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Article in *Tropical Animal Health and Production* · March 2021

DOI: 10.1007/s11250-020-02545-7

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# Efficacy of neem (*Azadirachta indica*) aqueous fruit extracts against *Sarcoptes scabiei* var. *suis* in grower pigs

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Received: 17 August 2020 / Accepted: 22 December 2020

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## Abstract

The acaricidal activity of *Azadirachta indica* (neem) aqueous fruit extracts was evaluated against *Sarcoptes scabiei* var. *suis* (mange mites) in an on-farm trial using grower pigs. Aqueous neem fruit extracts of three concentrations 5%, 10%, and 25% w/v and a commercial acaricide, 12.5% amitraz-based Triatix spray (positive control), were compared with pigs that received no treatment (negative control). Thirty grower pigs of the Dalland breed were allocated to the five treatments in a completely randomized experiment. Each experimental animal was sprayed on day 0 and again on day 7. Counts of mange mites, scoring of lesion index, and calculation of rubbing index were done weekly. Topical application of 25% aqueous neem fruit extract had a higher efficacy ratio ( $p < 0.05$ ) than the other fruit extract concentrations, and performed similarly to an amitraz-based acaricide, suggesting a dose-dependent response. Amitraz (positive control) cured clinical mange on grower pigs after 5 weeks and 25% aqueous neem fruit extract 6 weeks post-treatment. The results indicated that aqueous neem fruit extracts have acaricidal effects against mange mites and can provide a cheaper, safer, and more eco-friendly alternative for the control of *Sarcoptes* mange in pigs.

**Keywords** Ethnoveterinary plants · Acaricidal properties · Sarcoptic mange · Resource-poor farmers

## Introduction

As the demand for animal-based protein increases to feed the world's growing and increasingly discerning human population, without harming the environment, the need to improve the productivity and health of tropical livestock is imperative. Pig production offers a significant potential to attain food security, reduce poverty, and improve livelihoods for millions

of resource-poor farmers in the global south. However, pigs are threatened by endemic pathogens and parasites (Wanzala et al. 2005). In tropical and sub-tropical regions, parasites are major constraints to efficient pig production and estimated to be second to African swine fever in importance (Halimani et al. 2010). Firkins et al. (2001) reported a worldwide prevalence rate of 70–90% of sarcoptic mange mite infection in pigs with performance losses of up to US \$115 per sow per year.

The Sarcoptic mange mite (*Sarcoptes scabiei* var. *suis*) is a serious parasitic mite of pigs that relies on the host epidermis for its nourishment, reproduction, habitat, and survival. Their morphology and ecology are highly adapted to a life of intimate contact with their host, as there are no free-living development stages and no intermediate hosts in its life cycle. Thus, this contagious, host-specific, burrowing, and astigmatid mite remains an obligate parasite throughout its life cycle. Sarcoptic mange mites undergo four distinct stages in their life cycle: egg, larva, nymphs, and adults. Adult female mites mate only once on the skin surface, burrow into the skin, and lay two or three eggs per day. Eggs hatch as larvae within 2–4 days and larvae molts into adult mites in 4–7 days. Sarcoptic mange mites negatively impact grower pig production

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through rubbing and scratching of irritations that may result in bleeding, reduced feed intake and efficiency, sub-optimal growth, and longer days to market. *Sarcoptes* infestations often cause intense pruritus which in turn leads to chronic stress, compromised welfare, and growth potential of the pigs. There are two clinical forms recognized: a pruritic or hypersensitive form that affects mostly growing pigs; and a chronic hyperkeratotic form characterized by the presence of aural crusts and a large number of mites on the animal, and most commonly affects multiparous sows (Arlian and Morgan 2017).

To control sarcoptic mange, a number of commercial veterinary formulations are used, including those for subcutaneous injection, oral administration, and topical application: pour-on, spot-on, spray, or dips. Most of these systemic drugs work on mange mite nerve axons by modifying the kinetics of ligand-gated ion channels (Bernigaud et al. 2019). Prophylaxis and treatment rely on the repeated use of subcutaneous injections of the macrocyclic lactones, ivermectin, and doramectin, and topical applications of formamide acaricides such as amitraz. For effective control, repeated and blanket applications of the correct drug dosage must be done in order to break the life cycle of the mange mite, which usually lasts up to 10 days. Such regularity of use of these costly drugs is beyond the ability of resource-poor smallholder farmers, who form the bulk of pig producers in the tropical and subtropical developing world (Mwale and Masika 2009). As a result, most farmers cannot afford blanket control of mange in the whole pig herd, but tend to use symptomatic treatment and directed sprays, prioritizing sows moving to the farrowing house and breeding stock before sale.

Effective control of mange mites remains a big challenge due to incorrect use of drugs, and there are reports of emerging resistance and treatment failures (Gopinath et al. 2018). This makes it imperative to explore the potential use and efficacy of locally available plant materials to control this important livestock parasite. Botanicals are preferred for their organic nature and a broad spectrum of insecticidal and acaricidal activities, which help to prevent development of drug resistance. Plant-based biocides are preferred because they show effective and eco-friendly features, including little non-target effects, multiple mechanisms of action, low cost, and easy production in countries with limited industrial facilities (Benelli et al. 2017). The most widely used of these botanicals is the neem plant (*Azadirachta indica*), being effective against more than 350 species of arthropods, twelve nematode species, fifteen fungal species, three viruses, and some snail and crustacean species. As a result, the United Nations declared neem as “the tree of the 21st century” (Agbo et al. 2019).

Neem is a widely distributed tropical plant that originated from India. Almost all parts of the neem tree have varied insecticidal, larvicidal, and acaricidal effects, mediated by groups of phytochemical compounds such as cardenolide, azadirachtins, salannin, nimbin, and 6-desacetylnimbin.

Most of the acaricidal effects of neem fruit extract against mites, lice, and ticks are attributed to the triterpene and azadirachtin (George et al. 2014; Gopinath et al. 2018). Azadirachtins are known to exist in high concentrations in the fruits, which are produced in great quantity. The fruit is a small drupe, ellipsoid in shape, about 2 cm long, yellow-green when ripe with one stone inside, and one or two seeds (Benelli et al. 2017).

Smallholder pig producers in Zimbabwe are using extracts of the neem tree (*Azadirachta indica*) as a bio-acaricide against a number of ecto-parasites, including mange. Although various veterinary preparations from botanicals are relatively safe at the rate they are used by smallholders (Tabassam et al. 2008; Seddiek et al. 2013), their effectiveness and safety are not ascertained. Most studies on acaricidal efficacy of plant material tend to be laboratory-based, ignoring practical smallholder farmer realities (Madzimure et al. 2013). Validation of farmer claims and determination of the optimum dosage rates for botanicals that do not lead to mammalian toxicity and development of drug resistance are important. This study, therefore, sought to validate smallholder farmer claims on the efficacy of aqueous neem extracts against mange mites in pigs. The second objective was to determine appropriate application rates of *Azadirachta indica* (neem) fruit extract for effective control of mange mites (*Sarcoptes scabiei* var. *suis*) in grower pigs under conditions similar to those used by farmers.

## Materials and methods

### Research site

The trial was conducted at the Pig Industry Board (PIB) in Arcturus 25.5 km east of Harare, Zimbabwe. The farm is located on 17° 47' S and 31° 19' E at an altitude of 1385 m above sea level. Ecologically, the farm lies in the high rainfall high potential Natural Farming Region IIA (Vincent et al. 1960; Mugandani et al. 2012). It receives an average rainfall of between 900 and 1200 mm per annum, falling between November and March. Mean maximum temperatures range from 19 to 23 °C; mean minimum temperatures range from 10 to 13 °C with a mean annual temperature range of 16–19 °C. The pigs were housed in multiple-space pens with access to a standard concentrate grower diet supplied in trough feeders and free-choice access to water through nipple drinkers.

### Preparation of plant extracts

Ripe *Azadirachta indica* (neem) fruits were hand-harvested from neem trees in Arcturus, Zimbabwe. The fruits' skin was removed by hand during cleaning and the unskinned

fruits were left to dry in the sun for 48 h. The inner soft pulp and seeds were crushed in a mortar using a wooden pestle. The crushed fruits were then blended at 20,000 rpm for 2 min in a kitchen blender to form a homogenous but gummy, oily fluid. The fluid was weighed and diluted with tap water to come up with 5, 10, and 25% (w/v) concentrations and left to stand at room temperature for 48 h. The diluted fluid extracts were then filtered through a muslin cloth. To every 250 ml of the diluted fluid extracts, 2 ml of organic liquid soap (Sunlight Liquid, Unilever, Zimbabwe) was added as an emulsifier.

## Experimental procedure

The experiment was conducted during the hot-dry season (October–November 2016) for 6 weeks. The study was set up as a completely randomized design experiment with five treatments, each replicated six times. The treatments consisted of the three application levels of neem aqueous fruit extract: 5%, 10%, and 25% w/v; and two control treatments: 12.5% amitraz (positive control) and non-treated group (negative control). A total of thirty Dalland grower pigs aged 6 weeks and weighing  $12.5 \pm 0.5$  kg were randomly allocated to the five experimental pens, corresponding to the five treatments. Each pen had a total floor area of  $15.6\text{m}^2$ , measuring 4.35 by 3.59 m with a nipple drinker and a trough feeder. An empty pen was left between/separated experimental pens. Each pig was then infested with scabs with mites taken from naturally infested boars. The mites were applied on the dorsal area from the neck along the back towards the tail after scratching some fur. Pigs were sprayed at the start of the experiment using knapsack sprayer, and again 7 days later with treatments containing 5%, 10%, and 25% aqueous neem fruit extract or Triatix® spray (12.5% amitraz, Coopers Animal Health, Zimbabwe) at the recommended application and dilution rate of 0.2% v/v. The negative control group was left untreated. The spraying was done against the grain of the hair with 5 l of acaricide per animal, leaving it fully drenched.

The number of mange mites, lesions scoring, and rubbing index was determined for each pig every 7 days from the commencement of the experiment. The method described by Jensen et al. (2002) was used for lesion index scoring from 0 for no lesions to 4 for chronic lesions (Table 1). For mites, skin scrapings were taken using a sharp spoon until blood was visible. Skin scrapings were taken from the same position, parts, and of the same area ( $25\text{cm}^2$ ) on growers' lesions bordering healthy tissue. Scrapings were spread on a piece of black paper in a small cardboard box and left for 10 min. Mange mites rounded in shape and approximately 0.5 mm in length were visible to the naked eye; a hand lens was used to increase visibility. The number of live mange mites from each scraping of each grower was recorded weekly. The rubbing index was defined as the number of growers rubbing and

scratching in a pen over a period of 10 min, divided by the total number of growers in the pen observed. A rubbing index of  $\geq 0.5$  was denoted as a possible mange infection. The rubbing index was measured during periods of the day when pigs were not eating or sleeping.

## Statistical analyses

Weekly mite counts for each treatment were used to calculate the acaricide efficacy ratio using a formula adapted from O'Neill (1988) as follows:

$$\text{Acaricide efficacy} = 1 - \left( \frac{\text{treatment mite count}}{\text{untreated control mite count}} \right)$$

The acaricide efficacy ratios, expressed as a percentage, were subjected to arcsine square root transformation to normalize the data before analysis. However, untransformed least square means were used for reporting purposes. All data was analyzed by the general linear model procedure with and without repeated measures in Minitab 17 (Minitab 2014). Means were separated by Tukey's honest significant difference (HSD) at the 5% significance level.

## Results

The present study sought to evaluate acaricidal efficacy of neem-based fruit extracts in the control of sarcoptic mange in grower pigs. Time post-treatment had an effect ( $P < 0.05$ ) on acaricide efficacy ratio for all treatments except the untreated control (Table 2). The 25% aqueous neem fruit extract's acaricide efficacy ratio reached 96.6% efficacy 6 weeks post-treatment and was the most efficacious among the aqueous neem extract treatments. Amitraz treatment had the highest acaricide efficacy ratio (100%) after 5 weeks, but did not differ from the 25% Neem treatment at 6 weeks. The negative control (no treatment) had 0% efficacy ratio throughout the trial.

The mean number of mange mites counted was similar ( $P > 0.05$ ) for all treatments in the first and second week of the experiment (Fig. 1). However, the amitraz and 25% v/v neem fruit extract treatment had significantly lower mange mite counts from three to 6 weeks of the experiment. These two treatments did not differ from each other in mange mite counts from three to 6 weeks. The 5% and 10% neem fruit extract treatments did not differ from each other in number of mites recorded from the third week onwards. However, they had higher numbers of mites ( $P < 0.05$ ) compared to the other treatments. The untreated group had the mean number of mites sampled per pig increasing from about 10.5 to 14.8 mites between week 1 and week 6.

Mean rubbing index for each treatment across time is indicated in Fig. 2. The rubbing index for the non-treated

**Table 1** Description of clinical index scores for skin lesions due to mange in pigs (after Jensen et al. 2002)

Score	Classification	Description
0	No lesions	No lesions - no visible mange skin lesions, scab, or rubbing
1	Mild lesion	Only occasional, small (0–4 cm in diameter) visible mange body skin lesions, no bloody skin injuries, and good overall body condition; only occasional rubbing and scratching
2	Moderate lesion	Medium sized visible mange body lesions (diameter 4–8 cm) covering less than 2% of the body surface, no body skin injuries, good overall body condition, and more frequent rubbing and scratching
3	Severe lesion	Severe body mange skin lesions covering large areas of the body surface, bloody skin injuries due to extended rubbing, and reduced overall body condition
4	Chronic lesion	Thick asbestos-like scab in the ears and body, bloody skin injuries due to rubbing, and marked reduced overall body condition

group increased to 0.83 by the end of the fifth week, while it reduced to zero for the amitraz treatments and for pigs treated with 25% aqueous neem extract, rubbing index was 0.17 and 0.0 in weeks 5 and 6. The 5% and 10% aqueous neem extract treatments did not differ ( $P > 0.05$ ) from each in rubbing index in up to the fifth week. However, these two treatments differed in rubbing index in the sixth week of the experiment.

Figure 3 shows acaricidal treatment effects on the lesion index of experimentally infested grower pigs during the 6 weeks of the study. The lesion index for the untreated group kept on rising with time as lesion condition worsened in grower pigs. There was significant and progressive decline in mean lesion index for all treatment groups over time post-treatment. Significant interaction effects of time and treatment for lesion index were observed. At the end of the experiment, all treatments differed ( $P < 0.05$ ). The untreated group had the highest mean lesion index followed by 5% aqueous neem, 10% aqueous neem, 25% aqueous neem, and lastly the positive amitraz control. In weeks 2–4, treatments 5% and 25% aqueous neem had similar lesion indices. These results indicate that throughout the experiment, 25% aqueous neem fruit extract and amitraz treatment were the most effective acaricides against *Sarcoptes scabiei* var. *suis*.

**Table 2** Acaricide efficacy ratios (%) following topical applications of aqueous neem fruit extract and amitraz against mange mites in grower pigs

Treatment	Week					
	1	2	3	4	5	6
Untreated control	0.0	0.0	0.0	0.0	0.0	0.0
5% Neem fruit extract	1.7 <sup>b</sup>	25.0 <sup>a</sup>	46.4 <sup>b</sup>	56.6 <sup>d</sup>	65.9 <sup>d</sup>	69.7 <sup>c</sup>
10% Neem fruit extract	1.7 <sup>b</sup>	25.0 <sup>a</sup>	42.0 <sup>c</sup>	60.5 <sup>c</sup>	73.2 <sup>c</sup>	75.3 <sup>b</sup>
25% Neem fruit extract	1.7 <sup>b</sup>	25.0 <sup>a</sup>	71.0 <sup>a</sup>	82.9 <sup>b</sup>	93.9 <sup>b</sup>	96.6 <sup>a</sup>
0.2% Triatix (12.5% amitraz)	5.0 <sup>a</sup>	21.9 <sup>b</sup>	69.6 <sup>a</sup>	93.4 <sup>a</sup>	100.0 <sup>a</sup>	98.9 <sup>a</sup>

<sup>abc</sup> Means in the same column with different superscripts indicate significant differences ( $P < 0.05$ ) between treatments at that particular point in time

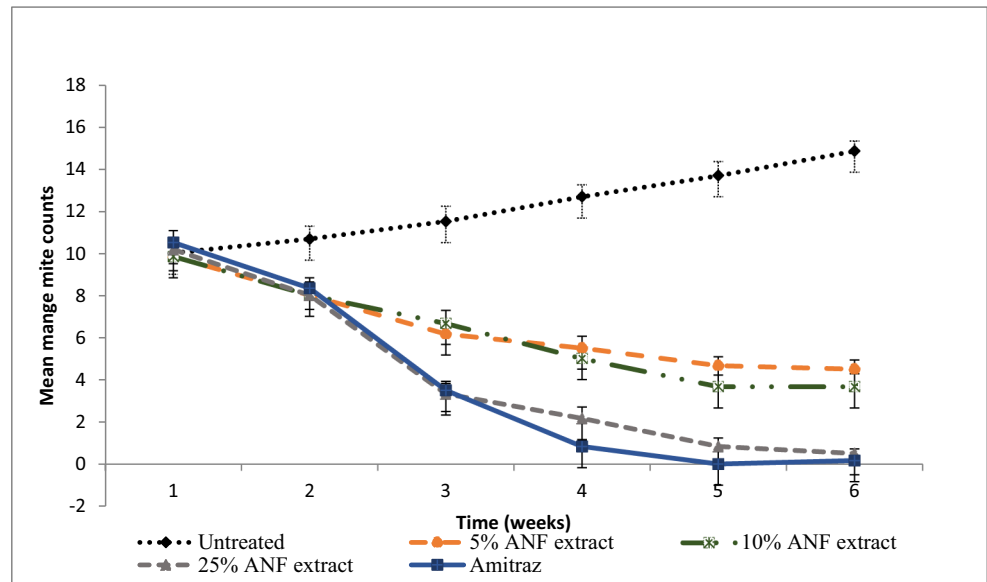
## Discussion

Growers infested with *Sarcoptes scabiei* var. *suis* and not treated showed sarcoptic mange on and inside the ears and along their backs; some growers had chronic lesions. Some affected regions had scabs with an asbestos appearance. These grower pigs had reduced body condition, and there was more frequent rubbing and scratching without any signs of recovery. In contrast, growers treated with aqueous neem fruit extracts showed improvement of clinical signs during the study; their lesion index was significantly reduced. Aqueous neem fruit extracts were also found to be safe for the grower pigs, as there were no observed signs of skin irritation and inflammation or restlessness during the time of application and afterwards. The safety of treatments with neem extracts against mange mites has been ascertained in studies by Tabassam et al. (2008) and Seddiek et al. (2013).

Results indicated that 25% aqueous neem fruit extract and amitraz treatment were the most effective acaricides against *Sarcoptes scabiei* var. *suis*. However, the rapid detachment of mange mites from growers within a short time period after treatment with amitraz was not pronounced. There could be resistance of mites against amitraz-based acaricides. In an experimental infestation of dogs with *Demodex* mites causing canine demodecticosis, Živičnjak (2005) observed that 10.3% of the mites were resistant to amitraz, but not ivermectin-based acaricides. In the present study, in vitro sensitivity tests of mange mites to amitraz and the different aqueous neem extracts were not carried out to prove this speculation, however. The low efficacy earlier in the trial (the first 3 weeks) as shown in Table 2 could be ascribed to severity of infestation and evaporation rates at the time of application. Cool, damp conditions favor survival of mites. *Azadirachta indica* (neem) fruit extracts did not show the classical dose dependence that normally occurs with conventional insecticides. The delay in effectiveness of the neem fruit extracts to the third and fourth weeks post-treatment supports the observed time and treatment interaction effect.

The acaricidal effects of aqueous neem fruit extracts could have been due to the presence of groups of phytochemical

**Fig 1** Mean mange mite counts following topical applications of aqueous neem fruit extract and amitraz based acaricides against mange mites in grower pigs. ANF, aqueous neem fruit extract; amitraz, Triatix spray (12.5% amitraz-based acaricide); Error bars indicate standard error of mean (SEM)

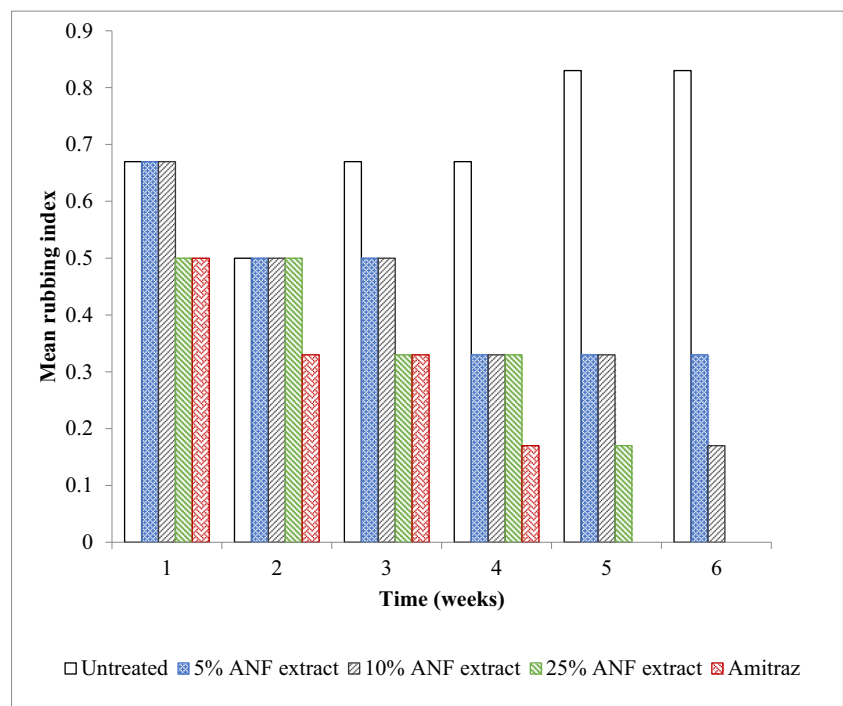


ANF – aqueous neem fruit extract; amitraz – Triatix spray (12.5% amitraz based acaricide); Error bars indicate Standard Error of Mean (SEM)

compounds with acaricidal, larvicidal, and pesticidal properties such as cardenolide, azadirachtin, salannin, nimbin, and 6-desacetyl nimbin (Atawodi and Atawodi 2009). Among these limonoids, azadirachtin is the most prominent constituent of the seed kernels of neem. Different sites have been identified as targets for azadirachtin, and it has generally been

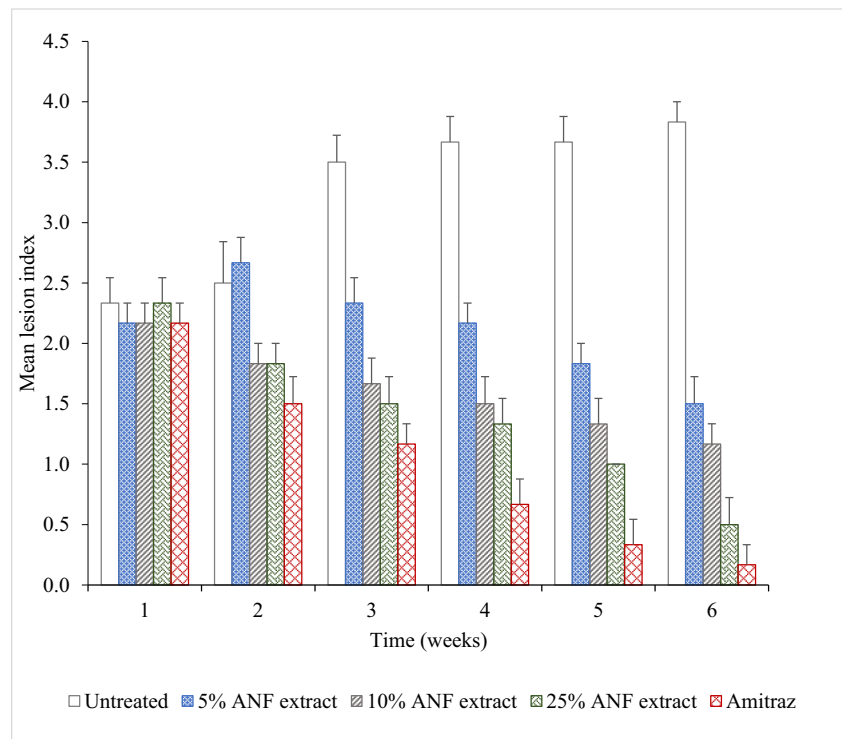
accepted that behavioral effects are through chemoreceptor mechanism, and growth-related effects are due to interference with the neuroendocrine control of molting and ecdysis. Azadirachtin is known to prevent apolysis and ecdysis, induces pharate mortality, or sometimes induces permanent larvae in several insect taxa (Raj 2014). Azadirachtin seems to

**Fig. 2** Mean rubbing index in grower pigs given acaricidal treatments of aqueous neem fruit extract and amitraz-based acaricides. ANF, aqueous neem fruit extract; amitraz, Triatix spray (12.5% amitraz-based acaricide)



ANF – aqueous neem fruit extract; amitraz – Triatix spray (12.5% amitraz based acaricide);

**Fig. 3** Mean lesion index in grower pigs given topical applications of aqueous neem fruit extracts and amitraz-based acaricides. ANF, aqueous neem fruit extract; amitraz, Triatix spray (12.5% amitraz-based acaricide); Error bars indicate standard error of mean (SEM)



ANF – aqueous neem fruit extract; amitraz – Triatix spray (12.5% amitraz based acaricide); Error bars indicate Standard Error of Mean (SEM)

have effect also on muscles, insect gut, central nervous system, immune system, etc. that cause death of insects (Atawodi and Atawodi 2009). Other plant-based products investigated for their efficacy against mange in a number of livestock species include tea tree oil (*Melaleuca alternifolia*), clove oil (*Syzygium aromaticum*), palmarosa (*Cymbopogon martini*), Lippia oil (*Lippia multiflora*), eucalyptus oil (*Eucalyptus radiata*), and rhizomes (turmeric) of *Curcuma longa* (Gopinath et al. 2018).

Neem-based preparations are not only effective for mange mites control in pigs. Various preparations of neem extracts have demonstrated in vitro or in vivo efficacy against sarcoptic mange in several other species such as sheep (Tabassam et al. 2008), dogs (Abdel-Ghaffar et al. 2008), and rabbits (Seddiek et al. 2013). Neem-based products are reported to efficiently control arthropods of medical and veterinary importance other than mange inducing mites, mosquitoes, tabanids, sand flies, and ticks through direct inhibition of the egg, larvae, and adult stages of the species or repellent action (Khater 2013; Agbo et al. 2019). Although acaricide chemicals such as ivermectin and amitraz are generally used for the treatment of sarcoptic mange in livestock, they are not safe to the user and the environment, are expensive, and may lead to development of resistance when not used well (Bernigaud et al. 2019; Sharun et al. 2019). On the other hand, plant-based compounds have several modes of action and are

more environmentally friendly, safer, and effective against a range of plant and animal parasites and pathogens (Khater 2013).

Koul et al. (1987) observed that the effects of neem extracts are dose and time-dependent and relative to the mode of application. The extraction method used in this study was not the best extraction method for biological compounds. Bio-active compounds in plants are usually partially soluble in water. Thus, extraction by different alcohols could have produced a more potent extract compared to simply using water and a detergent solution. Choice of the aqueous extraction method was based on cost and feasibility for use by resource-poor farmers.

## Conclusion

Effective control of mange mite based on current acaricidal agents is proving to be a huge challenge, with reports of emerging resistance and treatment failures. This study observed that aqueous fruit extracts of neem (*Azadirachta indica*) have acaricidal properties against mange mites (*Sarcoptes scabiei* var. *suis*) in grower pigs. Topical sprays of 25% aqueous neem fruit extract had the same efficacy as commercial amitraz drugs in mange mite control, without inducing adverse effects on treated pigs. Therefore, aqueous

neem fruit extracts offer a cheaper, safer, and more eco-friendly alternative to commercial drugs for control of arthropods harmful to human and animal health. Such extracts can be used to build novel biocides for use in crop and livestock protection.

**Acknowledgments** This research was supported by the Pig Industry Board (PIB), Zimbabwe, who donated the animals, drugs, and site used for the study. We are grateful to all staff and leadership of the PIB for the support rendered.

**Data availability** The data that support the findings of this study are available from the corresponding author on request.

### Compliance with ethical standards

The study was approved by the ethics committee of the Pig Industry Board (PIB, Zimbabwe), and all animal rights issues were appropriately observed during the experiment. Experimental treatments and sample collection procedures conform to European Union Directive 2010/63 regarding the protection of animals used in scientific experiments.

**Conflict of interest** The authors declare that they have no conflict of interest.

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