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Research Article

EVALUATION OF ACARICIDAL ACTIVITY OF *Carica papaya* SEEDS AND *Ricinus communis* LEAVES EXTRACT AGAINST SHEEP TICKS

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Abstract: The traditional method of tick control is mainly by use of synthetic acaricides. However, ticks are developing resistance to most of the available synthetic acaricides on repeated usage. In this regard, plant extracts have been attempted in this study in control of ticks. In this study the *in vitro* efficacy of *Carica papaya* seeds and *Ricinus communis* leaves extract in control of different species of ticks was evaluated by larval packet test (LPT) and adult immersion test (AIT). *Carica papaya* seed extract at 100mg/ml gave good results by inducing 100% mortality of either larvae or adult ticks whereas *Ricinus communis* leaves extract induced 60 to 75% mortality of larvae or adult ticks at 100mg/ml. In LPT, ticks exposed to *Carica papaya* extract showed less LC_{50} values indicative of good acaricidal activity whereas the ticks on exposure to *Ricinus communis* showed less acaricidal activity with high LC_{50} values.

Keywords: Plant extracts, Acaricidal activity, Sheep ticks

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Introduction

Ticks transmit economically important haemoprotozoan diseases which is a major threat to livestock population worldwide. Thereby the livestock development in several countries has hindered causing economic loss for small-scale farmers. The common control method involves the use of synthetic acaricides. However, the development of resistant strains in control of ticks in different parts of the world has rendered several chemical agents ineffective [1]. In addition, pollution and contamination of meat and milk are associated with this type of control [2]. The development of resistance has lead to huge economic loss in frequent usage of the chemical acaricides especially in the tropical and subtropical regions of the world. To combat the acaricide resistance problem, other alternative methods of tick control are being attempted using plant extracts with acaricidal properties [3]. The plant extracts are generally of low toxicity to mammals, soluble in water, have few side effects and are rarely or not at the origin of resistances within the tick populations. The present study evaluates the *in vitro* efficacy of the ethanolic extracts of *Ricinus communis* leaves and methanolic extract of *Carica papaya* seeds on field ticks collected from different places of Karnataka.

Materials and Methods

Preparation of *Carica papaya* seed / *Ricinus communis* leaves extract:

The *Carica papaya* seeds were collected from different fruit stalls located in Bangalore. The extract was prepared as per the procedure detailed by Shyma *et al*. 2014 [4].

The *Ricinus communis* plant leaves were collected from different farmer's field plots in different places of Karnataka. The extract was prepared as per the procedure detailed by Gosh, *et al*. (2013) [5]. The extracts prepared was mixed with methanol / ethanol to prepare dilutions of 6.25, 12.5,25,50 and 100mg/ml and were used to know the *in vitro* efficacy of *Carica papaya* and *Ricinus communis* extracts against ticks by adult immersion test (AIT) and larval packet test (LPT).

Collection of ticks

The ticks were collected from different sheep flockslocated in different places of Karnataka viz., Bangalore, Belgaum, Bellary, Chitradurga, Davangere, Mandya and Tiptur, they were identified based on the keys [6,7]. The collected ticks were put in glass vials and labeled with date of collection and place. The mouth of glass vials with ticks were wrapped in cotton muslin cloth for oxygen supply and transported to the laboratory for *in vitro* tests.

Larval Packet Test.

The larval packet test (LPT) was done as per FAO guidelines with minor modifications [8]. After making different dilutions of 6.25, 12.5, 25, 50 and 100mg/ml. Briefly, 0.7 ml of ethanolic *Ricinus communis* leaves extract / 0.7 ml of methanolic extract of *Carica papaya* seed was used to impregnate 3.75- cm by 8.5-cm Whatman filter paper rectangles. The compound was dried by keeping the filter paper for 30 min in incubator at 37 °C.

The rectangles were folded in half and sealed on the sides with adhesive tapes, forming an open-ended packet to place tick larvae. After the insertion of approximately 100 larvae (12-14days old), the top of each packet was sealed with adhesive tape, and the packets were placed in desiccators placed in BOD incubator maintained at 28±1 °C and 85±5 % RH. The packets were removed after 24 h, and larval mortality was calculated. Control group was treated with 0.7 ml of methanol / ethanol similar to the above mentioned procedure.

Adult Immersion Test

The adult immersion test (AIT) was conducted according to FAO guidelines [8]. The identified ticks were washed in distilled water and dried on an absorbent paper. The ethanolic *Ricinus communis* extract was mixed with ethanol to make different concentration of 6.26, 12.5, 25, 50 and 100mg/ml. For each concentration 5 ticks / petri dish were used along with a control group with 5 ticks. About three replications for each concentration was done for different species of ticks. The ticks used for this study were immersed for five minutes in different concentrations of the extract while control ticks were immersed in distilled water, later the ticks were transferred to the test petri dishes padded with Whatmann filter paper no.1. Every 24 hrs the mortality of ticks was recorded. After 48hrs, the ticks which were alive were transferred to glass vials covered with muslin cloth and kept in desiccators having 85±2 % relative humidity and placed in BOD incubator at 28±2°C. These ticks were observed for oviposition and death up to 15days. The percent adult tick mortality and the weight of the eggs laid by the treated ticks were recorded in comparison with the control.

The index of egg laying and percentage inhibition of fecundity were calculated with the formula wherein Reproductive Index =mean weight of eggs laid(g) / weight of females(g) and% Inhibition of egg laying = (IE control group – IE treated group / IE control group)X 100 respectively [7].This procedure is followed to test the *in vitro* efficacy of the ethanolic extract of *Ricinus communis* and methanolic extract of *Carica papaya* eeds against adult ticks of species against *R.haemaphysaloides, R.sanguineus, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum and H.m.isacci* ticks.

Statistical analysis

The dose – response data were analyzed by Probit method [9] using computer software, while differences in mean values of data among groups were analyzed by one way analysis of variance (ANOVA). The Fisher, Student, Kruskal-Wallis tests (at the 5% threshold) and correlation tests were used. The effect of plant extracts on the reproductive parameters of the fed-up female ticks was determined using the formulas of Goncalves, *et al.,* (2007) [10].

Results

Acaricidal efficacy of methanolic extracts of *Carica papaya* and ethanolic extract of *Ricinus communis* leaves extract on larval stages of ticks

The ticks collected for LPT and AIT were identified as R.*haemaphysaloides, R.sanguineus, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum and H.m.isacci* ticks. The crude methanolic extract of *Carica papaya* seeds and ethanolic extract of *Ricinus communis* extract was used against tick spp Haemaphysalis, Hyalomma and Rhipicephalus to check the *in vitro* efficacy of extracts in control of ticks. The results of the *in vitro* assay by larval packet test(LPT) on larval stages of different species of ticks *i.e.*, H.*bispinosa, H.kutchensis, H.intermedia, R.sanguineus, R.haemaphysaloides, H.a.anatolicum and H.m.issaci* at different concentration of *Ricinus communis* (6.25,12.5,25,50 and 100mg/ml) and *Carica papaya* seeds(6.25,12.5,25,50 and 100mg/ml) was recorded [Fig-1-2]. After 24hrs of exposure, 100 percent mortalityrate was recorded at 100mg/ml concentration of *Carica papaya* and at higher concentration of *Ricinus communis* at 200mg/ml against all species of ticks in this study.The details of larval mortality after 24hrs intervals are presented in the [Fig-1-2].

Lethal concentrations LC_{50} of methanolic extract of *Carica papaya* and ethanolic extract of *Ricinus communis*

In LPT varying LC_{50} values of both the extracts is classified as most effective to less effective according to their toxicity (LC_{50}) against different species of tick larvae [Table-1]. The lowest values of the LC_{50} was observed for all species of tick larvae in this study by for *Carica papaya* seed extract indicating higher acaricidal effect for larval control with LC_{50} values ranging from 23.07 to 26.85 [Table-1]. Whereas the *Ricinus communis* extract showed higher LC_{50} values ranging from 40.39 to 48.12 indicating of less acaricidal activity in control of tick larvae.

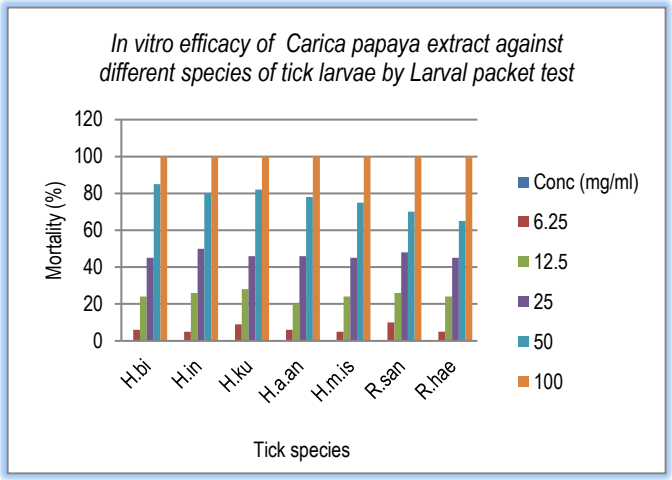

Fig-1 Larval mortality of different tick species H.bi:*Haemaphysalis bispinosa; H.in:Haemaphysalis intermedia; H.ku: Haemaphysalis kutchensis; H.a.an:Hyalomma anatolicum anatolicum; H.m.is: Hyalomma marginatum issaci; R.san: Rhipicephalus sanguineus; R.hae: Rhipicephalus haemaphysaloides* against methanolic extract of *Carica papaya* seeds.

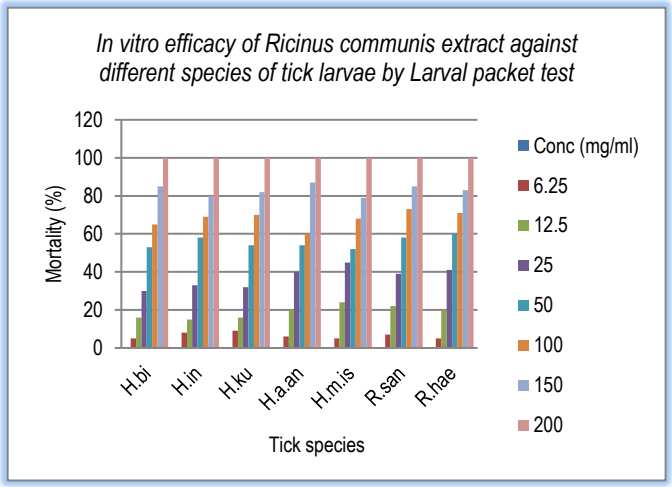

Fig-2 Larval mortality of different tick species *H.bi : Haemaphysalis bispinosa; H.in : Haemaphysalis intermedia; H.ku : Haemaphysalis kutchensis; H.a.an: Hyalomma anatolicum anatolicum;H.m.is:Hyalomma marginatum issaci;R.san : Rhipicephalus sanguineus; R.hae: Rhipicephalus haemaphysaloides* against ethanolic extract of *Ricinus communis* leaves.

Acaricidal efficacy of methanolic extract of *Carica papaya* on adult ticks

The percent adult mortality caused by the methanolic extract of *Carica papaya* was recorded at different concentrations *i.e.*, 6.25,12.5, 25,50 and 100mg/ml against H.*bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum , H.m.issaci, R.sanguineus and R.haemaphysaloides* [Table-2]. *Carica papaya* seed extract induced 100% mortality of all species of engorged ticks in this study at 100mg/ml concentration. Mortality at higher concentration was significantly higher than at lower concentrations. At highest concentration, egg laying was completely blocked and percentage inhibition of fecundity was more than 90.

Acaricidal efficacy of ethanolic extract of *Ricinus communis* on adult ticks

The percent adultmortality caused by the ethanolic extract of *Ricinus communis*was recorded at different concentrations *i.e.*, 6.25 ,12.5, 25,50 and 100mg/ml against H. *bispinosa, H. intermedia, H. kutchensis, H.a.anatolicum , H.m.issaci, R. sanguineus and R. haemaphysaloides* [Table-3].

Table-1 *Lethal concentration of Carica papaya and Ricinus communis extract*

Acaricide	Tick species	LC_{50}	Acaricide	Tick species	LC_{50}
Carica papaya	*H. bispinosa*	23.43	*Ricinus communis*	*H. bispinosa*	48.12
	H. kutchensis	24.05		*H. kutchensis*	44.77
	H. intermedia	23.07		*H. intermedia*	46.24
	H.a. anatolicum	26.03		*H.a. anatolicum*	47.01
	H.m. issaci	26.71		*H.m. issaci*	42.44
	R. haemaphysaloides	26.17		*R. haemaphysaloides*	41.67
	R. sanguineus	26.85		*R. sanguineus*	40.39

Table-2 *Mean adult mortality of ticks along with mean egg mass, reproductive index and inhibition of egg laying by Carica papaya seed extract*

Tick species	Conc mg/ml	Mean Tick weight	Mean adult mortality	Mean Egg mass	Reproductive index	Inhibition of egg laying
H.bispinosa	Control	134.28±12.2	0.0	48.5±0.004	0.43±0.40	-
	6.25	135.7±0.49	26.67±6.67	34.3±0.002	0.182±0.012	36.7
	12.5	129.48±0.91	43.33±11.54	23.3±0.001	0.149±0.06	44.2
	25	132.40±1.07	68.79±13.23	20.1±0.001	0.132±0.03	54.4
	50	129.76±0.90	72.43±	18.9±0.001	0.112±0.01	90.3
	100	135.7±0.49	100±0.0	0.0	0.0±0.0	100
H.intermedia	Control	133.26±9.8	0.0	44.54± 0.04	0.39±0.01	-
	6.25	133.35±0.57	25.57±5.57	30.15±0.003	0.035±0.01	34.3
	12.5	131.68±0.70	42.20±12.22	24.0±0.002	0.028±0.02	53.3
	25	131.78±0.66	69.82±6.60	20.1±0.001	0.026±0.02	95.1
	50	131.78±0.29	70.23±6.67	16.60±0.001	0.019±0.03	100
	100	132.51±0.63	100±0.0	0.0	0.0±0.0	
H.kutchensis	Control	134.64±6.8	0.0	46.56±0.002	0.48±0.21	-
	6.25	132.57±0.51	27.27±6.67	34.3±0.002	0.273±0.016	19.1
	12.5	132.26±0.46	43.45±13.33	23.3±0.001	0.198±0.009	39.4
	25	132.35±0.42	67.89±13.33	20.1±0.001	0.178±0.12	48.1
	50	132.6±0.45	73.36±6.62	18.9±0.001	0.152±0.006	45.1
	100	132.10±0.29	100±0.0	0.0	0.0±0.0	100
H.a.anatolicum	Control	133.34±9.2	0.0	49.2±0.006	0.43±0.01	-
	6.25	135.7±0.49	20±10.2	36.2±0.004	0.062±0.002	44.1
	12.5	129.48±0.91	38.32±10.55	28.7±0.002	0.058±0.002	53.1
	25	132.40±1.07	66.53±13.33	24.3±0.001	0.052±0.001	65.1
	50	129.76±0.90	75.25±11.55	15.45±0.001	0.042±0.001	80.1
	100	135.7±0.49	100±0.O	0.0	0.0±0.0	100
H.m.Issaci	Control	142.78±11.20	0.0	50.20±0.006	0.46±0.003	-
	6.25	140.52±	20.24±6.67	52.20±0.006	0.159±0.003	44.1
	12.5	142.32±0.29	32.32±11.26	34.43±0.004	0.153±0.004	56.4
	25	143.25±0.64	64.37±15.56	28.7±0.001	0.140±0.003	83.1
	50	142.81±0.19	79.39±7.62	29.3±0.001	0.128±0.001	95.1
	100	141.88±0.71	100±0.0	0.0	0.0±0.0	100
R.haemaphysaloides	Control	142.45±14.46	0.0	50.2±0.006	0.42±0.004	-
	6.25	141.19±0.56	21.13±6.27	36.68±0.006	0.243±0.18	32.13
	12.5	141.67±0.37	48.9±12.35	34.3±0.004	0.172±0.009	43.13
	25	141.55±0.60	65.67±13.53	23.3±0.002	0.156±0.012	80.0
	50	141.40±0.54	72.45±7.67	20.1±0.001	0.139±0.06	94.21
	100	141.52±0.49	100±0.0	100	0.0±0.0	100
R.sanguineus	Control	1412.76±11.46	0.0	51.12±0.009	0.420±	-
	6.25	142.55±0.29	22.23±7.33	54.2±0.008	0.273±0.16	33.33
	12.5	142.89±0.64	49.27±15.67	36.23±0.004	0.198±0.009	55.3
	25	143.67±0.19	68.16±14.92	20.01±0.002	0.178±0.012	73.33
	50	142.06±0.71	78.56±6.67	16.11±0.001	0.149±0.06	93.33
	100	142.44±0.21	100±0.0	0.0	0.0±0.0	100

Table-3 *Mean adult mortality of ticks along with mean egg mass, reproductive index and inhibition of egg laying by Ricinus communis leaves extract*

Tick species	Conc mg/ml	Tick weight	Mean mortality	Mean Egg mass	Reproductive index	Inhibition of egg laying(%)
H.bispinosa	Control	131.30±0.83	0.0	58±0.004	0.580±0.39	-
	6.25	132.23±0.54	6.76±0.76	42±0.003	0.215±0.016	60.16
	12.5	132.50±0.34	13.33±6.67	38±0.001	0.198±0.009	63.51
	25	132.40±0.23	26.67±6.67	26.7±0.0.01	0.187±0.012	65.50
	50	132.16±0.03	43.33±11.20	27.3±0.004	0.149±0.06	70.61
	100	134.2±0.0	68.79±3.0	26±0.005	0.132±0.03	73.15
H.intermedia	Control	133.33	0.0	48.9±0.004	0.520±0.27	-
	6.25	136.7±0.40	8.16±4.67	40.3±0.003	0.420±0.013	62.45
	12.5	135.48±0.90	15.23±6.34	38.2±0.001	0.437±0.013	68.45
	25	132.40±0.07	29.47±6.67	29.10±0.001	0.430±0.016	70.05
	50	134.76±0.90	45.62±11.32	23.0±0.001	0.410±0.040	70.50
	100	135.7±0.49	70.35±11.55	21.6±0.004	0.401±0.046	75.23
H.kutchensis	Control	132.34	0.0	45±0.004	0.526±0.27	-
	6.25	135.7±0.49	8.86±4.60	65±0.003	0.432±0.013	62.45
	12.5	133.48±0.91	15.33±6.34	60±0.001	0.427±0.013	68.45
	25	132.40±1.07	29.30±6.60	45±0.001	0.420±0.016	70.05
	50	129.76±0.90	47.32±11.55	32±0.001	0.402±0.040	70.50
	100	135.7±0.49	68.48±11.55	25±0.004	0.385±0.046	75.23
H.a.anatolicum	Control	135.7±0.49	0	54.02±0.005	0.436	-
	6	139.48±0.91	15.67±6.67	54±0.001	0.111±0.01	68.76
	12.5	132.40±1.07	21.13±8.76	49.2±0.001	0.101±0.03	68.94
	25	139.76±0.90	48.98±12.38	42±0.001	0.100±0.03	72.31
	50	135.7±0.49	65.67±11.02	32±0.01	0.064±0.01	75.24
	100	135.7±0.49	72.45±15.65	22±0.01	0.054±0.01	76.38
H.m.Issaci	Control	136.48±0.91	0	49.10±0.003	0.490±0.019	-
	6.25	135.40±1.07	16.70±7.76	54±0.001	0.100±0.01	58.26
	12.5	134.76±0.90	20.30±6.67	59±0.001	0.99±0.03	65.0
	25	135.7±0.49	45.18±12.56	42±0.001	0.99±0.03	68.67
	50	135.7±0.49	69.10±13.33	30±0.01	0.60±0.01	72.24
	100	133.48±0.91	70. 5±15.67	21±0.01	0.044±0.01	72.42
R.haemaphysaloides	Control	132.40±1.07	0	43.15±0.004	0.510±0.021	-
	6.25	134.26±0.90	7.67±2.46	40±0.003	0.392±0.027	43.8
	12.5	134.17±0.49	13.33±7.67	46.3±0.001	0.337±0.023	64.0
	25	134.71±0.49	26.67±11.56	38.12±.001	0.330±0.023	70.2
	50	134.58±0.91	60.17±13.46	29.64±0.0106	0.312±0.016	74.2
	100	134.42±1.07	75.87±11.0	28.76±0.004	0.329±0.040	80.0
R.sanguineus	Control	132.60±0.90	0	43.53±0.006	0.500±0.020	-
	6.25	134.27±0.59	9.20±5.67	50±0.003	0.410±0.030	43.68
	12.5	133.7±039	15.53±6.70	30±0.001	0.347±0.028	50.4
	25	134.48±0.81	28.50±6.67	30.43±.001	0.330±0.023	63.2
	50	132.40±1.03	62.0±12.55	29.18±0.010	0.322±0.012	69.0
	100	134.76±0.80	80±12.5	19.29±0.004	0.350±0.45	71.7

Ricinus communis leaves extract failed to induced 100% mortality of all species of engorged ticks in this study at 100mg/ml concentration. Mortality at higher concentration was not significantly higher than at lower concentrations. At highest concentration, egg laying was not completely blocked and percentage inhibition of fecundity was less than 90.

Discussion

In this study, the methanolic extract of *Carica papaya* seeds and ethanolic extract of *Ricinus communis* leaves were used to know the acaricidal efficacy against larval and adult stages of different tick species in the laboratory. After 24hrs, the larval mortality rate varied according to the concentrations of extracts. The methanolic extracts of *Carica papaya* seeds gave good results showing higher acaricidal effect with less LC_{50} values in control of larvae, even the seed extracts induced 100% mortality at concentration of 100mg/ml where egg laying was completely blocked and percentage inhibition of fecundity was more than 90 against adult ticks indicative of higher acaricidal effect. In this study the *Carica papaya* seeds extract showed higher acaricidal activity in control of larval and adult stages which is similar to the findings of Shyma, *et al.* (2014). They reported that papaya seed extract showed significant ($P<0.05$) adult mortality except at concentration 12.5 mg/ml ranging from 26.67 to 93.33%. Mortality at higher concentrations (100 and 50 mg/ml) was significantly ($P<0.01$) higher than at the lower concentrations.

At the highest concentration, egg laying was completely blocked, and percentage inhibition of fecundity was more than 90. Also, when LPT was performed, mortality of tick larvae was registered up to 82%. In this study, ethanolic extract of *Ricinus communis* leaves were used to know the acaricidal efficacy against larval and adult stages of different tick species in the laboratory. After 24hr, the larval mortality rate varied according to the concentrations and the ethanolic extracts of Ricinus *communis* leaves showed lower acaricidal effect with high LC_{50} values in control of larvae , even the extracts failed to induced 100% mortality at concentration of 100mg/ml where egg laying was not completely blocked and percentage inhibition of fecundity was less than 90. Not many studies have been reported with regard to *Ricinus communis* leaf extracts in control of ticks except Zahir, *et al*. (2009) and Ghosh, *et al*.,(2013). Zahir, *et al*. (2009), reported high acaricidal activity of *R. communis* leaf extracts against the larvae of *R. (B.) microplus* and calculated LC_{90} as 1829.94 ppm [11]. Zahir, *et al*. (2010), reported mortality of *Haemaphysalis bispinosa* in the range of 77.0 ± 2.1–100.0 ± 0.0% when treated with *R. communis* leaf extracts prepared in hexane, chloroform, ethyl acetate, acetone and methanol [12]. Arnosti, *et al*., (2012), reported inhibition in the development and maturation of oocytes of dog tick, *R. sanguineus* treated with ricinoleic acid esters in *R. communis* oil[13]. Ghosh, *et al*., (2013), reported that the *Ricinus communis* leaf extract at 6-10% concentration was highly efficacious and caused significant ($p<0.001$) mortality of ticks in the range of 55.0±9.6 – 95:0±5.0. The highest percentage of ticks died when treated at 10% concentration and the survived ticks laid fewer eggs and the RI values (0.171±0.0) was significantly different ($p<0.001$) from the control ticks (0.464±0.01). Although the ticks were treated with 2.5% concentration, the mortality was comparatively low(35-45%), the RI values significantly differed at 0.05-0.001 levels. The extract significantly affects the mortality rate of ticks in dose dependent manner ranging from 35.0 5.0 to 95.0 5.0% with an additional effect on reproductive physiology of ticks by inhibiting 36.4-63.1% of oviposition [6].

Conclusion

The results of this study showed that the larvae of tick species used in this study on exposure against methanolic extract of *Carica papaya* seeds, after 24hrs induced 100% mortality, along with less LC_{50} values indicating high larvicidal activity and also adulticidal activity inducing 100% mortality of adults at 100mg/ml with inhibition of egg laying and lower levels of egg laying inhibition whereas *Ricinus communis* showed less acaricidal effect by inducing 65-75% mortality at 100mg/ml. Thus, the phytoacaricides would be possible to produce solutions in combating the effective tick control and avoid the cases of resistance associated with the use of acaricides.

Application of research: To combat the chemical acaricides resistance problem, alternate method was explored to control ticks by herbal extracts.

Research Category: Herbal acaricides

Abbreviations:

cm: centimetre, mg/ml: milligram/ml, °C: degree Celsius, % RH: per centage relative humidity, LC: lethal concentration, FAO: Food and Agriculture Organization

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