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In-vitro CULTIVATION OF *Drosera capensis* AS AN INSECTIVOROUS PLANTS AND ITS EXTRACT EFFECT ON LARVA OF RED PALM WEEVIL

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ABSTRACT

Carnivorous or insectivorous plants belong to several botanical families, the most important of them is Droseraceae, which includes Drosera plants. Insecticide substances are extracted from Drosera. Tissue culture technique provides the best way to obtain as high and clean quantity as possible of the biomass needed to obtain these substances. This study aimed to propagate the Drosera capensis in-vitro and studying the effect of Drosera capensis residue on larva of red palm weevil. Shoots were visible on leaf explants, apparently forming directly on leaf surfaces without intermediate callus.

The best results of shoot number (13.8 shoots per explant) and length (2.93 cm) were obtained at 0.05 mg L^{-1} BA compared with the control, BA-free media, observed 2.8 shoots per explant and 2.27 cm in length. Shoots were sub-cultured on half strength of MS medium supplemented with four concentrations of IBA (0, 0.5, 1.0, 2.0 mg L-^{1}) in rooting stage. MS basal medium supplemented with 1.0 mg L^{-1} IBA achieved the best root formation where the root number was 47.3 per plant. The residue of Drosera capensis plants at different concentrations (0.0, 50.0, 100. 500.0 mg per liter) had been given to fully developed larvae of red palm weevil (Rhynchophorus ferrugineus Oliv.) through their feeding diet. Larvae were obtained from the field and were maintained on the stems of sugarcane prior to mass rearing, artificial diet, which was formulated from sucrose, molasses, potatoes and agar. The residue of Drosera capensis had toxicological effects on R. ferrugineus larvae. The lethal action of Drosera capensis residue had appeared clearly at 500 mg L^{-1} where the lethal percentage of red palm weevil larva was 65% after ten days.

Key words*:* *Drosera capensis;* Shoot multiplication; Benzyl adenine; Rooting stage; Indole butyric acid *; Rhynchophorus ferrugineus* Oliv.

INTRODUCTION

The use of compounds that are fabricated in a laboratory such as pesticide cause contamination to the environment in any agricultural, medical, or even at homes and companies, so it's necessary to replace these compounds with natural - vegetal compounds for their environmental safety and their importance that have not yet been lost over the years. Gen *et al.* (2010) mentioned that many phytochemicals function as noxious agents that protect plants against insects and other damaging organisms. Barasa *et al.* (2009) said that phytochemicals derived from plant sources can act as larvicide's, insect growth regulators, repellents and ovipositor attractants, as observed by many researchers. Abdullah (2009) mentioned that two natural biopesticides, (*Boxus chinensis* oil and precocene II) were tested against 10 days-old larvae of the red palm weevil, *Rhynchophorus ferrugineus*. He found that the two biopesticidse had toxicological and pathological effects on *R. ferrugineus* larvae. All palms are probably suitable for the development of the Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). It causes serious damage to the date palm. Pinhas *et al*. (2008) said that the red palm weevil (RPW) is a key pest of horticultural and ornamental palm species in Asia. Kaakeh *et al*. (2001) reported that a method for laboratory mass rearing of the red palm weevil (*Rhynchophorus ferrugineus* Oliv.) (RPW) were developed. Weevils, initially obtained from the field ,were maintained on the stems of sugarcane prior to mass rearing, several artificial diets were formulated and preliminary evaluated for development of the *Rhynchophorus ferrugineus*. Materials used for preparations of various diets were: oats, coconut cake, coconut fruit pieces, canned and/fresh pineapple, sucrose, molasses, egg yolk, salt, yeast, vegetable oil, potatoes, soybean flours, date palms leaves and palm fiber sheath, sugarcane fibers, bacto-agar, multi-vitamins, preservatives, and water. Oat and white bean diets were preferred by 1st to 3rd larval instars, while oats + fibers preferred by 4th to 5th larval instars. Larvae fully developed on artificial diets and molted four times during their development failed to construct cocoons because of the unavailability of fibers (palm or sugarcane).

The carnivorous, medicinal plant *Drosera capensis* (Cape Sundew), contains many of important substances that have a huge economic importance because their useful effect in agriculture and drug manufacture. Cape sundew (*Drosera capensis*) contains two major groups of pharmaceutically important substances, naphthoquinones: plumbagin , ramentaceone and flavinoids: myricetin, quercetin, which are considered to be responsible for antibacterial properties of preparations from their tissues (Krolicka *et al*., 2008). Shams El-Din (2002) reported that *Drosera capensis* contains plumbagin and juglone, that are naphthoquinone derivatives, the amount of

plumbagin is (1.04) mg $1gm^{-1}$ dry weight, while the amount of juglone is (31.37) □g $1gm^{-1}$ dry weight. Stary (1991) reported that both *Drosera capensis* and *D. rotundifolia* contain naphthoquinones derivatives. Plumbagin is a naphthoquinone derivative Fig.1 of commercial interest for its insecticide and pharmacological properties (Nahalka *et al*., 1996). Plumbagin can inhibit development of insects and parasitic nematodes (*Ascaris suum* and *Haemonchus contortus*) thereby prevent disease transmission (Fetterer and Fleming, 1991). Kubo *et al.* (1983) reported that plumbagin was reported to be an effective chitin-synthetase inhibitor therefore; it can be utilized for insect killing in agriculture. Satyanarayana and Gujar (1995) found that plumbagin showed the higher toxicity against the cotton stainer (*Dysdercus koenigii*) different age eggs with L C sub (50) ranging from 0.0044 to 0.0066%. Joshi and Sehnal (1989) reported that administration of 3–20 μg

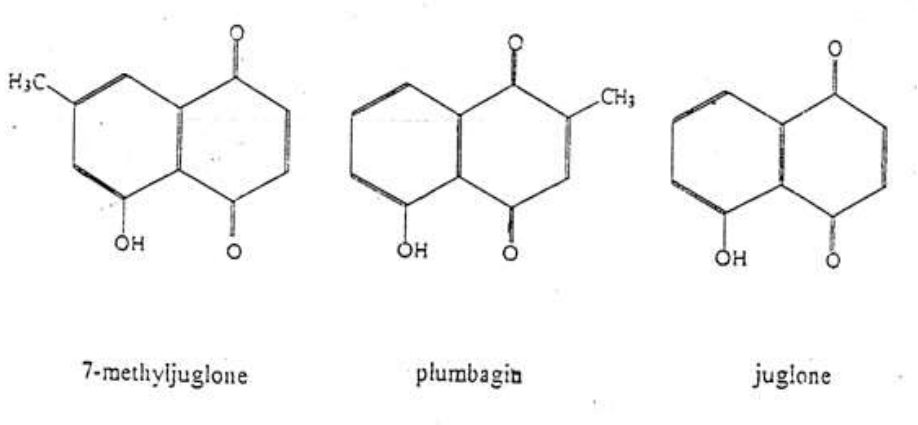


Fig. (1): Chemical structures of some of Naphthoquinone derivatives

plumbagin per last-instar larva of *Dysdercus cingulatus* caused a delay or inhibition of oviduct metamorphosis and of imaginal ecdysis.

The tissue culture technique provides the best way to obtain as big and as clean quantity as possible of the biomass needed to obtain these important compounds. The cytokinins are well known for their effect on stimulating multiplication of *in-vitro* cultured explants. El-Shamy *et al.* (2009) studied the effects of different concentrations of Kinetin (Kin) and benzyl

adenine (BA) on shooting behaviour of *Pyracantha fortumeana*. They found that the medium which supplemented with 3.0 mg L^{-1} BA gave the greatest number of shootlets for four subcultures. Kawiak *et al.* (2003) found that the highest number of plants that regenerated from *Drosera binata* explants was achieved on Vacin and Went medium without growth regulators, but in the case of *Drosera anglica* the highest proliferation rate was obtained on the Fast medium supplemented with 0.05 µ M (0.01 mg L^{-1}) BA and 0.005 µ M (0.0009 mg L^{-1}) NAA, whereas for *Drosera cuneifolia* the optimal regeneration was achieved on the ½ MS medium supplemented with 0.2 µ M (0.045 mg L^{-1}) BA and 0.2µ M (0.037 mg L^{-1}) NAA.

This study aimed to carry out some *in-vitro* experiments on propagation of *Drosera capensis* as an insectivorous plants to obtain enough amounts of plants to ethanolic extraction and its effect on larva of Red Palm Weevil.

MATERIALS AND METHODS

This study was conducted at the Central Laboratory of Development of Date Palm Research, Agricultural Research Center, Giza, Egypt and Genetic Engineering and Biotechnology Research Institute (GEBRI), Minufiya Univ. Sadat city during the period from 2006 to 2010.

Effect of benzyl adenine (BA) concentration on multiplication of *Drosera capensis*:

In-vitro propagation of *Drosera capensis* was conducted according the methods described by Shams El-Din (2002). Seedlings of *Drosera capensis* were obtained from *in-vitro* cultures. Whole leaves about 2cm in length (blade + petiole) were excised and used as explants. Leaf explants were cultured under aseptic conditions on half strength MS medium containing 30 g/l sucrose, solidified by 5 g L^{-1} Bacteriological agar and supplemented with 0.1 g L^{-1} myo-inositol and four concentrations of BA (0, 0.025, 0.05 and 0.1 mg L^{-1}) in a completely randomized block design experiment according to Snedecor & Cochran (1980). Each treatment contained three replicates, each replicate contained ten jars [glass jars of 200 ml capacity and plastic (polypropylene) caps], each jar contained one whole leaf. The medium pH was adjusted to 5.7. An amount of 35 ml of the medium was poured in each jar. Jars containing media were autoclaved under 1.2 kg/cm^3 pressure at 121°C for 20 minutes. After inoculation, jars were put in the incubation room under light intensity of 3000 lux, 16 hours daily photoperiod and 25+2ºC temperature. Shoot number and length were recorded after three and six weeks and the experiment was repeated three times.

Effect of indole butyric acid (IBA) concentration on root formation:

In this experiment, shoots of *Drosera capensis* about 2 cm long were cultured in aseptic conditions on half strength of MS medium supplemented with four concentrations of IBA (0, 0.5, 1.0, 2.0 mg L^{-1}) in a completely randomized experiment designed according to Snedecor and Cochran (1980). Each treatment comprised three replicates. Each replicate contained four tubes (150 x 25 mm), each tube contained 15 ml medium and one shoot. Tubes [glass tubes of 60 ml capacity and plastic (polypropylene) caps] were kept in the incubation room under the same conditions mentioned in the previous experiment for 1.5 month. The experiment was repeated three times and data obtained root number, root length and shoot length were recorded.

Effect of *Drosera capensis* residue on mortality percentage of larva of red palm weevil as an evaluation in bio-resistance:

The fresh sample of whole *Drosera capensis* plants *in-vitro* was freezdried and powdered. 20.0 g dry weight from this powder was extracted with 100ml of ethyl alcohol 95% at room temperature for three days. The extract was filtered and the solvent removed at low temperature (35- 40 °C) and reduced pressure on a rotary evaporator. The residue (1 g almost) was re-dissolved in three ml ethanol 95% and completed to 20 ml by sterilized water. This final solution was subjected to use in rearing media of Red Palm Weevil larva (Kaakeh *et al*.2001), at concentration of 0.0, 50.0, 100.0 and 500.0 mg residue per liter. The artificial diet, used in this study, was prepared in the laboratory and consisted of potatoes (57%), sugar (22%), molasses (11%), brewers yeast (9%), and salt (1%). The ingredients and water (1 - 2 liter of water for a diet weighting 500 - 1000 g) were blended for approximately 5 minutes. The diet also included bacto-agar, multi-vitamins, chemical preservatives (sodium benzoate). Bacto-agar was dissolved in water and added to other ingredients. The mixture of the diet was poured in glass jars (900 ml) with perforated covers then autoclaved for 20 min at 121ºC. Larvae were placed on diets after total coolness. Rearing of *R. ferrugineus* larvae was carried out in incubator at 25 ± 2ºC. In control medium, it has used solution of three ml ethanol completed to 20 ml by sterilized water to assess any possible effect that could be ascribed to ethyl alcohol. Each treatment was represented by three replicates, each replicate contained five larvae for completely randomized design. After four, seven and ten days from the onset of the experiment larval mortality percentage of *R. frrrugineus* was recorded. Data obtained were subjected to the analysis of vari-

ances of randomized complete design as recommended by (Sendecor and Cochran 1980).

RESULTS AND DISCUSSION

Effect of Benzyl Adenine (BA) concentration on multiplication of *Drosera capensis*:

In this experiment the shoot regeneration was obtained without intermediate callus formation. Data presented in Tables (1 and 2) show the effect of MS basal medium supplemented with different concentrations of BA on shoot formation of *Drosera capensis* estimated as shoot number (Table 1) and shoot length (Table 2). This result was confirmed by Bobak *et al*. (1995).

Table 1. Effect of BA on shoot number of *Drosera capensis* in multiplication stage:

Treatments		Shoot Number, after	
No. of Treatment	Concentration (mg L^{-1})	3 Weeks	6 Weeks
1	**0.000**	2	2.8
2	**0.025**	3.3	5.8
3	**0.050**	9.7	13.8
4	**0.100**	7.3	10.7
L.S.D at 0.05		**1.1**	**1.2**

Shoot number of *Drosera capensis* increased gradually and significantly as BA concentration increased from 0.00 mg L^{-1} to 0.025 and 0.05 mg L^{-1} where this character reached its utmost, 9.7 and 13.8 after three and six weeks, respectively. Significantly, raising BA concentration to 0.1 mg L^{-1} affected shoot number inversely, as it declined to 7.3 and 10.7 shoots after three and six weeks, respectively. These results are in agreement with that of Jambor-Benczur *et al.* (1995). They reported that the best result of inducing adventitious buds on *Dionaea* and *Drosera* leaf segments was obtained with the use of 0.05 mg L^{-1} kinetin. Also Jayaram and Prasad (2005) mentioned that the rapid *in-vitro* multiplication of *Drosera indica* and *Drosera burmanni* from leaf explants achieved by using MS basal medium with lower cytokinins levels.

Table 2. Effect of BA on shoot length (cm) of *Drosera capensis* in multiplication stage

Treatments		Shoot Length (cm), after	
No. of Treatment	BA Concentraion (mg L^{-1})	3 Weeks	6 Weeks
1	**0.000**	1.03	2.27
2	**0.025**	1.23	2.53
3	**0.050**	1.47	2.93
4	**0.100**	0.83	1.63
L.S.D at 0.05		**0.14**	**0.18**

Data presented in Table (2) show clearly that BA concentration had a significant effect on shoot length of *Drosera capensis*. The highest significant effect here was that of using BA at 0.05 mg L^{-1}, where shoot length was 1.47 and 2.93 cm after three and six weeks, respectively, followed by the use of BA at 0.025 mg L^{-1}, where shoot length was 1.23 and 2.53 cm after three and six weeks, respectively. When the BA concentration increased to 0.1 mg L^{-1}, shoot length decreased significantly to 0.83 and 1.63 cm after three and six weeks, respectively. In case of the control, half strength of MS medium without BA, the shoot length was 1.03 and 2.27 cm after three and six weeks, respectively. These findings are somewhat in agreement with what was reported by some workers whom approved that cytokinins have a stimulatory effect on shoot length, although the concentration used might differ according to plant species. The longest leaves of *Drosera capensis* were obtained on medium containing 0.05 mg L^{-1} Kinetin (Jambor-Benczur *et al*., 1995.) El-Shamy *et al*. (2009) studied the effect of BA at 0, 1, 2, 3, 4, and 5 mg L^{-1} on multiplication of *Pyracantha fortuneana*, Roem. Shrub. They found that using BA at 3 mg L^{-1} produced the largest number and tallest shootlets.

Effect of IBA on root number:

Data in Table (3) show clearly that IBA concentrations had a significant effect on root number of *Drosera capensis*. Root number of *D. capensis* increased from 4.3 and 9.3 after three and six weeks, respectively to 5.7 and 9.7 after three and six weeks, respectively and further to 31.3 and 47.3 after three and six weeks, respectively as IBA concentration increased from 0.0 to 0.5 and 1.0 mg L^{-1}, respectively. At the highest IBA

Table 3. Effect of IBA on root number of *Drosera capensis* in rooting stage:

Treatments		Root Number, after	
No. of Treatment	IBA Concentration(mg L^{-1})	3 Weeks	6 Weeks
1	**0.0**	4.3	9.3
2	**0.5**	5.7	9.7
3	**1.0**	31.3	47.3
4	**2.0**	16	26
L.S.D at 0.05		**2.1**	**1.5**

concentration (2 mg L^{-1}), root number of *Drosera capensis* decreased significantly to 16 and 26 after three and six weeks, respectively.

All differences between these treatments were significant except between treatments of IBA at 0.5 mg L^{-1} and IBA-free medium. Many workers were reported favoring the addition of IBA at 1.0 mg L^{-1} in the culture media to induce rooting. Examples include Kim-MeeSook *et al.* (1998) who mentioned that shoots of three green ash (*Fraxinus pennsylvanica*) clones were rooted *in-vitro*. The most effective treatment for root number, root length and shoot height was 1.0 mg L^{-1} IBA. Singh *et al*. (2009) said that a complete protocol for micropropagation of *Alternanthera sessilis* using leaf explants was developed. And they mentioned that for rooting, the optimal medium was half strength of MS medium supplemented with 1.0 mg L^{-1} IBA.

Effect of IBA on root length:

Data in Table (4) show that, a significant gradual increase of root length was obtained with increasing IBA concentration up to 1.0 mg L^{-1} after three and six weeks. However, increasing IBA to 2.0 mg L^{-1} had a negative effect. These results were in accordance with the findings of some researchers. Singh *et al*. (2009) reported that the optimal medium for rooting of *Alternanthera sessilis* plants was half strength of MS medium supplemented with 1 mg L^{-1}IBA.

Table 4. Effect of IBA on root length (cm) of *Drosera capensis* in rooting stage:

Treatments		Root Length (cm), after	
No. of Treatment	IBA Concentration (mg L^{-1})	3 Weeks	6 Weeks
1	**0.0**	0.97	1.4
2	**0.5**	1.07	2.1
3	**1.0**	1.27	2.9
4	**2.0**	1.17	2.3
L.S.D at 0.05		**0.01**	**0.3**

Effect of IBA on shoot length:

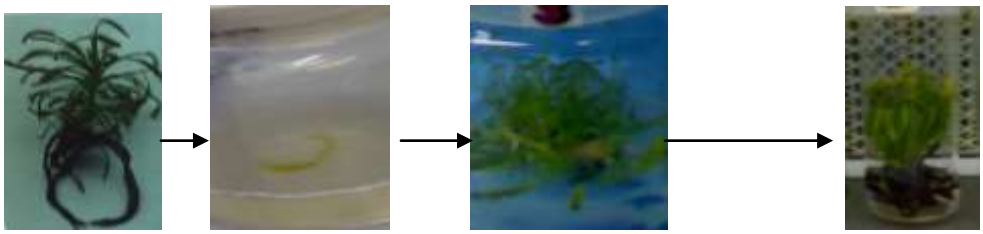
IBA as shown in Table (5) affected shoot length of *Drosera capensis.* In case of using medium without IBA, the shoot length was 2.47 and 3.1 after three and six weeks, respectively. When IBA added to medium at 0.5 mg L^{-1}, the shoot length increased significantly to 3.07 and 4.1 after three and six weeks, respectively.

Table 5. Effect of IBA on shoot length (cm) of *Drosera capensis* in rooting stage:

Treatments		Shoot Length (cm), after	
No. of Treatment	Concentration (mg L^{-1})	3 Weeks	6 Weeks
1	**0.0**	2.47	3.1
2	**0.5**	3.07	4.1
3	**1.0**	4.30	6.1
4	**2.0**	3.93	5.0
L.S.D at 0.05		**0.14**	**0.2**

Also, when IBA concentration increased to 1.0 mg L^{-1} the shoot length increased significantly to (4.30 and 6.1 after three and six weeks, respectively). But When IBA concentration increased to 2.0 mg L^{-1} the shoot length decreased significantly to (3.93 and 5.0 after three and six weeks, respectively). These results are in agreement with the following

reports. Jain and Nessler (1996) said that individual shoots of *Camototheca acuminate* were rooted on B5 medium supplemented with 4.9-19.6 (1- 4 mg L^{-1}) IBA and the lowest concentration (1 mg L^{-1} IBA) gave the highest percentage of rooting. Singh *et al*. (2009) reported that the optimal medium for rooting of *Alternanthera sessilis* plants was half strength MS medium supplemented with 1 mg L^{-1} IBA. Kim-MeeSook *et al.* (1998) said that shoots of *Fraxinus pennsylvanica* were rooted *in-vitro*, and the most effective treatment for shoot length was 1.0 mg L^{-1} IBA.



Mother plant | **Starting stage** | **Shoot formation ½ MS medium + 0.05 mg L^{-1} BA** | **Root formation ½ MS medium + 1.0 mg L^{-1} IBA**

In-vitro* propagation of *Drosera capensis

Effect of *Drosera capensis* residue concentration on lethal percentage of red palm weevil *in-vitro* as an evaluation of their using in bio-resistance:

Data in Table (6) clearly show that *Drosera capensis* residue concentration had a significant effect on lethal percentages of red palm weevil *in-vitro.* Data have been transformed according to Snedecor and Cochran (1980). Lethal effect calculated four days after treatment increased as the concentration used increased from 0.0 to 50, 100 and further to 500 where lethal percentages were 0.0, 18.4, 35.2 and 56.79% respectively.

Duration of the treatment had also a great effect in enhancing the lethal effect of the extract, whereas after seven days the lethal percentages were 0.0, 28.78, 46.9 and 60% respectively. While after ten days the lethal percentages were 0.0, 35.2, 52.8 and 65% respectively. A control medium made up of ethyl alcohol and water without *Drosera capensis* residue was used to detect any possible effect of the ethyl alcohol. These results are in accordance with the findings of some researchers. Kubo *et al*. (1983) reported that plumbagin's insecticidal activity as an ecdysiast inhibitor may make it more ecologically compatible than conventional neurotoxic

Table 6. Effect of *Drosera capensis* residue on lethal percentage of red palm weevil *in-vitro*

Treatments		Lethal percentage, after		
No. of treatment	**Concentration**	**4 days**	**7 days**	**10 days**
1	0.0 mg L^{-1} *Drosera capensis* residue	0.0	0.0	0.0
2	50.0 mg L^{-1} *Drosera capensis* residue	18.4	28.7	35.2
3	100.0 mg L^{-1} *Drosera capensis* residue	35.2	46.9	52.8
4	500.0 mg L^{-1} *Drosera capensis* residue	56.79	60	65
L S D at 0.05		**3.5**	**4.2**	**4.1**

insecticides because of its specificity for chitin bearing animals. Also, plumbagin, and its various derivatives, may be readily available due to the known synthesis. Therefore, plumbagin is a likely candidate for "leading compound" status in synthetic pesticide research. Satyanarayana and Gujar (1995) found that plumbagin showed the higher toxicity against the cotton strainer (*Dysdercus koenigii*) different age eggs with L C sub (50) ranging from 0.0044 to 0.0066%. Joshi and Sehnal (1989) reported that administration of 3-20 □g plumbagin per last-instar larva caused a delay or inhibition of oviduct metamorphosis and of imaginal ecdysis. Fetterer and Fleming (1991) reported that Plumbagin inhibited the motility and survival of *Haemonchus contonus* first-stage larvae (L1) with an ED_{50} of 1 □g ml^{-1}.

Conclusively, Results of this work indicated that, the residue of Drosera capensis had a toxicological effects on Rhynchophorus ferrugineus larvae. The lethal action of *Drosera capensis* residue appeared clearly at 500mg/l where the lethal percentage of larvae of red palm weevil was 65% after ten days.

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إنتاج نباتات دروسيرا كابنسيس (نبات آكل للحشرات) باستخدام تقنيات الزراعة النسيجية ودراسة تأثير المستخلص النباتي على يرقة سوسة النخيل الحمراء

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تنتمى النباتات آكلة الحشرات لعدة عائلات نباتية أهمها العائلة الدروسيرية التى ينتمى إليها نبات الدروسيرا . ويستخرج من نبات الدروسيرا عدة مواد فعالة تفيد فى المقاومة الحيوية ، وتعتبر تقنية زراعة الأنسجة النباتية أفضل وسيلة للحصول على المادة النباتية المطلوبة للحصول على المواد الفعالة التى تفيد فى صناعة الدواء .

ويهدف هذا البحث لدراسة تأثير احد النباتات أكلة الحشرات علي سوسة النخيل الحمراء وذلك بإكثارها في مزارع الأنسجة ثم دراسة تأثير المستخلص النباتي علي اليرقات وقد لوحظ زيادة فى عدد الأفرع لدروسيرا كابينسيس بازدياد تركيز البنزيل أدينين حتى ٠.٠٥ جزء فى المليون حيث وصل عدد الأفرع إلى أقصى عدد، إلا أن زيادة التركيز إلى ٠.١ جزء فى المليون كان له أثر سلبى على عدد الأفرع.

كما وجد أكبر أثر معنوى من إستعمال البنزيل أدينين بتركيز ٠.٠٥ جزء فى المليون على طول الأفرع أيضا.

كان لاستخدام أندول حامض البيوتيرك بتركيز ١ جزء فى المليون تأثير معنوى على تكوين الجذور حيث زاد عدد الجذور إلى ٤٧.٨ جذر وكذلك زاد طول الجذور إلى ٢.٩ سم وكان أيضا لنفس التركيز تأثير معنوى إيجابى على طول الأفرع.

تمت دراسة تأثير المستخلص الإيثانولى لنبات دروسيرا كابنسيس على يرقات حشرة سوسة النخيل الحمراء ، تبين أن لهذا المستخلص تأثير معنوى فى النسبة المئوية لموت اليرقات حيث تزداد بزيادة التركيز حيث إنه عند تركيز ٥٠٠مليجرام متبقى بلغت النسبة المئوية لموت اليرقات ٦٥ % بعد عشرة أيام من المعاملة .