. The AC treatment included, by volume, 1.2 g powdered palm shell AC (Motoki et al., 2006) of the potting (size: 10X10 cm^2) soil and mixed homogeneously. The without AC treatment included, no addition (–) of AC. Fifteen years old asparagus roots were obtained from an asparagus field in Nagano prefecture, Japan. After air drying, they were separated by cutting them into approximately 1 cm lengths. The roots were then powdered with a rotary shaker and mixed into the soil at the rate of 2 g·pot^{-1} (Blok and Bollen, 1993). Two sets of RR amended (+) and unamended (–) treatment was applied with and without AC treated soil.

d) *Replant Culture*

Prior to sowing, asparagus seeds were covered with a double layer of gauze and surface-sterilized in 70% ethanol and then rinsed in deionized water several times. The pre-germinated seedlings of the asparagus cultivar were grown in commercially available plastic black pot (size: 10X10 cm^2) in sand culture which was incorporated with or without AC and RR for the first times of planting. They were incubated at: 25° C; 12h light/12h dark; relative humidity 80% and 200 μmol m^{-2}·s^{-1} intensity of light in growth chamber (MLR-351H; Sanyo, Tokyo, Japan) for 56 days. To check the role of P to asparagus growth, 100 ml of a P0 (0), P1 (7.5), P2 (15.5) and P3 (22.5) solution (mg·l^{-1}) from KH_2PO_4 (Nuruzzaman et al., 2005) was applied weekly to each pot/plant, in different combination of AC and RR amended or unamended soils. The following combinational pattern was included: P0AC+RR+; P0AC+RR–; P0AC–RR+; P1AC+RR+; P1AC+RR–; P1AC–RR+; P2AC+RR+; P2AC+RR–; P2AC–RR+; P3AC+RR+; P3AC+RR–; P3AC–RR+. Each pot was irrigated with distilled water according to field capacity. Field capacity was calculated as (wet mass- dry mass)/ dry mass X 100% (Lambers et al., 2002). Nutrients were provided with 10-20 ml Hoagland's P free nutrient solution everyday to avoid immobilization of nutrients. The seedlings were then harvested after 56 days and new seeds were replanted again in the same pot by using the same soil for the second (first replanting for 56 days), third (second replanting for 56 days) and fourth plantings (third replanting for 56 days), respectively by following the same process as above. All treatments were replicated three times. Inhibition, expressed as a percentage, was calculated using the following equation:

$$\text{Inhibition (\%)} = (1 - Xt / Xc) \times 100 \qquad (1)$$

Table 2 : Characteristics of palm sell activated carbon

Activated Carbon	Palm shell
Raw and processed materials	Coconut shell
pH	9.9
Specific surface area	956 m^2· g^{-1}
Total pore volume	0.46 cc· g^{-1}
Micro-pore surface area	867 m^2· g^{-1}
Micro-pore volume	0.35 cc. g^{-1}
Meso-pore surface area	83 m^2· g $^{-1}$
Meso-pore volume	0.09 cc. g $^{-1}$
Meso+Macro-pore surface area	89 m^2· g $^{-1}$
Pore diameter	0.7 nm
Iodine absorption performance	1050 mg· g^{-1}
Methylene blue adsorption performance	180 mg· g^{-1}

16 meshes are equal to 1 mm size by Test methods (Ajinomoto Fine -Techno Co., Inc., Japan) for activated carbon in JAS K 1474-1991.

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Where, Xc denotes the dry mass of the roots for the first planting of each treatment and Xt represents the mean values of the corresponding dry mass of the treatments for the first, second and third replanting respectively.

e) Chemical Analysis of Soil after Subsequent Replanting

AC, RR and different levels of P amended or unamended soils pH, electrical conductivity (EC) and C: N ratio was determined by procedures already described in preceding sections. Total P was measured as described above. All treatments were replicated three times.

f) Plant Growt and P Uptake Measurements

After harvesting, all seedlings were thoroughly cleaned; blotted dry between absorbing paper and their dry mass (g pot^{-1}) were measured after oven drying at 70°C for 72h. All dry roots and shoots were combined and ground to a fine powder using a stainless ball mill, and analyzed for total P (mg g^{-1}) using standard procedures. Total P in the digested mixture was determined colorimetrically with a spectrophotometer (Model U-2001, Hitachi Co., Tokyo, Japan) using the phosphomolybdate blue method (Murphy and Riley, 1962). All samples were run in triplicate.

Inhibition, expressed as a percentage, for P uptake was calculated by using equation 1.

g) Statistical Analysis

Experimental data presented are the means of three replicates; statistical analyses were executed using Stat View software. The percentage data was $\log_e$-transformed before analysis where necessary to equalize variances between treatments (Lam et al. 2012). Tukey's protected multiple-comparison test at $P < 0.05$ was used to compare means.

III. Results and Discussion

a) Impact of AC and P on growth

When asparagus was grown in soils that had been or had not been amended with AC and RR with different levels of P, responded differentially to asparagus growth after the three subsequent replanting (Figure 1 a, b and c).

After the first, second and third replanting, the highest (23, 35 and 55%) and lowest (2, 3 and 8%) inhibitions for growth of root were found in the P0AC–RR+ and P3AC+RR–, respectively. On average, the application of AC increased asparagus growth with the increased level of P. Application of AC could reduce allelopathic effects and increased plant growth and yield (Ridenour and Callaway, 2001). In this study, the mixture of AC with the different concentration of P led to the production of asparagus seedlings with a high growth while reducing the effects of RR amendment. Therefore,

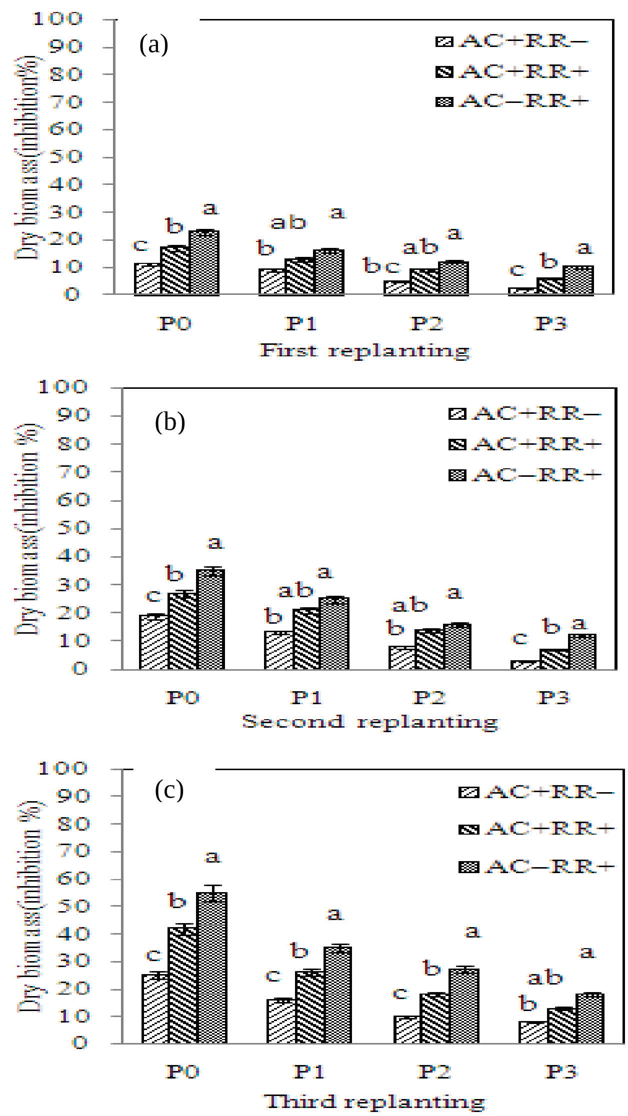

Figure 1 : Percentage of inhibition of asparagus seedling roots growth after the first, second and third replanting. Listed growth parameter suggests the values are actual dry mass (g· pot^{-1}). P0, P1, P2 and P3 denote 0, 7.5, 15.5 and 22.5 mg l^{-1} of applied P. Inhibition (%) was calculated by using equation 1. Bars presented as mean ± SE, Tukey's protected multiple-comparison test at $P < 0.05$ was used to compare means, replication (n= 3)

the positive effect of AC on growth could be a result of AC minimizing any negative growth effects of allelopathic compounds. Whatever the underlying mechanism(s), it was found that positive responses of combined AC and P influenced plant growth in the absence of the RR means that the biomass can be greater with AC and P than without AC and P.

b) P uptake

The highest inhibition ($P < 0.05$) was found for P uptake (52%) in P0AC–RR+ combination, compared to P3AC+RR– combination after the third replanting,

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followed by first and second replanting (Figure 2 a, b and c). Asparagus P uptake with RR amendment treatment showed the significant ($P < 0.05$) higher reduction in P uptake in compared to RR unamendment treatment. This nutrient decreased also could be due to a release of phytotoxic substances by the interaction between RR and microorganisms.

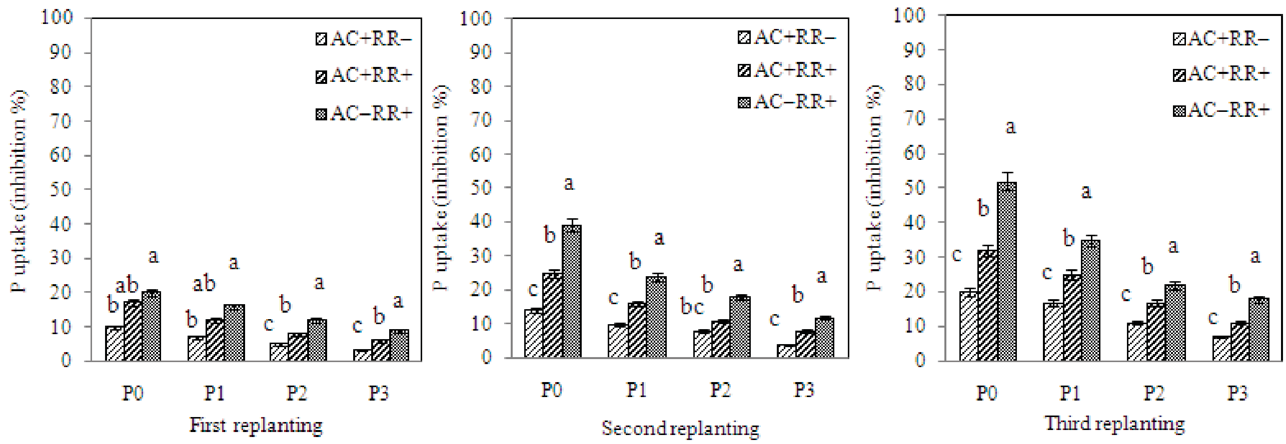

Figure 2 : Percentage of inhibition of asparagus seedling P uptake after the first (a), second (b) and third (c) replanting. Listed nutrient suggests the values are actual concentration (mg g-1). P0, P1, P2 and P3 denote 0, 7.5, 15.5 and 22.5 mg l-1 of applied P. Inhibition (%) was calculated by using equation 1. Bars presented as mean ± SE, Tukey's protected multiple-comparison test at $P < 0.05$ was used to compare means, replication (n= 3)

Allelochemical compounds including amino acids, organic acids, sugars, phenolic acids, and other secondary metabolites, serve as an important medium of root-based interactions with other microorganisms including bacteria, actinimycetes, pathogens, fungi, and insects in the growing media (Walker et al., 2003). AC also may reduce microbial activity by reducing concentrations of organic molecules (Kulmatiski and Beard, 2006). Inderjit and Callaway (2003) recommend fertilizing pots to minimize the effects of trace concentrations of nutrients contributed by AC. However, our results show that AC and P influenced P uptake in both the presence and the absence of RR. AC can increase nitrification, in part because AC may sorbs compounds that are inhibitory to nitrifying bacteria (Deluca et al., 2002, 2006). In particular, the plant allelochemicals changes the soil nutrients concentration. On the contrary, nutrients concentration also influences the concentration of plant allelochemicals. Wacker et al. (1990) informed about several allelochemicals were isolated and characterized from asparagus root tissue, including ferulic acids and which could inhibit P uptake in plant roots (McClure et al., 1978). Allelochemicals like benzoic, vanollic, cinamic and ferulic acids showed inhibition in P uptake; likewise, benzoic and trans cinnamic acids reduced growth, lowered the amounts of P, K, Mg, Mn, Cl^{-1}, and SO_4^{-2} (Baziramakenga et al., 2005). Since, the RR could enhance the microbial biomass that may be responsible for subsequent reduction of nutrient. However, in AC unamendment treatment, detect the effects of RR for total nutrient content; RR also affects the P uptake. RR trended for the highest reduction for P uptake depending on the different combinations with AC and P0, P1, P2 and P3. Therefore, AC and P can be a good tool for studies of allelopathy because it acts as an adsorbent for many large organic compounds and increased the P uptake.

c) *Effect of subsequent replanting on soil pH, EC and C: N ratio and P uptake*

Physico-chemical properties of the replanted soil were determined and the soil pH, EC and C: N ratio differed in with and between P3AC+RR– and P0AC–RR+ combination as a result of three subsequent replanting (Table 3). The soil contained AC significantly ($P < 0.05$) increased the soil pH and C: N ratio and decreased soil EC, but the magnitude of that increase depended upon the RR.

The influence of pH, EC and C: N ratio, to the soil properties revealed that application AC and P is an important factor for the soil property. With the increasing of planting time the soil pH was decreasing and soil was becoming more acidic. On the contrary to, EC was increasing with the increasing of plating time. Increases in soil pH (Lucas and Davis, 1961) may result in increases in bio- available P, and to often pH- related increases in nutrient availability (Lehmann et al., 2003). This contrasting difference is reflected in the significant effect of C: N ratio. The soil contained AC increased the soil pH and C: N ratio but the magnitude of that increase depended upon the RR with different combinational treatments with P. The effect could be attributed to the

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high surface area of AC. The reduction for total soil P uptake ranged from 5-63 % (Figure 3 a, b and c). After third replanting, the highest and lowest decreased was found for soil P uptake (12 and 63%) in P0AC-RR+ and P3AC+RR- combination, respectively. This result imply that the addition of AC and P to a sandy soil, making soil moisture and nutrients more available to plants growing to the soil, and eventually in improving crop productivity. Yeasmin et al. (2013) revealed that soil P uptake showed decreasing trend with the increasing of planting time.

Table 3 : Effects of phosphorus (P) and activated carbon (AC) application on soil physic-chemical properties after the first , second and third replanting

First replanting			Second replanting			Third replanting		
AC+RR–	AC+RR+	AC–RR+	AC+RR–	AC+RR+	AC–RR+	AC+RR–	AC+RR+	AC–RR+
			pH (H_20)					
5.9±0.04d	5.4±0.05d	4.8±0.11d	5.1±0.02d	4.7±0.09d	4.3±0.01c	4.7±0.04c	4.4±0.05d	4.0±0.23d
6.1±0.07c	5.8±0.03c	5.3±0.08c	5.6±0.01c	5.2±0.07c	4.8±0.06b	5.0±0.03bc	4.7±0.01c	4.4±0.65c
6.7±0.05b	6.3±0.01b	5.7±0.06b	6.0±0.04b	5.7±0.01b	5.5±0.02ab	5.3±0.09b	5.1±0.03b	4.9±0.71b
7.3±0.01a	6.7±0.02a	6.2±0.03a	6.5±0.07a	6.3±0.02a	5.7±0.02a	5.6±0.05a	5.5±0.00a	5.1±0.15a
			C:N ratio					
20.4±0.66d	17.9±0.11d	14.4±0.71d	16.8±1.29d	14.4±2.13d	11.5±0.93d	12.9±1.31d	9.7±2.61d	7.1±1.93d
24.8±1.02c	22.2±0.87c	18.2±0.94c	20.8±1.43c	18.8±1.21c	13.2±1.22c	15.2±2.14c	11.1±1.03c	9.9±1.43c
30.2±1.51b	24.8±0.25b	20.4±0.58b	23.2±2.81b	20.2±1.18b	16.8±0.81b	17.8±1.03b	15.4±3.03b	11.4±2.03b
35.7±2.78a	29.3±0.19a	25.8±1.92a	27.7±1.55a	24.7±3.27a	19.3±0.17a	21.3±1.23a	17.5±1.03a	14.8±1.03a
			EC(ds·m^1)					
0.45±0.03a	0.53±0.06a	0.89±0.11a	0.68±0.04a	0.95±0.01a	1.8±0.06a	1.3±0.02a	1.6±0.15a	2.0±0.01a
0.37±0.05b	0.44±0.04b	0.84±0.09b	0.65±0.06b	0.92±0.09b	1.4±0.02b	0.91±0.04b	1.3±0.03b	1.8±0.05b
0.24±0.01c	0.38±0.05c	0.72±0.05c	0.43±0.03c	0.78±0.05c	1.1±0.02c	0.83±0.09c	1.0±0.07c	1.6±0.17c
0.18±0.07d	0.27±0.01d	0.63±0.02d	0.40±0.01d	0.66±0.02d	0.98±0.09d	0.75±0.01d	0.83±0.01d	1.2±0.08d

Data presented as mean ± SE, Tukey's protected multiple-comparison test at $P < 0.05$ was used to compare means, replication (n= 3).

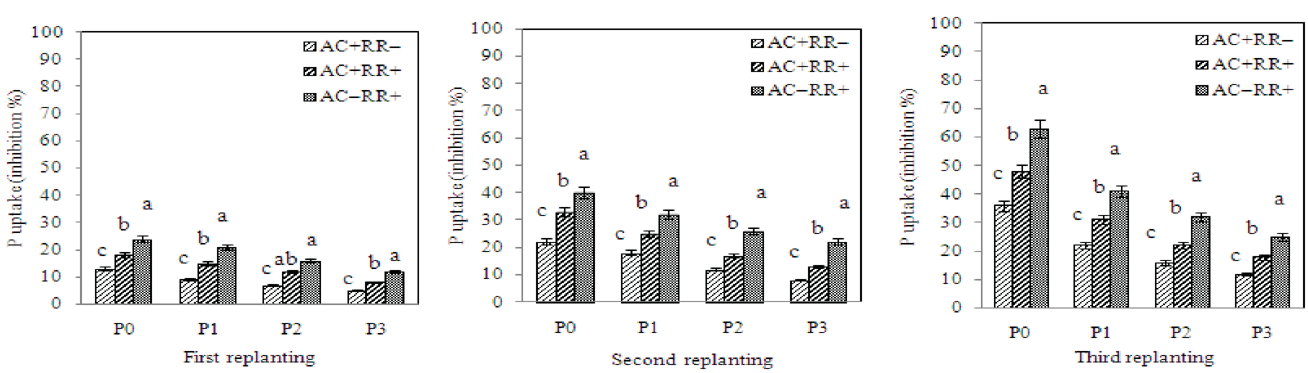

Figure 3 : Percentage of inhibition of soil P uptake after the first (a), second (b) and third (c) replanting. Listed nutrient suggests the values are actual concentration (mg g-1). P0, P1, P2 and P3 denote 0, 7.5, 15.5 and 22.5 mg l-1 of applied P. Inhibition (%) was calculated by using equation 1. Bars presented as mean ± SE, Tukey's protected multiple-comparison test at $P < 0.05$ was used to compare means, replication (n= 3)

Therefore, it is likely that allelochemical compounds could be readily leached from the residues. After entering into the soil, allelochemicals are influenced by microorganisms (Inderjit, 2001). However, the negative effect of allelochemicals depends greatly upon a variety of biotic and abiotic factors, soil type, presence of microorganisms and soil conditions, and further toxification and detoxification mechanisms in the soil (Blum et al., 1999). Based on the observed results, the present study evidences that root residues of Asparagus officinalis suppress the growth of asparagus by releasing allelochemicals into the soil rhizosphere through alteration of soil P which could be improve by the combined application of AC and P.

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IV. Conclusion

In summary, in soils without RR amendment, combined AC and P application increased asparagus growth and nutrient uptake, resulting in higher P recovery in the asparagus. RR incorporation, however, retarded the effects of fertilization on asparagus growth and P uptake. In this study, plant growth and P uptake was increased with the increasing of level of P, but it was not known the optimum level of P, until which level growth will be retarded, therefore, the present steps are currently validating to explore the exact quantity or level of P and to find out the mechanism and specific causes of these problems and how to improve the growth and P uptake under continuous replanting.

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