Parasitol Res (2013) 112:2319–2330
DOI 10.1007/s00436-013-3395-2

ORIGINAL PAPER

The acaricidal efficacy of aqueous neem extract and ivermectin against *Sarcoptes scabiei* var. *cuniculi* in experimentally infested rabbits

Shaker A. Seddiek · Hanem F. Khater · Mohamed M. El-Shorbagy · Ali M. Ali

Received: 8 March 2013 / Accepted: 12 March 2013 / Published online: 10 April 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract *Sarcoptes scabiei* var. *cuniculi* is one of the most important veterinary ectoparasites in rabbits and results in considerable loss of weight, productivity, and wool quality. The acaricidal activity of aqueous leaf extract of neem (CAN) and ivermectin (IVR) were evaluated in vitro and in vivo against *S. scabiei* var. *cuniculi.* Rabbits were classified into four groups (ten rabbits each). The first group (group 1) was designated as the negative control group. Each rabbit of the other groups was experimentally infested with 50 mites. One month post-infestation, the second group (group 2) was not treated and taken into account as the positive control group. The third group (group 3) was subcutaneously injected with 1 % IVR (200 μg/kg body weight, three times within a week interval). The fourth group (group 4) was treated topically with CAN (25 %) every 3 days for three consecutive weeks. Index scoring of lesions was described weekly. The number of live mites (larvae, nymphs, and adults) on each rabbit was counted on the 14th, 28th, and 42th day post-treatment (PT). Blood samples were taken 28 and 42 days PT for estimation of some chemical parameters. The body weight and cumulative body weight gain were recoded 14, 28, and 42 days PT. CAN (40 %) was highly efficacious against larvae of *S. scabiei* var. *cuniculi* as 100 % mortality was reached 24 h PT. On the other hand, all treated mites with CAN (20 %) and IVR died 48 h PT. The lethal values of CAN (LC_{50}, LC_{90}, LC_{95}, and LC_{99}) were 7.496, 14.67, 17.75, and 25.37 %, respectively, 48 h PT. Lesion scoring in groups 3 and 4 were significantly decreased ($P \leq 0.05$), reaching 0.20 and 0.40, respectively, when compared with that of group 2 (4.00), 42 days PT. Twenty-eight days PT, the reduction percentages of mites infesting rabbits were 93.38 and 93.09 % for IVR and CAN, respectively. However, complete mite reduction was reached 42 days PT. Rabbits treated with CAN did not show signs of restlessness or irritation, respiratory signs, or inflammation on the eye and/or skin at the time of application or afterwards. Regarding biochemical analysis, the levels of aspartate aminotransferase, alanine aminotransferase, creatinine, and total cholesterol in rabbits treated with CAN were decreased significantly ($P \leq 0.05$) than those of rabbits of the positive control group and those treated with IVR. On the other hand, the levels of total protein, albumin, and globulin of rabbits in group 4 were significantly ($P \leq 0.05$) increased when compared with the corresponding values of groups 2 and 3. The body weight and cumulative body weight gain of rabbits treated with CAN were significantly increased ($P \leq 0.05$) when compared with such values of groups 2 and 3, 28 and 42 days PT. The present data indicated that CAN had in vitro and in vivo acaricidal efficiency similar to that of IVR and improved the performance of rabbits without inducing adverse effects on treated rabbits; consequently, CAN could be suitable as a promising alternative acaricide for veterinary use.

S. A. Seddiek
Avian Diseases Department, Animal Health Research Institute, Benha Laboratory, Benha, Egypt

H. F. Khater (✉)
Faculty of Veterinary Medicine, Parasitology Department, Benha University, Benha, Egypt
e-mail: hafkhater@yahoo.com

M. M. El-Shorbagy
Faculty of Veterinary Medicine, Avian Diseases Department, Benha University, Benha, Egypt

A. M. Ali
Pharmacology Department, Animal Health Research Institute, Benha laboratory, Benha, Egypt

Introduction

Sarcoptic mange belongs to the family sarcoptidae and is a highly contagious and burrowing parasite (Wall and Shearer

1997; Walton and Currie 2007). *Sarcoptes scabiei* is an important ectoparasite in rabbits because of the possibility of zoonotic infection (Harrenstien et al. 1995) and considerable losses in weight, productivity, wool, and fiber quality (Aiello et al. 1998). In Egypt, mange (Sarcoptes and Psorptic) in rabbits is considered to be second to coccidiosis impotence, with high losses reported (Ezzat 1955). *Sarcoptes scabiei* var. *cuniculi* causes mange infestation in rabbits, affecting their ears, nose, feet, and areas around the genitalia, resulting in hypertensive reaction, body weight loss, and death (Aiello et al. 1998; Saha and Mukherjee 1998). *S. scabiei* can be difficult to eliminate in rabbits compared to other domestic animals (Aiello et al. 1998).

There is extensive evidence of the activity of macrocyclic lactones, such as ivermectin, doramectin, abamectin, and eprinomectin, when they are used as systemic parasiticides for controlling nematodes and arthropods (Marley and Conder 2002). In general, many of the chemical acaracides have limitations such as resistance (Currie et al. 2004) and toxicity (O'Brien 1999), environmental contamination, and persistence (Halley et al. 1993; O'Brien 1999). Such side effects of chemical acaricides have prompted a search for new alternatives (Khater 2011, 2012, 2013; Khater et al. 2013a, b).

Medicinal plants have been used as a source of remedies since ancient times. The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in the treatment of various diseases (Khater 2013). Egypt possesses an enormous diversity of plant resources that is used for herbal remedies for humans and animals (Hifnawy et al. 2001; Shalaby and Khater 2005; Khater and Shalaby 2008; El-Garhy and Mahmoud 2002; Khater and Khater 2009; Mohamed et al. 2010; Khater 2003, 2011, 2012, 2013; Seddiek et al. 2011; Khater et al. 2013a, b).

Botanicals have been in nature for millions of years without any adversative effects on the ecosystem. Botanical extracts kill and repel pests (Khater et al. 2009), affect insect growth and development (Shalaby and Khater 2005; Khater and Shalaby 2008; Khater and Khater 2009; Khater et al. 2009, 2011), and have anti-feedant and arrestant effects. As a consequence, botanical acaricides have become research hot spots because of their environmental safety and efficacy (Khater 2011, 2012, 2013).

Neem, *Azadirachta indica* A. Juss, has multipurpose medicinal properties, including antibacterial (El-Mahmood et al. 2010; Biswas et al. 2002), anti-fertility, antifungal, immunostimulant, antipyretic (Biswas et al. 2002), and acaricidal activities (Mulla and Su 1999). Neem was found to have biocidal activity against nearly 200 medical and veterinary arthropods, without any adverse effects toward most non-target organisms (Mulla and Su 1999; Khater 2011, 2012). As an acaricide, neem oil is effective against *S. scabiei* (Du et al. 2007), ticks (Abdel-Shafy and Zayed 2002), and poultry red mites (Lundh et al. 2005).

The aims of this work were to evaluate the in vitro and in vivo acaricidal efficacy of the crude aqueous extract of neem and ivermectin as acaricides against *S. scabiei* var. *cuniculi* and their effects on some biochemical parameters and growth performance of rabbits experimentally infested with sarcoptic mite.

Material and methods

Collection of mites

S. scabiei var. *cuniculi* larvae were collected from naturally infested rabbits in Qalyubia governorate, Egypt. The scabs containing mites were placed in Petri dishes which were incubated at 35 °C for 30 min. Under a stereomicroscope, the motile larvae were used in the experiments. Larvae have six legs, which makes them easily distinguishable from nymphs and adults which have eight legs. Mites were identified according to Soulsby (1982).

Plant material and extraction

Fresh leaves of *A. indica* were collected from Giza, Egypt, in May 2012. The crude aqueous leaf extract (CAN) was prepared according to Haussain (2002), with some modifications. Leaves of *A. indica* were dried indoors and then ground and weighed (5, 10, 15, 20, 30, and 40 g). Ground leaves were soaked in 100 mL distilled water for 24 h and then homogenized in an electric blender. The homogenate was filtered through a sterilized triple-folded piece of gauze. The filtrates (5, 10, 15, 20, 30, and 40 %) were use for in vitro bioassays.

Acaricidal activity in vitro

Regarding the concentration response bioassay, studying the acaricidal property was done according to Khater and Ramadan (2007). Five doses of CAN with four replications for each concentration were used in vitro. Twenty larvae per replicate were placed in a clean dry Petri dish with a filter paper, disc of Whatman no. 1 filter paper with surface areas measuring 62.63 cm^2, and impregnated uniformly with the used concentration of CAN on the bottom. The bioassay trails were carried out with increasing concentrations of CAN (5, 10, 20, 30, and 40 %) and contact time (24, 48, and 72 h). Two other groups were used: the first one (control) was treated with distilled water and the second group was treated with ivermectin 1 %. Bioassays were done at 27±2 °C and 75±5 % RH. Immobility of the larval mites, even when stimulated with a needle, the lack of a response, and persistent immobility were considered indicative of death (Khater and Ramadan 2007; Khater et al. 2013b).

Experimental animals

Forty New Zealand male rabbits (mean weight, 756.65± 2.17 g) were purchased from the Faculty of Agriculture, Benha University, Egypt. All rabbits were healthy and fed on balanced rations and a clean source of water ad libitum. These rabbits had not been treated with any anti-acariasis drug. The sampling procedures adhered to institutional ethical and animal care guidelines, and all methods were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, according to Nong et al. (2013).

Experimental design and treatment strategy

Rabbits were classified into four groups (ten rabbits each). The first group (group 1) was designated as the healthy or negative control group (non-infested and non-treated). Each rabbit in groups 2, 3, and 4 was experimentally infested with 50 mites. The infestation was carried out on the dorsal area after scratching the fur. One month post-infestation, the second group (group 2) was not treated and taken into account as the positive control group. The third group (group 3) was subcutaneously injected with ivermectin (IVR) 1 % (Ivomec®, Merk Sharp and Dohme Agvet Inc.) in a dose of 200 μg/kg body weight (bw), according to the producer, three times within a week interval. Finally, group 4 was treated topically with 25 % CAN (the concentration which showed an LC_{99} value 48 h post-treatment, PT) every 3 days for three consecutive weeks, according to Haussain (2002).

Clinical score value descriptions

Index scoring of lesions was described weekly according Jensen et al. (2002) as follows: 0=no lesion; 1=mild lesion—small visible mange body lesion (diameter, 0–4 cm), no bloody skin injuries, good overall body condition, only occasional rubbing; 2=moderate lesion—medium-sized visible mange body lesion (diameter, 4–8 cm), no bloody skin injuries, good overall body condition, more frequent rubbing; 3=severe lesion—severe body mange skin lesion, bloody skin injuries due to rubbing, reduced overall body condition; 4=chronic lesion—thick asbestos-like scab in the ears and body, bloody skin injuries due to rubbing, marked reduced overall body condition. Skin scrapings were taken from the part of the lesions bordering healthy tissue by scraping the infested arias. The number of live mites (larvae, nymphs, and adults) on each rabbit was counted on the 14th, 28th, and 42th day PT.

Chemical analysis

Blood samples were taken from the ear veins of five rabbits per group two times, 28 and 42 days PT, to separate the serum, which was stored at −20 °C until used for the estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activity (Reitman and Frankel 1957), cholesterol (Flegg 1973), creatinine (Henry 1974), total protein (Weichselbaum 1946), albumin (Doumas 1971), and globulin.

Rabbit performance

The body weight and cumulative body weight gain of rabbits were recoded 14, 28, and 42 days PT.

Statistical analysis

In bioassay tests, probit analysis was done on mortality data (Finney 1971) using a computer program (Biostat 2009) to calculate the lethal concentration (LC) and lethal time (LT) values. The biological data were subjected to analysis of variance (ANOVA) with Duncan's multiple range test (Duncan 1955) using a computer program (SPSS, 2001).

Results

CAN (40 %) was highly efficacious against *S. scabiei* var. *cuniculi* larvae as 100 % mortality was reached 24 h PT. On the other hand, all mites treated with CAN (20 %) and IVR (1 %) died 48 h PT (Table 1). The lethal values of CAN (LC_{50}, LC_{90}, LC_{95}, LC_{99}) were 4.50, 14.67, 17.75, and 25. 37 %, respectively, 48 h PT (Table 2). Lesion scoring in groups 3 and 4 were significantly decreased ($P \leq 0.05$), reaching 0.20 and 0.40, respectively, when compared with that of group 2 (4.00), 42 days PT (Table 3).

Rabbits infested with *S. scabiei* var. *cuniculi* and not treated (group 2) showed sarcoptic mange on the nose (Figs. 1 and 2). The affected regions showed scales, alopecia, and scale formation. Rabbits showed pruritis and were intermittently scratching the area with front paws. Later, hemorrhagic crusts with fissures developed, even becoming eroded in places. Rabbits showed minor signs of recovery. In contrast, rabbits treated with CAN exhibited improvement of clinical signs during the experiment, no inflammation was observed, and showed absence of macroscopic lesions on the nose, ears, and legs after 42 days PT. Similar results had been reported for the IVR-treated group, group 3 (Figs. 1 and 2).

Twenty-eight days PT, the reduction percentages of mites infesting rabbits were 93.38 and 93.09 % for IVR and CAN, respectively. However, complete mite reduction was reached 42 days PT (Table 4). Rabbits treated with CAN did not show signs of restlessness or irritation, respiratory signs, or inflammation on the eye and/or skin at the time of application or afterwards.

Regarding biochemical analysis 42 days PT, the levels of AST, ALT, creatinine, and total cholesterol of rabbits treated with CAN were decreased significantly ($P \leq 0.05$) than those

Table 1 In vitro mortality percentage of *S. scabiei* var. *cuniculi* after treatment with solutions of different concentrations of crude aqueous neem extract and the recommended dose of ivermectin

	Time post-treatment (h)								
	24 h			48 h			72 h		
	AV±SE	*D*/*T*	MO%	AV±SE	*D*/*T*	MO%	AV±SE	*D*/*T*	MO%
Control	0.50±0.50f	2/80	2.5	1.50±0.96d	6/80	7.5	2.50±0.50d	10/80	12.5
IVR (1 %)	17.50±0.50b	72/80	90	20.00±0.00a	80/80	100	20.00±0.00a	80/80	100
CAN (5 %)	3.50±0.50e	14/80	17.5	5.50±0.50c	22/80	27.5	7.00±0.58c	28/80	35
CAN (10 %)	8.00±0.83d	32/80	40	12.00±0.82b	48/80	60	15.00±0.59b	60/80	75
CAN (20 %)	14.00±0.82c	56/80	70	20.00±0.00a	80/80	100	20.00±0.00a	80/80	100
CAN (30 %)	17.00±0.58b	68/80	85	20.00±0.00a	80/80	100	20.00±0.00a	80/80	100
CAN (40 %)	20.00±0.00a	80/80	100	20.00±0.00a	80/80	100	20.00±0.00a	80/80	100
LSD*	2.50		–	4.00		–	4.50		–

Values within a column followed by different lowercase letters were significantly different ($P \leq 0.05$), while values within a column followed by the same lowercase letters were not significantly different ($P \leq 0.05$)

*LSD**=least significant difference at $P \leq 0.05$; *D*/*T*=(number of dead mites/total number of mites); MO%=Mortality%

AV ±SE = Average number of dead mites ±Stander Error

IVR ivermectin, *CAN* aqueous leaf extract of neem

of rabbits of the positive control group and those treated with IVR (Table 5). On the other hand, the levels of total protein, albumin, and globulin of rabbits in group 4 were significantly ($P \leq 0.05$) increased when compared with the corresponding values of groups 2 and 3 (Table 6). The body weight and cumulative body weight gain of rabbits treated with CAN were significantly increased ($P \leq 0.05$) when compared with such values of groups 2 and 3, 28 and 42 days PT (Table 7).

Discussion

In the present in vitro study, IVR (1 %) caused 90 and 100 % mortality 24 and 48 h PT. IVR completely reduced mite infestation 42 days PT of rabbits with 200 μg/kg bw, three times within a week interval. In spite of the applied dose for treating rabbits, such efficacy of IVR was in harmony with the reports of other researches. Rabbits that were naturally infected with *Psoroptes cuniculi* mites showed complete recovery after a single subcutaneous IVR injection of 200 μg/kg bw (Srivastava et al. 1991). Rabbits were treated with two doses of 1 % IVR solution (300–400 μg/kg bw) with an interval of 14 days between each injection for the treatment of dermatologic problems (Harrenstien et al. 1995). Subcutaneous injection of IVR in a dose of 200 μg/kg bw two to three times, with a mean interval of 11 days, was sufficiently effective in rabbits naturally infested with *Cheyletiella* spp. (Mellgren and Bergvall 2008). IVR (3 mL/L) was administered to rabbits for 24 h in drinking water and repeated every 6 months to treat and suppress the ear mange (Koopman et al. 1989). IVR (400 μg/kg bw, once) resulted in complete elimination of body mange in rabbits within 7 days after oral administration (El-Refaey 2008) and subcutaneous injection (Pandey 1989).

Similar to our in vivo results, IVR effectively treated mange in animals other than rabbits, such as rams and bucks (Magda and Fatma 2003), mice (Baumans et al. 1988), and in rats (Arise and Malomo 2009). In-feed, IVR was completely effective in field treatment of psoroptic mange in sheep (Foreyt 1993). IVR (200 μg/kg, single s.c. injection) efficiently controlled *S. scabiei* var *ovis* naturally infested sheep as 100 % mortality was reached after 10 days

Table 2 In vitro sensitivity of *S. scabiei* var. *cuniculi* to crude aqueous neem extract with determination of its effective lethal time against the mites

	Time post-treatment (h)		
	24 h	48 h	72 h
LC_{50}	11.680±1.354	7.496±0.834	6.389±0.379
LC_{90}	32.153±5.651	14.672±2.444	12.531±1.007
LC_{95}	42.850±9.343	17.751±3.630	15.169±1.488
LC_{99}	73.420±22.456	25.370±7.191	21.703±2.942
Slope[a]	2.9143±0.408	4.394±0.746	4.381±0.494

LT_{50} values for 5 and 10 % were 156.961±26.988 and 33.307±4.406, respectively

LC lethal concentration, *LT* lethal time

[a] Slope of the regression lines

Table 3 Index scorings (mean±SE)

	Days post-treatment					
	7	14	21	28	35	42
Group 1	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00b	0.00 0.00b
Group 2	2.00±0.00a	2.60±0.25a	3.40±0.25a	3.60±0.25a	3.80±0.20a	4.00±0.00a
Group 3	1.60±0.25a	1.60±0.25b	1.40±0.24b	1.20±0.20 b	0.40±0.25 b	0.20±0.20b
Group 4	1.60±0.24a	1.80±0.20b	1.60±0.25b	1.40±0.25b	0.60±0.24b	0.40±0.25b
LSD*	1.60	0.80	1.40	1.20	0.60	3.60

Group 1: negative control, not infested, not treated; group 2: positive control, infested, not treated; group 3: infested and treated with ivermectin (s.c.); group 4: infested and treated with neem extract locally

Values within a column followed by different lowercase letters were significantly different ($P \leq 0.05$), while values within a column followed by the same lowercase letters were not significantly different ($P \leq 0.05$). Index scores according to Jensen et al. (2002): 0=no lesion, 1=mild lesion—small visible mange body lesion (diameter, 0–4 cm), no bloody skin injuries, good overall body condition, only occasional rubbing; 2=moderate lesion—medium-sized visible mange body lesion (diameter, 4–8 cm), no bloody skin injuries, good overall body condition, more frequent rubbing; 3=severe lesion—obvious severe body mange skin lesion, bloody skin injuries due to rubbing, reduced overall body condition; 4=chronic lesion—thick asbestos-like scab in the ears and body, bloody skin injuries due to rubbing, marked reduced overall body condition (to be culled)

*LSD** least significant difference ($P \leq 0.05$)

PT (Tabassam et al. 2008). Moreover, in vitro trials indicated that IVR was effective against *S. scabiei* var. *S. scabiei* var. *homins* at a dose of 100–8,000 ng/g (Walton et al. 2000), and the lower threshold for the acaricidal effect of IVR was 50–500 μg/mL on *S. scabiei* var. *suis* larvae in vitro (Brimer et al. 1995).

Concerning the side effects of IVR, the first documentation of in vivo and in vitro ivermectin resistance in *S. scabiei* had been reported by Currie et al. (2004). In addition, IVR induced neonatal toxicity in rats (Lankas et al. 1989), and the use of IVR has deleterious effects on the male fertility of cattle (Avery and Schmidt 1995), goats (Tanyildizi and Bozkurt 2002), and rats (El-Nahas and El-Ashmawy 2008). Ecologically, most macrocyclic lactones have been shown to be highly toxic for the dung beetles (*Onthophagus taurus*), as a non-target organism (Wardhaugh et al. 2001; Lumaret and Errouissi 2002). Although treatment of sarcoptic mange with various acaricides like diazinon, fenvalerate, deltamethrin, and avermectin (Campbell 1989; Merck 2005) has been attempted with different grades of success, insecticides pollute the environment around animals (Gassner et al. 1997) and their side effects (Ahmad et al. 2012) overweigh their benefits. Consequently, healthcare providers now face a serious lack of new commercial acaricides, and new alternatives are urgently needed.

Botanicals could be an environment-friendly solution of this dilemma (Khater 2011, 2012, 2013). Our in vitro study signposted that 20 % CAN generated 70 and 100 % mortality of *S. scabiei* var. *cuniculi* larvae after 24 and 48 h, respectively. CAN comprised good effect against sarcoptic mite larvae in vitro as the LC_{50} values were 11.68, 7.50, and 6.39 %, respectively, after treatment for 24, 48, and 72 h. The LT values were 156.95 and 33.31 h after treatment with 5 and 10 % CAN, respectively.

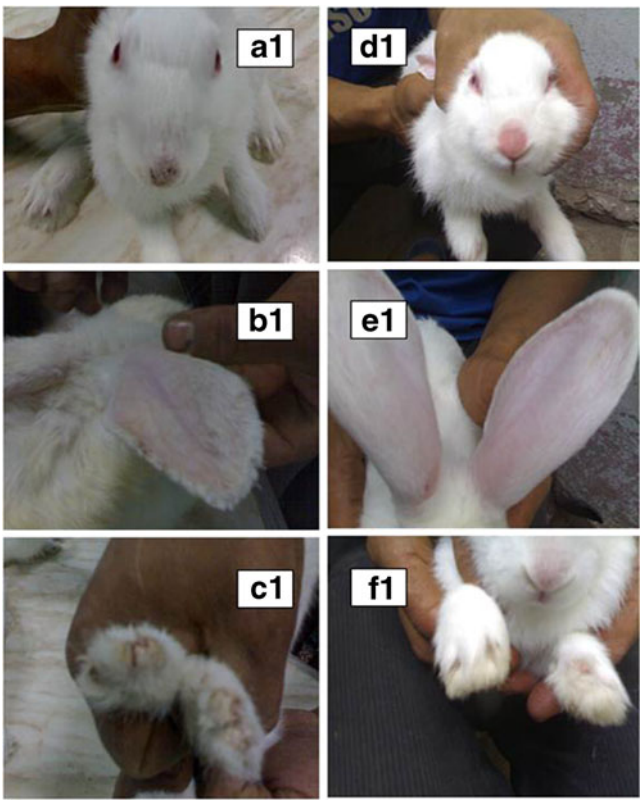

Fig. 1 Rabbits infested with *S. scabiei var. cuniculi* showed sarcoptic mange in the nose (*A1*), ears (*B1*), and legs (*C1*) before treatment. Rabbits infested with *S. scabiei* var. *cuniculi* showed recovery from mange in the nose (*D1*), ears (*E1*), and legs (*F1*) 42 days post-treatment with crude aqueous extract of neem

Fig. 2 Rabbits infested with *S. scabiei* var. *cuniculi* showed sarcoptic mange in the nose (*A2*), ears (*B2*), and legs (*C2*) before treatment. Rabbits infested with *S. scabiei* var. *cuniculi* showed treated mange in the nose (*D2*), ears (*E2*), and legs (*F2*) 42 days post-treatment with ivermectin

Similar efficacies of neem against *S. scabiei* var. *cuniculi* had been reported in vitro. The LC_{50} value of neem oil was 2.908 mL/L at 24 h PT, and the LT_{50} values of 500, 250, and 125 mL/L were 1, 2, and 5 h, respectively (Du et al. 2007). Chloroform extract of neem oil induced 100 % mortality after 4.5 h of exposure at a concentration of 200 mg/mL in vitro, with an LC_{50} value of 0.1 mg/mL at 24 h PT and LT_{50} of 15.3 h at a concentration of 7.5 mg/mL (Du et al. 2009). The LT_{50} values for neem oil (10 %) microemulsion and aqueous emulsion were 81.74 and 95.55 min, respectively (Xu et al. 2010). The LC_{50} value of the petroleum ether extract of neem (1.3 μL/mL) was about three times that of the chloroform extract (4.1 μL/mL) at 24 h PT. At a concentration of 500.0 μL/mL, the LT_{50} values of the petroleum ether extract and the chloroform extract were 8.4 and 9.6 h, respectively (Du et al. 2008). Petroleum ether extract of neem oil and its four fractions (F1–F4) possess useful acaricidal activity in vitro as the LC_{50} value was 70.9 mL/L 24 h after treatment. At concentrations of 500.0, 250.0, 125.0, 62.5, and 31.2 mL/L, the LT_{50} values of the petroleum ether extract were 8.7, 8.8, 10.8, 11.5, and 13.1 h, respectively. Acaricidal activities of 68.3 and 100.0 % in F2 and F4 were confirmed (Deng et al. 2012). In contrast to our results and the previous findings, Walton et al. (2000) reported that neem showed little acaricidal activity against *S. scabiei* var. *hominis*.

Our in vivo trial indicated that rabbits infested with *S. scabiei* var. *cuniculi* and not treated showed mange infestations on the nose, ears, and legs with minor signs of recovery. On the contrary, rabbits treated with IVR and CAN exhibited improvement of clinical signs during the experiment. The reduction percentage of *S. scabiei* mites on rabbits was 100 % after treatment with CAN or IVR for 42 days PT, which is represented clinically by the absence of macroscopic lesions. Likewise, neem oil had been reported to induce recovery of mange affecting other animals, such as sheep (Hirudkar et al. 1997; Tabassam et al. 2008) and dog (Abdel-Ghaffar et al. 2008a). Neem ointment (5 %) was effective against ear canker of rabbits, *P. cuniculi* (Joshi et al. 2000). Moreover, neem induced an effective in vivo

Table 4 Treatment of rabbits with ivermectin or crude aqueous neem extract

	Days post-treatment					
	14		28		42	
	No.±SE	*R*%	No.±SE	*R*%	No.±SE	*R*%
Group 1	0.00±0.00c	–	0.00±0.00c	–	0.00±0.00b	–
Group 2	98.60±1.97a	00.00	136.00±4.28a	00.00	181.80±6.04a	00.00
Group 3	35.20±2.22b	35.69	9.00±0.71b	93.38	0.00±0.00b	100
Group 4	39.00±1.52b	39.55	9.40±0.51b	93.09	1.00±0.45b	100
LSD*	35.20	–	9.00	–	181.80	–

Group 1: negative control, not infested, not treated; group 2: positive control, infested, not treated; group 3: infested and treated with ivermectin (200 μg/kg bw, s.c.); group 4: infested and treated with neem extract locally (25 %)

No.±SE=mean number of live mites (larvae, nymphs and adults); *R*%=reduction%; *LSD** = Least Significant Difference ($P \leq 0.05$)

Data were analyzed using one-way ANOVA. Means with different alphabetical letters in the same column are significantly different using LSD and Duncan's tests at $P \leq 0.05$

Table 5 Some serum biochemical parameters in rabbits treated with ivermectin (s.c.) or crude aqueous neem extract (locally)

Days PT	AST (U/L)		ALT (U/L)		Creatinine (mg/dL)		Total cholesterol (mg/dL)	
	28	42	28	42	28	42	28	42
Group 1	38.55±0.26c	37.10±0.26b	21.05±0.27a	18.94±0.09ab	0.90±0.01b	1.08±0.01c	48.71±0.50a	50.53±0.22b
Group 2	43.98±0.37a	43.75±0.69a	21.04±0.31a	19.13±0.18ab	0.94±0.01b	1.07±0.01c	48.89±0.42a	51.05±0.19ab
Group 3	40.94±0.43b	38.62±0.51a	21.37±0.18a	19.31±0.16a	1.18±0.02a	1.35±0.01a	49.02±0.56a	51.30±0.17a
Group 4	40.56±0.25b	37.21±0.31b	20.86±0.18a	18.73±0.16b	0.92±0.01b	1.12±0.01b	45.24±1.51b	49.47±0.35c
LSD*	2.01	6.54	–	0.58	2.48	0.04	3.47	0.77

Mean±SE, n=5

Values within a column followed by different lowercase letters were significantly different ($P \leq 0.05$), while values within a column followed by the same lowercase letters were not significantly different ($P \leq 0.05$)

*LSD** = Least Significant Difference (P ≤ 0.05), *PT* post-treatment

control of the poultry mite, *Dermanyssus gallinae* (Lundh et al. 2005; Abdel-Ghaffar et al. 2008b; Locher et al. 2010a, b).

Comparable to IVR, our in vivo data indicated that neem extract completely cured mange in rabbits. Similarly, the undiluted neem oil killed all *S. scabiei* var. *cuniculi* larvae in 25 min and was significantly higher than pyrethrins, but not significantly different from avermectin (Du et al. 2007). Crude aqueous methanol extract of neem seed kernel (20 %) was found as effective as IVR against sarcoptic mange infesting sheep under field conditions (Tabassam et al. 2008). An analogous effect was also observed for IVR when compared with that of Crofton weed (*Eupatorium adenophorum*) extracts against *P. cuniculi* (Nong et al. 2013).

Regarding index scoring, the index scores of rabbits infested with *S. scabiei* var. *cuniculi* and treated with CAN or IVR were significantly decreased; there was no significant difference between both treatments when compared with those of the infested and non-treated ones (group 2). Equivalent improvement of lesion scoring was reported for neem as an acaricide against mange in dog (Abdel-Ghaffar et al. 2008a) and sheep (Tabassam et al. 2008).

Neem-based products efficiently controlled arthropods of medical and veterinary importance other than mange-inducing mites (AL-Rubae 2009; Khater 2011, 2012, 2013). Neem seed oil induced in vitro acaricidal effect on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Abdel-Shafy and Zayed 2002). The plant-based compounds from neem oil such as limonoids may be an effective alternative to conventional synthetic insecticides for the control of *Anopheles stephensi* (Nathan et al. 2005). A neem extract proprietary product, AG1000, has been shown to be repellent to the biting midge *Culicoides imicola*, which can spread cattle diseases (Braverman et al. 1999). Neem oil is a low-cost alternative for personal protection against sand fly bites (Sharma and Dhiman 1993).

Neem is used commercially as an insecticide and repellent. The biological compound (MiteStop®), based on a neem seed extract, had a considerable repellent effect on bloodsucking mosquitoes, tabanids, ceratopogonids, simuliids, as well as on licking flies. The product has a very high and broad efficacy against a wide spectrum of insects, ticks, and mites that molest birds, animals, and humans (Abdel-Ghaffar et al. 2008a, b, 2009, 2010; Abdel-Ghaffar

Table 6 Serum total protein, albumin, and globulin in rabbits treated with ivermectin (s.c.) or crude aqueous neem extract (locally)

Days PT	Total protein (g/dL)		Albumin (g/dL)		Globulin (g/dL)	
	28	42	28	42	28	42
Group 1	5.55±0.10a	5.55±0.21b	2.62±0.04b	2.55±0.02a	3.09±0.16a	2.80±0.02b
Group 2	4.38±0.04c	4.11±0.03c	2.40±0.03c	2.17±0.02b	1.98±0.06d	1.95±0.03c
Group 3	4.89±0.03b	5.39±0.04b	2.63±0.04b	2.59±0.02a	2.26±0.05c	2.80±0.06b
Group 4	5.41±0.06a	6.11±0.01a	2.83±0.04a	2.57±0.03a	2.56±0.05b	3.54±0.03a
LSD*	0.52	0.56	0.20	0.38	0.28	0.75

Mean±SE, n=5

Values within a column followed by different lowercase letters were significantly different ($P \leq 0.05$), while values within a column followed by the same lowercase letters were not significantly different ($P \leq 0.05$)

*LSD** least significant difference ($P \leq 0.05$), *PT* post-treatment

Table 7 Mean body weight and body gain in rabbits treated with ivermectin (s.c.) or crude aqueous neem extract (locally)

		Days post-treatment					
		14		28		42	
		Body weights (g)	Cumulative body gains (g)	Body weights (g)	Cumulative body gains (g)	Body weights (g)	Cumulative body gains (g)
Group 1	757.00±1.46a	1059.50±2.49a	302.70±3.13a	1348.70±2.37a	591.70±1.88a	1646.60±5.50a	879.60±4.96a
Group 2	755.90±1.95a	968.00±2.89b	206.80±4.52b	1141.20±2.66d	385.30±2.65d	1377.50±3.38d	621.60±4.15c
Group 3	757.10±2.56a	969.70±3.08b	213.60±3.91b	1251.50±3.44c	493.40±3.67c	1567.40±3.53c	810.30±3.33b
Group 4	756.60±2.69a	964.00±3.15b	212.10±3.88 b	1261.40±3.94b	504.60±5.57b	1626.50±1.57b	869.90±3.41a
LSD*	–	89.80	90.10	9.90	11.20	20.10	59.60

Mean±SE, $n=10$

Values within a column followed by different lowercase letters were significantly different ($P\leq0.05$), while values within a column followed by the same lowercase letters were not significantly different ($P\leq0.05$)

*LSD** least significant difference ($P\leq0.05$), *PT* post-treatment

and Semmler 2007; Locher et al. 2010a, b; Schmahl et al. 2010; Walldorf et al. 2012; Mehlhorn et al. 2011, 2012; Al-Quraishy et al. 2012a, b).

The pesticidal activity of neem oil is generally thought to be due to the tetranortriterpenoid, azadirachtin, a well-known potent insecticide whose content could be a useful quality control criterion for neem oil insecticide products (Isman et al. 1990). Azadirachtin induces feeding and oviposition deterrence, growth inhibition, and fecundity and fitness reductions (Schmutterer 1990). Azadirachtin is a natural plant defense chemical affecting feeding through chemoreception (primary anti-feedancy) that consists in the blockage of the input from receptors that normally respond to phagostimulants or from the stimulation of specific deterrent cells or both (Chapman 1974; Dethier 1982) and through a reduction in food intake due to toxic effects if consumed (secondary antifeedancy), where food intake is reduced after the application of azadirachtin in ways which bypass the mouth part chemoreceptors (Mordue and Blackwell 1993). Anti-feedancy could be assessed from crude to refined neem extracts to neem-enriched extracts to pure azadirachtin.

Azadirachtin also has growth regulatory effects on larval insects like disruption of molting, growth inhibition, and malformation, which may contribute to mortality. This is attributed to a disruption of endocrine events such as the downregulation of hemolymph ecdysteroid level through the blockage of release of the prothoracicotropic hormone from the brain–corpus cardiacum complex or to a delay in the appearance of the last ecdysteroid peak showing complete molt inhibition. Furthermore, azadirachtin also affects allatropin and juvenile hormone titers (Mordue and Blackwell 1993). Dealing with reproduction, adverse effects on ovarian development, fecundity, and fertility have been reported (Karnavar 1987).

CAN was found safe for rabbits, as indicated by the chemistry analysis in the present study. There are no skin irritations or restlessness during the time of application and afterwards. Similar results had been reported (Tabassam et al. 2008; Schmahl et al. 2010). Along with the economic benefits, additional advantages of using neem pesticides are that they have low environmental persistence (Sundaram and Curry 1994), do not induce resistance readily in insects (Feng and Isman 1995; Jacobson 1995), and are relatively nontoxic to mammals (Jacobson 1995; Larson 1989) and non-target organisms (Cóndor_Golec 2007).

Biochemical analysis indicated that the levels of AST, ALT, and total cholesterol in rabbits significantly ($P\leq0.05$) increased in rabbits in the positive control and those treated with IVR. Such results indicated an adverse effect of IVR on the liver. Such results were in agreement with those mentioned for macrocyclic lactones for the treatment of rabbits (Eman and Abdella 2000), rats (Arise and Malomo 2009), and rams and bucks (Magda and Fatma 2003).

Repeated administration of IVR in rats (0.4 mg/kg bw for 15 consecutive days) led to significant increases in serum urea, creatinine, cholesterol, AST, and ALT activities, while albumin was significantly decreased (Arise and Malomo 2009). Liver function was negatively affected, which was monitored by increasing the transaminase enzymes (AST and ALT) after 28 days of IVR injection in rats (Ali et al. 1988) and in rabbits (El-Shaieb and Mohamed 2000; Eman and Abdella 2000). The serum AST, ALT, urea, and creatinine levels were significantly increased after 28 days of treatment with IVR in swine and cattle (Slantna et al. 1989). The toxic effects of IVR on liver and kidney functions were transient, and the treated rabbits required not less than 3 months after injection of IVR to regain their normality (Eman and Abdella 2000).

On the other hand, the levels of total protein, albumin, and globulin of rabbits in groups 2 and 3 were significantly ($P\leq0.05$) decreased compared to values in the negative

control and CAN-treated groups. However, the total protein, albumin, globulin, cholesterol, and urea did not significantly differ in rabbits treated with IVR in drinking water for 2 days (Adu et al. 2009). These finding disagreed with those of our findings, which may be due to the different route of administration of IVR (in drinking water); in our study, the route was subcutaneous injection.

Hypoproteinemia in rabbits treated with IVR may be due to the destructive and toxic effect of IVR on the hepatocytes and renal epithelia (Kalifa and Al-Elyani 1997). Similar results were reported after using IVR for the treatment of rats (Ali et al. 1988) and rabbits (Ghoniem and Mansour 1992; Eman and Abdella 2000) and using abamectin for the treatment of rams and bucks (Magda and Fatma 2003).

In the present study, on the 42th day PT, the levels of AST and ALT enzymes, creatinine, and total cholesterol in rabbits treated with CAN were decreased compared to those treated with IVR. Similarly, Ogbuewu et al. (2010a) reported that neem leaf meal-based diets decreased the serum cholesterol level in rabbit bucks.

Our data indicated that the total protein and globulin levels were significantly ($P<0.05$) increased in the CAN-treated group than the IVR-treated group. In contrast to our findings, Bawa et al. (2007) reported that the neem seed cake diets (20 %) resulted in low value for total protein in rabbits. Meanwhile, AST, ALT, total protein, albumin, and globulin were not significantly altered. Oral feeding of water extract of fresh leaves of *A. indica* at a dose of 250 mg/kg bw for 16 weeks resulted in improvement in serum cholesterol in diabetic rats (Hussain 2002).

In our study, CAN could be considered as safe because it had no side effect on the liver and kidney of the treated rabbits. Our data pointed out to the hepato- and renal-protective effects of the neem extract. Similar results were reported by Khalifa et al. (1998) as they found that water suspension of dried leaves of *A. indica* was hepatoprotective in rabbits infected with *Eimeria stiedae*, and also by Ezz-Din et al. (2011) who found that the use of *A. indica* leaf extract was a promising renal and hepatoprotective agent in rats due to its antioxidant effect and normalization of impaired kidney and liver function activities.

In contrast, the hepatobiliary toxic effect of *A. indica* leaf aqueous extract was reported after its use in high oral doses (2.34 g/kg bw) in rabbits (Akah et al. 1992) and after its repeated oral administration for five successive days which resulted in irregular hepatocytes, widened sinusoids, and degeneration of blood vessels in liver of the quail (Kalifa and Al-Elyani 1997).

Regarding growth performance of rabbits in the present study, the body weight and cumulative body weight gain of rabbits in the infested and non-treated group were significantly decreased compared to values of the treated groups. In the same way, the intense pruritus associated with sarcoptic mange in production herds interferes with milk production, weight gain, and leather quality and can inflict serious economic losses on primary industries (Elbers et al. 2000; Rehbein et al. 2003a, b)

The body weight and cumulative body weight gain of rabbits treated with CAN were significantly increased when compared with those for groups 2 and 3, 28th and 42th day PT. Similar results were reported (Wasanthakumar et al. 1999; Hussain 2002; Esonu et al. 2006; Bawa et al. 2007; Ogbuewu et al. 2010a, b). Neem seed kernel cake can be incorporated up to 10 % in rabbit diets to improve body weight gain without any adverse effect on palatability and performance to spare costs in developing countries (Wasanthakumar et al. 1999). Rabbits fed leaf meal (15 %) for 16 weeks showed no deleterious effects on body weight gain (Ogbuewu et al. 2010b). In addition, poultry diets treated with 15 % neem leaves may have increased body weight gain, hen-day egg production, and egg yolk color (Esonu et al. 2006).

It could be concluded that CAN had in vitro and in vivo acaricidal efficiency similar to that of IVR. It improved the growth performance (body weight and gain) of rabbits infested with *S. scabiei* var. *cuniculi*. No significant signs of side effects or adverse reactions were noticed throughout the study. These data provide a platform for the development of environment-friendly, non-toxic, non-accumulating medicines against acariasis which could be carried out in a large scale in rabbit farms. Botanicals could create a herbal remedy export market and thereby create more jobs in developing countries which will improve the national economy.

Acknowledgments The authors thank Prof. Dr. Azza A. Moustafa, Research Institute of Medical Entomology, Egypt, for her support and advice.

References

Abdel-Ghaffar F, Semmler M (2007) Efficacy of neem seed extract shampoo on head lice of naturally infected humans in Egypt. Parasitol Res 100:329–332

Abdel-Ghaffar F, Al-Quraishy S, Sobhy HM, Semmler M (2008a) Neem seed extract shampoo, Wash away Louse®, an effective plant agent against *Sarcoptes scabiei* mites infesting dogs in Egypt. Parasitol Res 104:145–148

Abdel-Ghaffar F, Sobhy HM, Al-Quraishy S, Semmler M (2008b) Field study on the efficacy of an extract of neem seed (Mite-Stop) against the red mite *Dermanyssus gallinae* naturally infecting poultry in Egypt. Parasitol Res 103(3):481–485

Abdel-Ghaffar F, Semmler M, Al-Rasheid KAS, Mehlhorn H (2009) In vitro efficacy of ByeMite® and Mite-Stop® on developmental stages of the red chicken mite *Dermanyssus gallinae*. Parasitol Res 105:469–471

Abdel-Ghaffar F, Semmler M, Al-Rasheid K, Klimpel S, Mehlhorn H (2010) Comparative in-vitro test on the efficacy and safety of 13 anti-head-lice products. Parasitol Res 106:423–249

Abdel-Shafy S, Zayed AA (2002) In vitro acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea, Ixodidae). Vet Parasitol 16:89–96

Adu OA, Ladipo MK, Adebiyi OA, Akinfemi A, Igbasan FA (2009) Performance and blood characteristics of pre-pubertal rabbits fed varied levels of dietary rare earth element (REE). World Appl Sci J 6(11):1489–1494

Ahmad L, Khan A, Khan MZ, Hussain I, Mahmood F, Sleemi MK, Akbar Lodhi LA, Abdullah I (2012) Toxico-pathological effects of cypermethrin upon male reproductive systemin rabbits. Pestic Biochem Physiol 103:194–201

Aiello SE, Mays A, Amstutz HE (1998) Rabbits. In: Aiello SE (ed) Merck veterinary manual. Merck Inc., Whitehouse Station, NJ, pp 1386–1396

Akah PA, Offiah VN, Onugu E (1992) Hepatotoxic effect of *Azadirachta indica* leaf extract in rabbits. Fitoterapia 63(4):311–319

Ali AA, Fahmy MFM, Edress NM (1988) Pathologic and clinico-pathologic studies on anti parasitic drug ivermectin toxicosis in albino rats. Zag Vet J 16:19–31

Al-Quraishy S, Abdel-Ghaffar F, Al-Rasheid KA, Mehlhorn J, Mehlhorn H (2012a) Observations on effects of a neem seed extract (MiteStop®) on biting lice (mallophages) and bloodsucking insects parasitizing horses. Parasitol Res 110(1):335–339

Al-Quraishy S, Abdel-Ghaffar F, Al-Rasheid KA, Mehlhorn J, Mehlhorn H (2012b) Effects of a neem seed extract (MiteStop®) on mallophages (featherlings) of chicken: in vivo and in vitro studies. Parasitol Res 110(2):617–622

AL-Rubae AY (2009) The potential uses of *Melia Azedarach* L. as pesticidal and medicinal plant, review. Am-Eurasian J Sustain Agric 3(2):185–194

Arise RO, Malomo SO (2009) Effect of ivermectin and albendazole on some liver and kidney function indices in rats. Afric J Biochem Res 3(5):190–197

Avery B, Schmidt M (1995) A dose–response study of ivermectin in a bovine in vitro production system. Theriogenology 43(1):163

Baumans V, Havenaar R, Van Herck H (1988) The use of repeated treatment with Ivomec and Neguvon spray in the control of murine fur mites and oxyurid worms. Lab Anim 22:246–249

Bawa GS, Orunmuyi M, Agbaji AS, Ladan Z, Okekeifi UO (2007) Effect of different methods of processing neem (*Azadirachta indica*) seeds on performance of young rabbits. Pakistan J Nutr 6(3):212–216

Biswas KI, Chattopadhyay A, Banerijee YA, Bandopadhyay U (2002) Biological activities and medicinal properties of neem (*Azadirachta indica*). Curr Sci 82:1336–1345

Braverman Y, Chizov-Ginzburg A, Mullens BA (1999) Mosquito repellent attracts *Culicoides imicola* (Diptera: Ceratopogonidae). J Med Entomol 36(1):113–115

Brimer L, Bonlocke L, Pontoppidan C, Henriksen SA, Gyrd-Hansen N, Rasmussen F (1995) A method for in vitro determination of the acaricidal effect of ivermectin using *Sarcoptes scabiei* var. *suis* as test organism. Vet Parasitol 59(3–4):249–255

Campbell WW (1989) Ivermectin and abamectin. Springer, New York

Chapman RF (1974) Chemical inhibition of feeding by phytophagus insects—a review. Bull Ent Res 64:339–363

Cóndor_Golec AF (2007) Effect of neem (*Azadirachta indica* A. Juss) insecticides on parasitoids. Rev Peru Biol 14(1):069–074

Currie BJ, Harumal P, McKinnon M, Walton F (2004) First documentation of in vivo and in vitro ivermectin resistance in *Sarcoptes scabiei*. Clin Infect Dis 39(1):e8–e12

Deng Y, Shi D, Yin Z, Guo J, Jia R, Xu J, Song X, Lv C, Fan Q, Liang X, Shi F, Ye G, Zhang W (2012) Acaricidal activity of petroleum ether extract of neem (*Azadirachta indica*) oil and its four fractions separated by column chromatography against *Sarcoptes scabiei* var. *cuniculi* larvae in vitro. Exp Parasitol 130(4):475–477

Dethier VG (1982) Mechanisms of host plant recognition. Entomologia Exp Appl 31:49–56

Doumas B (1971) Colourimetric method for albumin determination. Clin Chim Acta 31:87–92

Du YH, Yin ZQ, Pu ZH, Li W, Li JD, Yu SS (2007) Acaricidal activity of neem oil against *Sarcoptes scabiei* var. *cuniculi* in vitro. Vet Sci China 63(2):75–78

Du YH, Jia RY, Yin ZQ, Pu ZH, Chen J, Yang F, Zhang YQ, Lu Y (2008) Acaricidal activity of extracts of neem (*Azadirachta indica*) oil against the larvae of the rabbit mite *Sarcoptes scabiei* var. *cuniculi* in vitro. Vet Parasitol 157(1–2):144–148

Du YH, Li JL, Jia RY, Yin ZQ, Li XT, Lv C, Ye G, Zhang L, Zhang YQ (2009) Acaricidal activity of four fractions and octadecanoic acid-tetrahydrofuran-3,4-diyl ester isolated from chloroform extracts of neem (*Azadirachta indica*) oil against *Sarcoptes scabiei* var. *cuniculi* in vitro. Vet Parasitol 163(1–2):175–178

Duncan DB (1955) Multiple range and multiple *F* tests. Biometrics 11:1–42

Elbers AR, Rambags PG, van der Heijden HM, Hunneman WA (2000) Production performance and pruritic behaviour of pigs naturally infected by *Sarcoptes scabiei* var. *suis* in a contact transmission experiment. Vet Q 22:145–149

El-Garhy MF, Mahmoud LH (2002) Anthelmintic efficacy of traditional herbs on *Ascaris lumbricoides*. J Egypt Soc Parasitol 32:893–900

El-Mahmood AM, Ogbonna OB, Raji M (2010) The antibacterial activity of *Azadarichta indica* (neem) seeds extracts against bacterial pathogens associated with eye and ear infections. J Med Plants Res 4(14):1414–1421

El-Nahas AF, El-Ashmawy IM (2008) Effect of ivermectin on male fertility and its interaction with P-glycoprotein inhibitor (verapamil) in rats. Environ Toxicol Pharmacol 26(2):206–211

El-Refaey KEM (2008) Efficacy and safety of ivermectin administered orally to infected rabbits with mange. Mansoura Vet Med J 5(1):55–64

El-Shaieb AF, Mohamed KI (2000) Experimental pathological and clinical studies of ivermectin in male rabbits with reference to the protective role of vitamin E and selenium. Mansoura Vet Med J 2(1):137–153

Eman EE, Abdella OEL (2000) Effect of ivermectin and moxidectin on fertility and some biochemical parameters in male rabbits. Egypt J Agric Res 78(1):293–301

Esonu BO, Opara MN, Okoli IC, Obikaonu HO, Udedibie C, Iheshiulor OOM (2006) Physiological response of laying birds to neem (*Azadirachta indica*) leaf meal-based diets: body weight organ characteristics and haematology. Online J Health Allied Sci 5(2):1–7

Ezzat M (1955) External parasites of Egyptian animals. Second-Arab-Scientific-Congress, Cairo

Ezz-Din D, Gabry SM, Farrag AH, Abdel Moneim AE (2011) Physiological and histological impact of *A. indica* (neem) leaves extract in a rat model of cisplatin-induced hepato and nephrotoxicity. J Med Plants Res 5(23):5499–5506

Feng R, Isman MB (1995) Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. Experientia 51:831–833

Finney DJ (1971) Probit analysis. Cambridge University Press, Cambridge, 303 pp

Flegg HM (1973) Quantitative-enzymatic–colourimetric determination of total cholesterol and HDL-C in serum or plasma. Ann Clin Biochem 10:79–88

Foreyt MJ (1993) Efficacy of in-feed formulation ivermectin against *Psoroptes* spp. in Bighorn sheep. J Wildlife Dis 9(1):85–89

Gassner B, Wüthrich A, Lis J, Scholtysik G, Solioz M (1997) Topical application of synthetic pyrethroids to cattle as a source of persistent environmental contamination. J Environ Sci Health B 32:729–739

Ghoniem HM, Mansour HH (1992) Some studies on antiparasitic drug ivermectin in rabbits. Zagazig Vet J 20:753–761

Halley BA, Vandenheuvel WJA, Wislock PG, Herd R, Strong L, Wardhaugh K (1993) Environmental effects of the usage of avermectines in livestock. Vet Parasitol 48:109–125

Harrenstien L, Gentz EJ, Carpenter JW (1995) How to handle respiratory, ophthalmic, neurological and dermatologic problems in rabbits. Proceedings of the Symposium on Rabbit Medicine, 4 April, Lenexa, Kansas. Vet Med 90(4):373–380

Henry RJ (1974) Clinical chemistry, principles and techniques, 2nd edn. Harper and Row, Hagerstown, MD

Hifnawy MS, Rashwan OA, Rabeh MA (2001) Comparative chemical and biological investigations of certain essential oils belonging to families Asteraceae, Lamiaceae and Graminae. Bull Fac Pharm Cairo Univ 39:35–53

Hirudkar US, Deshpande PD, Narladkar BW, Vadlamudi VP (1997) Effect of herbal treatment with himax ointment and neem oil in sarcoptic mange in sheep. Indian Vet J 74:506–508

Hussain HEMA (2002) Reversal of diabetic retinopathy in streptozotocin induced diabetic rats using traditional Indian antidiabetic plant, *Azadirachta indica* L. Indian J Clinic Biochem 17(2):115–123

Isman MB, Koul O, Luczynski A, Kaminski J (1990) Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J Agric Food Chem 38:1406–1411

Jacobson M (1995) Toxicity of neem to vertebrates and side effects on beneficial and other ecologically important non-target organisms: toxicity to vertebrates. In: Schmutterer H (ed) The neem tree: source of unique products for integrated pest management, medicine, industry, and other purposes. VCH Weinheim, Germany, pp 484–495

Jensen JCE, Nielsen LH, Arnason T, Cracknell V (2002) Elimination of mange mites *Sarcoptes scabiei* var. *suis* from two naturally infested Danish sow herds using a single injection regime with doramectin. Acta Vet Scand 43:75–84

Joshi SS, Dakshinkar NP, Sapre VA, Sarode DB (2000) Evaluation of herbal medicaments in psoroptic mange of rabbits. Indian Vet J 77:706–708

Kalifa SAM, Al-Elyani RAA (1997) The effect of water suspension of dried leaves of *A. indica* on the liver of quail *C. conturnix*. Histological and histochemistry. J Egypt Soc Zool 24(C):1–14

Kalifa SAM, Al-Elyani RAA, Toulah FHS (1998) The effect of dried leaves water suspension of neem plant (*Azadirachta indica*) on some organs of rabbits (*Oryctolagus cuniculus* L.) infected by coccidiosis (*E. stiedae* K.). 1. Some cytological and histological studies on the liver. Egypt J Histol 21(1):19–32

Karnavar GK (1987) Influence of azadirachtin on insect nutrition and reproduction. Proc Indian Acad Sci (Anim Sci) 96:341–347

Khater HF (2003) Biocontrol of some insects. PhD thesis, Zagazig University, Benha Branch, Egypt

Khater HF (2011) Ecosmart biorational insecticides: alternative insectcontrol strategies. In: Perveen F (ed) Advances in integrated pest management. InTech, Croatia, pp 17–60

Khater HF (2012) Prospects of botanical biopesticides in insect pest management. Pharmacologia 3(12):641–656

Khater HF (2013) Bioactivity of essential oils as green biopesticides: recent global scenario. In: Govil JN, Bhattacharya S (eds), Recent progress in medicinal plants, vol. 37. Essentials oils II. Studium Press LLC, USA (in press)

Khater HF, Khater DH (2009) The insecticidal activity of four medicinal plants against the blowfly *Lucilia sericata* (Diptera: Calliphoridae). Int J Dermatol 48(5):492–497

Khater HF, Ramadan MY (2007) The acaricidal effects of peracetic acid against *Boophilus annulatus* and *Argas persicus*. Acta Sci Vet 35:29–40

Khater HF, Shalaby AA (2008) Potential of biologically active plant oils for control mosquito larvae *Culex pipiens* (Diptera: Culicidae) from an Egyptian locality. Rev Inst Med Trop S Paulo 50(2):107–112

Khater HF, Ramadan MY, El-Madawy RS (2009) Lousicidal, ovicidal, and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. Vet Parasitol 164:257–266

Khater HF, Hanafy A, Abdel-Mageed AD, Ramadan MY, El-Madawy RS (2011) Control of the myiasis-producing fly, *Lucilia sericata*, with Egyptian essential oils. Int J Dermatol 50(2):187–194

Khater HF, Seddiek SA, El-Shorbagy MM, Ali MM (2013a) The acaricidal efficacy of peracetic acid and deltamethrin against the fowl tick, *Argas persicus*, infesting laying hens. Parasitol Res 112(1):259–269

Khater HF, Ramadan MY, Abdel Mageid AD (2013b) In vitro control of the camel nasal botfly, *Cephalopina titillator*, with doramectin, lavender, camphor, and onion oils. Parasitol Res. (in press)

Koopman JP, Scholten PM, van Zutphen T, Hooghof JB (1989) The effect of ivermectin on psoroptes ear mange in rabbits. Tijdschr Diergeneeskd 114(15–16):825–828

Lankas GR, Minsker DH, Robertson RT (1989) Effects of ivermectin on reproduction and neonatal toxicity in rats. Food Chem Toxicol 27:523–529

Larson RO (1989) The commercialization of neem. In: Jacobson M (ed) 1988 Focus on phytochemical pesticides, vol. 1. The neem tree. CRC, Boca Raton, pp 155–168

Locher N, Al-Rasheid KA, Abdel-Ghaffar F, Melhorn H (2010a) In vitro and field studies on the contact and fumigant toxicity of a neem-product (Mite-Stop) against the developmental stages of the poultry red mites *Dermanyssus gallinae*. Parasitol Res 107(2):417–423

Locher N, Klimpel S, Abdel-Ghaffar F, Al-Rasheid KA, Melhorn H (2010b) Light and scanning electron microscopic investigations on MiteStop-treated poultry red mites. Parasitol Res 107(2):433–437

Lumaret J, Errouissi F (2002) Use of anthelmintics in herbivores and evaluation of risks for the non-target fauna of pastures. Vet Res 33:547–562

Lundh J, Wiktelius D, Chirico J (2005) Azadirachtin-impregnated traps for the control of *Dermanyssus gallinae*. Vet Parasitol 130(3–4):337–342

Magda MM, Fatma ESG (2003) Effect of abamectin on some biochemical parameters in rams and bucks infected with mange. Egypt J Agric 81(1):293–303

Marley SE, Conder GA (2002) The use of macrocyclic lactones to control parasites of domesticates wild ruminants. In: Vercruysse J, Rew RS (eds) Macrocyclic lactones to antiparasitic therapy. CABI Publishing, USA, 425 pp

Mehlhorn H, Abdel-Ghaffar F, Al-Rasheid KA, Schmidt J, Semmler M (2011) Ovicidal effects of a neem seed extract preparation on eggs of body and head lice. Parasitol Res 109(5):1299–1302

Mehlhorn H, Walldorf V, Abdel-Ghaffar F, Al-Quraishy S, Al-Rasheid KA, Mehlhorn J (2012) Biting and bloodsucking lice of dogs—treatment by means of a neem seed extract (MiteStop®, Wash Away Dog). Parasitol Res 110(2):769–773

Mellgren M, Bergvall K (2008) Treatment of rabbit cheyletiellosis with selamectin or ivermectin: a retrospective case study. Acta Vet Scand 50(1):1–17

Merck R (2005) The Merck veterinary manual, 9th edn. Merck & Co., Whitehouse Station, NJ

Mohamed AEH, El-Sayed MA, Hegazy ME, Helaly SE, Esmail AM, Mohamed NS (2010) Chemical constituents and biological activities of *Artemisia herba-alba*. Rec Nat Prod 4:1–25

Mordue AJ, Blackwell A (1993) Azadirachtin: an update. J Insect Physiol 39:903–924

Mulla MS, Su T (1999) Activity and biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc 15:133–152

Nathan S, Kalaivani K, Murugan K (2005) Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Acta Trop 96:47–55

Nong X, Ren Y, Wang J, Fang C, Xie Y, Yang D, Liu T, Chen L, Zhou Z, Gu X, Zheng W, Peng X, Wang S, Lai S, Yang G (2013) Clinical efficacy of botanical extracts from *Eupatorium adenophorum* against the scab mite, *Psoroptes cuniculi*. Vet Parasitol 192(1–3):247–252

O'Brien DJ (1999) Treatment of psoroptic mange with reference to epidemiology and history. Vet Parasitol 83(3–4):177–185

Ogbuewu IP, Uchegbu MC, Okoli IC, Iloeje MU (2010a) Toxicological effects of leaf meal ethnomedicinal plant—neem—on serum biochemistry of crossbred New Zealand white typed rabbit bucks. Rep Opin 2(2):54–57

Ogbuewu IP, Uchegbu MC, Okoli IC, Iloeje MU (2010b) Assessment of blood chemistry, weight gain and linear body measurements of pre-pubertal buck rabbits fed different levels of neem (*Azadirachta indica* A. Juss) leaf meals. Chilean J Agric Res 70(3):515–520

Pandey VS (1989) Effect of ivermectin on the ear mange mite, *Psoroptes cuniculi*, of rabbits. Br Vet J 145(1):54–56

Rehbein S, Visser M, Winter R, Trommer B, Matthes HF, Maciel AE, Marley SE (2003a) Productivity effects of bovine mange and control with ivermectin. Vet Parasitol 114:267–284

Rehbein S, Visser M, Winter R, Trommer B, Matthes HF, Maciel AE, Marley SE (2003b) Productivity effects of bovine mange and control with ivermectin. Vet Parasitol 114(4):267–284

Reitman S, Frankel S (1957) Transaminases in serum. Am J Clin Path 28:56

Saha SB, Mukherjee S (1998) Sarcoptic mange in domestic rabbits. Indian J Anim Health 37:73

Schmahl G, Al-Rasheid KA, Abdel-Ghaffar F, Klimpel S, Melhorn H (2010) The efficacy of neem seed extracts (Tre-San, MiteStop on a broad spectrum of pets and parasites). Parasitol Res 107(2):261–269

Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu Rev Entomol 35:271–297

Seddiek SA, Ali MM, Khater HF, El-Shorbagy MM (2011) Anthelmintic activity of the white wormwood, *Artemisia herba-alba* against *Heterakis gallinarum* infecting turkey poults. J Med Plant Res 5(16):3946–3957

Shalaby AA, Khater HF (2005) Toxicity of certain solvent extracts of *Rosmarinus officinalis* against *Culex pipiens* larvae. J Egypt-German Soc Zool 48E:69–80

Sharma VP, Dhiman RC (1993) Neem oil as a sand fly (Diptera: Psychodidae) repellent. J Am Mosq Control Assoc 9(3):364–366

Slantna PJ, Kuivinen C, Ohlsen C, Ekstrom LG (1989) Ivermectin residues in the tissues of swine and cattle. Effect of cooking and toxicological evaluation. Food Add Contam 6:475–481

Soulsby EJL (1982) Helminths, Arthropods and Protozoa of domesticated animals, 7th edn. Baillière Tindall, London

Srivastava CP, Maru A, Dubey SC (1991) Effect of ivermectin against mange mite infection in rabbits. Indian J Vet Med 11:74

Sundaram KMS, Curry J (1994) Initial deposits and persistence of azadirachtin in fir and oak foliage after spray application of Margosan-O® formulation. Pestic Sci 41:129–138

Tabassam SM, Iqbal Z, Jabbar A, Sindhu ZU, Chattha AI (2008) Efficacy of crude seed neem kernel extracts against natural infestation of *Sarcoptes scabiei* var. *ovis*. J Ethnopharmacol 115(2):284–287

Tanyildizi S, Bozkurt T (2002) An investigation of the effects of ivermectin on blood serum, semen hyaluronidase activities and spermatological characteristics in sheep. Turk J Vet Anim Sci 26:353–357

Wall R, Shearer D (1997) Veterinary entomology. Chapman and Hall, London

Walldorf V, Mehlhorn H, Al-Quraishy S, Al-Rasheid KAS, Abdel-Ghaffar F, Mehlhorn J (2012) Treatment with a neem seed extract (MiteStop®) of beetle larvae parasitizing the plumage of poultry. Parasitol Res 110(2):623–627

Walton SF, Currie BJ (2007) Problems in diagnosing scabies, a global disease in human and animal populations. Clin Microbiol Rev 20(2):268–279

Walton SF, Myerscough MR, Currie BJ (2000) Studies in vitro on the relative efficacy of current acaricides for *Sarcoptes scabiei* var. *hominis*. Trans R Soc Trop Med Hyg 94:92–96

Wardhaugh KG, Longstaff BC, Morton R (2001) A comparison of the development and survival of the dung beetle, *Onthophagus taurus* (Schreb.) when fed on the faeces of cattle treated with pour-on formulations of eprinomectin or moxidectin. Vet Parasitol 99(2):155–168

Wasanthakumar P, Sharma K, Sastry VRB, Agrawal DK (1999) Effect of replacing peanut meal by neem (*Azadirachta indica*) seed kernel cake on nutrient intake, digestibility and retention, and body weight in broiler rabbits. World Rabbit Sci 7(3):145–149

Weichselbaum PE (1946) Colourmetric determination of total protein. Am J Clin Path 16:40–47

Xu J, Fan QJ, Yin ZO, Li XT, Du YH, Jia RY, Wang KY, Lv C, Ye G, Geng Y, Su G, Zhao L, Hu TX, Shi F, Zhang L, Wu CL, Tao C, Zhang XY, Shi DX (2010) The preparation of neen oil microemulsion (*Azadirachta indica*) and comparison of acaricidal time between neem oil microemulsion and other formulations in vitro. Vet Parasitol 11(3–4):399–403