

قَالَ تَعَالَى: أَعُوذُ بِاللَّهِ مِنَ الشَّيْطَانِ الرَّجِيمِ

﴿ إِنَّ اللَّهَ لَا يَسْتَحْيِي ۚ أَنْ يَضْرِبَ مَثَلًا مَّا بَعُوضَةً فَمَا فَوْقَهَا فَأَمَّا
الَّذِينَ ءَامَنُوا فَيَعْلَمُونَ أَنَّهُ الْحَقُّ مِنْ رَبِّهِمْ ۗ وَأَمَّا الَّذِينَ كَفَرُوا
فَيَقُولُونَ مَاذَا أَرَادَ اللَّهُ بِهَذَا مَثَلًا ۖ يُضِلُّ بِهِ كَثِيرًا وَيَهْدِي
بِهِ كَثِيرًا ۗ وَمَا يُضِلُّ بِهِ إِلَّا الْفَاسِقِينَ ﴿٢٦﴾

سورة البقرة

Dedication

To those who inhabit the un favourable habitats struggling under favourable environmental conditions for disease vectors to breed freely and provide them with untapped pools of infections and suffers. To those who conceal their complaints and tears behind their disabilities to reach a nearest public health centre. To those who are victims of mosquito bites ,I dedicate this work for them.

Throughout my life to those persons who have always been there during those difficult and tiring times. I would like to dedicate this thesis and everything I do to my dearest parents , brothers , sisters and daughters. In addition to them I have always been surrounded by strong supportive women. I would not be who I am today without the love and support of my wife Neimat and daughters for their patience and contributions to my life will be felt forever.

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Abstract

The study was conducted to evaluate the larvicidal activity of aqueous and organic solvent extracts of four indigenous plant species in Sudan , root of *Balanites aegyptiaca* (L) Del. (Higleeg) , shoots of *Solenostemma argel* Del Hyne (Argel) , leaves of *Eclipta prostrata* L. (False daisy , Swamp daisy , eclipta) , and seeds of *Azadirachta indica* A . Juss (Neem) , against larvae of three predominant mosquito species , *Anopheles arabiensis* , *Culex quinquefasciatus* , and *Aedes aegypti* , vectors of many important diseases in Sudan.

Larvae of each mosquito species were firstly treated with aqueous extracts of each of the four above mentioned plant species . Then the same numbers of larvae of each mosquito species were put under bioassay testing of methanolic extracts of *Balanites aegyptiaca* , *Solenostemma argel* , and *Eclipta prostrata* , and hexane extract of *Azadirachta indica* seeds. The synthetic organophosphorous larvicide , Abate® (Temephos) , was used as a standard .

Statistical analysis showed significant differences between the tested concentrations of each plant , mostly with activity increases progressively with dosage rates . The root aqueous extract of *Balanites aegyptiaca* , gave the best larval mortality results of the three mosquito species which was comparable with that of the standard larvicide Temephos , and showed LC₅₀ of (0.000357ml/L) for *Anopheles arabiensis*, followed by(0.001335 ml / L) for *Culex quinquefasciatus* , and lastly (0.001542 ml / L) for the *Aedes aegypti*. The highest concentration (500 ppm) of the root aqueous extract caused 100% larval mortality of the three mosquito species after 24 hours , which was comparable with that of the standard larvicide Temephos.

The methanolic extract of *Balanites aegyptiaca* caused LC₅₀ of 0.000028 ml /L for *Anopheles* larvae , 0.000313 ml /L for *Culex* larvae and 0.001227 ml / L for *Aedes* larvae , respectively. Also, the highest concentration (500 ppm) of this extract caused 100% mortality of the larvae of the three mosquito species after 24 hours , which was a comparable result with that of the standard larvicide Temephos.

The Hargal shoot water extract showed also good results against the larvae of the three mosquito species compared with that of the standard larvicide , and showed an LC_{50} of (0.008748ml/L) for *Aedes aegypti* , (0.002706 ml / L) , and (0.002568 ml / L) for *Anopheles arabiensis* , and *Culex quinquefasciatus* larvae , respectively. The highest concentration of this extract (750 ppm) , caused 100% larval mortality of the three mosquito species after 24 hours ,which was comparable result with that of the standard larvicide Temephos.

The methanolic extract of Hargal leaves gave comparable effects on the three mosquito species and showed LC_{50} of (0.001905 ml /L) for *Aedes aegypti*,(0.001463 ml /L) for *Anopheles arabiensis* and (0.001479 ml / L) for *Culex quinquefasciatus* larvae , respectively

Aqueous extracts of *Eclipta prostrata* at the highest concentration (2500 ppm) gave more than 70% larval mortality of the three mosquito species after 24 hours.The probit analysis for the aqueous extract of the plant showed LC_{50} of (0.003862 ml / L) for the *Aedes aegypti* , (0.004151 ml /L) for *Anopheles arabiensis* and (0.004343 ml / L) for *Culex quinquefasciatus* mosquito larvae.

The methanolic extract of *Eclipta prostrata* leaves, caused more than 90% larval mortality of the *Anopheles arabiensis* and *Culex quinquefasciatus* and 78.8% of the *Aedes aegypti* mosquito species, respectively, after 24 hours and showed an LC_{50} of (0.002241 ml /L) for the *Aeds aegypti* , (0.001251 ml / L) for the *Anopheles arabiensis* and (0.001345 ml / L) for the *Culex quinquefasciatus* larvae .

It was also observed that , solvent extract of the plant leaves was more active on the larvae of the three mosquito species than the aqueous leaves extract.

Neem seed aqueous extract at the highest concentration gave mortality percentages, ranging between 80% -98% against the larvae of *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively , which are comparable results with that of the standard larvicide Temephos on the larvae of the three mosquito species .The LC₅₀ of this extract was found to be (0.00 8732 ml /L) for *Aedes aegypti* , (0.003942 ml / L) for *Anopheles arabiensis* and (0.008699 ml / L) for *Culex quinquefasciatus* , respectively.

Neem seed hexane extract gave comparable results on the larvae of the three mosquito species with regard to the increased time of exposure . It was observed that , in the case of exposing mosquito larvae to hexane extracts of Neem seed , the larval mortality percentage increased progressively with longevity of time . Moreover, solvent extracts of Neem seed in general were highly effective than aqueous extract.

The highest concentration of Neem seed hexane extract , gave 87.5% , 99.8% and 98.25% larval mortality of the *Aedes aegypti*, *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively after 24 hours . Application of the probit analysis showed an LC₅₀ of (0.002641 ml / L) , (0.004932 ml / L) , and (0.003323 ml / L), for the *Aedes aegypti* , *Anopheles arabiensis* , and *Culex quinquefasciatus* mosquito species, respectively.

The detected superior insecticidal activities of Higlieeg root water and methanol extracsts after the bioassay on mosquito larvae are due to presence of these bioactive compounds as saponin ,triterpene , flavonoids that were normally found in the different parts of the tree.

Phytochemical test of the shoot aqueous extract of *Solenostemma argel* revealed the presence of various chemical groups at variable levels. Polar and intermediately polar ingredients were shown in wide

ranges . Alkaloids , saponins , flavones and amino acids were the major chemicals detected. Methanol extracts of the plant revealed more or less the same previous compounds , plus some embedded trace amounts of tannins , sterols and triterpenes . The detected groups were different according to the type of the extracts (viz., water , methanol).

Laboratory analysis of the aqueous extract of the leaves of *Eclipta prostrata* , revealed the presence of biologically active secondary metabolites such as saponins , phytosterols , phenols , flavonoids , tannins, terpenoids and carbohydrates. Besides , there were other phytochemicals estimated but were present only in very low concentrations. The methanolic extract yielded tannins , flavonoids coumestans,saponins and alkaloids , etc.,.

In the present study , it can be concluded that , the results of the tested plant extracts against the larvae of the 3 mosquito species showed that , root extracts of *Balanites aegyptiaca* were found to be most toxic thus have the highest larvicidal potency on the larvae of the three mosquito species , even when tested at the lowest concentration (40 ppm) , which credited *Balanites aegyptiaca* root extracts to be superior upon all the other tested plant extracts , moreover , the order of larvicidal potencies among the tested plant extracts was roots of *Balanites aegyptiaca* > shoots of Hargal > neem seed > leaves of Tamr Al-ghanam.

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الخلاصة

اجريت هذه الدراسة لتقييم فعالية المستخلصات المائية والعضوية لاربعة انواع من النباتات في السودان وهي : الهجليج (جذور) ، الحرجل (الاوراق) ، تمر الغنم (الاوراق) والنيم (البذرة) ، ضد يرقات ثلاثة انواع من البعوض السائدة او الاكثر انتشارا ، وهي : الانوفيليس، الكيولكس والايبيدس الناقلة للكثير من الامراض الهامة في السودان .

يرقات كل نوع من البعوض تمت معاملتها اولا بالمستخلصات المائية لكل نوع من النباتات المذكورة بصورة منفصلة ، ثم بعد ذلك استخدم نفس عدد اليرقات لكل نوع من البعوض ووضعت لاختبارات السمية باستخدام المستخلص الميثانولي لكل من الهجليج ، الحرجل وتمر الغنم ، والمستخلص الهكسيني لبذور النيم في تجارب اخري . استخدم مييد اليرقات الابييت (تيميغوس) كمبيد قياسي في التجارب .

اظهر التحليل الاحصائي فروقات معنوية بين التركيزات المختبرة لكل من المستخلصات المائية والعضوية لكل نبات حيث يزداد النشاط غالبا مع زيادة معدل الجرعات .

المستخلصات المائية لجذور الهجليج اعطت أكفا الجرعات النصفية القاتلة ليرقات كل من الانوفيليس (0.000357 مل / ل) ، الكيولكس (0.001335 مل / ل) والايبيدس (0.001542 مل / ل). التركيز الاعلي (500 جزء من المليون) للمستخلص المائي لجذور الهجليج اعطي نسبة موت 100% ليرقات الانواع الثلاثة من البعوض بعد 24 ساعة ، وهي مقارنة لنسبة الموت بواسطة المبيد القياسي التيميغوس . والمستخلص الميثانولي لجذور الهجليج اعطي جرعات نصفية قاتلة ليرقات الانوفيليس (0.00028 مل / ل) ، الكيولكس (0.00313 مل / ل) والايبيدس (0.001227 مل / ل) ، علي التوالي . ايضا التركيز الاعلي للمستخلص الميثانولي (500 جزء من المليون) لجذور الهجليج ، اعطي نسبة موت 100% ليرقات انواع البعوض الثلاثة بعد 24 ساعة ، والتي كانت ايضا مقارنة لنسبة الموت لمبيد اليرقات القياسي التيميغوس .

المستخلص المائي لاوراق الحرجل اعطي افضل النتائج بالمقارنة مع نتائج المبيد القياسي علي يرقات انواع البعوض الثلاثة . واعطي جرعات نصفية قاتلة ليرقات الانواع الثلاثة من البعوض

كالاتي : (0.008748 مل / ل) للايبديس ، (0.00 2706 مل / ل) للانوفيليس و (0.002568 مل / ل) للكولكس ، علي التوالي . التركيز الاعلي للمستخلص المائي لاوراق الحرجل (750 جزء من المليون) اعطي نسبة موت 100% ليرقات الانواع الثلاثة من البعوض بعد 24 ساعة ، وهي مقارنة بنسبة الموت بواسطة المبيد القياسي التيميفوس .

المستخلص الميثانولي لاوراق الحرجل اعطي جرعات نصفية قاتلة لكل من الايبديس (0.001905 مل / ل) ، الانوفيليس (0.00 1463 مل / ل) والكولكس (0.00 1479 مل / ل) .

اظهرت نتائج الدراسة ان مستخلصات الهجليج اكثر كفاءة في مكافحة يرقات انواع البعوض الثلاثة المدروسة وتلية مستخلصات الحرجل في المرتبة مع عدم وجود الفروق المعنوية بينهما ، وياتي النيم بعدهما ثم يليهم نبات تمر الغنم .

المستخلص المائي لاوراق تمر الغنم في التركيز الاعلي له (2500 جزء من المليون) اعطي نسبة موت اكثر من 70% ليرقات البعوض الثلاثة بعد 24 ساعة . واعطي جرعات نصفية قاتلة ليرقات الايبديس (0.003862 مل / ل) ، الانوفيليس (0.00 4151 مل / ل) والكولكس (0.00 4.343 مل / ل) .

المستخلص الميثانولي لاوراق تمر الغنم اعطي نسبة موت اعلي من 90% ليرقات الانوفيليس والكولكس ، 78.8% ليرقات الايبديس علي التوالي بعد 24 ساعة . واعطي جرعات نصفية قاتلة (0.00 2241 مل / ل) ليرقات الايبديس ، (0.001251 مل / ل) ليرقات الانوفيليس و (1345 0.00 مل / ل) ليرقات الكولكس .

التركيز الاعلي للمستخلص المائي لبذور النيم اعطي نسبة موت تراوحت ما بين اكثر من 80% الي اكثر من 98% ليرقات الايبديس ، الانوفيليس والكولكس علي التوالي بعد 24 ساعة وهي مقارنة لنسبة الموت بواسطة المبيد القياسي التيميفوس ، كما اعطي جرعة نصفية قاتلة ليرقات الايبديس (0.008732 مل / ل) ، الانوفيليس (0.003942 مل / ل) والكولكس (0.008699 مل / ل) علي التوالي .

والمستخلص الهكسيني لبذور النيم اعطي تركيزه الاعلي اكثر من 87% نسبة موت ليرقات الايبديس ، 100% تقريبا نسبة موت ليرقات الكولكس والانوفيليس ، بعد 24 ساعة ، وهي مقارنة لنسبة

الموت بواسطة المبيد القياسي التيميفوس ، كما اعطي جرعات نصفية قاتلة ليرقات الابيدس) (0.002641 مل / ل) ، الانوفيليس (0.004932 مل / ل) والكيلوكس (0.003323 مل / ل) ، علي التوالي .

التحليل الكيميائي للمستخلص المائي لبذرة النيم اظهر وجود المواد ذات النشاط الحيوي الاتية : القلويدات ، الصابونين ، الفلافونويدات ، الاسترويدات ، الفينولات وثلاثي التيربينات . في حين ان المستخلص الهكسيني اظهر بالاضافة للمواد السابقة الذكر الفلافونات ، الاحماض الامينية مع قليل من الاسترات والفينولات . كما ان هناك مركبات مختلفة من عديمة التيربينويدات ، وكذلك الهيدروكربونات والمواد العطرية ، الفينولات ، الكومارين ، الايسوكومارين ، الفلافونات ، الاحماض الدهنية واستراتها .

التقصي الكيميائي للمستخلصات المائية والعضوية لكل الاجزاء (الاوراق ، الثمرة ، اللحاء ، الجذور) لنبات الهجليج اظهر مركبات ثانوية عدة ذات نشاط حيوي شملت الصابونين ، القلويدات ، الفلافونات ، الكاربوهيدرات ، كومارين واسترويدات .

اظهر التحليل الكيميائي للمستخلص الميثانولي لاوراق الهجليج مركبات كل من البروتين ، ثلاثي التيربين والاحماض العضوية بالاضافة لما ذكر سابقا من المركبات ذات النشاط الحيوي .

كشفت الدراسات ان النشاط الابادي الحيوي للمستخلصات المائية والكحولية لجذور الهجليج ناتج عن وجود مركبات الصابونين ، ثلاثي التيربين ، الفلافونات والتي وجدت في الاجزاء المختلفة لشجرة الهجليج.

التحليل الكيميائي للمستخلص المائي لاوراق الحرجل اظهر وجود مجموعات كيميائية مختلفة ذات مدي واسع . من تلك المكونات ، مكونات قطبية موجبة الشحنة الكهربائية ومركبات ذات شحنة وسط قطبية ومركبات سالبة الشحنة الكهربائية ، مثل القلويدات ، الفلافونات والاحماض الامينية ، حيث انها تعتبر هي المركبات الكيميائية الرئيسية. المستخلص الميثانولي لاوراق الحرجل ، اظهر وجود نفس المركبات السابقة ذكرها ، زائدا وجود بعض اثار قليلة متداخلة من المواد الدباغية ، الاسترويدات وثلاثي التيربينات . تختلف تلك المركبات في مكوناتها حسب نوع مذيبي الاستخلاص (الماء ، الميثانول).

التحليل المعملّي للمستخلص المائي لاوراق تمر الغنم ، اظهر وجود نشاط بايولوجي حيوي لبعض المركبات الثانوية مثل الصابونين ، الفايستيروسول ، الفينولات ، الفلافونويدات ، المواد الدباغية ، التيربينات والكاربوهيدرات . بالاضافة لذلك توجد هناك بعض المستخلصات الكيميائية لكنها بتركيزات منخفضة جدا. كما ان المستخلص الميثانولي انتج مركبات كالمواد الدباغة ، الفلافونويدات ، الكومستان ، الصابونين والقلويدات .. الح .

يمكن تلخيص نتائج هذه الدراسة في ان المستخلصات المختبرة للنباتات الاربعة ضد يرقات انواع البعوض الثلاثة ، قد اوضحت ان مستخلصات جذور الهجليج قد وجدت هي الاكثر كفاءة في فعاليتها كمبيد يرقي علي الانواع الثلاثة ، حتي عند اختبارها علي اقل تركيز (40 جزء من المليون) . وبذلك تميزت بان كانت هي الاكفأ علي كل المستخلصات النباتية الاخرى . بالاضافة لذلك ، فان ترتيب الكفاءة الابدائية لليرققات فيما بين مستخلصات تلك النباتات كان كالآتي : جذور الهجليج < اغصان واوراق الحرجل < بذرة النيم < اوراق تمر الغنم .

ان نتائج هذه الدراسة تعتبر واعدة للاستمرار في اجراء الدراسات في المستقبل عن طرق استخدام وتطبيق المواد ذات الفعالية النشطة في مكافحة انواع البعوض الناقلة للأمراض ، حيث انها صديقة للبيئة كما تعتبر انها تمثل بدائل جيدة لمبيدات اليرقات الكيميائية (او المصنعة) .

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