International Journal of Acarology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/taca20

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To cite this article: Adolat U. Mirzaeva, Udipta Chakraborti, Kh. R. Tukhtaev, F. D. Akramova, D. A. Azimov, E. I. Musabaev, S. Sh. Rabbimov & Kakali Bhadra (2022): Acaricidal efficacy of bitter almond against two tick species, *Argas persicus* and *Haemaphysalis darjeeling* – an *in vitro* study, International Journal of Acarology, DOI: 10.1080/01647954.2022.2108494

To link to this article: https://doi.org/10.1080/01647954.2022.2108494

INTERNATIONAL JOURNAL OF ACAROLOGY
https://doi.org/10.1080/01647954.2022.2108494

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Acaricidal efficacy of bitter almond against two tick species, *Argas persicus* and *Haemaphysalis darjeeling* – an *in vitro* study

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ABSTRACT

Use of plant extracts as acaricides for controlling ticks is an essential area of research concerning economic security and environmental health. The aim of this study was to focus on the acaricidal efficacy of bitter almond powder and bitter almond extracts (aqueous and alcoholic) against the nymph and adult stages of two tick species. It was evaluated at five different concentrations (1% to 5%) with bitter almond powder, bitter almond extracts, and alpha-cypermethrin (1 ml/100 ml) at four different exposure times (24, 48, 72, and 96 hours). The effectiveness of bitter almond alcoholic extract was significantly higher than the aqueous extract, and effectiveness was lowest with the powder at all concentrations at different exposure times for both species. Furthermore, aqueous and alcoholic extracts showed a higher mortality rate among both species than alpha-cypermethrin. LC_{50} value showed that the toxicity level of bitter almond acaricides increased with the longer exposure time and higher concentration. Toxicity level decreased in the order of alcoholic > aqueous > powder on both species. For both species, the highest toxicity level of bitter almond alcoholic extract was shown for the adult than nymph. Therefore, extracts of bitter almond can be used as an excellent acaricidal alternative to alpha-cypermethrin against these two species.

ARTICLE HISTORY
Received 16 February 2022
Accepted 25 July 2022
Published online 11 August 2022

KEYWORDS
Acaricide; *Argas persicus*; bitter almond; toxicity; *Haemaphysalis darjeeling*

1. Introduction

Ticks are highly specialized arthropods characterized as temporary obligate vertebrate ectoparasites. Worldwide, Guglielmone et al. (2010) reported 193 tick species from the family Argasidae and 702 tick species from the family Ixodidae.

Ticks are reported as vectors of various rickettsial, protozoal, fungal, bacterial, and viral diseases in humans and animals (Ghosh and Nagar 2014; Taha and Ali 2020; Udayan et al. 2020). Most of these tick-borne diseases are zoonotic in nature, infective to the human population closer to the forest and farm animals. The majority of tick species utilize a new host during each of their developmental stages of life; therefore, the infections by these ectoparasites in animals cause distressing economic losses (Nwanade et al. 2020; Udayan et al. 2020). Hence, tick and tick-transmitted diseases are major livestock constraints around the world. Tick-borne human diseases like Lyme and Crimean Congo haemorrhagic fever (CCHF) cause serious problems for public health in European and American countries. Tick-borne diseases like Theileriasis and Babesiosis are significant veterinary problems in numerous countries (Geevarghese and Mishra 2011). These destructive ectoparasites cause food insecurity and economic loss, with an estimated global loss of ~7 billion USD per year against control and productivity (Kemal et al. 2020).

In the context of controlling tick population and tick infestation, use of chemical acaricides is the most common method (Rodriguez-Vivas et al. 2006), but application of these acaricides is hazardous to the environment, causing environmental contamination, while, biological pesticides are environmentally friendly and more effective (Pourseyed et al. 2010). Various chemical acaricides such as organophosphate compounds (like Comaphous, malathion), amitraz, synthetic pyrethroids, and carbamate carbaryl are being used (Ghosh et al. 2007; Davey et al. 2008; Pourseyed et al. 2010; Krishna et al. 2014). For controlling the tick population and disease transmission, synthetic pyrethroids are also frequently and widely used as acaricides (Sharma et al. 2012). With increasing number of farms and continuous application of chemical acaricides, issues of acaricidal resistance (Kumar et al. 2011; Sharma et al. 2012; Singh et al. 2014), food contamination, and toxicity in human health are developing (Fernandez-Salas et al. 2011; Krishna et al. 2014; Kemal et al. 2020). Hence, recent researchers are focusing on the alternatives, with the main focus on the application of biological pesticides as acaricides (Moawad et al. 2015; Ebadah et al. 2016; Moawad and Sadek 2018). These biological pesticides are eco-friendly, less expensive, and in some cases, are reported to be more effective than chemical pesticides (Alonso-Díaz et al. 2008; Pourseyed et al. 2010; Fernandez-Salas et al. 2011). Earlier studies on various plant extracts were reported with different acaricidal impacts (Srivastava et al. 2008; Krishna et al. 2014; Nwanade et al. 2020). About 119 plant species from 47 families have been reported to have anti-parasitic secondary metabolites, acting as a repellent and acaricide on various tick species.

Bitter almond, *Amygdalus communis* L., belongs to the Rosaceae family, distributed throughout the world. The presence of flavonoids and different phenolic compounds make the extract of whole almond possess free radical scavenging ability (Sfahlan et al. 2008). Amygdalin is a natural cyanogenic glycoside found in the seeds of bitter almond (Jaszczak-Wilke

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/01647954.2022.2108494.

Published online 11 Aug 2022

et al. 2021). This cyanogenic glycoside was reported to be hazardous for bees and reported to have an LC_{50} of 30 ppm against adult bees (Johnson 2014). Yu et al. (2018) reported that three toxic beta glucosides, namely phlorizin, santonin, and amygdalin, have an impact on insect carbohydrate metabolism and suggested using these beta glucosides as a guided compound for pesticides.

Previous studies reported the acaricidal efficacy of various chemical pesticides such as cypermethrin and permethrin against *Argas persicus* (Oken, 1818) (Dusbabek et al. 1997). As an alternative to these chemical pesticides, plant extracts of *Azadirachta indica* (Khan et al. 2001), essential oil from *Haplophyllum tuberculatum* and *Artemisia monosperma* (Abdel-Shafy et al. 2006), and extracts from different cryptogam plants (Taha and Ali 2020) and different strains of entomopathogenic fungi, *Metarhizium anisopliae* (Pourseyed et al. 2010), were reported as biological acaricides against *A. persicus*. However, to the best of our knowledge, no detailed studies have been found on biological acaricides against *Haemaphysalis darjeeling* Hoogstraal and Dhanda, 1970. A previous report by Ronghang and Roy (2016) stated the prevalence of *H. darjeeling* infection in *Bos frontalis* (Mithun) in the Indian state of Arunachal Pradesh and also revealed that this tick species has seasonal fluctuation in their occurrence with peak period during the rainy season (June to August). Geevarghese and Mishra (2011) reported the adult stage of *H. darjeeling* from goat, antelope, wild boar, barking deer, and humans.

The present study highlights the acaricidal efficacy of bitter almond powder and aqueous and alcoholic extracts along with their toxicity level on different developmental stages of *A. persicus* and *H. darjeeling* at different concentrations and exposure times. This study further evaluates the impact of alpha-cypermethrin (1 ml/100 ml) as a synthetic acaricide on these tick species and compares its acaricidal activity with the bitter almond powder and aqueous and alcoholic extracts.

2. Material and methods

2.1. Tick species collection

Argas persicus is a soft-bodied tick belonging to the family Argasidae. *Argas persicus* was collected from henhouses and cattle farms in Tashkent and Sirdarya regions located in the northwestern part of Uzbekistan. *Haemaphysalis darjeeling* is a hard tick species belonging to the family Ixodidae. *Haemaphysalis darjeeling* was collected from the body of Jersey cow from the animal farms located at Ashoknagar area in the state of West Bengal, India. For both species, nymph and adult stages were collected (Figure S1).

2.2. Bitter almond extracts preparation

Bitter almonds (*Amygdalus communis* L.) were collected from Bostanlyk district of Tashkent region. For the experimental analysis, a total amount of 1 kilogram bitter almond was taken, and seeds were isolated from bitter almond. Further, the amniotic membrane was separated by immersion for 1–2 minutes in boiling water. Thereafter, the seeds were cold pressed, and the oil was removed. The cake was crushed mechanically in a mortar to produce the bitter almond powder used in this study.

Bitter almond aqueous extraction was carried out with a water bath at a temperature of 40–50°C for 40 minutes. The ratio of raw material and extractant was 1:10. Further, the raw materials were kept in the extractant for a day, and after removing the water content, the mixture was light brown in colour. The extract is soluble in water.

Bitter almond alcoholic extraction (Ethanol with a purity of 90%) was carried out in a water bath at a temperature of 70–80°C for 40 minutes. The ratio of raw material and extractant was 1:10, and the raw materials were kept in alcohol for a day. The extract was presented in the form of milk powder which was dried at a temperature of 40°C in a vacuum cabinet.

Detailed experimental procedure for extractions was followed according to the technique adopted by Khamidov et al. (2021). Each of the extraction procedures for bitter almond powder, aqueous and alcoholic extracts have been replicated two times.

For the first set of extractions (SET A), 1 kg of bitter almond was converted into powder, the aqueous extraction was performed with powder and extractant in 1:10 ratio and the yield was 1.31 ± 0.06 gm or 13.12 ± 0.6%; the alcoholic extraction was conducted with powder and extractant in 1:10 ratio and the yield was 1.07 ± 0.01 gm or 10.7 ± 0.01%. For the second set of extraction SET B, 1 kg of bitter almond was converted into powder, the aqueous extraction was performed with powder and extractant in 1:10 ratio and the yield were 1.35 ± 0.02 gm or 13.52 ± 0.62%; the alcoholic extraction was conducted with powder and extractant in 1:10 ratio and the yield was 1.1 ± 0.01 gm or 11.2 ± 0.16%. The SET B extraction showed a comparatively higher range of yield than SET A for aqueous and alcoholic extracts; therefore, further experiments for acaricidal activities for SET A include three replicates (n = 3) and SET B include four replicates (n = 4).

2.3. Determination of acaricidal efficiency of bitter almond extracts

The experiments were performed for the quantification of the acaricidal efficiency of bitter almond extracts on nymph and adult stages of *A. persicus* and *H. darjeeling* tick species.

Each experiment was conducted with 40 tick samples placed in a clean petri-dish and monitored for 24, 48, 72, and 96 hours with respective acaricides. Two sets of experimental set-up were prepared, respectively, for both the bitter almond extraction replicates, in which one set (SET A) includes three replicates and another (SET B) comprises four replicates for each of the concentrations and each exposure time. As treatment representatives, bitter almond powder, bitter almond aqueous extract, bitter almond alcoholic extract, and alpha-cypermethrin were used, and besides this, one experimental setup was maintained as control with no exposure. The experimental set-up for bitter almond powder and both the extracts (aqueous and alcoholic) were sprayed with five concentrations (1%, 2%, 3%, 4%, and 5%) of respective acaricides, and for alpha-cypermethrin, the exposure concentration was 1 ml/100 ml.

All the experiments were carried out under relative humidity 81 ± 1.5% and temperature 26 ± 3°C.

2.4. Statistical analysis

t-Test analysis was performed to show the acaricidal efficacy of two extraction replicates for all the bitter almond extracts among different exposure times. One-way ANOVA was used to understand the significant variation in the mortality of tick species among different bitter almond acaricides (powder, aqueous extract, and alcoholic extract) for all the concentrations and alpha-cypermethrin (1 ml/100 ml) at each exposure time. Tukey's pairwise post hoc test was performed for respective ANOVA analysis to visualize the significant differences among the efficiency of different acaricides. All the data

were tested for homogeneity of variance by performing Levene's test (untransformed data). Non-significant test values were assumed to be homogeneous (equal variance). Non-homogeneous (unequal variance) data or significant test values were subjected to the Welch's ANOVA test analysis. *t*-Test and ANOVA analysis were performed by using PAST software version 4.05 (Hammer et al. 2001). All the test values were regarded as significant when $P < 0.05$.

For the quantification of toxicity of different bitter almond acaricides among different exposure time, LC_{50} value was calculated by using probit analysis. The analysis was performed with Ecotox package (Hlina et al. 2021), and the graphical representations of probit analysis were performed by using ggplot2 package in R software (R studio Team version 2020, 1.3.1).

3. Results

t-Test analysis between the acaricidal efficacy of SET A and SET B showed non-significant variation for all concentrations and exposure times regarding both the tick species in their different developmental stages (Tables S1–S5).

The experiments regarding the acaricidal efficacy of bitter almond powder and both the extracts were performed on the pooled datasets of SET A and SET B; therefore, each experiment comprises seven replicates. The results showed the highest mortality at 5% concentration and furthermore, these experiments also profiled that both tick species showed the highest mortality at 96 hours of exposure time (Table 1 and Figure S2). The mortality rate against different stages of both species was higher for both aqueous and alcoholic extracts compared to alpha-cypermethrin (Table 1 and Figure S3). Whereas bitter almond powder with 1% extract for 48 hours exposure time (mortality 16.4 ± 1.7) and 1%, 2%, and 3% extract for 72 hours exposure time (mortality 1% = 19.8 ± 1; 2% = 21.8 ± 1; and 3% = 23.4 ± 0.5) showed a lower range of mortality than alpha-cypermethrin among respective exposure times for the nymphal stage of *A. persicus*, the efficiency of bitter almond powder on the mortality rate of adult stages of *A. persicus* species was higher than alpha-cypermethrin throughout all concentration and exposure time. Regarding *H. darjeeling*, bitter almond powder with 1% extract for 24 hours exposure time (mortality 6 ± 0.8); 1% extract for 48 hours exposure time (mortality 12 ± 0.8), 1% and 2% extract for 72 hours exposure time (mortality 1%, = 4.1 ± 1 and 2% = 18.4 ± 0.2), and 1% and 2% extracts for 96 hours exposure time (mortality 1% = 17.2 ± 2.3 and 2% = 20.8 ± 1.5) showed a lower range of mortality than alpha-cypermethrin for the nymphal stage among the respective exposure times. In the case of the adult stage of *H. darjeeling*, the mortality rate with bitter almond powder showed a lower range only in 1% concentration with 96 hours exposure time (mortality-23.4 ± 1.3) than the mortality with alpha-cypermethrin with 96 hours exposure time

ANOVA analysis accompanied by the post hoc analysis concluded that alcoholic extract of bitter almond showed significantly higher mortality than bitter almond powder and alpha-cypermethrin, for both tick species in both the nymphal and adult stages for all the concentrations of bitter almond acaricides during all the exposure time (Table S6).

Regarding the evaluation of toxicity level, probit analysis explained that the LC_{50} value for both the bitter almond extracts and bitter almond powder was at its peak at 96 hours of exposure time. For both stages of the studied tick species, alcoholic extracts of bitter almond showed the highest level of toxicity, whereas powder showed the lowest toxicity level. For both tick species, the highest toxicity level showed by bitter almond alcoholic extract was for the adult at 96 hours (Adult: *A. persicus*: LC_{50} value = 0.018; Adult: *H. darjeeling*: LC_{50} value = 0.119) (Figures 1–4).

Mortality of different developmental stages of both tick species increased with concentration, and the highest concentration (5%) of the respective acaricides showed the highest mortality rate. In relation to the exposure times, the mortality rate of both stages in *A. persicus* and *H. darjeeling* increased with exposure time and was the highest at 96 hours.

4. Discussion

Previous studies relating to the impact of bitter almond reported that aqueous and alcoholic extracts showed potential antimicrobial action against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Salmonella typhi* (Abtahi et al. 2014). Essential oil extracted from bitter almond contains 21 different compounds, among which benzaldehyde, benzoic acid, and hexadecane were reported to be abundant, and showed antifungal activity on plant pathogenic fungi (Geng et al. 2016). Bitter almond essential oil also exhibits antifeedant and insecticidal activities against the egg and larval stages of the cotton leafworm insect *Spodoptera littoralis*, (Moawad and Sadek 2018).

The results of the present study demonstrated that both tick species showed a higher mortality rate for the alcoholic extract of bitter almond than bitter almond powder, aqueous extract, and alpha-cypermethrin. Ali and Taha (2022) reported that the alcoholic extracts from *Adiantum capillus-veneris* and *Funaria hygrometrica* plants showed acaricidal activity against *A. persicus* nymphs; moreover, their study also mentioned that methanol extract of *A. capillus-veneris* at a concentration of 4% showed the highest mortality rate against this tick species. In terms of mortality rate of nymph and adult stages of *A. persicus* and *H. darjeeling*, our study showed that 5% alcoholic extract of bitter almond with 96 hours of exposure showed the highest acaricidal activity in both the soft and hard tick, respectively. In relation to the chemical pesticides, the use of Cypermethrin and permethrin against *A. persicus* is very common. In search of a substitute for these chemical pesticides, plant extracts of *Azadirachta indica* (Khan et al. 2001), *Haplophyllum tuberculatum*, and *Artemisia monosperma* (Abdel-Shafy et al. 2006) and different strains of entomopathogenic fungi (Pourseyed et al. 2010) were reported as biological acaricides against *A. persicus*. (Dusbabek et al. 1997).

Our study prominently explained that in comparison to the acaricidal effect of the chemical pesticide alpha-cypermethrin, bitter almond extracts are much more considerable for both species. LC_{50} value of the acaricides (except alpha-cypermethrin) showed that the toxicity level of all three bitter almond acaricides was ordered as alcoholic extract > aqueous extract > powder and increased with the longer exposure time. For both species, toxicity level was higher for adult stages than for nymphal stages for alcoholic extract. The present study clearly implies that the alcoholic extract of bitter almond showed the highest toxicity for both developmental stages in both species at 96 hours and during this exposure time, alcoholic extracts showed more toxicity on *A. persicus* than *H. darjeeling*. The toxicity level of bitter almond extracts may depend on the quantity of amygdalin. Since the amount of amygdalin was very insignificant in the aqueous extract of bitter almond compared to its ethanolic extract (Khamidov et al. 2021), this may be the reason for low mortality and low toxicity of bitter almond aqueous extract than the alcoholic extract.

Table 1. Comparative study on the effect of bitter almond powder and extracts (aqueous and alcoholic) for different concentrations (1–5%) along with alpha-cypermethrin (1 ml/100 ml) on the mortality (mean ± se) of tick species among different exposure time.

		Mortality (mean ± se)											
		Nymph						Adult					
					Alpha-cypermethrin 1 ml/100 ml						Alpha-cypermethrin 1 ml/100 ml		
Exposure time (hours)	Concentration (%)	Powder	Aqueous extract	Alcoholic extract	I	II	Control	Powder	Aqueous extract	Alcoholic extract	I	II	Control
Argas persicus													
24	1%	12 ± 2.1^{a}	18.1 ± 0.9^{ab}	19.7 ± 0.9^{ac}	bc	7.5 ± 0.2	0 ± 0.0	10.1 ± 0.7^{a}	20.1 ± 0.6^{ab}	20.2 ± 0.8^{ac}	abc	6.5 ± 0.2	0 ± 0.0
	2%	13.43 ± 1.4^{a}	19 ± 1.6^{ab}	21 ± 1.2^{ac}	abc			12.7 ± 1.1^{a}	21 ± 0.6^{ab}	23.2 ± 0.5^{ac}	abc		
	3%	15.4 ± 1.7^{a}	19.7 ± 1.6^{b}	21.4 ± 0.9^{ac}	abc			15.1 ± 0.9^{a}	21.7 ± 0.4^{ab}	24.2 ± 0.4^{abc}	abc		
	4%	16.7 ± 1.4^{a}	20 ± 0.9^{b}	22 ± 0.9^{ac}	abc			16 ± 1.6^{a}	22.4 ± 1^{ab}	25.2 ± 0.8^{ac}	abc		
	5%	18.1 ± 1.4^{a}	20.4 ± 0.2^{ab}	22.7 ± 0.1^{ac}	abc			18 ± 0.3^{a}	23.2 ± 0.2^{ab}	26.4 ± 0.2^{abc}	abc		
48	1%	16.4 ± 1.7^{a}	25.1 ± 0.9^{ab}	25.2 ± 1^{ac}	bc	16.8 ± 0.3	0 ± 0.0	15 ± 0.6^{a}	25.2 ± 0.9^{ab}	26.5 ± 1.3^{ac}	bc	12.1 ± 0.2	0 ± 0.0
	2%	17.2 ± 1.3^{a}	25.8 ± 1.5^{ab}	27.8 ± 1.7^{ac}	bc			17.4 ± 1.3^{a}	25.8 ± 1^{ab}	27.4 ± 1.3^{ac}	abc		
	3%	18.4 ± 1.7^{a}	26.2 ± 0.2^{ab}	28.5 ± 0.20^{abc}	bc			20 ± 0.3^{a}	26.5 ± 0.3^{ab}	28.2 ± 0.2^{abc}	abc		
	4%	20 ± 1.7^{a}	26.7 ± 1.2^{ab}	29.5 ± 1.2^{ac}	bc			20.2 ± 1.8^{a}	27.2 ± 1.5^{ab}	29.1 ± 1.7^{ac}	abc		
	5%	21.5 ± 1.9^{a}	27.8 ± 0.2^{ab}	30.2 ± 0.1^{ac}	abc			20.7 ± 0.2^{a}	28.7 ± 0.2^{ab}	30.4 ± 0.2^{abc}	abc		
72	1%	19.8 ± 1^{a}	28 ± 0.8^{ab}	28.7 ± 1.1^{ac}	abc	23.5 ± 0.2	0 ± 0.0	20.1 ± 0.8^{a}	30.1 ± 0.7^{ab}	33 ± 0.5^{abc}	bc	18.7 ± 0.2	0 ± 0.0
	2%	21.8 ± 1^{a}	29 ± 1.6^{ab}	30.5 ± 0.4^{ac}	bc			20.8 ± 0.9^{a}	31.5 ± 2^{ab}	33.8 ± 1.7^{ac}	bc		
	3%	23.4 ± 0.5^{a}	30.7 ± 0.2^{ab}	31.4 ± 0.2^{ac}	bc			25.2 ± 0.6^{a}	32 ± 0.6^{ab}	35.4 ± 0.4^{abc}	abc		
	4%	24.1 ± 0.8^{a}	31.5 ± 1.1^{b}	32.4 ± 0.5^{ac}	bc			26.1 ± 1.4^{a}	33 ± 0.9^{ab}	35.8 ± 0.4^{ac}	abc		
	5%	24.7 ± 0.3^{a}	32.2 ± 0.2^{ab}	33.2 ± 0.1^{ac}	abc			27.5 ± 0.2^{a}	33.7 ± 0.2^{ab}	36.2 ± 0.2^{abc}	abc		
96	1%	28.1 ± 1.2^{a}	31.5 ± 0.9^{b}	33 ± 1.4^{ac}	bc	25.7 ± 0.2	0 ± 0.0	30.4 ± 1.4^{a}	33.4 ± 0.6^{b}	36 ± 0.5^{ac}	abc	26 ± 0.3	0 ± 0.0
	2%	31 ± 0.6^{a}	32.4 ± 0.9^{b}	33.8 ± 0.7^{ac}	abc			31.8 ± 1^{a}	33.8 ± 0.9^{b}	36.1 ± 0.6^{ac}	abc		
	3%	32.2 ± 0.4^{a}	33.2 ± 0.1^{b}	34.2 ± 0.2^{ac}	abc			34 ± 0.3^{a}	34.8 ± 0.6^{b}	37.7 ± 0.3^{abc}	abc		
	4%	33.2 ± 0.5^{a}	35.1 ± 0.5^{ab}	36.7 ± 0.2^{ac}	abc			34.5 ± 0.8^{a}	35.8 ± 0.6^{b}	38.1 ± 0.2^{ac}	abc		
	5%	34.5 ± 0.3^{a}	36.2 ± 0.2^{ab}	37.4 ± 0.3^{ac}	abc			35.5 ± 0.2^{a}	36.8 ± 0.2^{b}	38.4 ± 0.5^{abc}	abc		
Haemaphysalis darjeeling													
24	1%	6 ± 0.8^{a}	17.1 ± 1.3^{ab}	21.8 ± 1^{abc}	bc	9 ± 0.9	0 ± 0.0	9.2 ± 1.7^{a}	17.2 ± 1.4^{ab}	23.1 ± 1.8^{ac}	bc	7.2 ± 0.9	0 ± 0.0
	2%	11.2 ± 0.9^{a}	20.8 ± 0.3^{ab}	23.2 ± 0.9^{ac}	bc			10.1 ± 1.2^{a}	18.2 ± 1.3^{ab}	24.4 ± 1.9^{abc}	bc		
	3%	15.2 ± 1.5^{a}	23 ± 1.7^{ab}	25.4 ± 1.2^{ac}	abc			11 ± 1.5^{a}	18.7 ± 0.3^{ab}	25.4 ± 0.2^{abc}	bc		
	4%	17.2 ± 1.3^{a}	26. ±1.4^{ab}	27 ± 1.5^{ac}	abc			16.7 ± 1.4^{a}	19.8 ± 1.3^{b}	26.2 ± 1.8^{abc}	abc		
	5%	18.7 ± 1^{a}	27.8 ± 1.3^{ab}	28.8 ± 1.2^{ac}	abc			19.1 ± 1^{a}	22.4 ± 1.3^{b}	27.2 ± 0.9^{abc}	abc		
48	1%	12 ± 0.8^{a}	21.8 ± 1.1^{ab}	27.1 ± 1^{abc}	bc	13.2 ± 1.1	0 ± 0.0	16.1 ± 1.5^{a}	22.7 ± 1.2^{ab}	28.5 ± 1.8^{abc}	bc	15.1 ± 0.9	0 ± 0.0
	2%	15.5 ± 0.2^{a}	24.8 ± 1.5^{ab}	29.5 ± 0.7^{abc}	bc			16.7 ± 1.6^{a}	25.2 ± 1.4^{ab}	31.8 ± 1.4^{abc}	bc		
	3%	19.5 ± 1.6^{a}	27.2 ± 1.8^{ab}	31.4 ± 0.9^{ac}	abc			17.4 ± 0.2^{a}	26.5 ± 0.2^{ab}	32 ± 0.6^{abc}	abc		
	4%	21.5 ± 0.5^{a}	29.7 ± 0.6^{ab}	32.4 ± 0.8^{ac}	abc			19.2 ± 1.5^{a}	28 ± 1.7^{ab}	33.2 ± 0.8^{abc}	bc		
	5%	26 ± 1.8^{a}	32.4 ± 1.3^{ab}	34.1 ± 0.7^{ac}	abc			21.5 ± 2.1^{a}	29 ± 0.8^{ab}	34.5 ± 1^{abc}	abc		
72	1%	14.1 ± 1^{a}	26.1 ± 1.1^{ab}	29.5 ± 0.9^{ac}	abc	21.1 ± 1.1	0 ± 0.0	19 ± 1.1^{a}	27.5 ± 0.9^{ab}	31 ± 1.5^{ac}	bc	17.5 ± 0.8	0 ± 0.0
	2%	18.4 ± 0.2^{a}	28.7 ± 0.8^{ab}	31.5 ± 0.5^{ac}	bc			19.8 ± 1.6^{a}	31.2 ± 0.9^{ab}	32.4 ± 1.1^{ac}	bc		
	3%	23.2 ± 1.2^{a}	30.4 ± 1.4^{ab}	32.5 ± 0.9^{ac}	bc			20.7 ± 0.3^{a}	32.8 ± 0.2^{ab}	34 ± 0.4^{ac}	abc		
	4%	25.5 ± 0.7^{a}	31.5 ± 1.1^{ab}	35.1 ± 0.9^{ac}	abc			23 ± 1.2^{a}	33.8 ± 1^{ab}	34.8 ± 1.5^{ac}	abc		
	5%	27.4 ± 1.6^{a}	33.8 ± 1^{ab}	36.2 ± 0.6^{ac}	abc			24.1 ± 1.5^{a}	34.5 ± 0.8^{ab}	36.7 ± 0.7^{ac}	abc		
96	1%	17.2 ± 2.3^{a}	29 ± 1.7^{ab}	31.5 ± 1.3^{ac}	ac	25 ± 0.3	0 ± 0.0	23.4 ± 1.3^{a}	30.1 ± 1.4^{b}	32.1 ± 1.2^{ac}	c	25 ± 1.5	0 ± 0.0
	2%	20.8 ± 1.5^{a}	31.7 ± 0.2^{ab}	33.8 ± 0.9^{ac}	abc			27 ± 1.7^{a}	32.4 ± 0.9^{b}	33.1 ± 1.4^{ac}	bc		
	3%	29.8 ± 1^{a}	33.2 ± 0.7^{ab}	35.5 ± 0.6^{ac}	abc			27.7 ± 0.3^{a}	33.5 ± 0.2^{ab}	34.1 ± 0.4^{ac}	bc		
	4%	31.5 ± 0.4^{a}	35.1 ± 0.5^{ab}	36.8 ± 0.3^{abc}	abc			28.8 ± 1^{a}	34.8 ± 0.7^{ab}	35.8 ± 0.6^{ac}	bc		
	5%	33.2 ± 0.6^{a}	36.8 ± 0.4^{ab}	38.1 ± 0.2^{ac}	abc			30.1 ± 1.6^{a}	36.8 ± 0.4^{ab}	37.7 ± 0.4^{ac}	abc		

- Each analysis was done with pooled data set ($n = 7$; for SETA $n = 3$ and SET B $n = 4$) for respective bitter almond extracts among different concentrations and different times.
- Means followed by the same letter on the same row are subjected to be significant at $p < 0.05$
- Alpha-cypermethrin (1 ml/100 ml) is compared with different extracts of bitter almond (powder, aqueous, and alcoholic) at different concentrations (1%, 2%, 3%, 4%, and 5%) among 24, 48, 72, and 96 hours of exposure, where the significance level ($p < 0.05$) is denoted by column (I) and mean value of dead tick species is denoted by column (II).
- Please see Table S6 for Levene's test values.

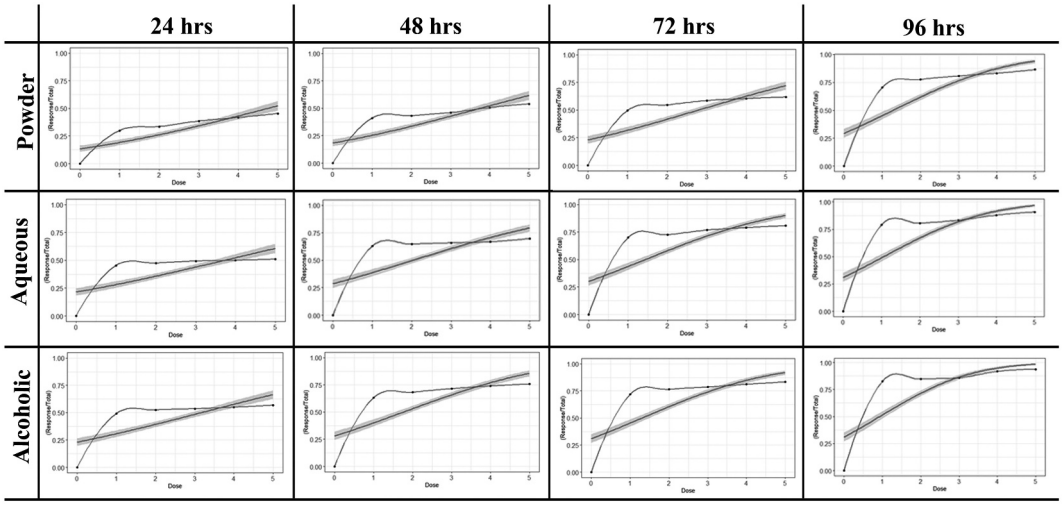

Figure 1. Graphical representation of bitter almond powder, aqueous and alcoholic extracts LC_{50} for each time point against *Argas persicus* nymph. Graphs were prepared using probit analysis and represented using "glm" method.

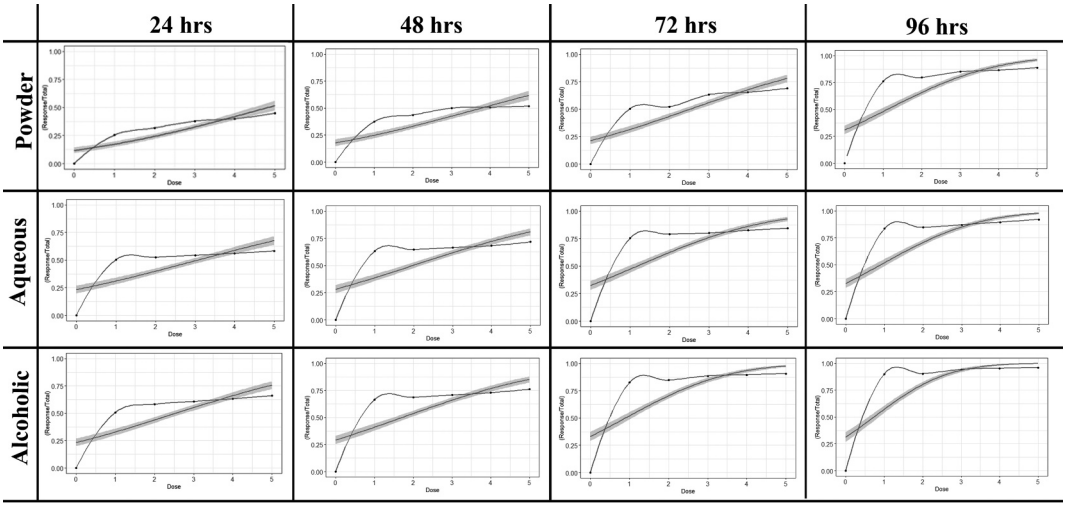

Fig 2

Figure 2. Graphical representation of bitter almond powder, aqueous and alcoholic extracts LC_{50} for each time point against *Argas persicus* adult. Graphs were prepared using probit analysis and represented using "glm" method.

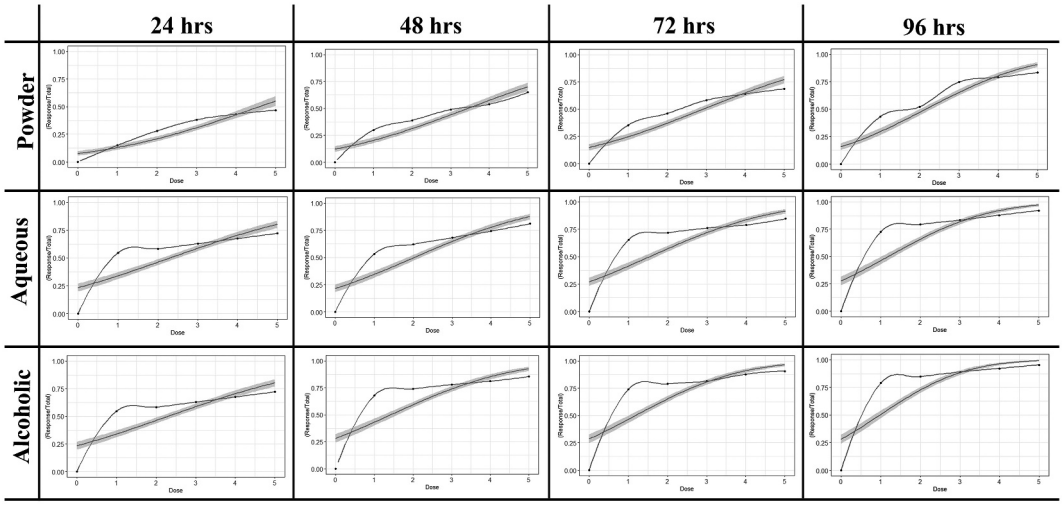

Figure 3. Graphical representation of bitter almond powder, aqueous and alcoholic extracts LC_{50} for each time point against *Haemaphysalis darjeeling* nymph. Graphs were prepared using probit analysis and represented using "glm" method.

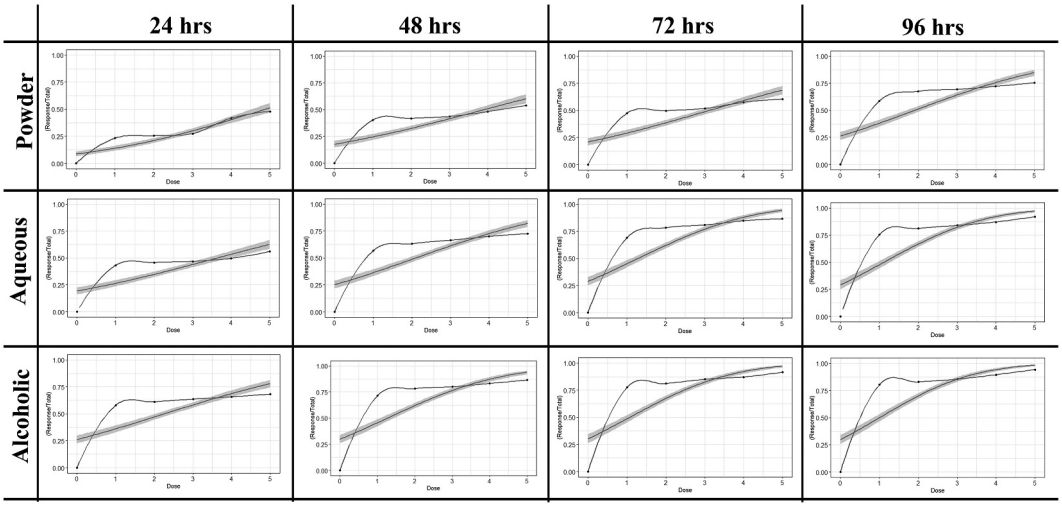

Figure 4. Graphical representation of bitter almond powder, aqueous and alcoholic extracts LC_{50} for each time point against *Haemaphysalis darjeeling* adult. Graphs were prepared using the probit analysis and represented using "glm" method.

5. Conclusion

In summary, the present study indicated that bitter almond powder and aqueous and alcoholic extracts showed more effectiveness in terms of their acaricidal activity against different developmental stages (nymph and adult) of *A. persicus* and *H. darjeeling* than a common synthetic chemical pesticide (alpha-cypermethrin). Though 1% suspension of bitter almond powder showed equal efficiency with alpha cypermethrin, 1% aqueous and alcoholic extracts of bitter almond profiled better efficacy in terms of mortality in both tick species. Efficacy of alcoholic extract of bitter almond has been found to be higher on *A. persicus* compared to *H. darjeeling* for adults at 96 hours. However, further investigation is required to better understand the potential of bitter almond acaricidal activity and to more precisely formulate it as an eco-friendly acaricide for veterinary practice and healthcare.

Acknowledgments

Corresponding author, KB, is grateful to the Department of Science & Technology-Uzbek (INT/UZBEK/P-09, 2021), Grants-in-Aid under RUSA component 10 (IP/RUSA(C-10)/03/2021) and Department of Science & Technology, W.B., Govt. of India (865/ST/P/S&T/1G-1/2015) for the support. AUM is grateful to the Ministry of Innovation development (grant № UZB-IND-2021-86) for the support. UC received financial assistance from a grant from DST, West Bengal. PRG, University of Kalyani & DST-PURSE 2022-23 are acknowledged for partial financial support.

Disclosure statement

No potential conflict of interest was reported by the author(s). The work embodied in this manuscript has not been published previously or is under consideration for publication in any other journal.

Funding

This work was supported by the Ministry of Innovation development [UZB-IND-2021-86]; Department of Science & Technology-Uzbek [INT/UZBEK/P-09, 2021]; and RUSA component 10 [IP/RUSA (C-10)/03/2021].

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