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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

The acaricidal effect of clove leaf plant extract *Eugenia caryophyllus* against *Argas* spp. soft ticks in Iraq.

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Manuscript Info	Abstract
Manuscript History:	Eugenia caryophyllus Clove leaf different oil extracts concentrations 1%,
Received: 22 July 2015 Final Accepted: 15 August 2015 Published Online: September 2015	5%,10% and 15% were evaluated In vitro for acaricidal activity against adults of <i>Argas</i> spp. soft ticks collected from local poultry flocks in Diyala province, Iraq, during 2014. Adult immersion tick test was used. <i>Eugenia</i> <i>caryophyllus</i> 15% was efficient, killed 80% in 24 hours than other
Key words:	concentrations but without significant difference $p \ge 0.449$. Eugenia caryophyllus Lc ₅₀ = 3.15% and Lt ₅₀ =47.96 hours were obtained
Clove, extracts, acaricidal, Argas spp.	showing probably highly plant non synthetic safety acaricide with low Lc $_{50}$ and Lt $_{50}$ could be used to control poultry soft tick .
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INTRODUCTION

The environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and public in recent years. It has been estimated that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches \$100 billion annually. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment ^[1]. Clovely Scientific classification : Survivium gramaticum (L.) Marrill & Parry with supervised of the classification of the supervised of the super

Clove's Scientific classification : *Syzygium aromaticum* (L.) Merrill & Perry; *with* synonyms of : *Caryophyllus aromaticus* L. ;*Eugenia aromatica* (L.) (Baill.) ; *Eugenia caryophyllata* (Thunb.) ; *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison . <u>Eugenol</u> comprises 72-90% of the <u>essential oil</u> extracted from cloves, and is the compound most responsible for the cloves'aroma ^[2]. The major component of clove oil is usually considered to be eugenol, with β -caryophyllene and eugenyl acetate, being present although in lower concentrations ^[3].

Eugenia caryophyllata (Clove) (EC) has been used in traditional public medicine to relieve nasal obstruction and musculoskeletal pain which imply anti-inflammatory activity for the plant .The plant has a strong phenolic smell and sharp acrid taste. It contains tanene 13% fixed oil and essential oil at 15-20%, in which the main constituents are eugenol (80-90%), β -caryophyllene (9%), eugenyl acetate (7%). Analgesic, anesthetic, spasmolytic and antibacterial effects of EC were demonstrated by several scientific studies^[4]; (EC) was found to be effective against egg and adult of *Pediculus capitis*^[5]. It has antiseptic as well as bacteriostatic and bactericidal activity against several bacteria including *Escherichia coli, Staphylococcus aureus*^[6]; ^[7]. (EC) used as nematicidal, and acaricidal ^[8].

Argas spp. primarily an Old World ectoparasite, although it does exist in the New World, along with similar species, all parasites of domestic fowl and other birds. Under favorable conditions, these ticks may build up a huge population in a henhouse, and their nocturnal depredations can exhaust a flock or even kill individuals Their bites to humans are painful, often with toxic after effects, but such attacks on humans are rare^[9].

The aim of this work is to evaluate the in vitro acaricidal effects of, *Eugenia caryophyllata* (Clove) extract oil against *Argas* spp. adults based on adult immersion test (AIT) in order to determine the Lc_{50} and Lt_{50} values of this chemical plant.

materials and methods :

Study area :

The climate in Baqubah is called a desert climate. Throughout the year, there is virtually no rainfall in Baqubah. The Köppen-Geiger climate classification is BWh. The average annual temperature in Baqubah is 22.8 °C. The average annual rainfall is 186 mm; Coordinates: $33^{\circ}45'N 44^{\circ}38'E / 33.750^{\circ}N 44.633^{\circ}E^{-(13)}$. Fig.(1).

Soft ticks :

Adults of *Argas* spp. soft ticks collected during march and april 2014 from under small stones around poultry flocks premises houses, roost, and nest construction in Diyala province , Iraq ;Samples were sent to the parasitology lab. for , Classified and reard in an insectary, maintained in big glass containers at $27\pm2^{\circ}$ C and a relative humidity of $77\pm3\%$

In vitro bioassays :

In vitro acaricidal activity of adult immersion test was carried out according with modification to ^[10]; and ^[11]; Ticks were divided randomly in petridishs (10 adults/ dish / replicate), Subjected to exposure for 2 minutes in Different extract concentrations 1%, 5%, 10% and 15% OF *Eugenia caryophyllus* Clove leaf ;removed and examined after 24 hours and for successive 5 days period to calculate dead ticks for evaluation the acaricidal activity ; The negative control group was treated with distilled water , 5replica were used for each concentration ; according to Hanem etal 2013 with modification .

preparation of the phytoextraction:

method of ^[12] and ^[13] were used for preparation of the Ethyl phytoextraction of Eugenia caryophyllus Clove leaf powder buds by weighing 100gm from it placed in glass baker with addition of 500ml of absolute Ethyl alcohol with blending on magnetic stirrer for 3 hours then left to stagnate for 20 minutes then re -blends for another 1 hour then left to stagnate; after that mixture were filtered by filter paper (Whattman No.1); The solvents from the extracts were removed using a rotary vacuum evaporator to collect the crude extract which weighted finally; From this stock solution, different concentrations 1%, 5%, 10% and 15% were prepared, preserved in sterile bottles and these solutions were used for acaricidal bioassays.

Statistical analysis:

Groups were compared using one-way anova analysis ;regression test for Lc_{50} (Lethal concentration) and Lt_{50} (Lethal time), using SPSS software (IBM SPSS statistics 20); A value of p < 0.05 was considered as statistically significant.



Results :

Fig (1): Baquba's Metrological data



Fig (2): shows different clove leaf buds plant concentrations.

Results revealed that *Eugenia caryophyllus* 15% was efficient , killed 80% in 24 hours of adult immersed tick than other concentrations but without significant difference $p \ge 0.449$. Fig.(2); table (1);Fig.(3).



Fig.(3) shows killed soft ticks post immersion test.

Table (1): shows no. of dead ticks according vs. clove concent.

Control	concentration				Day
	%15	%10	%5	%1	
0	8	2	0	0	1
0	1	0	0	0	2
0	0	4	0	0	3
0	0	0	0	0	4
0	0	0	0	0	5



Fig.(4): shows Regression Curve Fitting of Lc $_{50}$ clove concentrations Vs. no. of killed ticks. Lc $_{50}$ 3.15% and Lt $_{50}$ =47.96 hours were obtained *for Eugenia caryophyllus*, showing highly plant non synthetic safety acaricide with low Lc $_{50}$ and Lt $_{50}$ could be used to control poultry soft tick .table(2);Fig.(4,5).

Table (2) :show no. of ticks mortality in time vs. clove.

Concnt	hours			
15%	10%	5%	1%	
dead Ticks	dead Ticks	dead Ticks	dead Ticks	
8	2	0	0	24
1	0	0	0	48
0	4	0	0	72
0	0	0	0	96
0	0	0	0	120



Fig.(5) : shows Regression Curve Fitting of Lt_{50} in hours Vs. no. of killed ticks.

Discussion :

Argas spp. Soft ticks is of great medical and veterinary importance because of its role as vector of several pathogens. Control of *Argas* spp. was mainly done using available synthetic chemical acaricides e.g. kerosene, kerosene emulsion, benzene, nicotine sulfate , gasoline; carbolated lime; lime whitewash ,sulfur, carbolineum, crude petroleum, creosote oil, lindane, chlordane, toxaphene , dieldrin, aldrin , malathion diazinon , carbaryl and others . Adult Immersion Test (AIT) is a bioassay, which can be used to determine the relative effectiveness of acaricides in

addition to diagnosis of resistance [10].

By using Adult immersion tick test for adulticidal assay in this study, it was found that clove leaf oil 15% concentration was highly effective as compared to other concentrations 1%,5% and 10%. It was also found that as the dose of essential oils were increased the % mortality was also increased. Results in this study also revealed that Lc $_{50}$ 3.15% and Lt $_{50}$ =47.96 hours were obtained *for Eugenia caryophyllus*, showing highly plant non synthetic safety acaricide with low Lc $_{50}$ and Lt $_{50}$ could be used to control poultry soft tick.

In fact, it is difficult to make exact comparisons for our study results with other studies , may be according to ^[14] due to large variation in oil composition, target insect, mode/scale of experimentation, different exposure times and concentrations employed. So it reflected by different affectivity of same oil in various assays. Clove oil toxicity to different types of insects varies considerably. Other studies indicate that clove oil and eugenol can be effective at controlling mites, termites and mosquitoes at lower application rates

The mechanism of action of essential oils to insects is not clear but is reported to be diverse ; Plant extracts can be used to control certain species of ticks such as *Hyalomma anatolicum excavatum*^[16], *Amblyomma americanum*, *Dermacentor variabilis*^[17] and even *Rhipicephalus sanguineus*^[18], ^[19], ^[20], ^[21]. Among the advantages of phytotherapics that currently justify their use are the synergistic effects of its compounds, the combination of mechanisms for substances acting on different molecular targets, the lowest risk of side effects and lower costs in research^[22].

The authors awake that No previous reports testing natural clover leaf oil extract against adults of *Argas* spp. Using Adult Immersion Test (AIT) were found in the literature, precluding comparison with the results presented in this study, making it the pioneer.

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