Journal

J. Biol. Chem. Environ. Sci., 2010, Vol. 5(2): 489-502
www.acepsag.org

STUDIES ON ACCLIMATIZATION AND SOME BIOLOGICAL ACTIVITIES OF PLECTRANTHUS AMBIGUUS IN EGYPT.

Mohammad. M.EL-Zefzafy, Gouda T. M. Dawoud*. and Islam M. El-Garawani**

National Organization for Drug Control and Research (NODCAR) Medicinal Plants Dept., Phytochemistry Dept. and **Biology Dept., Applied Research Center for Medicinal Plant (ARCMP).*

ABSTRACT

The present work was carried out to study the effect of the temperature on acclimatization and the growth of *Plectranthus ambiguus* Family Lamiaceae (Labiatae) in a greenhouse of controlled temperature, as will as studying the effect of different seasons in and out normal greenhouse conditions, The results clearly showed the effect of temperature on the survival percentage of plants during acclimatization with significant difference ($p<0.05$), as the highest significant percentages of survival were obtained with the degrees 30 and 35 ºC compared to the other treatments. Interestingly, the results indicated that the summer and autumn showed higher percentages of survival and growth rate compared to the other seasons. However, the best result was observed in the summer. The antioxidant activity, total phenolic compounds and flavoniod contents were investigated in plants grown in and out the normal greenhouse. The obtained results outside the greenhouse were 30 %, 70 µg and 30 µg %, while the results inside the greenhouse were 25 %, 55 µg % and 28µg %, respectively. The genotoxic investigation on the cultivated plants outside the greenhouse was also carried out by feeding rats with 20 % dried leaves containing diet daily for four weeks. The results indicated that there were no significant genotoxic effects on the level of DNA damage.

INTRODUCTION

The name *of Plectranthus* Family Lamiaceae (Labiatae) from the Greek *plektron,* a spur and *anthos,* flower, referring to the spur that is found at the base of the corolla tube of the species type of the genus, *P. fruticosus.* As it turned out, this was not the best name for the genus as few other *Plectranthus* species have the spur. The species name *ambiguus* means doubtful or uncertain, because botanists initially struggled to classify it, placing it in another genus *P. ambiguus* . It was first described as *P. coloratus* or *Orthosiphon ambiguus* which transferred to *Plectranthus* in 1964. The other species that were studied are *P. ambiguus*. (Bessi 1924, Codd 1975, and Catherine *et al* 2005).

The genus *Plectranthus* belongs to the mint and sage family (Lamiaceae) and consists of annual or perennial herbs or shrubs with herbaceous stems and leaves, sometimes semi-succulent or succulent, with usually terminal, spike-like inflorescences of two-lipped flowers. There are ± 350 species spread throughout the tropical and warm regions of the old World, in Africa, Madagascar, Asia, India, Australia and a few Pacific islands. There are 53 species in southern Africa, occurring mainly in the southeastern and eastern parts and absent from the Northern Cape It is a good plant for containers and hanging baskets and can be grown indoors. *P. ambiguus* leaves are used in traditional medicine to treat colds. (Potgieter, et al 1999). An antioxidant activity-resulted in the isolation of 5, 6- dihydroxy -7, 4_-dimethoxyflavone (ladanein) (Liu and Peter Ruedi, 1996). In South Africa, tea made from the leaves of *Plectranthus laxiflorus* is taken for coughs and colds (Rabe and van Staden, 1998) the plant was perennial with a productive life of about 2 to 3 years and it was propagated mainly by rooted cuttings. and an infusion of the crushed leaves of *P. ambiguus* was mixed with a little hot water and sipped for coughs (Hulme, 1954). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. (Cook and Samman, 1996). Monoterpenoids, sesquiterpenoids, diterpenoids and phenolics have been reported in species of *Plectranthus*. The abietane diterpenoids are the most diverse of the diterpenoids isolated from species of *Plectranthus* (Liu and Peter Ruedi, 1996) . The labdane diterpenoid,

forskolin, occurs in *Plectranthus barbatus* and that could explain some of the traditional uses of this species (Catherine *et al* 2005). Additionally, the effect of certain substance of medicinal plant on the DNA was great concern. As well traditionally known, *Plectranthus* species were used as medicines, food, flavors and ornamentals (Lukhoba et al., 2006), the genotoxic effect and safety limits of higher doses were not investigated widely. Consequently, the effect of daily feeding of 20% content of the dried *P. ambiguus* leaves for four weeks on rat' liver DNA was investigated through this study.

In continuation of the research concerning biologically active constituents of African, Asian and Australia medicinal plants, this study was achieved on acclimatization, some biological activities and the possible genotoxicity of *P. ambiguus* in Egypt.

MATERIALS AND METHODS

This study was conducted during the years 2008, 2009 and 2010 in greenhouse under control, phytochemistry and biological labs. of the Applied Research Center of Medicinal Plants(ARCMP) at Abo-Elhol region, National Organization for Drug Control and Research (NODCAR).

I- Acclimatization and plant growth of *P. ambiguus*.

A-Effect of various degrees of temperature on acclimatization and growth of *P. ambiguus* in greenhouse under control.

Plantlets obtained from rooting stage in average tall (6 – 7cm) and fresh weight (6. 60) g /plant and root number (8) were washed from phytagel under running tap water and soaked in solution of fungicide (0.2 % Benlet), then transferred to the greenhouse under control in plastic containers (5 cm) full of peat moss and sand (1/1– V/V) for acclimatization to study the effect of various degrees of temperature (20 ºC , 25 ºC ,30 ºC and 35 ºC) on acclimatization of plants. Data were recorded after six weeks of incubation. Plants were covered with plastic bags and maintained in the greenhouse at different temperature degrees. Twenty five replicates were made for each treatment Data (survival percentage, plant height (cm), fresh weight g / plant, root number, root fresh weight g/plant and plant growth rate) were recorded after six weeks from culturing at greenhouse under control.

B- Effect of various seasons on plant growth and development of *P. ambiguus* cultured in and out normal greenhouse.

This experiment was conducted to study the effect of various seasons on vegetative growth and development of P. ambiguus cultured in greenhouse. Twenty five replicates of acclimatized plants with average tall 15.77cm, fresh weight 21.88 g/plant and root number 9.89/plant, were transferred to plastic containers (50 cm) full of peat moss/sand at equal volumes for more growth and development in uncontrolled greenhouse. While other twenty five individuals were grown out the greenhouse conditions to study the effect of various seasons (spring, summer, autumn and winter). Data were recorded after the end of each season. Twenty five replicates of each group were estimated for survival %, plant height (cm), fresh weight (g) and plant growth rate. These data were recorded after three months at the end of each season.

All data were statistically analyzed among one factorial randomized complete design package (SAS, 1988). The least significant difference among levels of each treatment was compared using L.S.D. test at 5 % and 1% levels according to Steel and Torrie (1980).

II- Total antioxidant activity determination:

A- Free radical scavenging assay

The free radical scavenging effect of the plant extract was assessed by the discoloration of a methanolic solution of DPPH(1,1 diphenyl -2- picryl hydrazyl) according to Viturro *et al.* (1999) and Astudillo *et al*. (2000).

B-Determination of total phenolic contents:

The concentration of total phenolic compounds in the different extracts were determined spectrophotometrically using the Folin-Ciocalteu reagent which is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic antioxidants and polyphenol antioxidants according to (Frankle and Meyer 2000, Donald *et al.,* 2001 and Julkunen, 1985).

C- Determination of total flavonoids.

Determination of the total flavonoid contents in the different extracts were done colorimetrically by using aluminum chloride solution. Standard curve was done using different concentrations of rutin in methanol (six serial 2 fold dilution to give 100-3.2 μg/ml).

100µl of each extract (previously prepared) were added to a 96 micro well plate and then 100µl of 2% aluminum chloride solution in methanol. After 10min, their absorbance was measured using HP spectrophotometer at 415nm using methanol as blank and the concentration of total flavonoids was calculated according to (Karawy and Aboutable, 1982; Lamaison and Carisat, 1990, and Chiang *et al*., 2002).

III- Evaluation of possible genotoxicity of *P. ambiguus*:-

Experimental animals:

The present study was carried out using adult female western rats of an average body weight about 120 -150 gm. The experimental animals were divided into two groups, (-ve) control, 20 rats, and *P. ambiguus* dosing, 20 % dried leaves containing diet daily for four weeks, 8 rats.

Genetic analysis (DNA damage) of liver:

DNA extraction and apoptosis detection:

DNA extraction and detection of apoptosis were done according to "salting out extraction method" of Aljanabi and Martinez (1997) with modification introduced by Hassab El-Nabi (2004).

Gel preparation

Gel was prepared using 1.8% electrophoretic grade agarose obtained from (Hispangar D-1 LE,Spain).The agarose was boiled with 1X tris borate EDTA buffer (pH 8.3), and then, 0.5 microgram /ml ethidium bromide was added to agarose mixture at 40°C . Gel was poured and allowed to solidify at room temperature for 1h before samples were loaded and horizontally separated. The analysis was done using Biogene software as a maximum optical density values (height) where the height is a maximum intensity at 256 grey levels.

RESULTS AND DISCUSSION

A-Effect of various degrees of temperature on acclimatization and growth of *P. ambiguus* in greenhouse under control.

Shoots produced from multiplication stage by tissue culture without roots were transferred from planting media to the greenhouse under control in plastic containers (5 cm) full of peat moss and sand (1/1-V/V) at different temperature degrees for six weeks for root formation and data of the plant growth were shown in Table (1) and Fig. (1). Data shows that, temperature degree levels (20, 25, 30, and 35 ºC) improved the acclimatization and plants growth in greenhouse

under control. In case of temperature degrees 30 and 35 ºC significantly recorded the maximum values of survival percentage which was 100% with both of them. The same degrees observed the best plant height (15.77, and 19.96 cm), fresh weight plant (21.88 and 23.77 g/plant), root number (9.89 and 11.46) root fresh weight (3.38 and 4.85 g/plant) and plant growth rate (150 and 172 %). The lower degree temperature showed lower effects on all treatment as shown in Table (1) and Fig. (1).

Table (1): Effect of temperature degree levels on acclimatization and growth of in vitro obtained P. *ambiguus* after 5 weeks in greenhouse under control.

Temperature ºC	Survival %	Plant height cm	Fresh weight g / Plant	Root number	Root fresh weight	Plant growth rate
20	5	6.80	8.72	2.00	0.99	0.0
25	65	9.98	12.97	6.46	1.95	48
30	100	15.77	21.88	9.89	3.38	150
35	100	19.96	23.77	11.46	4.85	172
LSD at 5%	**……**	**1.851**	**1.693**	**1.973**	**1.274**	**……**

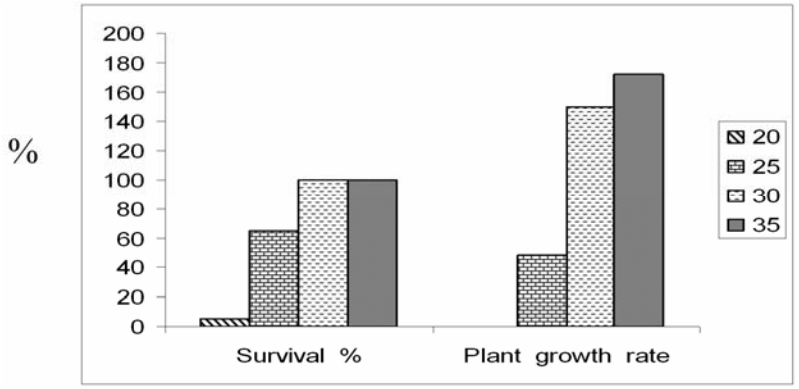


Fig. (1) The histogram of table (1) records.

Amat (1982) which reviewed that *Stevia rebaudiana* growth requires mild temperature between 15 and 38 degree and relative humidity of about 80 %, supposed that at the higher temperature, respiration exceeded Figsynthesis. The formation of adventitious roots on shoots is temperature dependant, for example no_roots formed on

young shoot tips of Asparagus at 0, 10 or 15 ºC, but 22 % of cuttings rooted at 20 ºC, and 45 % at 25 ºC (Gorter, 1965), Most *plectranthus* are adapted to live in the summer rainfall forest, and have to deal with low light, trampling, winter drought, and root competition. They are fast-growing, which enables them to move quickly into areas of good light and /or nutrients **(**Bessi. 1924 and Bowden, 1978).

B- Effect of various seasons on growth and development of *P. ambiguus* cultured in and out normal greenhouse conditions.

This experiment was conducted to study the effect of various seasons (spring, summer, autumn and winter respectively) on the survival percentage, vegetative growth and development for those plants cultured in and out normal greenhouse conditions. As shown in table (2) and Fig. (2) The recorded data revealed that the maximum values in plant height cm, (27.42, 69.88, 48.44 and 21.55 respectively), fresh weight g/ herb of plant (31.63, 144.55, 121.44 and 26.97 respectively), and plant growth rate (33 %, 150.8 %, 126.7 and 13.00 % respectively). These results have a significant differences ($P<0.05$) among the various seasons (spring, summer, autumn and winter respectively). While out greenhouse conditions. the maximum effect of various seasons (spring, summer, autumn and winter) on growth, development and survival percentage was recorded as showed in table (2) and Fig. (2).

Table (2): Effect of various seasons (spring, summer, autumn and winter) on survival percentage and vegetative growth of *P. ambiguus* cultured in and out normal greenhouse conditions.

Seasons	Survival %	Av. Plant height cm	Av. fresh weight g / herb of plan	Plant growth rate
Plant growth inside normal greenhouse (uncontrolled)				
Spring	100	27.42	31.63	33
Summer	100	69.88	144.55	150.8
Autumn	100	48.44	121.44	126.7
Winter	98	21.55	26.97	13.00
LSD at 5%	.....	4.851	4.693	.....
Plant growth in outside greenhouse				
Spring	85	23.55	26.77	27.90
Summer	100	43.66	163.99	170.90
Autumn	100	35.22	132.94	138.5
Winter	94	18.99	23.80	0.00
LSD at 5%	.....	5.437	4.942	.....

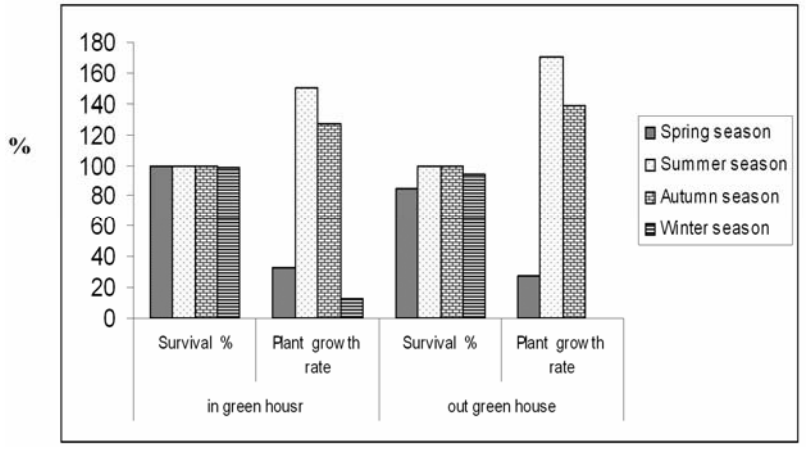


Fig. (2) The histogram of table (2) records.

And the recorded data has a significant differences ($P<0.05$) in various seasons (spring, summer, autumn and winter respectively)

The good plant growth requires mild relatively high temperature and enough light which is always available between summers to autumn**.** (Van Jaarsveld, 2006)

As shown in table (3), the antioxidant activity, total phenolic compounds and flavoniod contents were investigated through out greenhouse and in greenhouse cultivated *P. ambiguus* groups. The obtained results were appeared 30 %, 70 µg and 30 µg %, while the results of in greenhouse were 25 %, 55 µg % and 28µg % respectively.

It has been mentioned that, the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Frankel and Meyer 2000).Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic

antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990).

Total antioxidant activity determination of *P. ambiguus* :-

Table (3): % Antioxidant activity (by using of DPPH method), Total phenolic compounds (Measured by Folin Ciocalteu and flavonoids (Measured by $AlCl_3$) in different extracts of the studied plants as percentage of extracts of *P. ambiguus*.

Name of extract	%Antioxidant activity	TotaL Phenolic contents µ g %	TotaL Flavonoids contents µ g %
P. ambiguus out greenhouse	30 %	70	55
P. ambiguus In greenhouse	25 %	30	28

In continuation of our current research the evaluation of the possible genotoxic effect due to the dietary intake of the *P. ambiguus* leaves cultivated out greenhouse was carried out via feeding rats 20 % dried leaves containing diet daily for four weeks and the results indicated that there were no significant genotoxic effects on the level of DNA damage over this period and the obtained results were as the following.

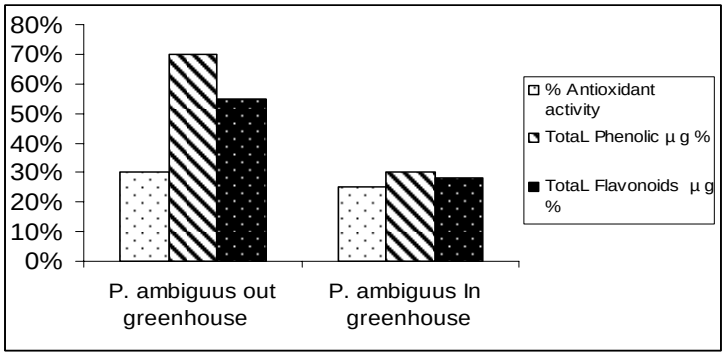


Fig. (3): The histogram of table (3) records.

Determination of DNA damage and apoptosis in liver of *P. ambiguus*:-

The electrophoretic pattern of DNA in each lane shows one form of DNA, intact DNA, and the measured height values were (14803425) for L2 which resembles two weeks period of treatment and (15066597) for L3 which resembles four weeks period of

treatment. Although a little decrease in the value of L2 than that of L3 was observed, it can be neglected when compared with the control value (14935011). This means that no significant DNA damage occurred among this experiment when compared with control leading to a conclusion, the intake of the plant leaves may be safe but further research is recommended to prove the complete safety of this plant.

Photo (1) *P. ambiguus* grown in the green house for two month.

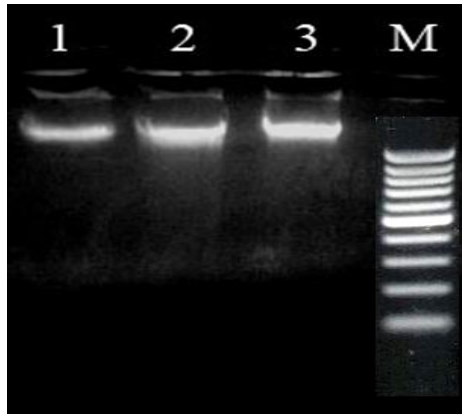
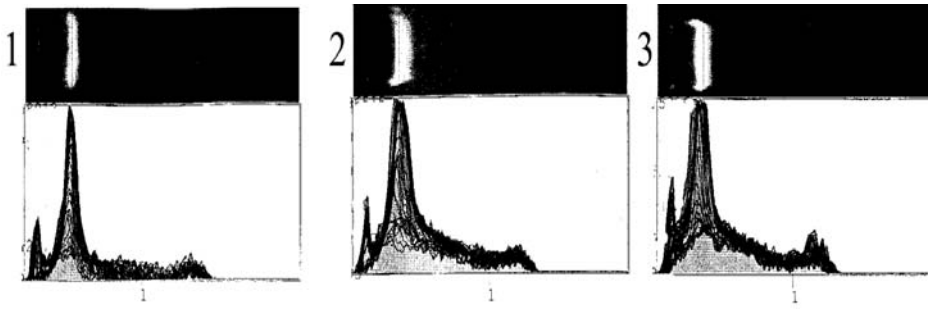


Photo. (2): Electrophoresis of DNA shows the effect of *P. ambiguus* feeding for four weeks, lane 1 (L1) resembles control liver; L2: after two weeks. L3: after four weeks and M: 100 bp ladder.



The analytical peaks for DNA lanes from L1 to L3 which resemble the same lanes in (photograph: 2) using biogene software.

REFERENCES

Aljanabi, S.M. and Martinez, I. (1997): Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res., 25: 4692-4693.

Amat, A.G. (1982): Losprincipios edulcorantes de *Stevia rebaudiana*, Bert Estado actual de su conoci miento Acta Farm Bonaer 1: 121-123.

Astudillo. *L*.; Schmedu-Hischmmann, G.; Herrea, J.P. and Cortes, M. (2000): Proimate composition and biological activity of Chilean

Prosopis species.J. Sci. Food Agri., 80: 567-573.

Barlow, S.M. (1990): Toxicological aspects of antioxidants used as food additives in food antioxidants", Hudson BJF (Ed) Elsevir, London, 253-307.

Bessi, M. (1924): The South African *Nemestrinidae* (*Diptera*) as represented in South African Museum. Ann. S. African Mus.19: 164-190.

Bowden, J. (1978): *Diptera*. In: Werger M. J. A. (ed) Biogeography and ecology of southern Africa. Junk. The Hague. 775-796.

Catherine W. Lukhoba, Monique S.J. Simmonds and Alan J. Paton (2005)*Plectranthus*: A review of ethnobotanical uses Copyright © 2005 Elsevier Ireland Ltd .

Chiang, L.C.; Chaiang, W.; Chang, M.Y.; Ng, L.T. and Lin, C. (2002): Antiviral activity of Plantago major extracts and related compounds *in-vitro.* Antiviral Res., 55: 53-62.

Codd L.E. (1975) *Plectranthus* (Labiatae) and allied genera in southern Africa. Bothalia 11:371-442.

Cook, N.C. and Samman, S. (1996). Flavonoids Chemistry, metabolism, cardio-protective effects, and dietary sources, Nutrition Biochemistry, 7: 66-76.

Donald, S., Prenzier, P.D., Autolovich, M. and Robard, K., (2001).Phenolic content and antioxidant activity of olive extracts, Food Chemistry, 73: 73-84.

Frankle, E. and Meyer, A. (2000): The problem in using one dimensional methods to evaluate multifunctional food and biological antioxidants', J. Sci. Food Sci. Agric., 80, 1925-1941.

Gorter, C.J. (1965): Vegatative propagation of *Asparagus officinalis* by cuttings.J . Hort. Sci., 40, 177- 179.

Hassab El-Nabi, S.E. (2004): Molecular and cytogenetic studies on the antimutagenic potential of eugenol in human lymphocytes culture treated with depakine and apetryl drugs. J. Egypt. Ger. Soc. Zool., 43(c): 171-196.

Hulme, M.M.(1954): Wild Flowers of Natal. Shulter & Shooter, Pietermaritzburg.

Julkunen-Tiito. R. (1985): Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. J. Agric Food Chem., 33, 213-217.

Karawya, M.S. and Aboutabl, E.A. (1982): Phytoconstituents of *Tabernaemontana cornari* Jac Q. Willd and Dichotoma roxb, growing in Egypt Univ. XXI(1): 41-49, 41-650.

Lamaison, J. L. and Carisat, A. (1990): Tencur en principaux flavonoides des fleurs et des feuilles de *Crataegus monocyna* Jacq. et de *Crataegus laevigata* (Poirot) D.C. (*Rosaceae*). Pharm. Acta Helu, 65, 315-217.

Liu, G. and Ruedi, P. (1996): Phyllocladanes (13beta-kauranes) from *Plectranthus ambiguus*. Phytochemistry, 41, 1563–1568.

Lukhoba C.W.; Simmonds, M.S.J. and Paton, A.J. (2006): Plectranthus: A review of ethnobotanical uses. J. of ethnopharmacology, 103, 1-24.

Potgieter, C. J.; Edwards, T.J.; Miller, R. M. and Staden, J. V. (1999): Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. Plant Systematics and Evolution. 218: 99- 112.

Rabe, T. and van Staden, J. (1998): Screening of *Plectranthus* species for antibacterial activity. South African Journal of Botany, 64, 62–65.

S.A.S. (1988): Statistical analysis system SAS Users Guide: Statistical SAS Institute Inc. Editors, Cary, N.S.

Steel , G.D. and Torrie, J.H. (1980) : Principles and procedures of statistics,Mc. Grow Hill Boot –Col . New York.

Van Jaarsveld, E. (2006): The southern African *Plectranthus* and the art of turning shade into glade. Fernwood Press, Simon's Town, South Africa.

Viturro, C; Moina, A. and Schmeda-Hirchmann, G (1999): Free radical scavengers from Mutisi friesiana (Asteraceae) and Sanicula gravelens (Apiaceae) Phytother.Res., 13:422-424.

دراسات على اقلمة و بعض الأنشطة البيولوجية لنباتِ بلكترنسس امبجيس في مصر.

محمد محمود احمد الزفزافي * و جوده طلعت داود **و اسلام الجروانى*

الهيئة القومية للرقابة و البحوث الدوائية. *

مركز الدراسات التطبيقية لبحوث النباتات الطبية – ابو الهول – الهرم **

اجريت هذه الدراسة داخل و خارج الصوبة الزراعية و معامل كيمياء النبات والسموم (معامل البيولوجي) بمركز الدراسات التطبيقية لبحوث النباتات الطبية – ابو الهول – الهرم التا بع للهيئة القومية للرقابة والبحوث الدوائية – بهدف دراسة تأثير اختلاف درجات الحرارة على اقلمة و نمو نباتِ بلكترنسس امبجيس المنزرع داخل وخارج الصوبة تحت التحكم في درجات الحرارة وكذلك تاثير فصول السنة على نمو النباتِ المنزرع داخل الصوب العادية.أما بالنسبة لصوبة ذات التحكم في الحرارة اوضحت النتائج إن درجات الحرارة 30،35 أعطت أعلى تأثير في نسبة النباتات الحية وكذلك في جميع القياسات الأخرى عند فرق معنوي 5% أما بالنسبة للنتائج داخل وخارج الصوبة العادية وجد أن فصلى الصيف و الخريف أعطت اعلي نسبة في النباتات الحية و معدل النمو- تم تقدير مضادات الأكسدة و المواد الفينولية وكذالك الفلافونيدات للنباتات النامية خارج وداخل الصوبة العادية و كانت النتائج التالية % 30 µg and 70 µg, % خارج الصوبة و 25 % and 28µg % 55 µg, % داخل الصوبة على التوالى.- كما تم عمل اكتشاف للسمية الجينية للنبات المنزرع خارج الصوبة وذلك بإطعام الفئران وجبات يومية تحتوى على 20% من مسحوق النبات المجفف لمدة أربعة أسابيع وكانت النتيجة عدم وجود أثار سمية على مستوى (الحمض النووي DNA) خلال هذه الفترة