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ORIGINAL ARTICLE

Toxicity of *Millettia ferruginea darasana* (family: Fabaceae) against the larvae and adult ticks of *Amblyomma variegatum* Fabricius a three-host tick in cattle

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Abstract The in vitro toxicity of *Millettia ferruginea darasana* (family: Fabaceae) was tested against the larvae adult male and female of a three-host tick, *Amblyomma variegatum* Fabricius (family: Ixodidae or hard tick), known as 'tropical bont tick' parasitic mainly to cattle found in Ethiopia and other equatorial Africa. The 20, 40, 60, 80 and 100 % concentrations of the seed oil extracted with petroleum ether were found to kill all (100 % mortality) larvae after 12, 9, 6, 3 and 1.5 h respectively. The results summarized in the Table 1 was found to be statistically significant at the probability level of $p = 0.05$. The 100 % concentration of the oil caused 100 % mortality of adult male, adult female and fully engorged female tick after 5, 7 and 12 h respectively. The root and root bark showed less toxicity. The leaves did not show any toxicity.

Keywords Toxicity · In vitro · *Millettia ferruginea* · Fabaceae · *Amblyomma variegatum* Fabricius · Tick · Cattle

Introduction

The plant *Millettia ferruginea darasana* (family: Fabaceae) is a large tree growing up to 25 m (~75 feet) high. It is widely distributed all over Ethiopia within the agro climatic zones of 1,000–2,500 m (~3,000–7,500 feet) above the sea level. The tree is commonly known as 'Berebera' (in Amharic), Sotallo, Kotalu, Sari, Yego (in Afan Oromo), Enghediksho (in Sidama), Zaghia (in Wolaita), Dhadhato (in Gedoffa) languages (Getahun 1976). The mature seeds are used for catching fish due to its toxicity is a common practice in the country. It was believed that 'Rotenone' present in the seeds is responsible for fish poisoning. Moreover, it serves as shade tree for coffee (*Coffea arabica*) plantation in Eastern and Southern parts of Ethiopia. The plant is traditionally used to treat skin infection (Mesfin et al. 2009) and for dressing 'mujele' an infection caused by an insect present in the soil (Teklelehaymanot and Giday 2007). The seed extract of this plant has been observed to be effective in controlling storage insect pests as adzuki bean beetle, *Callasobruchus chinensis* (Mulatu 2007), maize weevil, *Sitophilus zeamais* (Jembere 2002; Jembere et al. 2007), bean bruchid, *Zebrotes subfaciatus* (Habeeb 2010) and mosquito larvae (Asegid et al. 2007). The aqueous suspension of the seeds showed high toxicity against the larvae of maize stalk borer *Busseola fusca* (fuller) (Lepidoptera: *Noctuidae*) on the first day after hatching of eggs (Tilahun et al. 2009).

Various parasitic diseases are transmitted by different types of ticks present in the environment that stick to the bodies of different animals like cattle, sheep, goat, horse, donkey, dog, rabbit etc. The cattle and sheep are mostly affected due to large infestation of different types of ticks. The ticks not only suck blood from the cattle and sheep but also transmit various kinds of diseases to the animals (Shaw et al. 1970; Soulsby 1982).The cattle become anaemic and ill-health due to loss of blood causing a low production of milk and meat. Severe infestation usually leads to premature death of the infected animals after a long period of suffering. Infected mammals bite the sites of infection or rub their bodies against hard objects thus damaging the skin. These infected animals therefore do not attract good prices in livestock market, their skin become worthless in the leather industry and also due to the presence of a large number of holes in it caused by the tick-bite.

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We were interested to find a cheap, readily available and better substitute natural product for synthetic insecticide. The other information led us to work on this plant is its acaricidal activity which was not explored so far.

Amblyomma variegatum is also known as 'variegated tick' or 'tropical bont tick' (family: Ixodidae or hard tick) is a three-host tick that originated in Africa (Yonow 1995). It is an African species generally distributed throughout the Ethiopian region, south-western Africa and southern Africa. Since then it has spread to several countries, including the Caribbean island, where it is known as the Senegalese tick. It is commonly found in cattle, sheep and goats and rarely on birds. The tropical bont tick is of greatest economic significance and has had a huge effect on the livestock industry, primarily through its transmission of *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*, Allan et al. 1998) causing heart water disease (Nairobi sheep disease) and *Coxiella burnetii* in cattle and sheep (Soulsby 1982). In addition, it is associated with streptothricosis, the actinomycete infection of the skin of cattle, caused by *Dermatophilus congolensis* (Sewell and Brocklesby 1992). The fully engorged female may lay up to 20,000 eggs (Shaw et al.1970).

A disease of human health concern transmitted by the tropical bont tick is African tick-bite fever, caused by *Rickettsia africae*, which results in fevers, headaches and swollen lymph nodes (Parola et al. 1999). The distribution of African tick-bite fever is considered widespread in the Caribbean but there have been only a few positive human cases reported (Kelly et al. 2010; (NTHNC) 2008). Although the tick is not considered a vector of yellow fever, it may serve as a potential concern for human infection, as well as a reservoir, for the disease as the virus has been isolated from it (CDC 2001; Merck 2011).

The isolation of three rotenoids namely rotenone, deguelin and tephrosin from the seeds were previously published (Clark 1943), many isoflavones namely ferrugone, durmillone from the seeds (Highet and Highet 1967), ferrugone, jamaicin, ichthynone, 7-hydroxy-5,6-dimethoxy-3′,4′-methylenedioxy isoflavone, flemichapparin-B from the bark, durmillone, 5-methoxy-durmillone, 5-hydroxy durmillone, calopogonium isoflavone-A, calopogonium isoflavone-B, 4-hydroxy-lonchocarpine, barbigerone, preferrugone, predurmillone, prebarbigerone, pre-5-methoxy durmillone reported earlier from this plant (Dagne et al. 1989; Dagne and Bekele 1990).

The toxicity of neem seed oil against the larvae of *A. variegatum*, a three-host tick in cattle (Ndumu et al. 1999), toxicity of neem seed oil against the larvae of *Rhipicephalus sanguineus*, a three-host tick in dog (Choudhury 2001), toxicity of neem leaf against the larvae of *Boophilus decoloratus*, a one-host tick in cattle (Choudhury 2003), toxicity of neem seed oil against the larvae of *B. decoloratus*, a one-host tick in cattle (Choudhury 2009) were published earlier.

The most interesting part of this article is that besides its toxicity against fish and some other insects, the present article reports the in vitro toxicity of the crude petroleum ether extract (oil) of the seeds of *Millettia ferruginea* (Berebera) for the first time against the larvae and both mature male and female ticks of *A. variegatum* Fabricius.

Materials and methods

Seed oil

The mature seeds were collected from a place close to Dilla University campus in the month of December–January and

Table 1 Toxicity of the seed oil extracted with petroleum ether against the larvae of *Amblyomma variegatum* at different concentrations and every 1.5 h interval of time

Plate No.	Amount of oil taken (ml, %)	Total number of dead larvae with SD and % mortality							
		1.5 h	3 h	4.5 h	6 h	7.5 h	9 h	10.5 h	12 h
1	Water (1 ml,0 %) control	0	0	0	0	0	0	0	0
		0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %
2	0.2 (20 %)	0	2 ± 0.5	5 ± 0.82	8 ± 0.82	11 ± 0.58	12 ± 0.82	13 ± 0.96	15 ± 1.41
		0 %	13 %	33 %	53 %	73 %	80 %	87 %	100 %
3	0.4 (40 %)	2 ± 0.82	3 ± 0.5	6 ± 0.96	9 ± 0.58	12 ± 0.82	15 ± 0.96		
		13 %	20 %	40 %	60 %	80 %	100 %		
4	0.6 (60 %)	5 ± 0.96	8 ± 0.82	11 ± 0.58	15 ± 0.5				
		33 %	53 %	73 %	100 %				
5	0.8 (80 %)	9 ± 0.82	15 ± 1.29						
		60 %	100 %						
6	1.0 (100 %)	15 ± 0.5							
		100 %							

15 Larvae were used in each replicate. Values are given as mean of 4 replicates

powdered using a mortar and pestle. The powdered seeds (20 g) was extracted (Soxhlet) with petroleum ether (b.p. 60–80 °C) for 6 h. The solvent after extraction was removed by distillation on a water bath when viscous yellow coloured oil obtained (7.52 g, 37.6 % yield).

Collection of ticks

The larvae, adult male, adult female and fully engorged female tick of *A. variegatum* were used in this study. The engorged female (full of blood), mature male and female ticks were collected from the bodies of the cattle in Oromiya regional state, Borana zone, Abaya woreda, Samaro kebele near Dilla town, Ethiopia. Each fully engorged female tick was carefully detached from the body of the cattle by hand picking, placed separately in a clean test tube plugged with cotton and incubated at 30 °C with a relative humidity of about 80 %. After the complete laying of eggs (it took only a few days for laying eggs) the ticks died (completion of life cycle) and the dead female ticks were discarded.

The eggs laid in each tube were transferred into a clean tube with a cotton plug. The eggs were kept under the same incubating conditions until they hatched into larvae (it took almost 2 months) and then starved in the incubator at 30 °C for 1 week before use. The adult male and female ticks were collected in a similar way from the bodies of cattle and kept in a clean glass tubes plugged with a cotton plug and the bioassay carried out soon after 30 min of collection. All the ticks were properly identified at Holeta Research Centre, Ministry of Agriculture and Rural Development, National Animal Health Diagnostic and Investigation Centre, Addis Ababa, Ethiopia.

In vivo test

It is practically impossible to carry out the in vivo experiment due to mobile character of the larvae as well as the adult ticks attached to the animal. The movement of the animal also cannot be controlled that also adds to the difficulties towards the in vivo experiment. Therefore, as usual the in vitro experiment was carried out which is very convenient and easy to perform.

In vitro test

Petri dishes (9 cm diameter) with one sheet of filter paper inside of the same size were used for the experiment. Five different amounts of seed oil, i.e., 1 ml (100 %), 0.8 ml (80 %), 0.6 ml (60 %), 0.4 ml (40 %), 0.2 ml (20 %) and 1 ml distilled water (0 %, control) were evenly soaked (spread with a spatula) in different filter papers of each Petri dish. Fifteen (15) larvae were put inside in each plate with the help of a soft brush and then monitored at certain interval of time. The number of larvae found dead in each plate was recorded and the results summarized in Table 1. The experiment was repeated four times.

Results and discussion

The experiment was conducted according to the procedure cited in the literature (Choudhury 2001, 2003, 2009; Ndumu et al. 1999). From the Table 1, it is clear that the mortality of larvae was concentration and time dependent. With 0 % concentration (control) there was no mortality at any time. 100 % mortality was observed with 20, 40, 60, 80 and 100 % seed oil after 12, 9, 6, 3 and 1.5 h respectively. Table 2 showed the 100 % mortality of the adult male, adult female and fully engorged female after 5 h (LT_{50}, 2.45 h), 7 h (LT_{50}, 3.46 h) and 12 h (LT_{50}, 5.48 h) respectively with the seed oil. The results showed that the fully engorged females were most resistant to toxic oil followed by adult females and adult males. Table 3 showed that the toxic constituent(s) is/are totally absent in the leaves. The toxicity was found highest in the seeds (100 % mortality in 2 h) followed by the root (100 % mortality in 2.5 h) and root bark (100 % mortality in 3 h). The engorged female delayed laying eggs when came in contact with the oil for a short time.

For a particular concentration, the statistical analysis showed that there was a significant difference with the increase of time regarding the mortality at 95 % level ($p = 0.05$). Similarly, for a particular time, the statistical analysis showed that there was a significant difference with increase of concentration regarding the mortality at 95 % level ($p = 0.05$).

Eradication of ticks

In Africa, birds are the primary means of biological control of ticks, but there is a potential risk to cause additional skin damage as they peck at the host's skin (Samish 2006). In regions such as the Caribbean, birds are not as successful in controlling the ticks (Popham et al. 1996). An attempt made using a parasitic wasp, *Ixodiphagus* sp., was shown to reduce the host nymph populations for ~2 years but a sustained and effective population of the wasp could not be maintained. Other methods of biological control such as nematodes, bacteria and fungi have also not been very successful to date (Samish 2006). Eradication campaigns are currently under way in the Caribbean to end the economic losses and perhaps prevent further spread of *A. variegatum* (Barre and Garris 1990; Barre et al. 1995; Pegram and Eddy 2002).

Table 2 Toxicity of the petroleum ether extract (free of solvent) of the seeds against the adult male, adult female and fully engorged female of *Amblyomma variegatum*

Volume of PE extract put (ml)	Nature of tick	Number of ticks under investigation	100 % mortality of ticks after (h)	LT_{50} (h)
1	Adult male	20	5	2.45
1	Adult female	20	7	3.46
1	Fully engorged female	20	12	5.48

Table 3 Toxicity of the petroleum ether extract (free of solvent) of leaf, seed, root and root bark against the larvae of *Amblyomma variegatum*

Plant material, amount of extract put	Total number of larvae died after 1/2 h interval of time with % mortality			
	1.5h	2 h	2.5h	3 h
Leaf (1 ml)	0	0	0	0
	0 %	0 %	0 %	0 %
Seed (1 ml)	14.8	15		
	99 %	100 %		
Root (1 ml)	11.8	14.8	15	
	79 %	99 %	100 %	
Root bark (1 ml)	11.0	13.3	14.8	15
	73 %	89 %	99 %	100 %
Water (control, 1 ml)	0	0	0	0
	0 %	0 %	0 %	0 %

15 Larvae were used in each replicate. Values are given as mean of 4 replicates

Primary control of tropical bont tick is through the use of acaricides (Norval et al. 1992). Some work has been done using a combination of pheromone and acaricides to help reduce the costs associated with the chemical as well as improve kill efficacy (Allan et al. 1998; Norval et al. 1992). Footbaths containing acaricides have been used with incomplete success, as the ticks often will attach between the hooves (Stachurski and Lancelot 2006). The use of neem seed oil can be effective but is very dependent on the concentration and contact time (Ndumu et al. 1999).

The comparative studies of our present findings with that of neem seed oil (*Azadiracta indica*) against *A. variegatum* revealed the fact that the oil obtained from the seeds of this plant, *M. ferruginea* possesses much higher acaricidal activity (efficacy) than neem seed oil. The 100 % concentration of neem seed oil took 48 h for 100 % mortality of the larvae (Ndumu et al. 1999) whereas 100 % concentration of this plant seeds took only 1.5 h, almost 30 times more active than neem seed oil.

The seed extract (oil) was found to be safe to the skin of the cattle. When the oil (100 % concentration) was applied to the body (skin) of the cattle and kept under close observation for more than a week, no irritation or inflammation on the skin of the cattle was observed. The cattle did not show any uncomfortable feeling. No larvae or adult male/female were found within the close vicinity of the oil applied. This again showed the toxic nature of the oil.

The present article reports the acaricidal activity of the seed oil from this plant for the first time. Since the seed oil of *M. ferruginea* is inexpensive and readily available; the oil can safely be used for the animals. The natural source will be a much better substitute for the synthetic insecticide due to its very low cost, easy availability and bio-degradability. Eventually there is a more risk of using synthetic insecticide causing an environmental pollution. Two isoflavonoid compounds were isolated and characterized by the present authors during the phytochemical investigation of the seed but their detailed studies as regard to acaricidal activities could not be carried out due to very small amount (few mg) of the compounds. It needs lots of compounds to perform such type of experiment. The phytochemistry part is a very tenacious, hard work, long drawn process; it needs plenty of chemicals, man power and money. The isolation and characterization of bioactive compound (s) is in progress.

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