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Acaricidal Activity of *Azadaritcha indica* L. (Meliaceae) Essential Oil against the *Dermanyssus gallinae* of Poultry: *In Vitro*

I. R. M. Al-Shaibani[1*], M. M. Al-Sharhi[1], A. M. A. Al-Khadher[1], S. M. Awam[1], S. M. Salah[1] and S. F. Thwabah[1]

[1]Faculty of Agriculture and Veterinary Medicine, Thamar University, Dhamar, Yemen.

Authors' contributions

This work was carried out in collaboration among all authors. Author IRMAS designed the study, wrote the protocol, performed the laboratory analysis and wrote the first and final draft of the manuscript. Author MMAS performed the statistical analyses. Author AMAAK contributed in writing of the protocol, the final version of the manuscript and managed the literature searches. Authors SMA, SMS and SFT collected the samples, performed the laboratory analysis and field works. All authors read and approved the final manuscript.

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ABSTRACT

Dermanyssus gallinae, red mite, has been considered one of the most serious problems to poultry industry worldwide. The acaricidal activity of the *Azadaritcha indica* essential oil against the red mites of poultry was investigated. The effect of *A. indica* essential oils was assessed by contact and fumigation bioassays in laboratory. Freshly collected adults red mites from poultry houses were exposed to the *A. indica* essential oils at concentrations of 0.5%, 1% and 2% in Petri dishes/ vials in laboratory at 27 ^{O}C and 60-70% relative humidity over a period of 24 hrs. Post treatment, the red mites were collected and counted to determine the mortality rates for each concentration in different interval periods. The result of contact and fumigation bioassays revealed that, the *A. indica* essential oil possesses significant ($P<0.05$) acaricidal activity at different concentrations against *D.gallinae* under laboratory conditions and cause significant ($P<0.05$) mortality rate of red mites compared to

**Corresponding author: E-mail: dr_ibra67@yahoo.com;*

control group. The mortality rate of red mites researched the peak (100%) with concentration of 2% at 24 hrs post treatment. In conclusion, the study demonstrated that *A. indica* essential oil possesses acaricidal activity and could be used as potential alternative for controlling of *D. gallinae* in poultry farms.

Keywords: Acaricidal; Azadaritcha indica; Dermanyssus gallinae; In vitro; poultry.

1. INTRODUCTION

Infestation of laying hen farms with *Dermanyssus gallinae* (red mites) causes major economic losses for the egg producing industry worldwide [1,2]. The poultry red mite is an important blood sucking ectoparasites of laying hens [3]. In commercial egg production farms, red mite attacks laying hen at rest time mainly at night for a short blood meal [4,5]. After feeding, the mites leave the hen and hide in cracks and crevices, where they also mate and lay their eggs. Under favorable warm and moist conditions, the life cycle can be completed within one week [6,7].

D. gallinae is a serious problem, not only as a potential vector of several pathogens as the fowl spirochaetosis, chicken pox virosis, Newcastle disease, pullorum disease, fowl typhoid, and fowl cholera [8], but more important as a direct parasite which affects hen negatively, growth rate, egg quality, eggs production and welfare [6,9]. Hens infected with red mites may induce a number of symptoms, depending on the severity of infestation, including irritation, restlessness, anemia and occasionally death [9].

Infestations of poultry with *D. gallinae* were reported worldwide [10-15] with different prevalence rates. In general, the prevalence of poultry red mites on laying flocks varies from 20 to 100 %, depending on the country and poultry management used [16,15].

Eradication of *D. gallinae* from poultry farms present a serious challenge for poultry industry, because of their biological characteristics, such as short life cycle, development, growth, high resistance to starvation, and suspected resistance to acaricides [16,17]. Despite the damages and economic losses they cause, still no efficient control methods capable of eradicating this pest [18].

Physical cleaning of poultry houses and conventional control methods mostly relies on the use of synthetic chemical products such as organophosphorous compounds, carbamates and pyrethroids are frequently used as caricides for controlling mites. The use of these products, however, has become increasingly hampered by more strict legislation [19], emerging drug resistance due to frequent incorrect usage [20,21], human health hazards due to chemical residues in food products and environmental contamination [22]. Therefore, the present situation has prompted researchers to develop new effective, safe and environmentally acaricidal agents for controlling red mites [23,24].

Plant-derived have been in nature for many years without any adversative effects on the ecosystem and showed significant interfering with physiological and growth process of Arthropoda. As a consequence, botanical acaricides have become research interest of workers worldwide [25,26].

Azadirachta indica tree (neem) is indigenous to India and other countries including Yemen; it belongs to the family maliceae. All the parts of the *A. indica* tree is used in medicine [27-29].The purified product of every part of this plant, particularly the leaves, bark, seeds are widely used for treatment of parasitic, bacteria and fungi infections [30]. Over 60 different types of biochemical products including, Nimbolide, Margolone, Mahoodin, Margolonone, Azadirachtin have been purified from *A. indica* [30-32].

Many researchers have investigated and assessed the effectiveness of *A. indica* and other plant extracts against red mites [20,23,33-37] in different countries of the world and little data exists regarding to effectiveness of *A. indica* essential oil on red mites of poultry at Dhamar, Yemen. Therefore, the aim of the present study was to assess the acaricidal activity of the *A. indica* essential oil of local origin tree against *D. gallinae* of poultry under laboratory conditions in Dhamar governorate, Yemen.

2. MATERIALS AND METHODS

2.1 Study Setting

The study was conducted in the year 2020 in laboratory department of Veterinary Parasitology, Faculty of Agriculture &Veterinary Medicine,

Thamar University and the poultry farm in Dhamar governorate.

2.2 Collection of Mites

The populations of red mites were collected from laying hen (Ross 308) farms in suburban area of Dhamar. Those farms include thousands of birds as large flocks in single room with good management. Mites were collected by brushing from typical infested areas into plastic containers (200 ml) with screw top bearers from the infested areas in poultry farm or by cardboard trap (150×100 mm) as described by Bahadori et al. [38]. The samples were placed in sealed bags and labeled with necessary information and brought to the laboratory. In laboratory, all trapped mites were weighed using electronic microgram balance. Then one gram of the mite weight was counted and the total number calculated according to the weight following the guidance of Bahadori et al. [38]. Only recently fed adult mites of both sexes to ensure consistency of results were selected for investigation and analysis. Mites were held at 26 ± 1 ^{O}C and 55 ± 5% relative humidity under a photoperiod of 16:8 hrs (light/dark) prior to bioassays. Mites were tested within 1 day after collection. No chemical acaricidal treatment had been used in the sampling farms for at least two months prior to mite's collection.

2.3 Plant Materials and Essential Oil Extraction

The *A. indica* seed materials were obtained from the Moor valley area in Alhodieda governorate, Yemen, in fresh condition. Identification of plant materials were carried out by the botanical specialist in the Faculty of Agriculture & Veterinary Medicine, Thamar University, Dhamar, Yemen. The *A. indica* essential oil was extracted with the technique mimic the tradition methods and following the guidance of Aziz et al. [39] and Okese [40]. In brief, the mature *A. indica* seeds were cleaned, skin removed and dried under air conditioning, 500 g de-coated seeds were weight with electronic balance (SF-400, China) and crushed in a ceramic mortar and pestle (J maker, India), and then ground by electric blender machine (Sonya, Osaka, Japan). The powder was streamed by placing it over boiling water for 15-20 minutes. This turned the materials into dough from which the oil was extracted. The dough was enclosed in fine cloth and pressed to flow out/extract the oil to clean container. The obtained oil was filtered and refined with centrifuge machine (DMC Company, Model LC-045, China). The oil was weighed to calculate the concentration required and kept at refrigerator (FR-330, Daewoo electronics, Korea) until used.

2.4 Laboratory Bioassay Methods

In laboratory, contact and fumigation bioassays were used to assess the acaricidal activity of *A. indica* essential oil on *D. gallinae* of poultry.

2.4.1 Contact assay

In this assay, the guidance given by Locher et al. [23], Paramasivam and Selvi [41] and Chen et al. [42] were followed, in brief, 300 µl(0.3 ml) of undiluted *Azadirachta indica* essential oils were used to impregnate filter papers (Whatman No. 2, 4.25cm diameter) at concentrations of 0.5%, 1% and 2%,whereas; filter papers received distilled water only used as controlled group. The treated filter papers were left to dry at room temperature conditions. The treated and control papers were placed in Petri dishes (4.8cm ×1.5cm diameter, Aptaca spa, Italy). Approximately, 20 adult of mites were added to each dish, and then sealed with a layer of parafilm in order to prevent any mites from escaping. Mortality rate was assessed under dissecting microscope with magnification 40× (FX 4, China Made) after 2hrs, 4hrs, 6hrs, 12hrs and 24hrs. The mites were considered as dead if they exhibited no movement after repeated agitation with an entomological pin. Three replicates for each concentration were applied. The experiments described were conducted in a climate-controlled at 27^{O} Celsius and 60-70% relative humidity.

2.4.2 Fumigation assay

Fumigation assay was conducted to investigate the vapor phase toxicity according the techniques described by Kim et al. [20] and Locher et al. [23], with some modifications. In this assay, batch of 20 mites were placed into each Eppendorf cup (2 ml, Citotest lab ware Co., China). The lid of the cup had been cut off to seal the opening with a special foil allowing the entrance of vapors. Each filter paper was treated with 300 µl (0.3 ml) of undiluted *A. indica* essential oils with one of 0.5%, 1% and 2% concentrations; whereas control treated with distilled water. The screened tube containing the adult mites, treated and control filters were placed into the containers. This prevented direct contact of adult mites with the tested oil. Each

container was then covered with lid to investigate the potential vapor phase toxicity of the tested oils. Adults' mortality rate of mites was observed at 2hrs, 4hrs, 6hrs, 12hrs and 24hrs post treatment. The number of dead mites was determined under a dissecting microscope with 40× magnification. Mites were considered dead if showed no movement. Three replicates for each concentration were applied. The experiments described were conducted in a climate-controlled at 30 ± 2 °C and 60 ± 5 % relative humidity.

2.5 Statistical Analysis

Statistical package SAS institute Inc. Cary, NC was used to carry out all statistical analysis. Duncan's multiple range tests was applied for the separation of means. Probit analysis was used to calculate the LD_{50} values. The differences among group means were considered significant at $P < 0.05$.

3. RESULTS

3.1 Extraction of *Azadirachta indica* Essential Oil

The yield extraction of *A. indica* essential oil in milligram is presented in Table 1.

3.2 *In vitro* Bioassays

The results for assessment acaricidal activity of *Azadirachta indica* essential oil against *Dermanyssus gallinae in vitro* by contact and fumigation bioassays are presented in Table 2 &3. As shown, *A. indica* essential oil exhibited high efficacious acaricidal activity against red mites at different concentrations. Higher mortality of mites ($P < 0.05$) was observed in treated groups compared to the control. The *A. indica* essential oil showed clear a dose-dependent for residual toxicity; as *A. indica* essential oil dose concentration increased, the mortality of red mites increased. There were correlation between time exposure and *D. gallinae* mortality ($P<0.05$). In contact assay, the lower morality mean value (16±2.89) of red mites was recorded at the concentration of 0.5% at 2hrs post treatment; whereas, the higher mean value (100.0±0.0) at the concentration of 2% at 12hrs post treatment. Similarly, in fumigant assay, the lower mortality mean value (23.3±2.89) of red mites was recorded at the concentration of 0.5% at 4hrs post treatment; whereas, the higher mean value (100.0±0.0) at the concentration of 2% at 24hrs post treatment.

Table 1. Yield of essential oils from *Azadirachta indica* seeds extraction

Plant species	Fresh weight/Raw materials(g)	Volume of oil obtained	% yield
Azadirachta indica	1kg	15 ml	1.5%

Table 2. Mean ± SD mortality rate of *Dermanyssus gallinae* exposed to different concentrations of *Azadirachta indica* essential oil according to the contact bioassay

A. indica essential oil concentrations	2hrs	4hrs	6hrs	12hrs	24hrs
0.5%	$16±2.89^{e}$	$43.3±2.89^{e}$	$65.0±0.0^{e}$	75.0^{e}	$78.0±0.0^{e}$
1%	$25.0±0.0^{d}$	$53.3. ±2.89^{d}$	$75.0±0.0^{d}$	$90.0±0.0^{d}$	$95.0±0.0^{d}$
2%	$38.3±2.89^{c}$	$73.3±2.89^{c}$	$98.3±2.89^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$
Control	0.0^{a}	0.00^{h}	0.00^{h}	0.00^{f}	0.00^{f}

Means with different superscripts in column are differ significantly (P<0.05)

Table 3. Mean ± SD mortality rate of *Dermanyssus gallinae* exposed to different concentrations of *Azadirachta indica* essential oil according to the fumigation bioassay

A. indica essential oil concentrations	2hrs	4hrs	6hrs	12hrs	24hrs
0.5%	$0.0±0.0^{g}$	$23.3±2.89^{e}$	$40.0±0.0^{g}$	75.0^{e}	$77.0±0.0^{e}$
1%	$11.6±0.0^{f}$	$20.0 ±0.0^{g}$	$71.7±2.89^{e}$	$76.6±2.89^{e}$	$90.0±0.0^{e}$
2%	$75.0±5.0^{b}$	$80.0±0.0^{b}$	$85.0±0.0^{c}$	$93.3±2.89^{c}$	$100.0±0.0^{a}$
Control	0.0^{a}	0.00^{h}	0.00^{h}	0.00^{f}	0.00^{b}

Means with different superscripts in column are differ significantly (P<0.05)

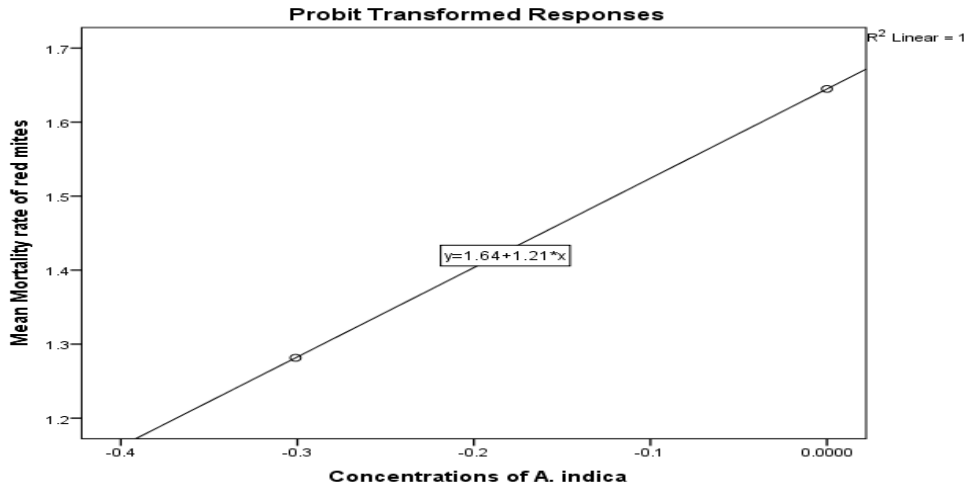

Fig. 1. Linear relationship between red mite's mortality and *A. indica* essential oil concentration (%) according to contact bioassay

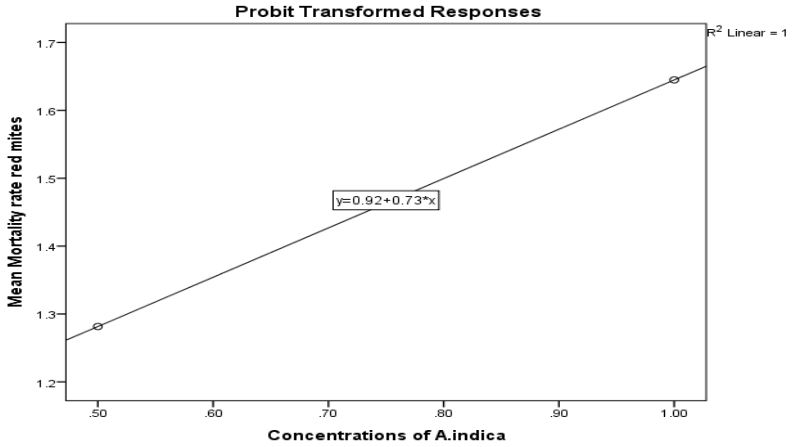

Fig. 2. Linear relationship between red mites' mortality and *A. indica* essential oil concentration (%) according to fumigation bioassay

Regarding to $LD_{50,}$ in contact bioassay the *A. indica* essential oil showed LD^{50} value as 0.126 %; whereas, The LD_{50} value in Fumigant bioassay was 0.353 % at 24hrs post treatment. as depicted in Fig. 1 & 2.

4. DISCUSSION

Until now, the control of the poultry *D. gallinae* has been largely dependent upon the use of sprayed acaricides compounds, such as, carbamates, macrocyclic lactones, pyrethroids, organophosphates and spinosyns [43,44]. The continuous usage of these compounds has increased the resistance of mites to these compounds [21,35]. Furthermore, the lack of new acaricides and increasingly stringent requirements for chemicals used on food animals greatly limit the options for controlling this pest [9,45,46]. Many efforts have been done on the development of natural acaricides of plant-derived origin with main aim to decrease the negative impact of synthetic acaricides, such as chemical residues in food and undesirable effect in environment. One of the alternative methods for the controlling pests is the use of plant essential oils. Plant essential oil have been recognized for their properties such antibacterial, antifungal, antiviral, insecticidal, anticaricidal and antioxidant, Plant extracts are widely used in

medicine and the food industry since centuries [46,47]. In addition, previously studies [48-51] reported that, plant-derived bioactive ingredients are effective for controlling of Arthropoda parasites including *D. gallinae* and less harmful to the ecosystem.

Presently, considerable number of plant extracts like thyme, neem, and garlic and eucalyptus essential oils have been assessed *in vitro* [35,50,52] for their acaricidal activities against *D. gallinae* and reported that essential oil of screened plants showed variety degree of efficacy against *D. gallinae* of poultry under laboratory conditions. Furthermore, Lundh et al. [33] and Du et al. [53] reported that *A. indica* possess biocidal activity against nearly 200 medical and veterinary arthropods including *D. gallinae*, without any adverse effects toward most non-target organisms in nature.

In this study, contact and fumigation bioassays were used to assess the effectiveness of *A. indica* on *D. gallinae in vitro*. The results showed that the *Azadirachta indica* essential oil is efficacious against the poultry red mites at different doses and concentrations which were comparable to the commonly used acaricides such as carbamates, organophosphates, organochlorines, pyrethroids, carbamates, amitraz and natural products such as fluralaner, *Conocarpus erectus*, *Portulaca oleracea*, *Pistacia atlantica, Ocimum basilicum*, *Coriandrum sativum*, *Menthax piperita*, *Satureja hortensis,* Metarhizium anisopliae, Saccharopolyspora spinosa, *Thymus vulgaris*, *Pelargonium graveolens* and *Ferula assafoetida* [13,24,36,37,54-56]. Furthermore, Kim et al. [20] screened acaricial activity of 56 plant essential oils against poultry house-collected adult *Dermanyssus gallinae* in Korea and reached to similar findings. However, the efficacy rates of those plants were varied against the red mites. This variation may be attributed to many factors such as geographic origin, seasonality, method of oil extraction, year of harvest and even storage conditions, composition of essential oils [57].

The results of this study revealed that the mean mortality rates of red mites were affected significantly with increasing dose concentrations of *A. indica* essential oil and exposure time. These results are in agreement with findings of other researchers [23,33,58] who investigated the acaricidal activity of neem on red mites *in vitro* and *in vivo*. Sariosseiri et al. [59] also investigated the acaricidal effectiveness of *Melia azedarach* ripe fruit extract against *D. gallinae* (Acari: Dermanyssidae) *in vitro* in Iran and reported similar results. The higher mortality rate recorded of mites with higher concentrations and longer exposure period may be due to increased susceptibility of mites to treatment.

In this study, the LD_{50} values confirmed that *A. indica* essential oil was more efficacious acaricidal, at different concentrations used and 50% *D. gallinae* mortality occurred with concentration of 0.126 % and 0.353 % according to contact and fumigation respectively. These results are partially in agreement with findings of Locher et al. [23]. The differences could be attributed to source of plant materials and amount of dose concentration used.

In the current study, the acaricidal activity of *A. indica* against *D.gallinae* may be due to excitation the nervous system of arthropods by A. *indica bioactive* ingredients, and this could be explained in view of Pritchard et al. [60] who suggested that, Acetylcholine is essential for neurons excitatory signal transmission, inhibition of signal termination by acaricides, overloads receptors with too much acetylcholine preventing recovery of post-synaptic neuron potential. Post-synaptic acetylcholine receptors are targeted by naturally derived essential oils via competitive inhibition. Conversely, these compounds hinder acetylcholine binding so no post synaptic signal is produced which leading to subsequent paralysis and death arthropoda. Moreover, Agbo et al. [30] suggested that *A. indica* possessed the antifeedant and growth inhibitory activities on D. *gallinae.*

5. CONCLUSION

It could be concluded that, *Azadirachta indica* essential oil has potential acaricidal activity against *D. gallinae*, Therefore, could be used as alternative acaricidal agents to control *D. gallinae* in poultry farm. Further research work is needed to study the acaricidal activity of *A. indica in vivo.*

ETHICAL APPROVAL

This study was conducted after approval from Animal Ethics Committee (AEC2020/05) in Faculty of Agriculture & Veterinary Medicine, Dhamar University, Yemen.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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