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Chemistry

Five Flax Genotypes

Reduction of Cyclohexanones

Highlights

Asparagus Officinalis L

Continuously Replanted Asparagus

Discovering Thoughts, Inventing Future

Volume 13 Issue 5 Version 1.0

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

By Rumana Yeasmin, Satoru Motoki, Sadahiro Yamamoto & Eiji Nishihara

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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.

Keywords : *asparagus; activated carbon; growth; nutrient uptake; phosphorus; root residue.*

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Keywords : asparagus; activated carbon; growth; nutrient uptake; phosphorus; root residue.

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 spectrophotometer (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

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.

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:

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

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,

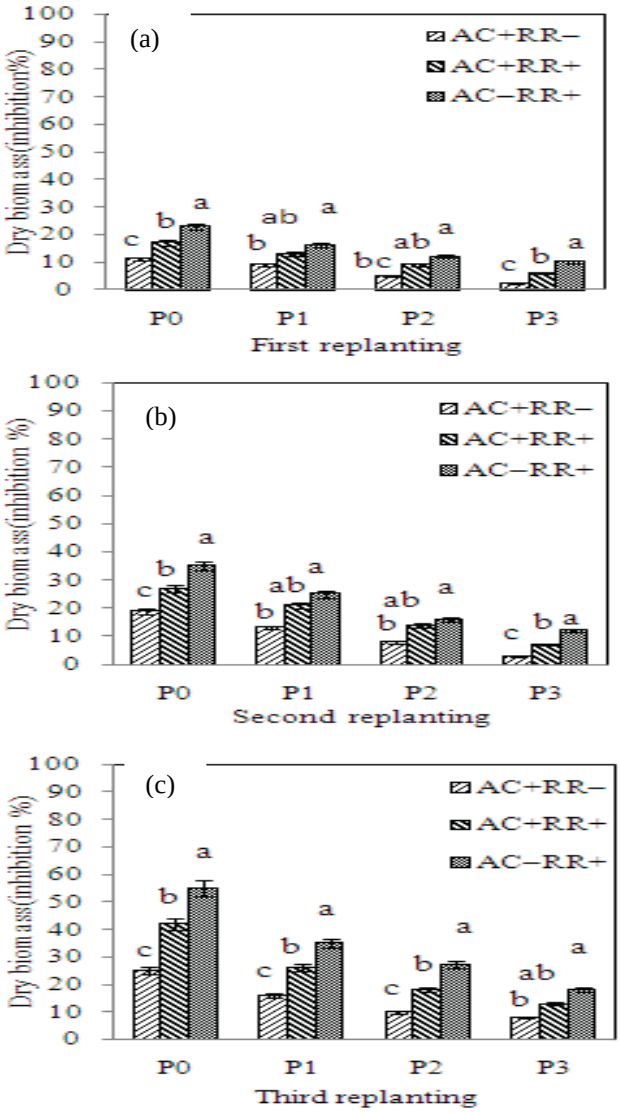

Figure 1 : Percentage of inhibition of asparagus seedling roots growth after the first, second and third replanting. Listed growth parameter suggests the values are actual dry mass (g· pot^{-1}). P0, P1, P2 and P3 denote 0, 7.5, 15.5 and 22.5 mg l^{-1} of applied P. Inhibition (%) was calculated by using 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,

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.

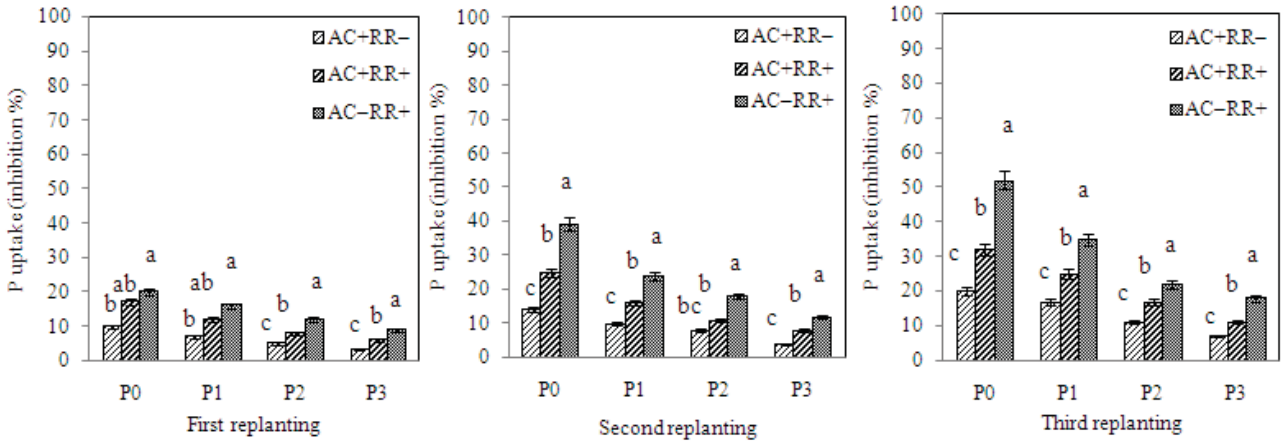

Figure 2 : Percentage of inhibition of asparagus seedling P uptake after the first (a), second (b) and third (c) replanting. Listed nutrient suggests the values are actual concentration (mg g-1). P0, P1, P2 and P3 denote 0, 7.5, 15.5 and 22.5 mg l-1 of applied P. Inhibition (%) was calculated by using 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

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($ds \cdot m^1$)					
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).

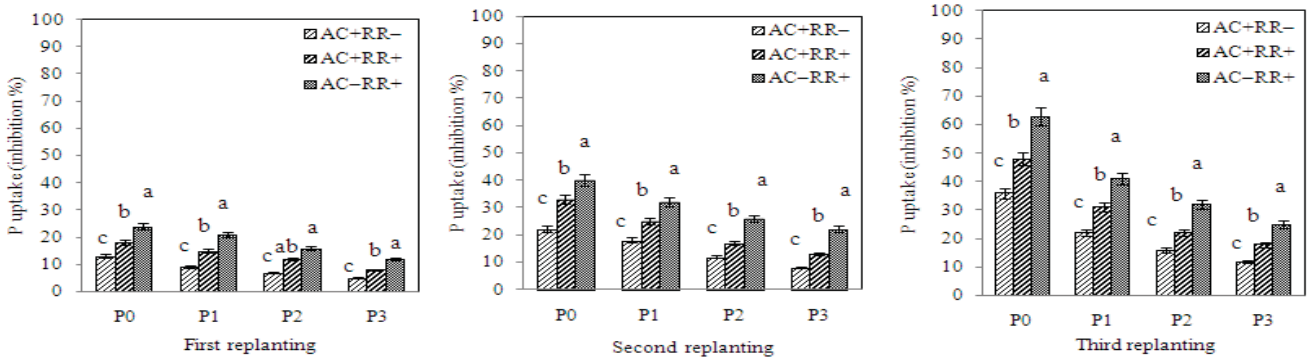

Figure 3 : Percentage of inhibition of soil P uptake after the first (a), second (b) and third (c) replanting. Listed nutrient suggests the values are actual concentration (mg g-1). P0, P1, P2 and P3 denote 0, 7.5, 15.5 and 22.5 mg l-1 of applied P. Inhibition (%) was calculated by using equation 1. 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.

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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Synthesis of 6,6-Dimethyl-2-Cyclopentadienylet-Hylbicyclo [3.1.1] Hept-2-Ene and of 6,6-Dimethyl-2-Indenylethylbicyclo [3.1.1] Hept-2-Ene

By Dr. (Mrs.) Gloria Ukalina Obuzor & Dr. Brian L. Booth
University of Port Harcourt, Nigeria

Abstract - The New Compounds 6,6-Dimethyl-2-Cyclopentadienylethylbicyclo [3.1.1] Hept-2-Ene (4) And 6,6-Dimethyl-2-Indenylethylbicyclo [3.1.1] Hept-2-Ene (5) Were Synthesized In 82% And 60% Yield Respectively Using 6,6-Dimethyl-2-PToluenesulphonyloxomethylbicyclo [3.1.1] Hept-2-Ene (2). However, Compounds - (4) And (5) Were Obtained In 71% And 81% Yield Respectively Using 6,6-Dimethyl-2- Sulphonyloxomethylbicyclo [3.1.1] Hept-2-Ene (3). Allcompounds Were Characterized By NMR, Infra-Red, Micro Analysis And Mass Spectroscopy.

Keywords : *ligand, metallocene, cyclopentadienyl, indenyl, organ metallic, n-buthyllithium.*

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Strictly as per the compliance and regulations of :

Synthesis of 6,6-Dimethyl-2-Cyclopentadienylet-Hylbicyclo[3.1.1] Hept-2-Ene and of 6,6-Dimethyl-2-Indenylethylbicyclo[3.1.1] Hept-2-Ene

Dr. (Mrs.) Gloria Ukalina Obuzor [α] & Dr. Brian L. Booth [σ]

Abstract - **The New Compounds 6,6-Dimethyl-2-Cyclopentadienylethylbicyclo[3.1.1] Hept-2-Ene (4) And 6,6-Dimethyl-2-Indenylethylbicyclo[3.1.1] Hept-2-Ene (5) Were Synthesized In 82% And 60% Yield Respectively Using 6,6-Dimethyl-2-*P*-Toluenesulphonyloxomethylbicyclo[3.1.1] Hept-2-Ene (2). However, Compounds - (4) And (5) Were Obtained In 71% And 81% Yield Respectively Using 6,6-Dimethyl-2-Sulphonyloxomethylbicyclo[3.1.1Hept-2-Ene (3).Allcompounds Were Characterized By NMR, Infra-Red, Micro Analysis And Mass Spectroscopy.**

Keywords : ligand, metallocene, cyclopentadienyl, indenyl, organ metallic, n-buthyllithium.

I. Introduction

The different types of cyclopentadienyl ligands being synthesized have re-emphasized the importance of this class of legend in organ metallic chemistry. The first report of the synthesis of enantiomerically–enrimono substitutedcyclopentadienyl legends was by the reduction of fulvene with $LiAlH_4$ in the presence of (-)-quinine or (+)-cinch nine to producephenylethyl cyclopentadienenidelithium in low enantiomeric purity; Leblanc and Moose, 1976 and Erker *et al.,* 1991.Thereafter, several types of cyclopentadienyl ligands with exotic variations have emerged such as the followings: synthesis of new silyl-bridged bis(cyclopentadienyl) ligands and complexes, Huhmann *et al.,* 1998; cyclopentadienyl ligands as perfect anion receptors: teamwork between π-anion interaction and C-H·anion hydrogen bonds, Amouri *et al.,* 2009;optically active cyclopentadienyl ligands from the chiral pool, the synthetic utility of a camphor-derived pentafulvene, Quindt *et al.,* 2002 etc. These and other types of cyclopentadienyl ligands have equally been synthesized and carbometalated such as 4-(2-cyclopentadienylethyl) morph line and their transition metal complexes, Obuzor and Booth, 2010; 1-(2-indenylethyl) pyrrolidine, Obuzor and Booth, 2010;4-(2-indenylethyl) morph line, Obuzor and Booth, 2011 and amongst others. Despite these ligands, there is still shortage of facile routes to cyclopentadienes with optically active substituent's originating from easily available starting materials Huhmann *et al.,* 1998.The attachment of substituents to cyclopentadienyl ligands affects metalcomplexes in several ways. Substituents may affect the molecular structure, physical and chemical properties, spectroscopic properties, electrochemical properties, and catalytic activity and selectivity of a complex. Controlling the size and position of substituents on a metallocene catalyst affects the molecular weight and tacticity of polyolefins. Marks *et al.,* 1997, found that by increasing the "wingspan" of one of the η^5 ligands of a metallocene, the average molecular weight and isospecificity could both be increased. Polymerization studies of propylene using catalysts with varying ligand sizes; it was reported that the catalyst which contains a bulkier ligand increased average molecular weightfrom 2,170 to 18,900 g/mol and that the isotacticity increased from 35% to85%. The authors concluded that the catalyst with the larger "wingspan" allowed propylene to coordinate in only one direction, leading to stereoregular, isotactic polypropylene Marks *et al.,* 1997 andBurkey *et al.,* 1997.The work presented in this paper shows the synthesis of two different compounds (Fig. 1) of varying molecular sizes and it is hoped that when metallated; will polymerize ethylene to different molecular weight sizes based on the ligand size as experienced by Marks *et al.,* 1997.

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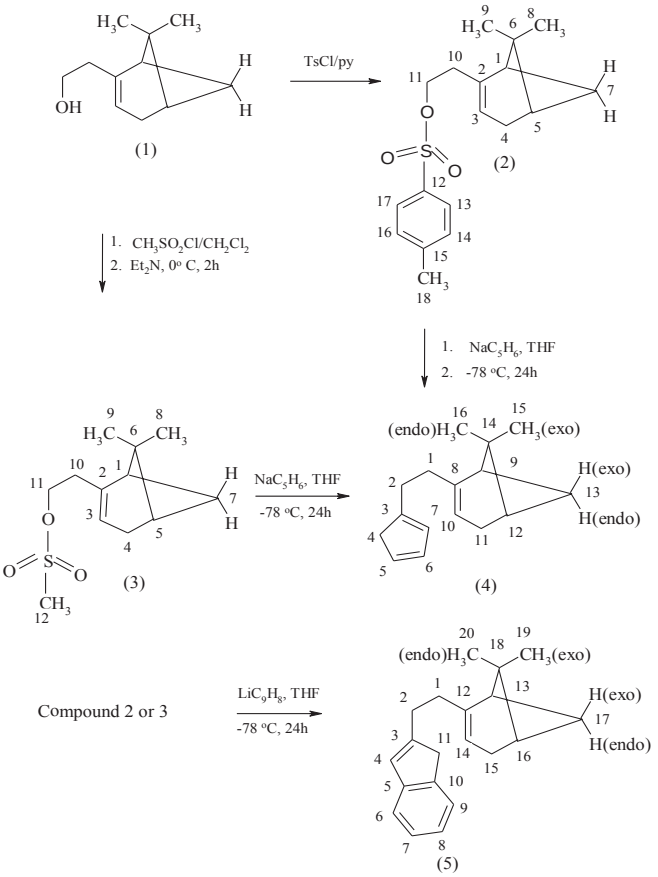

Figure 1 : Synthesis of substituted 6,6-dimethy1-2-ethylbicyclo[3.1.1.]hept-2-ene ligands

II. Materials and Methods

a) General Procedures

All manipulations were carried out under argon or nitrogen and carefully dried solvents. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AMX 400 spectrometer with the protiosolvent signal (^{1}H NMR) or solvent signals (^{13}C NMR) used as a reference; chemical shifts are quoted on the δ scale (downfield shifts are positive) relative to tetramethylsilane. Elemental analyses (micro analysis) were performedwith an Elemental Analyzer 240 (Perkin Elmer). All reactions were done at The University of Manchester, Manchester, England.

b) Preparation of 6,6-Dimethyl-2-P-Toluenesulphonyloxomethylbicyclo[3.1.1] Hept-2-Ene (2), Obuzor and Booth 2005

A 1-litre, three-necked flask equipment with a magnetic stirrer, internal thermometer and nitrogen inlet was charged with 6, 6-dimethylbicyclo [3.1.1] hept-2-ene-2-ethanol (5.0g, 0.030mol) and pyridine (100.00ml). The solution was stirred and cooled to -10°C in an ice-salt bath under argon and *p*-toluenesulfonyl chloride (7.3g, 0.045mol) was added in one portion under argon. The temperature rose to 40°C for 20mins before returning to 5°C where it was maintained for 2h. Ten 1ml portions of water were slowly introduced without allowing the temperature to exceed 5°C. The reaction mixture was poured into 1 litre of diethyl ether and extracted with ice-cold 5M sulfuric acid until the aqueous layer remained acidic, then with saturated $CuSO_4$ solution until the layer remained blue. The ethereal phase was washed with two 100ml portions of each water and sodium bicarbonate solution prior to drying over magnesium sulfate and evaporating the solvent. A white solid residue was obtained which was recrystallized by dissolving in hot hexane (100.00ml) and cooling to -78°C. Six such recrystallizations gave the product (**2**) as a white powder with m.p.48-49°C and $[\alpha]_D^{24}$ -25.7 (C_2H_5OH, $C_{0.03}$). The yield was 8.5g, 0.027mol, 89%. IR (Nujol) ν_{max} 2923m, 2854m, (-CH str.vib.), 1597m, 1461m, 1385 (O=S=O asym. str.)1306m, 1230 (O=S=O asym. str.) 1188m, 1174m, 1095m, 1088m, 1020m, 980m, 961s, 912m, 814s, 773m (S–O–C str.), 722m 665m.cm^{-1}. ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.83 (s, 3H, H_9), 1.2 (d, J 7.7Hz 1H, $H_{7\text{-endo}}$), 1.38 (s, 3H, H_8), 2.06 (t, 1H, J 3Hz, H_5), 2.18 (m, 1H, H_1), 2.3 (d, 1H, J 7.7Hz, H_{7exo}), 2.3-2.49 (m, 4H, H_4 and H_{10}), 2.59 (s, 3H, H_{18}), 4.18 (t, 2H, J 5Hz, H_{11}), 5.39 (s, 1H, H_3), 7.45 (d, 2H, J 8.0Hz, aromatic), 7.9 (d, 2H, J 8.0Hz, aromatic) pap. ^{13}C NMR (70MHz, $CDCC1_3$) δ:20.4 (C_9), 26.1(C_8), 31.2(C_{18}), 31.8(C_7), 37.7(C_4), 40.3(C_{10}), 40.8(C_6), 102.8(C_5), 109.7(C_1) 124.2(C_{11}), 128.0(C_{14}), 129.7(C_{13}), 130.6(C_{17}), 131.3(C_{16}), 137.5(C_3), 142.5(C_{15}), 144.7(C_{12}), 146.7(C_2) pap. [Found: C, 67.2; H, 7.9; S, 10.4, M 320. $C_{18}H_{24}O_3S$ requires C, 67.6; H, 5.6; S, 10.0 MS (FAB) m/z 320 (M+1)].

c) Preparation of 6,6-Dimethyl-2-Sulphonyloxomethyl-bicyclo [3.1.1] Hept-2-Ene (3), Obuzor and Booth 2005

To a 1-litre flame-dried, three-necked flask equipped with a dropping funnel under dry argon was added a solution of 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol (**1**) (5.0g, 0.030mol) in dichloromethane (100.00ml) and dry triethylamine (3.04g, 4.20ml 0.030mol) at 0 °C under dry argon. A solution of freshly-distilled methanesulphonyl chloride (4.3g, 2.10ml, 0.045mol) in CH_2Cl_2 (50.00ml) was added and the mixture was stirred at 0 °C. After 2h, TLC (CH_2Cl_2) showed complete reaction and a 10% w/w of sodium carbonate solution (20.00ml) was added and the mixture was extracted with diethyl ether (100.00ml). The ether layer was washed with sodium carbonate solution (2x20.00ml), water (20.00ml), followed by brine (20.00ml) and was dried over $MgSO_4$. Removal of solvent afforded the mesylate as pale yellow viscous oil in (7.17g, 0.029mol, 98%). IR (Nujol); ν_{max} 2923m, 2854m (–CH str. vib.), 1597m, 1461m, 1385s (O=S=O asym. str.), 1306m, 1230s (O=S=O asym. str.) 1188m, 1174m, 1095m, 1088m, 1020m, 980m, 961s, 912m, 814s, 773m (S–O–C str.), 722m, 665m cm^{-1}. 1H NMR

(300MHz, $CDCl_3$) δ: 0.79 (s, 3H, $CH_{3\text{-endo}}$ H_9), 1.10 (d, 1H, J 7.7Hz, $H_{7\text{-endo}}$), 1.20 (s, 3H,$CH_{3\text{-exo}}$ H_8), 1.99 (t, 1H, J 3Hz, H_5), 2.05 (m, 1H, H_1), 2.19 (d, 1H, J7.7Hz, H_{7exo}), 2.30-2.4 (complex, 4H, H_4 and H_{10}), 2.99 (s, 3H, H_{12}), 4.19 (t, 2H, J 5Hz, H_{11}), 5.30 (s, 1H, H_3), pap; ^{13}C NMR (70MHz, $CDCl_3$) δ: 20.7(C_9), 26.3(C_8), 31.2(C_{12}) 31.8(C_7), 37.7(C_4), 40.3(C_{10}), 40.9(C_6), 102.8(C_5), 109.5(C_1), 124.5(C_{11}), 137.8(C_3), 146.7(C_2) pap. [Found: C, 59.2; H, 8.4; S,13.6, M.244$C_{12}H_{20}SO_3$ requires C,59.0; H,8.2; S,13.1. MS (FAB) m/z 244 (M+1)].

d) Preparation of 6, 6-Dimethyl-2-Cyclopentadienyl-ethylbicyclo [3.1.1] Hept-2-Ene (4).

A 250ml three-necked flask equipped with a magnetic stirrer, internal thermometer, and argon inlet, was charged with 6,6-dimethyl-2-*p*-toluenesulphonyl oxyethylbicyclo[3.1.1] hept-2-ene (**2**) (3.0g, 0.009mol) and a fresh, dry sample of tetrahydrofuran (THF) (50.00ml) under argon. The solution was stirred at -78 °C for 4h and cyclopentadienyl sodium (1.2g, 0.014mol) in THF (20.00ml) was annulated into the mixture. It was then stirred at room temperature for 24h. Water (20.00ml) was added and the aqueous layer was extracted with diethyl ether (3 x 20.00ml). The ether layers were combined, dried and concentrated. Thin layer chromatography (TLC, hexane/ethyl acetate 3:1) showed it to contain two spots at a $R_e$value of 0.48 and a second spot at R_f value of 0.32. Column chromatography (silica gel) gave the desired products pale yellow oil after solvent removal (1.6g, 0.007mol, 82% yield), bop. 118-120 °C. IR (Nujol) ν_{max}3040m, 2930m, 2923m, 2854m, (-CH str. vibe.), 2695w,2340w, , 1600w, 1597m, 1461m, 1188m, 1174m, 1100m, 1095m, 1088m, 1020m, 980m, 961s, 912m, 814s, 722m, 665m.cm^{-1}.^{1}H NMR (300MHz, $CDCl_3$)δ: 0.81 (s, 3H, CH_3, $H_{16\text{-endo}}$), 1.20 (d, 1H, J 7.7Hz, $H_{13\text{-endo}}$), 1.52 (s, 3H,CH_3, $H_{15\text{-exo}}$), 1.95 (t, 1H, J 3Hz, H_{12}), 2.0 (m, 1H, H_9), 2.3(d, 1H, J7.7Hz, H_{13endo}), 2.40-2.65 (complex, 4H, H_1 and H_{11}), 2.8 (d, 2H, J 3.4Hz, H_4]),3.9 (t, 2H, J 5Hz, H_2), 5.0 (s, 1H, H_{10}),5.71-6.6 (complex, 3H, $H_5H_6$and H_7)ppm;^{13}C NMR (70MHz, $CDCl_3$) δ: 21.7(C_{16}), 24.3(C_{15}), 30.5(C_{13}), 34.7(C_{11}), 39.8(C_1), 41.9(C_{14}), 42.6 (C_4), 101.4 (C_{12}), 107.5(C_9), 125.5(C_2), 126.6 (C_5), 130.7 (C_6), 134.8 (C_7), 139.8(C_{10}), 146.1(C_8), 147 3 (C_3)ppm. [Found: C, 89.83; H, 9.52, M.213 $C_{16}H_{21}$ requires C, 90.14; H, 9.86. MS (FAB) m/zz 213 (M+1)].

e) Preparation of 6,6-Dimethyl-2-Indenylethyl-bicyclo [3.1.1] Hept-2-Ene (5).

In a dry, 1-litre three-necked flask fitted with internal thermometer, argon inlet and outlet, a magnetic stirrer, was suspended freshly-distilled indene (2.84g, 0.032mol) in THF (20.00ml) at -78 °C. The solution was stirred for 30mins and n-buthyllithium (14.1ml of 2.5M solution, 0.032mol) was added by syringe. The solution turned yellow and stirring was maintained for another 30mins at -78 °C. A solution of 6, 6-dimethyl-sulphonyloxyethylbicyclo [3.1.1] hept-2-ene (**2**) (5.0g, 0.021mol) in fresh, dry sample of tetrahydrofuran (THF) (100.00ml) under argon was added cautiously added (exothermic). It was then stirred at room temperature for 24h. Water (100.00ml) was cautiously added and the aqueous layer was extracted with diethyl ether (3 x 100.00ml). The ether layers were combined dried ($MgSO_4$) and concentrated. Thin layer chromatography (TLC, hexane/ethyl acetate 3:1) showed it to contain two spots at a $R_e$value of 0.36 and a second spot at R_f value of 0.28. Fractionally distillation of the crude oil (5.5g) through Vireo column under vacuum (in a sand-bath) gave (4.3g, 0.017mol, 81%) of (**5**) as brown oil bop. 104-106°C/1mm Hg. IR (Nucor); ν_{max} 2923m, 2856m (–CH str. vibe.)3065w, 3020,2980, 2860, 2710,1597m, 1186m, 1174m, 1095m, 1088m, 1020m, 980m, 961s, 912m, 814s, 722m, 665m cm^{-1}. 1H NMR (300MHz, $CDCl_3$) δ: 0.79 (s, 3H, $CH_{3\text{-endo}}$ H_{20}), 1.10 (d, 1H, J 7.7Hz, $H_{17\text{-endo}}$), 1.20 (s, 3H,$CH_{3\text{-exo}}$ H_{19}), 1.99 (t, 1H, J 3Hz, H_{16}), 2.05 (m, 1H, H_{13}), 2.19 (d, 1H, J7.7Hz, H_{17exo}), 2.30-2.4 (complex, 4H, H_{15} and H_1),3.5 (s, 2H H_{11}),4.19 (t, 2H, J 5Hz, H_2), 5.30 (s, 1H, H_{14})6.3 (s, 1H, H_4), 7.2-7.65 (complex, 4H, aromatic)pap; ^{13}C NMR (70MHz, $CDCl_3$) δ: 21.7(C_{20}), 26.7(C_{19}), 32.8(C_{17}), 37.6 (C_{11}), 37.9(C_{15}), 40.3(C_1), 44.9(C_{18}), 106.8 (C_{12}), 109.5(C_{13}), 120.6 (C_9), 123.6 (C_6), 124.5 (C_8),124.5(C_2), 125.9 (C_7), 128.3 (C_4),137.8(C_{14}), 142.0 (C_3), 143.0 (C_{10}), 144.0 (C_5), 146.7(C_{12}) pap.[Found: C, 90.60; H, 10.30; M 251; $C_{19}H_{23}$ requires C, 90.84; H, 9.2; MS (FAB) m/z: 251 (M + 1)].
4.19 (t, 2H, J 5Hz, H3) corresponding to H_2

III. Results and Discussion

The synthesis of the new legends 6,6-dimethyl-2-cyclopentadienylethylbicyclo[3.1.1] hept-2-ene (**4**) and 6,6-dimethyl-2-indenylethylbicyclo[3.1.1] hept-2-ene (**5**) were achieved by a three step procedure Figure 1. The starting material 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol (**1**) (an alcohol) which is commercially available, was treated with *p*-toluenesulphonyl chloride in pyridine to give the tosylate (**2**) in 89% yield as a white powder with m.p. 49-50 °C while treatment of the alcohol (**1**) with methanesulfonyl chloride and triethylamine gave the corresponding mesylate (**3**) as viscous, pale yellow oil in 98% yield. The tosylate(**2**) and mesylate (**3**) were fully characterized Ouzo and Both, 2005.6,6-Dimethyl-2-cyclopentadienylethylbicyclo [3.1.1] hept-2-ene (**4**) was synthesized by treating the corresponding tosylate (**2**) or the maculate (**3**) with cyclopentadienylsodium (1:1.5) Figure 1 in THF at-78 °C for 4h then at room temperature for 24hr to give 82% and 71% yield of (**4**) respectively. The IR spectrum of 6,6-dimethyl-2-cyclo-pentadienyle-thylbicyclo [3.1.1] hept-2-ene(**4**) showed the absence of the sulfonyl group with bands at 1385s (O=S=O say. str.), 1230 (O=S=O say. str.), 961s, 814m, 773m (S-O-S) cm^{-1} when compared with the maculate or the tosylate. The^{1}H NMR spectrum of (**4**) showed additional

peaks with the methylene protons of the cyclopentadienyl ring at δ 2.8 pap (J 3.4Hz). The vinyl protons appeared as complex signals in the range δ 5.71 – 6.6 pap. The absence of mixture of isomers was confirmed by the ^{13}C NMR; there were additional four signals only to (**3**) and on decoupling showed the methylene carbon (C_4) of the cyclopentadienyl group at 42.6 paps. There were three signals for the vinyl carbon atoms in the range δ126.6 – 134.8 paps as well as an additional quaternary carbon 147.3 paps. Micro analysis and mass spectrum fast atomic bombardment (MS FAB) confirmed the elemental composition and a molecular mass of 213 a. m. u.

The reaction of the maculate (**3**) and indenyllithium at -78 °C and then at room temperature for 24hr gave the legend new 6,6-dimethyl-2-indenylethylbicyclo [3.1.1] hept-2-ene (**5**) Figure 1as brown oil after purification by fractional distillation in 81% yield while the to slate gave 60% yield. The characteristic (O=S=O say. str.) and (S-O-S) bands were absent in the IR spectrum. The ^{1}H NMRof compound (**5**) which is an indene complex as opposed to a cyclopentadienyl complex (indene is a cyclopentadiene containing a fused benzene ring) has an additional benzene ring (aromatic) to compound (**4**)with peaks at δ7.2-7.65 ppm (complex) integrating for four protons. The ^{13}C NMR of (**5**) gave twenty lines with a total of five quaternary carbons. Complex (**5**) has a molecular mass of 251 a. m. u. which was confirmed by micro analysis and mass spectrum (FAB).

IV. Conclusions

The synthesis of two different ligands of varying molecular sizes (cyclopentadiene and indene substituted complexes) with their corresponding sodium/lithium salts were successfully synthesized.NMR analysis revealed the structures, IR identified and indicated changes in functional groups, and MS (FAB) spectroscopygave their molecular weights while micro analysis gave the percent compositions of all new compounds.

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Keywords : *bioreduction; rhizopussp.; aspergillussp.; biocatalysis; cyclohexanones.*

GJSFR-B Classification : *FOR Code: 100301p*

Strictly as per the compliance and regulations of :

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I. Introduction

The study of the growth of microorganisms is important in many fields, forexample clinical analysis and agriculture. Meletiades et al. used the growth curves of filamentous fungi in various nutrient media to optimize amethod for antifungal susceptibility testing [1]. Several parameters involved in testing filamentous fungi for their susceptibilities, such as inoculum preparation, incubation conditions (time and temperature) and the type of the nutrient mediumwere investigated. In addition, the use of microorganismsto improve the availability of nutrients to plants is a necessary and important practice in agriculture. Burity et al. investigated the inoculation of mycorrhizal fungi as an aid to the cultivation of *Mimosa caesalpiniifolia*. The nodulation of the plants roots was favored by appropriate inoculation of mycorrhizal fungi under specific growth conditions [2]. Growth of filamentous fungi in liquid culture is usually measured as an increase in dry weight, using either stationary or orbital shaker cultures in Erlenmeyer flasks. However, in large-scale experiments, for instance when testing the effects of various compounds on the growth of fungi, such experiments can become very space-demanding and laborious, limiting the number of treatments. Langvad developed an efficient method to measure the growth of filamentous fungi, using 96-well microtiter plates and a microplate reader [3]. In recently years, microorga-nism-shave been usedexhaustively inbiocatalytic processes [4]. Such processes require significant control and manipulation of the organisms during the growth phase.

Microorganisms are capable of growing on a wide range of substrates and can produce a broad spectrum of products. When a nutrient medium is inoculated with a microorganism, the growing culture passes through a number of the phases. Initially, growth does not occur and this period is referred to as the lag phase, while may be considered a period of adaptation. Following an interval during which the growth rate of the cells gradually increases, the cells divide at a constant, maximum rate and this period is referred to as the log or exponential phase. Exponential growth cannot continue indefinitely, because the medium is soon depleted of nutrients and enriched with metabolites. Several enzymes are synthesized in the exponential phase, while many secondary metabolites emerge in the ensuing stationary phase, when there is no net growth [5].

Microorganisms can be cultured to produce biomass that may be used to catalyze the conversion of axenobiotic compound into a structurally similar one. The enzymes obtained from microorganisms can behave as chiral catalysts with high regio-andstereo-specificity, in contrast to purelychemical catalysts, enabling the addition, removal, or modificationof functional groups at specific sites on a complex molecule, without the need for chemical protection. Enzymes generally work more effectively within the microbial cell, especially if co-factors such as reduced pyridine nucleotide need to be regenerated [5,6].

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Prochiral cycloalkanones have been shown to be convenient substratesfor asymmetric reduction to enantiomericallypure cycloalkanol derivatives [7-9]. Herein, we present a study of the growth of several species of filamentous fungi and their use in biocatalytic reduction reactions of cyclohexanones **1-3**.

II. Materials and Methods

a) General Methods

The reagents 2-(*tert*-butyldimethylsilyloxy) cyclohexanone (**1**), 3-methylcyclohexanone (**2**) and 4-methylcyclohexanone (**3**) were purchased from a commercial source (Sigma-Aldrich). All manipulations involving the fungi *Aspergillussydowii* CBMAI 933, *Aspergillus* sp. Pd1, *Penicilliummiczynskii* CBMAI 930, *Rhizopus* sp. Cs1 and *Trichoderma* sp. CBMAI 932 were carried out under sterile conditions in a Veco laminar flow cabinet. Technal TE-421 or Superohm G-25 orbital shakers were employed in the biocatalysed transformation experiments. Products of the reduction reactionscarried out with sodium borohydride were purified by column chromatography over silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and EtOAc. The column effluent was monitored by TLC on pre-coated silica gel 60 F254 layers (aluminium-backed: Sorbent) eluted with *n*-hexane and EtOAc. Reaction products were analyzed with a Shimadzu model 2010 GC gas chromatograph equipped with an auto-injector AOC20i flame ionization detector (FID) and a Varian Chiral CP-Chiralsil-DEX (β-Cyclodextrin), column (25 m x 0.25 mm i.d.; 0.39 μm). The chromatographic conditions were: oven temperature initially 50 °C (3 min), rising to 185°C (5 min) at 3°C/min, giving a total run time 53 min; injector temperature 250°C; detector temperature 250°C; injector split ratio 1:20; carrier gas N_2at a pressure of 50 kPa. The retention times were: 3-methylcyclohexanone (**2**), 19.5 min; 4-methylcyclohexanone (**3**), 20.8 min; *cis*- and *trans*-cyclohexane-1,2-diol (**4**), 35.0 min; *cis*- and *trans*-3-methylcyclohexanol (**5**), 24.3min; *cis*-4-methylcyclohexanol (**6**), 25.0 min; *trans*-4-methylcyclohexanol (**6**),24.3 min.

Gas Chromatography-Mass Spectrometry (GC-MS): a Shimadzu GC 2010 Plus gas chromatography system coupled to a mass selective detector (Shimadzu MS 2010 Plus) in electron ionization (EI, 70 eV) mode was used, with a DB5 fused silica column (J&W Scientific; 30 m x 0.25 mm x 0.25). The chromatographic conditions were: oven temperature initially 50°C (0 min) rising to 270°C (10 min) at 10°C/min; run time 32.5 min; injector temperature 250°C; detector temperature 250°C; injector split ratio 1:20; carrier gas He at a pressure of 88.2 kPa. 3-methylcyclohexanone (**2**), 4.54 min; 4-methylcyclohexanone (**3**), 4.62 min; *cis*-cyclohexane-1, 2-diol (**4**), 6.48 min; *trans*-cyclohexane-1, 2-diol (**4**), 6.62 min, *cis*- and *trans*-3-methylcy-clohexanol (**5**), 4.43 min; *cis*- and *trans*-4-methylcy-clohexanol (**6**), 4.47min.

*b) Preparation of cyclohexane-1, 2-diol (**4**), 3-methylcyclohexanol (**5**) and 4-methylcyclohexanol (**6**)*

The cyclohexane-1,2-diol (**4**), 3-methylcyclohexanol (**5**) and 4-methylcyclohexanol (**6**) were synthesized by reduction of the ketones **1-3**(100 mg) withexcess of sodium borohydridein methanol. The spectroscopic data (^{1}H and ^{13}C NMR and MS) of alcohols **4-6** were in agreement with those reported in the literature [10-13].

trans-cyclohexane-1, 2-diol (**4**): (GC-MS) retention time 6.62 min, (*c* = 30%,*Aspergillus* sp.; *c* = 48%, *Rhizopus* sp.); MS: *m/z* (%) 116 (M^+, 12), 98 (52), 83 (45), 70 (100), 57 (74);^{1}H NMR (200 MHz, $CDCl_3$, ppm)1.26-12.24 (m, 4H), 1.60-1.98 (m, 4H), 3.34 (m, 2H), 3.85 (br s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$, ppm) 25.3, 34.9, 77.8.

3-methylcyclohexanol (*cis* and *trans* mixture) (**5**): (GC-FID) retention time: 24.3 min, (*c* = 83%; *Aspergillus* sp.;*c* = 99%, *Rhizopus* sp.); (GC-MS) retention time 4.40 min, MS: *m/z* (%) 114 (M^+, 1), 96 (51), 81 (91), 71 (100), 57 (45); ^{1}H NMR (200 MHz, $CDCl_3$, ppm) 0.82 (d, 3H), 0.7-1.9 (m, 9H), 3.48 (m, 0.7H, *cis*-CHOH), 3.95 (m, 0.3H, *trans*-CHOH), 3.9 (broad singlet, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, ppm) 26.5, 31.4, 35.4, 41.5, 44.6, 66.8, 70.7.

trans-4-methylcyclohexanol (**6**): (GC-FID) retention time: 24.3 min, (35.5%*Aspergillus* sp., 55%, *Rhizopus* sp.);(GC-MS) retention time 4.50 min, MS: *m/z* (%)114 (M^+, 2), 96 (14), 81 (46), 70(21), 57 (100), 41 (48);^{1}H NMR (200 MHz, $CDCl_3$, ppm) 0.97 (d, 3H), 1.20-1.50 (m, 5H), 1.65-1.75 (m, 2H), 1.90-2.00 (m, 2H), 3.5 (tt, 1H, *J* = 4.5, 11.0 Hz).

cis-4-methylcyclohexanol (**6**):(GC-FID) retention time: 25.0 min, (42.5% *Aspergillus* sp., 42.2%, *Rhizopus* sp.); (GC-MS) retention time 4.50 min, MS: *m/z* (%) 114 (M^+, 2), 96 (14), 81 (46), 70(21), 57 (100), 41 (48); ^{1}H NMR (200 MHz, $CDCl_3$, ppm) 0.95 (d, 3H), 1.20-1.50 (m, 5H), 1.65-1.75 (m, 2H), 1.90-2.00 (m, 2H), 3.90 (q, 1H, *J* = 3 Hz).

c) Isolation and preservation of filamentous fungi

The marine fungal strains, *A. sydowii* CBMAI 933, *P. miczynskii* CBMAI 930 and *Trichoderma* sp. CBMAI 932 were isolated from the sponge, *Geodiacorticostylifera* [14]. The stock cultures of marine fungi were previously preserved at 4°C on solid media containing 2% malt extract in Petri dishes. The equivalent malt extract liquid culture medium was inoculated with stock fungal mycelium and inoculated at 32°C until 240 h, and then growth curves were constructed using dry myceliaweight in accordance with procedure described below (Figure1).

The fungi *Aspergillus* sp. Cs1 and *Rhizopus* sp. Pd1 were isolated during the germination of seeds of

Chorisiaspeciosa and *Peltophorumdubium*, collected on the soil surface. Ten seeds of *C. speciosa* and *P. dubium* were washed with distilled water (2 x 10 mL) and subsequently with solution of 0.1% sodium hypochlorite (2 x 10 mL). Then, the seeds were transferred to the Petri dishes containing a sterilized sheet of What man filter paper soaked in water, and incubated for seven days in a chamber at 37°C. After the required incubation period, from the seeds from *C. speciosa*, furry colonies of filamentous fungus were obtainedon the agar surface. The isolated fungus was identified as *Rhizopus* sp. Cs1. In addition, on the seeds of *P. dubium* colonies of a dark-sporing strain were obtained and during the growth of fungus on the agar, the seeds were completely destroyed. The purified black fungus was identified as *Aspergillus* sp. Pd1. The fungi *Rhizopus* sp. and *Aspergillus* sp. were used to inoculate liquid culture medium with 2% malt extract and cultured at 32°C for 240 h, to obtained dry weight growth curves (Figure 1). Each fungal isolation was conducted in duplicate. The fungi used in this study were identified by both conventional and molecular methods at the Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA) at UNICAMP, São Paulo, Brazil (http://www.cpqba.unicamp.br/) [14].

d) Preparation of culture media

Solid mediumfor the growth of fungi was prepared by adding 20 g malt extract and 10 g agar (Acumedia) to1 L distilled water and autoclaving at 121 °C for 15 min. The culture medium was dispensed into sterile Petri dishes, allowed to cool and inoculated with fungi. Liquid culture medium (100 mL) was prepared in 250 mL Erlenmeyer flasks, as the solid medium but without addition of agar.

e) Procedure to obtain the growth curves

Two small slices of solid medium (0.4×0.4 cm) bearing mycelium of each fungus (*A. sydowii* CBMAI 933, *Aspergillus* sp. Pd1, *P. miczynskii* CBMAI 930, *Rhizopus* sp. Cs1, *Trichoderma* sp. CBMAI 932) were cut from the stock solid culture and used to inoculate liquid culture medium (100 mL) contained in 250 mL Erlenmeyer flasks. The fungal cultureswere incubatedat 32°C on an orbital shaker (140 rpm). For each microorganism, 5 flasks were used. During the growth of strains in liquid medium, replicate Erlenmeyer flaskswere withdrawn from the orbital shaker one by one at 5 different times (96, 144, 192, 240 h). In addition, the mycelia were filteredon a Buchner apparatus and their dry weights determined after drying at 70°C for 24 h. The growth curves of fungi were constructed from the dry cell weights (g/100 mL) plotted *versus* incubation time (h). The experiments were conducted in triplicate (Figure1).

f) Biocatalytic conversion of cyclohexanones **1-3** *by Aspergillus sp. and Rhizopus sp.*

The fungi *Aspergillus* sp. Pd1 and *Rhizopus* sp. Cs1cultured in 250 mL Erlenmeyer flasks as described above. The fungal mycelia were incubated at 32°C on an orbital shaker (140 rpm) at 72 h. The cyclohexanones (**1-3**, 50 μL) previously diluted in ethanol (50 μL) were added at the time of exponential growth determined for each microorganism; in these cases, at 72 h. After a satisfactory time for, the fungal cultures were withdrawn from the orbital shaker and the mycelia were harvested by Buchner filtration and washed with ethyl acetate. Subsequently, the filtrates and washing were combined and extracted with ethyl acetate (3 x 30 mL) and the organic phase dried over Na_2SO_4, filtered and evaporated. The residues extracted were dissolved in EtOAc and analyzed by GC-FID and GC-MS (Figure 2).

III. Results and Discussion

a) Growth curves of filamentous fungi

In these studies, to optimize the use of filamentous fungi as biocatalysts for the reduction reactions of cyclohexanones**1-3**, growth curves were recorded for the dry weight of mycelia cultured over different periods of time. A total of five growth curves were obtained for the five strains of filamentous fungi (Figure 1). The exponential growth phases were characteristicofeach of the fungal strainsgrownin the malt extract liquid medium. These experiments were conducted in triplicate.

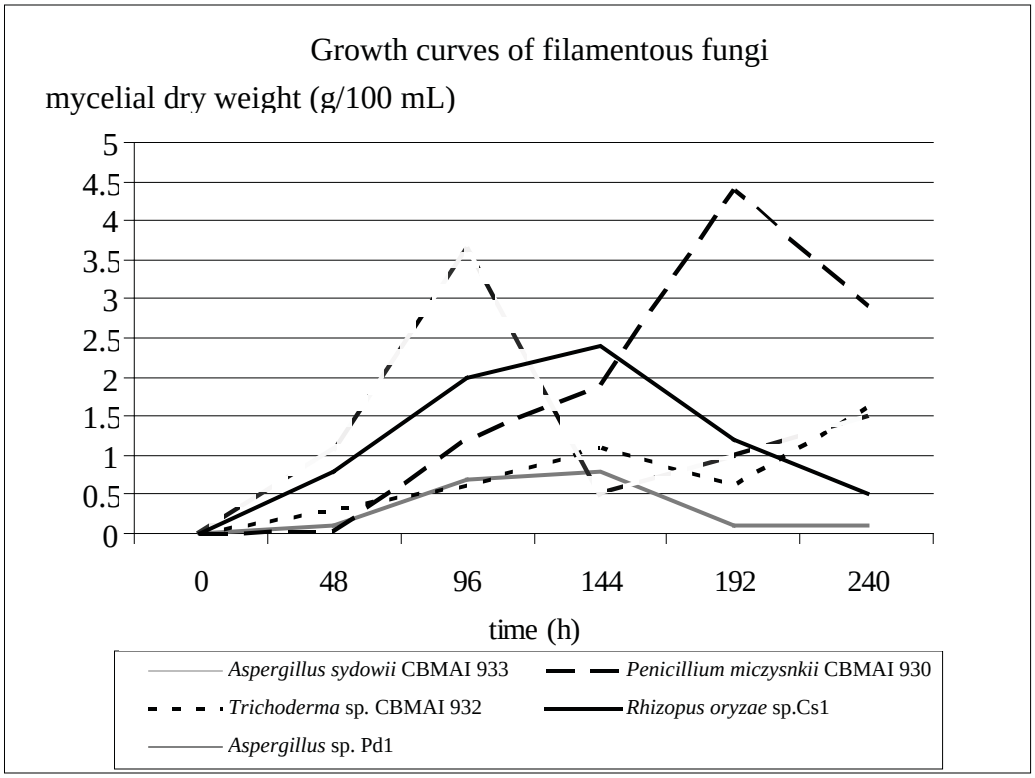

Figure 1 : Growth of filamentous fungi in 2% malt extract liquid medium at 32 °C for 10 days

For *A. sydowii* CBMAI 933, the growth curve showed a log phase that occurred in the period from 48 to 144 h. After this time, an accentuated fall in the mycelial mass until 192 h was observed, possibly because the nutrient had been consumed, leading to the death phase. *Aspergillus* sp. Pd1, a strain isolated from seeds of *Peltophorum dubium*, showed a log phase similar to that of *A. Sydowii* CBMAI 933, from 48 to 144 h. Afterwards, the massdeclined acutely from 240 h. This fungal strain showed a highermycelialproduction than *A. Sydowii* CBMAI 933 and an accelerated growth metabolism. Therefore, it was an interesting fungus to be tested in the biocatalytic process (Figure 1).

For marine fungus *P. miczynskii* CBMAI 930, the log phase in the growth curve contained to a maximum mass at 192 h, after which the growth curve had a negative slope at 240 h. In this case, also good yields of dry mycelium were obtainedin the log phase (Figure 1).

Trichoderma sp. CBMAI 932showed a log phase growth period from 48 to 144 h, which was similar to that of strains of the genus *Aspergillus*. However, following the decline phase at 192 h, a large mycelial growth occurred. Possibly, the release of metabolites produced in the primary growth phase fed a new phase of fungal growth.

The strain of *Rhizopus* sp. Cs1 showed the shortest log phase, where the highest production of mycelial mass occurred from 48 to 96 h of incubation. After this time, a decrease in mass of the myceliumwas observeduntil 144 h. When the incubation period of the fungus was prolonged, mode rate growth was observed after 144 h. However, the amount of mycelium remained significantly lower than in the log phase (Figure 1).

In conclusion, these experiments revealed a broad spectrumof behavior in the growth of filamentous fungi, which could be utilized as biocatalysts in the reduction of cyclohexanones **1-3**

*b) Biocatalytic reduction of cyclohexanones **1-3***

After the growth curves of the filamentous fungi were recorded. The best strains were selected for the biocatalytic reduction reactions of cyclohexanones**1-3**. According to the results shown in (Figure 1), the fungi *Aspergillus* sp. Pd1 and *Rhizopus* sp. Cs1 were chosen for assessment of their biocatalytic reduction capacity. The strain of *Aspergillus* sp. showed a fast growth and a long exponential phase, while the *Rhizopus* sp. showed an acceleratedlog phase and a high production of dry mycelial mass.

Initially, the fungi were cultivated in 100 mL of malt extract for 72 h. After this time, the cyclohexanones**1-3** (50 µL)diluted in ethanol (50 µL) were added, during the log phase of growth, and aliquots were collected at 96 and 240 h and analyzed by chromatographic methods (GC-FID and GC-MS). The mycelia of *Aspergillus* sp. and *Rhizopus* sp. catalyzed the biotransformation of 2-(*tert*-butyldimethylsilyloxy) cyclohexanone (**1**) into cyclohexane-1,2-diol (**4**) at 240 h. GC-MSanalysis showed that ketone **1** was consumed

partially, producing the *trans*-cyclohexane-1,2-diol (**4**) in modest concentrations ($c<50\%$), by both fungi (Figure 2). The cyclohexanone derivative **1** was also reduced with sodium borohydrideto yield the 2-TBS-cyclohexanol as intermediate. In this condition, the labile silyloxy group of the ketone **1** was notdeprotected. The deprotection of the TBS group was then accomplished by treatment with1M HCl in CH_2Cl_2 at room temperature for 24 h, leadingto the corresponding *cis*-diol **4**.On the other hand, the whole mycelia of *Aspergillus* sp. and *Rhizopus* sp. achieved good bioreduction of ketone **1** when it was added in the log phase of growth yielding the *trans*-diol **4**. This implied that silyloxy group was deprotected in water for 5 days. Finally, GC-MS analysis incapillary columns showed the separation of a racemic mixture of *cis*- and *trans*-cyclohexane-1,2-diol (**4**), with retention timesof 6.48 min (*cis*-**4**) and 6.62 min (*trans*-**4**).

The ketones **2-3** showed excellent bioconversion by the mycelia of *Rhizopus* sp. and *Aspergillus* sp. *Rhizopus* sp. catalyzed the reduction of**2** to yield 3-methylcyclohexanol (**5**) at a high conversion rate (c=99%), at 96 h. While *Aspergillus* sp. converted 83% to alcohol **5** at 96 h of incubation. The conversion of 3-methylcyclohexanol (**5**) by the fungi was measured by GC-MS analyses and compared with the amount of synthetic product **5** obtained by sodium borohydride reduction. The GC-FID and GC-MS analyses showed no separation of the stereoisomers of **5**.

Both *Rhizopus* sp. and *Aspergillus* sp. catalyzed the reduction of 4-methylcyclohexanone (**3**) to the corresponding *cis*- and *trans*-4-methylcyclohexanol (**6**) at high conversion rates,at 96 h (Figure 2). The fungi produced a higher proportion of *cis*-diastereoisomerthan in the product of sodium borohydride reduction, which was mainly the *trans*-diastereoisomer. The ratio of *cis*- and *trans*-alcohol **6**, determined by GC-FID on the chiral chromate graphiccolumn, was 1.0: 1.3 for *Rhizopus* sp. and 1.2 : 1.0 for *Aspergillus* sp. The biocatalytic reduction produced around 50% of the *cis*-diastereoismer **6**, which is thermodynamically and kineticallyless stable than the stable *trans*-diastereoismer **6** (Figure 3). In contrast, the *cis*- and *trans*-diastereoisomers **6** were synthetized by sodium borohydride reductionin the ratio 1.0: 4.5. The biocatalyticreduction of 4-methylcyclohexanone **3** could be used for the preparation of the thermodynamically andkinetically less stable *cis*-isomer **6**.

Finally, these have been a few reports in the literature of the microbial reduction of cyclohexanones. For example, the prochiralcouponds 1, 2-cyclohexanedione and (±)-2-hydroxycyclohexanone were reduced by, *Proteus mirabilis* bacteria leading to *trans*-cyclohexane-1,2-diol in high yields [6]. In addition, the reductionof cyclohexanone derivatives by a bacteria culture of *Serratiarubidaea* CCT 5742 and by the yeast *Saccharomyces cerevisiae* afforded cyclohexanols in good yields and selectivities [12,15]. However, these single-cell organisms are less readily separated from the culture medium than fungal mycelia.

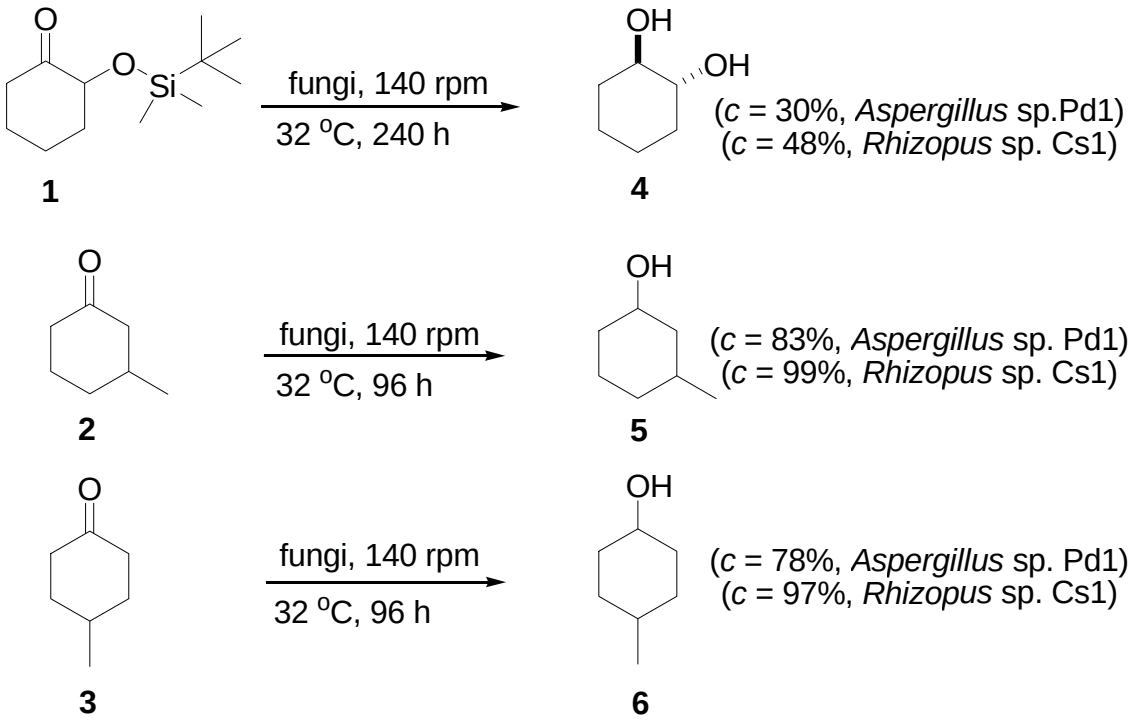

Figure 2 : Reduction of cyclohexanones **1-3** by *Aspergillus* sp. and *Rhizopus* sp. The conversions *(c)* were determined by GC-MS analyses

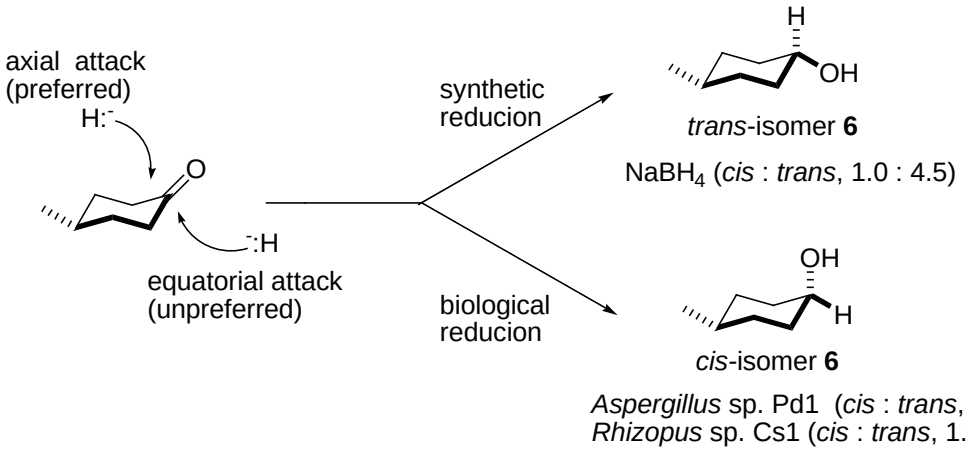

Figure 3 : Reduction of 4-methylcyclohexanone (**3**) by synthetic and biological methods. The ratio of *cis*- and *trans*-alcohol **6** was determined by GC-FID on a chiral chromatographic column

IV. Conclusion

According to the results reported here, the preliminary determination of growth curves of filamentous fungi was important to optimize the conditions for the biocatalytic reduction of cyclohexanones **1-3**. The addition of the substrates in the log phase of growth led to the biotransformation of cyclohexanones **1-3** to the corresponding cyclohexanols **4-6** in good yields. Therefore, during the exponential phase, enzymatic production was maximized. The bioreduction of 4-methylcyclohexanone (**3**) by *Aspergillus* sp. and *Rhizopus* sp. yielded a greaterpro-portion of *cis*-4-methylcyclohexanol (**6**), thethermody-namically and kinetically less stable isomer, thandid synthetic reduction. Finally, the bioreduction of 2- (*t*-butyldimethylsilyloxy) cyclohexanone (**1**) by either fungus led to a higherproportion of *trans*-cyclohexane-1, 2-diol (**4**) than synthetic reduction by $NaBH_4$.

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Effects of Zinc and Nitrogen on Yield Components of Five Flax Genotypes

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Abstract - Flax is one of the earliest plants that has been domesticated by humans. Due to high amounts of omega-3 fatty acid, its cultivation and consumption is increasing as a healthy oil resource. In nutritional point of physiology zinc and nitrogen are two important elements in plant biosynthesis. So in this experiment, effects of three levels of nitrogen (40, 60 and 80 kg/ha) and two levels of zinc sulfate (control and 3/1000) on five flax cultivars in split factorial based on randomized complete block design was investigated. The results showed that genotypes in terms of height, capsul number, seed yield and biological yield revealed significant differences ($P \leq 0.01$). In addition the results showed that the highest yield was obtained from treatment of 80 kg nitrogen and (3/1000) zinc sulfate spray.

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Gholamhosein Homayouni [α], Mohammadkazem Souri [σ] & Mohammad Zarein [ρ]

Abstract - **Flax is one of the earliest plants that has been domesticated by humans. Due to high amounts of omega-3 fatty acid, its cultivation and consumption is increasing as a healthy oil resource. In nutritional point of physiology zinc and nitrogen are two important elements in plant biosynthesis. So in this experiment, effects of three levels of nitrogen (40, 60 and 80 kg/ha) and two levels of zinc sulfate (control and 3/1000) on five flax cultivars in split factorial based on randomized complete block design was investigated. The results showed that genotypes in terms of height, capsul number, seed yield and biological yield revealed significant differences (P≤0.01). In addition the results showed that the highest yield was obtained from treatment of 80 kg nitrogen and (3/1000) zinc sulfate spray.**

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I. Introduction

Flax is an annual plant from Linacaea family. It is one of the most important medicinal plants due to its pharmaceutical active substances. It has different uses in pharmacy and cosmetics industries. So its cultivation is expanding all over the world. The origin of this plant was reported to be the west of Mediterranean region [10]. Flax is one of the oil seed plants and demand for its valuable oil and fatty acids, is increasing all over the world. The seed contain 40-45% oil and 23-34 % protein. Besides its valuable oil, seed meal with high percent of protein 42-46 percent, is also used in the animal diet [13]. Suitable fertilizing program is necessary for optimizing flax cultivation and its qualitative and quantitative characteristics [5]. Besides genotype and other environmental factors, plant nutrition and adequate levels of nutrient elements is prerequisite for maximum yield and quality. Surveys show that there is an increase of about 50% in food production due to use of chemical fertilizers [1]. Regarding plant production, effects of N fertilizers is higher than other fertilizers, however the efficiency of this fertilizers is low and frequently resulted in lodging and environmental pollution [19]. The amount of fertilizers that a crop needs depends to many factors including climate conditions, plant species and cultivar, and soil fertility levels [4]. However for micronutrients this can change a little bit, mainly due to their application method as in many cases they are used as foliar sparays [15]. Flax is a high sensitive plant to zinc deficiency [2] mostly resulted in pollen grains sterility, little leaf, chlorosis and dwarfing [8]. It was shown that foliar spray of zinc sulfate in canola increased seed yield, capsule number in plant, seed number in capsul, thousand weight, seed oil and protein [3,11]. Similarly it was shown that foliar application of zinc has significant increased seed yield, thousand weight and oil content, seed number in capsul, oil and seed yield in canola [16]. So according to incredible role of nutrient elements in quantity and quality of crops, this study was conducted to evaluate yield and quality response of five flax cultivars to different concentrations of nitrogen and zinc.

II. Materials and Methods

This experiment was performed in thirty treatment and three replication in research farming of Shahid Bahonar University of Kerman, Iran. So different amounts of nitrogen and zinc on five flax cultivars was studied in factorial split design. The experiment region has a dry climate with hot and dry summers and cold winters. The experimental site has Loam and clay soil, and pH of 8.7. The first factor was nitrogen with three levels of (40- 60- 80 kg/ha) from urea source (40 kg as control) and second factor including zinc element with two level (0 and 3/1000) zinc sulfate and third factor including five flax cultivars, two native line with complete combination of brown seed color (Birjand and Courdestan) and three new line with yellow complete color (R_{11} ،R_4 and R_{24}). To provide phosphorous, 20 kg of phosphorous was added as strip application just 2.5 cm in side and 2.5 cm in below the seed. Nitrogen was divided in 3 applications, one before planting, one part after planting, another part before flowering in N treatments. Experimental plots were three lines with 2m length and a space of 40cm. Plants were irrigated every 7-10 days depending to plant requirement. Zinc sulfate was sprayed on leaves before flowering. During growing season different plant parameters were measured such as time of flowering, maturity, plant height, number of lateral shoots, number of capsule, number of seeds in capsule, weight of one thousand seed, seed yield for each genotype. Seed yield per plot as well as per plant was determined.

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Data then were analyzed by SAS 9.1 statistical software, and comparison of means was performed by Duncan multiple comparison at 1% level.

III. Results and Discussion

Analysis of variance (Table1) revealed that flax cultivars in terms of height, number of capsule, seed yield and biologic yield showed significant differences (0.01%). Nitrogen levels (40, 60 and 80 kg/ha) in terms of height, capsule number, seed yield and biologic yield led to significant difference (0.01%). Zinc spray also in terms of height, capsule number, seed yield and biologic yield resulted in significant difference (1%) compared to control.

Table 1 : Analysis of variance for some physiological traits

SOV	Df	Plant height (cm)	No of branches	No of capsules	No of Seed in capsule	Biological yield(g)	Seed yield(gr)
Replication	2	5/3*	0/43^{ns}	0/81^{ns}	1/37^{ns}	0/04^{ns}	80/93^{ns}
Nitrogen	2	47.71**	00.3^{ns}	27.41**	1.11^{ns}	0.51**	2013.6**
Zinc	1	30.62**	0.17^{ns}	92.01**	2.5^{ns}	0.42**	3973.3**
Nitrogen× Zinc	10	2.11^{ns}	1.54^{ns}	0.69^{ns}	0.35^{ns}	0.01^{ns}	37.74^{ns}
Genotype	4	6.96**	2.37^{ns}	13.52**	1.65^{ns}	0.15**	988.36**
nitrogen× genotype	8	8.06**	1.43^{ns}	0.78^{ns}	1.12^{ns}	0.07*	285.74^{ns}
Zinc ×genotype	4	4.31*	1.67^{ns}	3.26^{ns}	0.30^{ns}	0.07^{ns}	65.40^{ns}
nitrogen× zinc× genotype	8	8.34**	2.73*	5.93**	0.83^{ns}	0.03^{ns}	564.55**
Error	48	1.57	1.17	2.11	0.78	0.03	140.86
Total	89	425.71	126.40	424.05	72.98	5.55	29910
C.V	2.96	2.96	22.27	9.87	14.27	7.03	14.89

ns: not significant, * and ** significant at level of 5% and 1% respectively

Analysis of variance revealed that interaction effects of genotype and nitrogen in terms of plant height and biological yield, and also between genotype, nitrogen and zinc in terms of plant height, number of capsules and seed yield showed significant differences (1%). The highest plant height was in 80 kg/ha N, and lowest was in 40 kg/ha N treatment. The highest number of capsule was in 80 kg/ha N treatment, and the lowest capsule number was in 40 kg/ha N treatment. In terms of seed yield, 80 kg/ha led to higher amount compared to two other N levels. Availability of nitrogen is an important factor on distribution of photosynthetic assimilates between vegetative and reproductive organs. While increasing nitrogen consumption will enhance vegetative growth. This could be due to leaf expansion [21]. [6] reported that higher number of branches, number of capsule and seed yield obtained with nitrogen amount of 90 kg/ha.

Table 2 : Differences between N levels regarding some physiological traits

Zinc	Height (cm)	Branch number	Capsul number	Seed number in capsul	Biologic yield (gr)	Seed yield (gr)
(Z_0)	41.74^{b}	4.91^{a}	13.71^{b}	6.04^{b}	2.60^{b}	73.02^{b}
(Z_1)	42.90^{a}	4.82^{a}	15.7^{a}	6.37^{a}	2.74^{a}	86.31^{a}

Means with the same words in each column are not significantly different

Comparison of means (Table 3) shows that zinc application led to significant effect on plant height, capsule number, seed number in capsule, seed yield and biologic yield ($P \leq 0.01$). Zinc foliar application increase seed and biomass yield compared to control plants. Zinc is an important element in protein and oil biosynthesis in seeds [12]. In flax it also increases plant height, branches number, capsule number and seed yield [20]. It has been shown that spraying of zinc on flax led to higher growth rate and oil percentage [18]. Zinc with increasing lateral shoots, helps for more capsules. The results showed that both vegetative and flowering stages promoted by Zn spray, probable by increasing of photosynthesis rate and plant metabolism [14]. It has been shown also that application of zinc sulfate on canola leaves had significant role in seed yield improvement [11]. The role of zinc on improvement of yield component has been documented by other researches [16]. Application of zinc on some plants such as lentil [9], safflower [8], soybean [6] led to enhancement of seed yield components.

Table 3 : Difference of zinc levels for some physiological traits. z_0= control, z_1= 3/1000

Zinc	Height (cm)	Branch number	Capsul number	Seed number in capsul	Biologic yield (gr)	Seed yield (gr)
(Z_0)	41.74^b	4.91^a	13.71^b	6.04^b	2.60^b	73.02^b
(Z_1)	42.90^a	4.82^a	15.7^a	6.37^a	2.74^a	86.31^a

Means with the same words in each column are not significantly different

Table 4 : Comparison of cultivars regarding some growth factors

genotype	Height (cm)	Branch number	Capsul number	Seed number in capsul	Biologic yield (gr)	Seed yield (gr)
C1	41.40^b	5.27^a	15.83^a	6.38^a	2.81^a	90.88 ^{a}
C2	42.76 ^{a}	4.83^{ab}	15.05^{ab}	6.11^{ab}	2.65^b	78.61^b
C3	42.26^{ab}	4.38^b	14.72^b	6.38^a	2.67^b	79.55^b
C4	42.17^{ab}	4.66^{ab}	14.55^b	5.72^b	2.55^b	70.05^c
C5	43.02^a	5.16^{ab}	13.44^c	6.44^a	2.67^b	79.22^b

Means with the same words in each column are not significantly different

IV. Conclusion

Genotypes showed significant differences regarding growth factors, and R_{24} genotype showed significant higher amounts compared to other genotypes. So, it can be concluded that R_{24} genotype with 80 kg/ha N+ foliar spray of zinc is suitable for Kerman province in Iran.

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(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

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(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

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(h) Brief Acknowledgements.

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- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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