ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

SYNTHESIS, CHARACTERIZATION AND INSECTICIDAL ACTIVITY OF ECOFRIENDLY CHITOSAN-BASED COMPOSITES

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ABSTRACT

Evaluation of some medicinal plants extracts which mixed with eco-friendly polymeric materials. Some medicinal plants have been extracted (*Achillea fragrantissima* and *Cleome droserifolia*) and the obtained crude extracts have been blended with suitable eco-friendly polymeric materials, namely, Chitosan and some cellulose derivatives. The blended materials have been characterized and their insecticidal activity has been also tested. In formulations F2 - F13 and F19 – F23 the extracts potency was decreased to less than 50% while showed more than 50% in case of F1 and F14 – F18. The conventional chemical and spectroscopic techniques have been used in the characterization in addition to other necessary techniques needed for their biological activity. The series of concentrations from Chitosan were prepared and tested against *Culex pipiens* and *Musca domestica* larvae mixed with both extracts at LC_{50} level. The obtained results have been collected; tabulated and possible justification has been included as well. While the increasing of Chitosan concentration in polymer mixtures increasing the potency of tested plant extracts. The temporal effect of mixtures number 4 and 5 revealed that the effect of mixtures continues for more than 15 days against *Culex pipiens* while their effect is almost stopped after 6 days in case of *Musca domestica*. From the last experiment we found that mixture No 4 is more persistent than mixture No 5 in fields. The results of the current work reveal that the potency of utilization of many polymers is due to their absorptive activity towards the plant extracts and also for their compatibility toward them. Besides, there is a synergistic effect of the polymer components enhancing the effect of the plant extracts.

Keywords: Chitosan; Ecofriendly; Insectidicidal activity; Composite; *Culex pipiens Musca domestica*

1. INTRODUCTION

Green composites are materials having eco-friendly attributes that are technically and economically feasible while minimizing the generation of pollution. In this project it refers to the combination of fully degradable materials mostly cellulosic materials and natural resins to develop green composite materials. In the past decade, overdependence on petroleum products (synthetic polymers, resins, etc.) has consistently increased and on account of this, the researchers are now focusing more on green materials specially carbohydrate polymer composites.

Cellulosic fibers in micro and nano-scales are attractive to replace man-made fibers as reinforcement to make environmentally friendly green products. Cellulosic nanocomposites are currently considered as one of the most promising areas of scientific and technological development [1 -5].

Carbohydrate Polymers cover the study and exploitation of the industrial applications of carbohydrate polymers in areas such as food, textiles, paper, wood, adhesives, pharmaceuticals, oil field applications and industrial chemistry. Carbohydrate polymer (Chitin, chitosan, hyaluronic acid cellulosic materials and natural resins) composited with extracted compound from natural product are biopolymers having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications especially in biomedical area. Chitin and chitosan are effective materials for biomedical applications because of their biocompatibility, biodegradability and non-toxicity, apart from their antimicrobial activity and low immunogenicity, which clearly points to an immense potential for future development [6]. These candidate biopolymers can be easily processed into gels, sponges, membranes, beads and scaffolds forms.

Some insects transmit serious human and animal diseases, causing millions of deaths every year. Among these diseases are malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis, bacterial diseases. Musca domestica salivary gland hypertrophy virus (MdSGHV) has a worldwide distribution and Rift Valley fever at endemic and epidemic

areas in many countries [7-10]. The extensive uses of chemical pesticides or insecticides are responsible for development of resistance to these insecticides which rebounding vectorial capacity. Plants may be alternative sources of mosquito control agents [11-15].

It is known that the developed countries were working since years in such new fields especially in the basic science, chemistry and engineering. So; it is needed to introduce these new fields in our work and in our university such as postgraduate studies; publications and research projects as well to go parallel with the well developed world.

It is already known also that the high polymers containing functional groups have attracted much attention since the beginning of the polymer chemistry on both academic and commercial levels. Also numerous natural or naturally occurring polymers such as cellulose, starch, Chitin and alginate have been chemically modified either through introduction of new functionalities or through chemical transformation of the already present functional groups. Such chemical modifications were aiming to modify their mechanical and/or physical properties to be suitable for certain applications [16-19].

Among the most attention-attracting applications of the polymers is the application of the modified polymers in the wastewater treatment as well as for the isolation and separation of heavy metal ions from their aqueous solutions. Such polymers with certain functional groups are very important in the environmental applications [1, 3, 5].

2. MATERIALS & METHODS

Chitosan (CS) with M_w= 300000 was obtained from Aldrich, USA. Carboxymethylcellulose sodium salt (CMC) of molecular weight 900000 supplied by ACROS, New Jersey, USA and Soluble starch from Aldrich, USA. Acetic acid and ethanol were obtained from Aldrich, Germany. *Culex pipiens* (*Culicidae: Diptera*), *Musca domestica* (*Muscidae: Diptera*), provided by collecting from Tabuk area and transferred to the research laboratory of Biology Department, Science College, Tabuk University where self-perpetuating colonies were established and maintained during the present study. The experimental work has been conducted in Chemistry Department, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia.

2.1. Preparation polymer solutions

2.1.1. Solutions of CS/CMC/S

One gram of CS was dissolved in 100 mL of 2 % aq. acetic acid solution. Five solutions of different concentrations of CMC in its sodium salt in distilled water were prepared. They are namely 1, 1.5, 2.0, 3.0 and 4.0 %. Also five solutions of different concentrations of soluble starch (S) in distilled water were prepared. They are namely 1, 1.5, 2.0, 3.0 and 4.0 %. Solutions of both CS and CMC were filtered off to remove the trace insoluble fractions before mixing. The different formulations prepared for the work are shown in Table 1.

Table 1: The different formulations of CS/CMC/S polymeric blends

Formula (F)	Components		
	CS	CMC	S
F1	1.0	0.0	0.0
F2	0.0	1.0	0.0
F3	0.0	0.0	1.0
F4	1.0	1.0	1.0
F6	1.0	2.0	2.0
F7	1.0	3.0	3.0
F8	1.0	4.0	4.0
F9	1.0	1.0	0.0
F10	1.0	1.5	0.0
F11	1.0	2.0	0.0
F12	1.0	3.0	0.0
F13	1.0	4.0	0.0
F14	1.0	0.0	1.0
F15	1.0	0.0	1.5
F16	1.0	0.0	2.0
F18	1.0	0.0	4.0
F19	0.0	1.0	1.0
F20	0.0	1.5	1.5
F21	0.0	2.0	2.0
F23	0.0	4.0	4.0

2.1.2. Solutions of M

Ten grams of chitosan (CS) was dissolved in 1000 mL of a 2 vol % aqueous acetic acid solution, and 10 g of soluble starch (S) was dissolved in 1000 mL of distilled water.

Table 2: The different formulations of M

Formula No	Chitosan	Strach	*Achillea*	*Cleome*
1	50 ml	0	+ 0.3 ml	0.5 ml
2	40	10		
3	30	20		
4	20	30		
5	10	40		
6	0	50		

2.2. Colony maintenance of tested insects

2.2.1. Laboratory maintenance of the tested mosquitoes

Mosquitoes were maintained in a walk-insectaries under controlled conditions of temperature (27±2°C), relative humidity, R.H. (70%-80%) and light - dark period (16:8 hrs.) under a fluorescent light. Larvae of the tested mosquito species were reared in white enamel pans (35-40 cm diameter and 10 cm depth) containing about 1.5 L of de-chlorinated tap water. Larvae were provided with tetra-amine (tropical fish food) sprinkled twice daily over the water surface of the breeding pans. The water containing larvae was gently transferred every 2 days into clean enamel pans to avoid formation of scum on the water surface or on the walls and bottoms of pans. The breeding water was gently aerated for about 5 minutes every day by means of a small air pump.

Developed pupa were collected and transferred daily to plastic cups containing saline water then introduced into the breeding screened wooden cages (30x30x30 cm^3). Emerged adults were fed on 10% sugar solution. After three days, adults were fed on blood to lay egg batches where transferred to the white enamel pans containing de-chlorinated tap water for hatching. When mosquito larvae developed to the 2nd instars, they were poured into clean pans and observed daily. Late third larval instars were used for toxicological studies as described previously for *Culex pipiens* [20].

2.2.2. Laboratory maintenance of the tested house flies

Larvae of house fly can be reared in a gallon plastic container with a cloth top. The container was filled with 3-4 inches of shredded paper or wood chips (cedar, redwood, or pine were avoided as they contain insecticidal chemicals). A cup of powdered milk was mixed with 2 cups of water and poured over the wood or paper. The wood/paper should be thoroughly wet while they are about 0.5 inch above the milk level. At 25 °C - 32 °C the larvae are ready to pupate in about five to six days. It is best to keep the container in the dark if the larvae are to be observed, as they will crawl away into the center of the medium because of the light. The culture was checked daily and the larvae are ready to pupate when they are crawling on the sides of the container. To collect the pupae, the container of the larvae was transferred to a shallow pan. The medium containing the larvae was spread so it is within 1 inch of the top of the pan. Wetting the medium thoroughly with no water standing in the pan the larvae will be driven out of the pan. The larvae can be collected by placing the small pan containing the larvae and medium inside a larger pan with paper toweling along the bottom of the large pan. Using two paper towel or toilet paper tubes support the smaller pan above the paper toweling. The larvae will crawl out of the inner pan and pupate under the paper toweling in the dry outer pan. Collect the pupae and place them in a well-ventilated cage to await adult emergence.

Larvae will eat the paper/wood/milk medium throughout their larval development. Adult flies are fed on a 1:1 mixture of granulated sugar and powdered milk. A bowl filled with wood chips and water serves as a source of water [21].

2.3. Tested compounds

The tested plants were washed to remove dusts and dirt then left to dry under shade in the laboratory. Dried plant (whole plant) was cut into small pieces and ground in an electric grinder. Hundred grams of the resulting powdered materials of each plant were exhaustively extracted with absolute ethanol by means of a Soxhlet apparatus. The solvent extracts of each plant were evaporated and dried under vacuum using a rotary evaporator at 60 °C. The dry crude extracts were stored at 4 °C in screw capped vials until use.

2.4. Toxicological studies:

Preliminary toxicological bioassay tests were carried out to the selected plant extracts on tested insects according to a cited method after modification [22]. Evaluation of new compounds for *Musca domestica* was carried out according to the method reported earlier [23].

2.5. Different formula (F*) with the tested plant extracts

The prepared solutions CS/ CMC/ S of different formulations were mixed with the tested plant extract (*Achillea fragrantissima* and *Cleome drosrefolia*) and then the bioassay was carried out on the tested insect.

2.6. Temporal effect of selected formula against *Culex pipiens* and *Musca domestica* larvae:

Series of experiments were carried out to determine the stability of the larvicidal activities of the selected selected polyer mitures with plant extracts at LC_{50} level on temporal bases. In this experiment stock solutions and stock beast from selected materials for mosquitoes and house fly respectively according method as described [12].

2.7. Statistical analysis:

The data were statistically analyzed by Log Propit and Excel programs.

3. RESULTS AND DISCUSSION

3.1. Characterization of the polymer blends

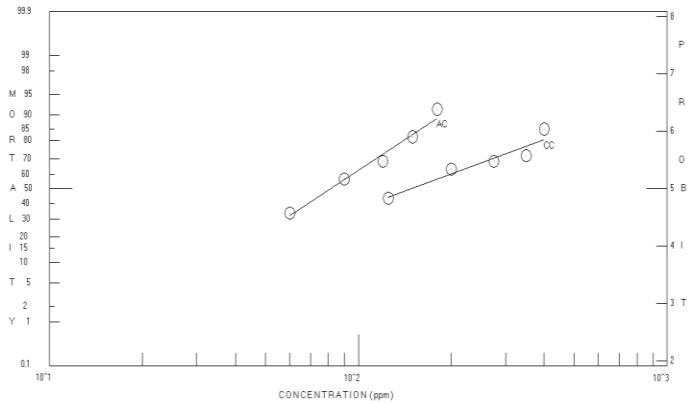
The blended polymers have been characterized with FT-IR spectroscopy and the obtained spectra showed all the characteristic absorptions of the components included. This is expected because there was no chemical modification been achieved. It was simple physical mixing or blending. The important target was investigation the insecticidal activity of the blended Chitosan as carrier mediating for the plant extract on some insects.

3.2. Larvicidal activity of plant extracts against *Culex pipiens* larvae

The insecticidal activity of two ethanolic plant extracts was bio-assayed against the 3rd instars of the *Culex pipiens* larvae in the laboratory. The results are shown in Table 3 and represented in Fig. 1. The confidential limits of each of the tested plant extract were statistically calculated for LC_{50} and LC_{95} at P= 0.05. The LC_{50} values of the ethanolic extracts of *Achillea fragrantissima* and *Cleome droserifolia* are 82.15 and 150.27 ppm, respectively.

Table 3: Larvicidal activity of some plant extracts against *Culex pipiens* larvae

Plant	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope Function
A. fragrantissima	82.15 (72.71-92.82)	237.6 (186.6-302.8)	1.905
C. droserifolia	150.27 (114.2-97.73)	997.2 (539.9- 1846.9)	3.38



AC = A. fragrantissima against C. pipiens. / CC = C. droserifolia against C. pipiens

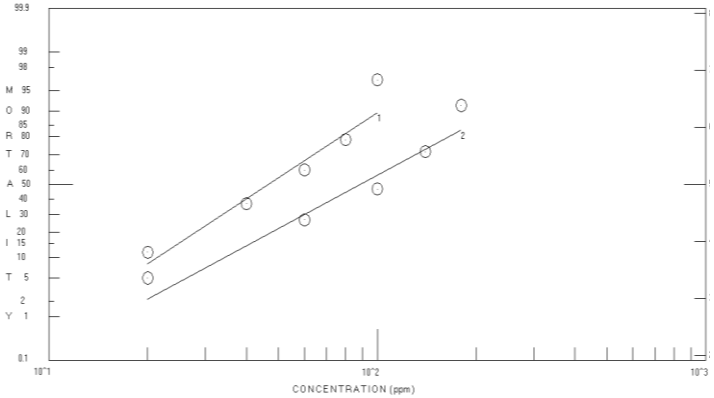
Fig. 1: Susceptibility of *Culex pipiens* larvae to *Achillea fragrantissima* and *Cleome droserifolia* ethanolic extract

3.3. Larvicidal activity of plant extracts against *Musca domestica* larvae

The insecticidal activity of two ethanolic plant extracts was bio-assayed against the 3rd instars of the *Musca domestica* larvae in the laboratory and the results are shown in Table 4 and represented in Fig. 2 and Fig. 3. The confidential limits of each of the tested plant extracts were statistically calculated for LC_{50} and LC_{95} at P= 0.05. The LC_{50} values of the ethanolic extracts *Achillea fragrantissima*and *Cleome droserifolia* are 46.61 and 89.02ppm, respectively.

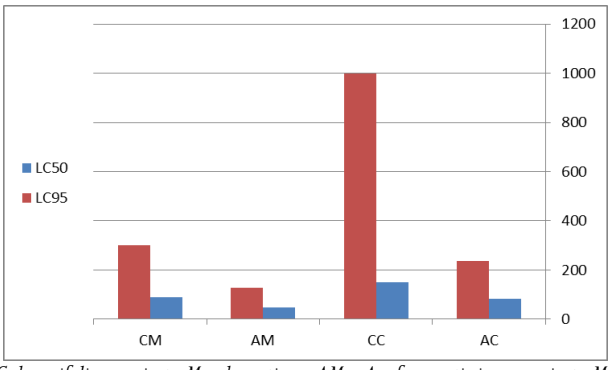
Table 4: Larvicidal activity of some plant extracts against *Musca domestica* larvae

Plant	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope Function
A. fragrantissima	46.61 (42-51.71)	126.54 (103.43-155)	1.905
C. droserifolia	89.02 (78.6-100.8)	300.86 (227.69-398.18)	3.38



[1]*A. fragrantissima against M. domestica.* [2]*C.droserifolia against M. domestica.*

Fig. 2: Susceptibility of *Musca domestica* larvae to *Achillea fragrantissima* and *Cleome droserifolia* ethanolic extracts



CM: C.droserifolia against M. domestica, AM: A. fragrantissima against M. domestica, CC: C. droserifolia against C. pipiens, AC: A. fragrantissima against C. pipiens.

Fig. 3: Larvicidal activity of *Achillea fragrantissima* and *Cleome droserifolia* ethanolic extracts against *Culex pipiens* and *Musca domestica*.

3.4. Evaluation of polymer blends with plant extracts against *Culex pipiens* and *Musca domestica* larvae

Different polymeric formulations containing extracts of *Achillea fragrantissima* and *Cleome droserifolia* at LC_{50} level were used against *C. pipiens* and showed various degrees of potency as represent in Tables 5-7.

Table 5: Larvicidal activity of polymer blends with LC_{50} level of *Achillea fragrantissima* extract on *Culex pipiens*

Formula	% Mortality	Formula	% Mortality
F4	0.0	F14	46.67
F5	46.67	F15	63.33
F6	40	F16	53.33
F7	6.67	F17	51
F8	13.33	F18	50
F9	30	F19	6.67
F10	3.33	F20	10
F11	3.33	F21	26.67
F12	6.67	F22	28.33
F13	0	F23	13.33

Table 6: Larvicidal activity of *Cleome droserifolia* extract at LC_{50} level mixed with different formula of polymers on *Culex pipiens*

Formula	% Mortality	Formula	% Mortality
F4	33.3	F14	46.67
F5	36.67	F15	56.67
F6	33.3	F16	50
F7	33.3	F17	50
F8	26.67	F18	60
F9	16.67	F19	0.0
F10	33.3	F20	26.67
F11	26.67	F21	20
F12	46.67	F22	26.67
F13	33.3	F23	10

Table 7: larvicidal activity of *Achillea fragrantissima* and *Cleome droserifolia* extracts at LC_{50} level mixed with blank solution of polymers on *Culex pipiens*

Formula	% Mortality	
	A. fragrantissima	*C. droserifolia*
F1	70	46.67
F2	0	0
F3	3.33	0

Results showed decrease of *Achillea fragrantissima* and *Cleome droserifolia* extract potency than 50% in formulations F2-F13 and F19-F23 while showed 50% or more in case of F1 and F14-F18 only in both extracts. Hence, the formula of mortality less than 50% was neglected. Clearly, the presence of material M give good result when mixed with the tested plant extracts. The previous mixtures not tested against *Musca domestica* for their pouring application and results. Therefore, the series of concentrations from M were prepared and tested against *Culex pipiens* and *Musca domestica* larvae mixed with both extracts at LC_{50} level showed also different degrees of potency which were represented in Tables (8 & 9). Hence, samples 4 and 5 have been selected to investigate their persisting effect in field after application. While the increasing of Chitosan concentration in polymer mixtures increasing the potency of tested plant extracts.

Table 8: Larvicidal activity of *Achillea fragrantissima* at LC_{50} level mixed with different concentrations of M polymer on *Culex pipiens* and *Musca domestica*

Mix No.	% Mortality *C. pipiens* ± SE	% Mortality *M.domestica* ± SE
1	100 ± 0.0	100 ± 0.0
2	100 ± 0.0	98.7 ± 0.0
3	86.67 ± 0.0	83.3± 0.0
4	70 ± 0.0	54.7 ± 0.0
5	3.33 ± 0.0	3 ± 0.0
6	0 ± 0.0	0 ± 0.0

Table 9: Larvicidal activity of *Cleome droserifolia* at LC_{50} level mixed with different concentrations of M polymer on *Culex pipiens* and *Musca domestica*

Mix No.	% Mortality *C. pipiens* ± SE	% Mortality *M.domestica* ± SE
1	100 ± 0.0	100 ± 0.0
2	100 ± 0.0	100 ± 0.0
3	93.33 ± 0.0	90.7 ± 0.0
4	83.33 ± 0.0	79.7 ± 0.0
5	53.33± 0.0	50 ± 0.0
6	0 ± 0.0	0 ± 0.0

Different formula from *Achillea fragrantissima* and *Cleome droserifolia* were evaluated against mosquitoes and house fly to improve their properties as natural insecticides. Results show different degree of potency. Although the mixtures (F4 – F13) containing chitosan but their potency less than 50% may be due to the presence of (CMC) compound make the mixture very viscous and cannot homogenate well with water. These results may be attributed to preparation of polyelectrolyte complexes. There are many factors affect the complexation reaction such as PH, temperature and ionic strength. Any of the previous factors affect the biocompatibility of the complex [23]. The tested M mixtures show increasing their potency while increasing chitosan concentration. These results are in a good agreement with those of testing chitosan against lepidopterous and homopterous insects [24-26].

3.5. Temporal effect of the selected polymer mixtures mixed with *Achillea fragrantissima* against *Culex pipiens* and *Musca domestica* larvae on larvicidal activities

The purpose of this study was to determine the stability of the larvicidal activities of the selected polymer mixtures with ethanolic extract of *Achillea fragrantissima*at LC50 level on temporal bases. Selection of these mixtures based on overcoming the low potency of some mixtures polymers by how long it persistent in the field. The obtained results revealed differences in the stability at LC50 levels of the selected mixtures against *Culex pipiens* & *Musca domestica*. The results shown in Tables 10 and represented in Figure 4 revealed that the effect of mixtures continues for more than 15 days against *Culex pipiens* while their effect is almost stopped after 6 days in case of *Musca domestica*.

The persistent effect of *Achillea fragrantissima* which mixed with polymer mixture (4 &5) in the field was extent to 15 days (tested against mosquito larvae) may be attributed to slow release of active ingredient due to the presence of starch in mixtures [27]. The potency of extract stopped after 6 days in case of *Musca domestica* may be due to the differences between the application sites for both target insects.

4. CONCLUSION

Finally, it can be concluded that the results of the current work reveal that potency of utilization of many polymers of natural origin can using as carrier mediating media due to many characters including their absorptive activity towards the plant extracts and also for their compatibility toward them. Besides, there is a synergistic effect of the polymer components enhancing the effect of the plant extracts and also help to prolong their activity due to their controlled release characteristics.

5. ACKNOWLEDGEMENT

The authors would like to acknowledge financial support for this work, from the Deanship of Scientific Research (DSR), University of Tabuk, Tabuk, Saudi Arabia, under grant no. S-1434-0192. This article does not contain any studies with animals performed by any of the authors.

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