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## ORIGINAL ARTICLE

# Bioefficacy evaluation of certain oleoresins against the larvae of *Rhipicephalus microplus* (Acari: Ixodidae)

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**ABSTRACT**
*The present study was aimed towards the evaluation of certain oleoresins isolated from Myristica fragrance (Nutmeg), Zingiber officinale (Ginger), Elettaria cardamomum (Cardamom), Piper nigrum (Black pepper), and Syzgium aromaticum (Clove) for larvicidal efficacy against the common cattle tick, Rhipicephalus microplus. The results indicated that the most potent oleoresin of larvicidal nature against R. microplus was Black pepper, having the $LC_{50}$ and $LC_{90}$ values 3.079 and 55.374 ppm and the efficacy was followed by Ginger, Cardamom, Nutmeg and Clove. The least effective oleoresin observed was Clove having $LC_{50}$ and $LC_{90}$ values 945.323 and 14177.036 ppm. The result thus suggests that oleoresins have acaricidal potential to be used as an eco-friendly approach for the control of ticks.*
**Key words:** *Rhipicephalus microplus, Larvicide, Oleoresins, Acaricide.*

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## INTRODUCTION

Ticks are ectoparasites of Mammals, Birds, Reptiles, and Amphibians and are reported worldwide specially in tropical and subtropical regions including Bangladesh, India and Pakistan. They constitute the order Parasitiformis and subclass Acari. Ticks are vector for many zoonotic diseases and play a vital role in transmitting the pathogens Bacteria (*Anaplasma marginale*), Viruses (*Arbovirus)*, Protozoans (*Babesia bovis and B. bigemina*) which affect the animal as well as human health worldwide. Approximately 10% of the currently known 867 tick species act as vectors for a broad range of pathogens for domestic animals and humans [21]. There are two types of ticks - hard ticks (Ixodidae) and soft ticks (Argasidae). The characteristics which make them an efficient vector are as wide host range, habit of feeding on different hosts during their life cycle, hardness and longevity makes them to accommodate in the unfavorable environment [11]. Therefore, these are considered to be second most important vector after the mosquitoes for transmission of vector borne diseases.

In India, about 70% of the population depends on agriculture for their income and survival. India have 16.5% of world cattles, 57% of buffaloes, 16.3% of goats, 57% of sheep [5]. Ticks infect the animals more severely as compared with the humans and multispecies infections are also present along with one species infestation on cattles and buffaloes which are responsible for the damage to the livestock and production of the milk and also responsible for the transmission of diseases like Anaoplasmosis, Rabesiosis, Theilariosis. There is a very high damage to the livestock from the infection of the ticks. In India, about US $ 498.7 million are required annually to control the ticks and tick born diseases [18]. There is a need to control the ticks as they affect the livestock and also responsible for transmitting the pathogens so widely. For this purpose many synthetic acaricides have been applied for tick management. They are effective but also have some drawbacks such as they are responsible for environmental pollution because of their very slow degradation and thus get accumulated in the environment leading to biomagnifications [9] causes contamination in the quality of milk with chemical residues and also affect the health of host animals and development of resistance [17]. Plant extracts are the mixture of different compounds, which act more effectively as compared to chemical acaricides and have less chance to develop resistance [3]. The chemicals which are obtained from plants have their distinguished way of action, such as they alter the concentration of hormones which control the growth and disrupt the development of eggs, inhibiting the sexual communication, reduction in chitin formation and repellent action against the target organisms [11**]**.

India is very rich in production of various kinds of plant spices and also exports them to different parts of the world. In Indian kitchen, spices are the essential ingredient of the food and other eatables, but their use is not restricted for food only they also serve for other purposes. Oleoresins which are obtained from spices have the same characteristics as the spices and serves several functions along with providing taste to the food such as they show antifungal, antimicrobial, antiviral, preservative, antioxidant, insect (beetle etc.) repellent activity [28]. Oleoresin are concentrated extracts of the spices having complete profile of the spice from which they are obtained, they have certain characters which render the taste including pungency. Oleoresins contain volatile essential oils as well as non-volatile resinous fraction consists of taste compounds, fixatives, anti-oxidants, pigments, and fixed oils that are naturally present in spices in a highly concentrated form. There are various mechanical and chemical processes for extraction of oleoresins such as solvent extraction, steam distillation, high hydrostatic pressure extraction, pulse electric field process, high pressure process [27].

Spice oleoresins exhibit sensitivity to light, heat and oxygen, and have short storage lives if not stored properly [23]. During prolonged storage some chemical and organoleptic changes can also occur in the oleoresin. On exposure to oxygen, destruction of pigments take place and hydroxylic groups convert to ketones, which then converts to colorless compounds of short Carbon skeleton.

Oleoresins are preferred over whole spice because they have microbiological advantages, they are easy to store, they have uniform flavor and pungency, they can be stored for a long period of time of about 12 months, they are concentrated forms, thus they reduce space for storage and transport requirements. Many studies have been done on Capsicum (*Capsicum annum*), Black pepper (*Piper nigrum* L.), Ginger *(Zinger officinale)*, Turmeric (*Curcuma longa*) oleoresins for their different biological properties [26; 12; 14]. Some species of piper and their bioactive constituents are reported to have remarkable larvicidal activity against various mosquito species such as *Culex* and *Aedes* [20; 16; 19]. Some workers have been reported the properties of oleoresins as antifungal, antiviral, antimicrobial, but very less studies has been done on the use of oleoresins in vector control or in insect pest management. Therefore, the present work is focused on the evaluation of certain oleoresins as tick larvicide against *Rhipicephalus microplus.*

## MATERIALS AND METHODS

*Rhipicephalus microplus* is found in tropical and subtropical regions and affects the cattles and buffaloes. Unfed adults are small (3-5 mm), on the other hand, engorged female can reach upto 1.2 cm in length. It is a single host tick and spends all its life stages on one animal and can survive for as long as 3-4 months without feeding. They can survive upto six months in cooler temperature conditions. The body is oval to rectangular and the shield is wider at front and oval in shape.

### Oleoresins:

### *Piper nigrum* (Black pepper)

It is good for stomach, helps in losing weight, good for skin, respiratory relief, antibacterial, antioxidant, enhances bioavailability, cognitive impairment, and in peptic ulcer. Oleoresin of black pepper is the natural extract of dried tender berries of *Piper nigrum*. It yields about 1-2.5% volatile oil which contain mainly terpenes, sesquiterpenes and oxygenated compounds. 'Piperine' is the major constituent of black pepper oleoresin [2]. Pepper plant has been prescribed for pest control as they contribute potential insecticidal compounds [7; 15; 29] (Figure 1a).

*Figure1 (a)*

 

***Elettaria cardamomum* (Cardamom)**

It is useful in the treatment of mouth ulcers, digestive problems, cold & flu, cancer, blood pressure, diuretics, cancer and even in depression and act as detoxifying agent. The oleoresin is dark brown viscous liquid with a sweet-spicy, warming fragrance. It is non-toxic in nature and is widely used in food materials. 'Cineole' contributes to pungency, while 'Terpinyl acetate' contributes towards the pleasant aroma (Figure 1b).

*Figure1 (b)*

***Syzgium aromaticum* (Clove)**

It has different uses such as pain relive, control nausea & vomiting, improve digestion, protects against internal parasite and are strongly antiseptic. It is also useful for treating rheumatoid arthritis and has anti-spasmodic properties. This oleoresin is extremely concentrated product which contains more flavoring ingredients that can be soluble in the particular solvent used, as it turns much close to original clove flavor and odor. Shelf life is 24 months from the date of manufacture when stored below 25$^{o}$C in closed containers away from direct light and not under humid conditions (Figure 1c).

*Figure 1 (c)*

*Zingiber officinale (Ginger)*

It has several health benefits like in Stroke & heart diseases, indigestion & nausea, malabsorption, immunity & respiratory functions, bacterial & fungal infection, pain, cancer, and in diabetes. It is dark brown viscous liquid. Flash Point is 168.00 °F and the Shelf Life is about 12 months or longer if stored properly. Ginger oleoresins contain monoterpenes and sesquiterpenes. The non-volatile pungent oil is obtained by solvent extraction of the spice named 'gingerol'. Pungent group includes gingerols, shogaols, paradols, and zingerone that produce a hot sensation in the mouth (Figure 1d).

**Figure 1(d)**

***Myristica fragrance* (Nutmeg)**

It has Pro-oxidant and antioxidant activity and also found to have psychoactive effect due to presence of hallucinogenic compounds. It is a yellow coloured viscous liquid having volatile oil content 30-

 

35mg/100g. Two largest components in nutmeg oleoresin are 'myristicin' and 'eugenol'. Myristicin has been studied extensively for its psychoactive effects [25]. High level of myristicin have been reported to effect hepatic function in test organisms, producing hypertrophy [10], increased microsomal enzyme activity [8] and fat degradation [31] (Figure 1 e).

*Figure1 (e)*

**Rearing of target organism:**

Ticks were collected from the farmer's flocks of the area nearby the institute and identification of the target organism was done according to the tick identification key [30]. Fully engorged females were rinsed with water and then place on the filter paper and were kept inside the tick rearing flat bottom glass tubes of 30ml capacity and covered by muslin cloth with rubber band. The glass tubes were kept inside the desiccator having 10% KOH solution for providing proper relative humidity (70-80%) to the ticks for laying eggs and left till complete oviposition takes place in BOD incubator. After 10 -14 days the hatching took place and larvae get emerged out, and larvae were subjected to experimentation at 27± 2ºC temperature.

**Bioassay:**

The oleoresins were procured from Elixir Extractions Pvt. Ltd. Kinfro Park, Nellad, Muvattupuzha Kochi-686669, Kerala, India and subjected to evaluate their larvicidal activity against *Rhiphicephalus microplus* by using **LIT (larval immersion test).** The stock solutions of oleoresins were prepared by dissolving them in ethanol/acetone separately. Different desired test concentrations were prepared by diluting the stocks in water. The experiments were conducted in triplicates along with control, each of these test concentration containing 100-150 larvae. These were then exposed to the test concentrations for 2-3 minutes in 10 ml of respective dilution in experimental flat bottom glass tubes. After 2-3 minutes, solutions were removed out from the vials or experimental tubes and were incubated for 24 hours in desiccators kept in BOD incubator at 27± 2ºC temperature and mortality data were observed. Mortality data were observed and corrected by using Abbot's formula [1] to remove the factors other than the oleoresins and corrected data (corrected % mortality) were subjected to calculate the $LC_{50}$ and $LC_{90}$ values by using Probit Analysis [6] along with other statistical parameters at 95% confidence level by the software developed by [22].

## RESULTS AND DISCUSSION

This study was conducted to evaluate the larvicidal activity of certain spice oleoresins of *Myristica fragrance* (Nutmeg), *Zingiber officinale* (Ginger), *Elettaria cardamomum* (Cardamom), *Piper nigrum* (Black pepper), and *Syzgium aromaticum* (Clove). The results revealed that the *Syzgium aromaticum* oleoresin was found the least effective having $LC_{50}$ and $LC_{90}$ values 945.32, 14177.0 ppm, the second least effective was *Myristica fragrance* oleoresin which is 1.26 times more effective than *Syzgium aromaticum* oleoresin. Followed by *Elettaria cardamomum* and *Zingiber officinale* oleoresin which were 2.66 and 5.50 times more effective than *Syzgium aromaticum* oleoresin. The most effective oleoresin was *Piper nigrum*, having the $LC_{50}$ and $LC_{90}$ values 3.07, 55.37 ppm and was 309.02 times more effective than the clove oleoresin (Table 1, Figure 2). Table-1 and Figure-2 shows the relative values of $LC_{50}$ and $LC_{90}$ of all the tested oleoresins. Thus, the present study revealed that *Piper nigrum* oleoresin was the most effective against larvae of *R. microplus*. Therefore, we can project it in a future prospective as an effective acaricide for controlling ticks and tick borne diseases. Many studies have been conducted with the aim of identifying substances of botanical origin, which can be used in the development of insecticides, especially for vectors of human and animal pathogens. The use of chemical acaricides causes environmental hazards; affect the host health, and development of resistance in ticks. In continuation, of the search of phyto-potentials as an effective acaricides. The extracts of *Datura stramonium*, *Azadirachta indica*, and *Calotropis procera* leaves, *Allium sativum* cloves and *Carica papaya* seeds were tested against on adult tick *R. Microplus* and evaluated their acaricidal properties, the percent adult mortality, reproductive index, percentage inhibition of oviposition, hatching of laid eggs, and percentage larval mortality at concentrations of 12.5, 25, 50, and 100 mg/ml and observed the adult tick mortality 66.67, 73.33, 80.00, and 93.33 % for *C. procera*, *D. stramonium*, *A. sativum*, and *C. papaya* extracts, respectively at the concentration of 100 mg/ml. However, for *Az. indica*, mortality was noticed low and estimated to be 33.33 % and inhibition of oviposition was 20.73, 71.34, 77.17, 85.83, and 100.00 % respectively [25]. Larvicidal activity of *Copaifera* sp. oleoresin against *Aedes aegypti* was studies by [13]. Some other similar results have been recorded with other plant extracts, *Acanthus ebracteatus, Acarus calamus, Annona squamosa, Luffa autangula,* and *Stemona collinsae* which induced a higher larvicidal effect (90–100%) against *B. microplus* [4]. A lot of work has been done on antimicrobial, antifungal, antiviral, properties of oleoresins but a very less work has been done regarding their acaricidal activity.

**Table 1:** Shows the relative values of $LC_{50}$ and $LC_{90}$ of all the tested Oleoresins, having their relative toxicity.

| Oleoresin | Exposure Period (Hr) | Chi-Square | Regression equation | $LC_{50}$ ±SE (UL-LL) | Relative toxicity | $LC_{90}$±SE UL- LL | Relative toxicity |
|---|---|---|---|---|---|---|---|
| Black pepper *(Piper nigrum)* | 24 | 21.933 | Y=1.02X-3.47 | 3.079±0.359 (3.78-2.374) | 309.022 | 55.374±20.031 (94.63-16.112) | 256.041 |
| Cardamom *(Elettaria cardamomum)* | 24 | 26.3169 | Y=1.06X-1.06 | 354.19±31.985 (416.89-291.503) | 2.66 | 5603.9 ±1586.80 (8714.08-2493.79) | 2.5298 |
| Clove*(Syzgium aromaticum)* | 24 | 24.9247 | Y=1.08X-0.66 | 945.32±149.66 (1238.65-651.98) | 1.00 | 14177.0±149.66 (27873.2-480.85) | 1.00 |
| Ginger (*ZingiberOfficinale)* | 24 | 77.880 | Y=2.03X+1.59 | 171.59 ±7.39 (186.09-157.09) | 5.509 | 729.58 ± 52.92 (833.32-625.844) | 19.4317 |
| Nutmeg (*Myristica fragrance)* | 24 | 12.6804 | Y=2.90X-6.27 | 745.86±48.327 (840.59-651.14) | 1.267 | 2055.73±315.8 (2674.84-1436.63) | 6.8963 |

Therefore, the present work was conducted to find out the potentiality of certain selected oleoresins against the larvae of *Rhiphicephalus microplus*. The mortality data showed that all the oleoresins have effective larvicidal activity and Black pepper oleoresin was found to be the most effective with $LC_{50}$ and $LC_{90}$ value as 3.097 and 55.374 ppm followed by Ginger, Cardamom, and Nutmeg Oleoresins, and Clove oleoresin was the least effective with $LC_{50}$ and $LC_{90}$ values 945.32 and 14177.0 ppm, respectively.
The result of the present study concluded that all the Oleoresins showed mortality against larvae of *Rhiphicephalus microplus* which was dose dependent. Compounds derived from plants are new effective tools of interest against vector management. Since the oleoresins are eco-friendly in nature and reduces the risk of resistance development, and they can potentially play a vital role in the management of ticks.

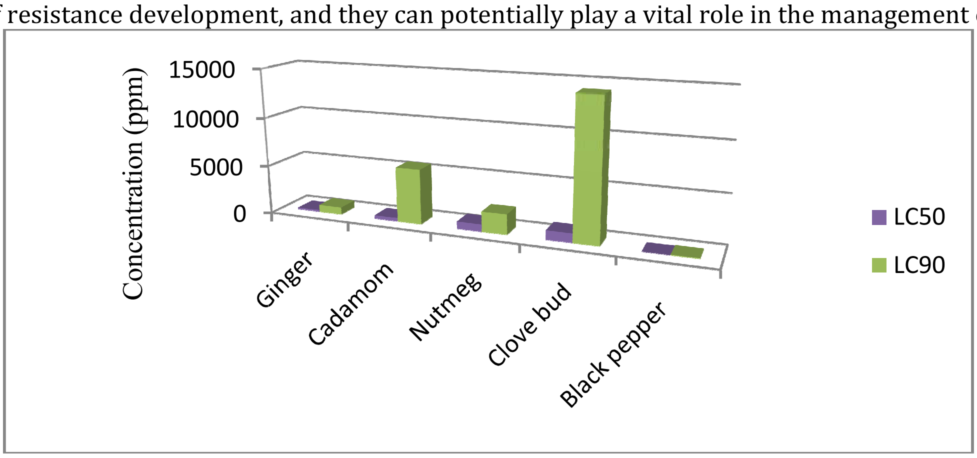


**Figure 1:** Relative activity of selected Oleoresins against larvae of *R. microplus.*

## REFERENCES


1. Abbott, W.S., (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomolology*, 18, 265–267.
2. Borges, P. and Pino, J. (1993). Preparation of black pepper oleoresin by alcohol extraction. Journal of Food/Nahrung, 37(2), 127–130.
3. Chungsamarnyart N., S. Jiwajinda, and w. Jansawan. (1991). Larvicidal effect of plant crude- extracts on the tropical cattle tick *Rhiphicephalus (Boophilus) microplus*. Kasetsart Journal of Natural Science Supplement*, 25, 80-89*.
4. Chungsamarnyart, N., Jiwajinda, S., Jansawan, W., Kaewsuwan, U., Burnasilpin, P., (1988). Effective plant crude-extracts on the tick (Boophilusmicroplus) larvicidal action Kasetsart Journal of Natural Science Supplement, 22, 37–41.
5. FAO (2004). Resistance Management and Integated Parasite Control in Ruminants Guidelines. Journal of Animal Production and Health Division, *FAO,* 25-77.
6. Finney DJ. Probit analysis 3rd Edition Cambridge (1971). *Cambridge University Press*
7. Freeborn, S. B., & Wymore, F. H. (1929). Attempts to Protect Sweet Corn from Infestations of the Corn Ear Worm, *Heliothis Obsoleta (Fabr.)*. *Journal of Economic Entomology*, *22*(4), 666-671.
8. Fuhremann, T.W.. Lichtenstein. P.E.. and Stratman. R.W. 1978. Effects of naturally occurring food plant components on insecticide dearadation in rats. Journal of Agricultural and ***Food Chemistry*** 26: 1068.
9. Furlong, J., Martins, J. R. S., & Prata, M. D. A. (2004). Controle estratégico do carrapato dos bovinos. *A Hora Vet*, *23*(137), 53-56.
10. Gershbein, L. L. (1977). Regeneration of rat liver in the presence of essential oils and their components. Journal of *Food and Cosmetics Toxicology*, *15*(3), 173-181.
11. Ghosh, S., Azhahianambi, P., & Yadav, M. P. (2007). Upcoming and future strategies of tick control: a review. *Journal of vector borne diseases*, *44*(2), 79.
12. Jayaprakasha, G. K., Negi, P. S., Anandharamakrishnan, C., & Sakariah, K. K. (2001). Chemical composition of turmeric oil-a byproduct from turmeric oleoresin industry and its inhibitory activity against different fungi. *Zeitschrift für Naturforschung C*, *56*(1-2), 40-44.
13. Kanis, L. A., Prophiro, J. S., da Silva Vieira, E., do Nascimento, M. P., Zepon, K. M., Kulkamp-Guerreiro, I. C., & da Silva, O. S. (2011). Larvicidal activity of *Copaifera sp*. (Leguminosae) oleoresin microcapsules against *Aedes aegypti* (Diptera: Culicidae) larvae. Journal of Parasitology research*,* 110(3), 1173-1178.
14. Kapoor, I. P. S., Singh, B., Singh, G., Isidorov, V. & Szczepaniak, L. (2008). Chemistry, antifungal and antioxidant activities of cardamom (*Amomum subulatum*) essential oil and oleoresins. International Journal of Essential Oil Therapeutics, *2*(1), 29.
15. Lathrop, F. H., & Keirstead, L. G. (1946). Black pepper to control the bean weevil. *Journal of Economic Entomology*, *39*(4), 534-534.
16. Lee, S. E. (2000). Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, Piper longum. *Journal-American Mosquito Control Association*, *16*(3), 245-247.
17. Miller, R. J., Davey, R. B., & George, J. E. (2007). First report of permethrin-resistant Boophilus microplus (Acari: Ixodidae) collected within the United States. *Journal of medical entomology*, *44*(2), 308-315.
18. Minjau, B. & McLeod, A (2003). Tick and tick borne diseases and poverty: The control of ticks and tick borne diseases on the livelihood of small scale and marginal livestock owners in India and Eastern and Southern Africa. Research Report DFID-AHP.UK. Centre for Tropical Veterinary Medicine, University of Edinburg,116.
19. Park, I. K., Lee, S. G., Shin, S. C., Park, J. D., & Ahn, Y. J. (2002). Larvicidal activity of isobutylamides identified in Piper nigrum fruits against three mosquito species. *Journal of Agricultural and Food Chemistry*, *50*(7), 1866-1870.
20. Pereda-Miranda, R., Bernard, C. B., Durst, T., Arnason, J. T., Sánchez-Vindas, P., Poveda, L., & San Román, L. (1997). Methyl 4-hydroxy-3-(3 ‘-methyl-2 ‘-butenyl) benzoate, major insecticidal principle from piper guanacastensis. *Journal of natural products*, *60*(3), 282-284.
21. Peter, R. J., Van den Bossche, P., Penzhorn, B. L., & Sharp, B. (2009). Tick, fly, and mosquito control–lessons from the past, solutions for the future. Journal of the South African ***Veterinary Association***, *80*, 121.
22. Shaikh, J., Bhosale, R., & Singhal, R. (2006). Microencapsulation of black pepper oleoresin. Journal of *Food chemistry*, *94*(1), 105-110.
23. Shulgin, A. T., Sargent, T., & Naranjo, C. (1967). The chemistry and psychopharmacology of nutmeg and of several related phenylisopropylamines. *Ethnopharmacologic Search for Psychoactive Drugs*, 202-214.
24. Shyma, K. P., Gupta, J. P., Ghosh, S., Patel, K. K., & Singh, V. (2014). Acaricidal effect of herbal extracts against cattle tick Rhipicephalus (Boophilus) microplus using in vitro studies. Journal of *Parasitology research*, *113*(5), 1919-1926.
25. Singh, G., Maurya, S., Catalan, C., & De Lampasona, M. P. (2005). Studies on essential oils, Part 42: chemical, antifungal, antioxidant and sprout suppressant studies on ginger essential oil and its oleoresin. *Flavour and Fragrance Journal*, *20*(1), 1-6.
26. Sofyana, S., Supardan, M. D., Zuhra, Z., Maulida, C. A., & Haura, U. (2013). Ultrasound Assisted Extraction of Oleoresin from Nutmeg (Myristia Fragrans Houtt). *International Journal on Advanced Science, Engineering and Information Technology*, *3*(4), 281-284
27. Steele, C. L., Lewinsohn, E., & Croteau, R. (1995). Induced oleoresin biosynthesis in grand fir as a defense against bark beetles. *Proceedings of the National Academy of Sciences*, *92*(10), 4164-4168.


 

28. Su, H. C., & Horvat, R. (1981). Isolation, identification, and insecticidal properties of Piper nigrum amides. *Journal of Agricultural and Food Chemistry*, *29*(1), 115-118.
29. Walker, A. R. (2003). *Ticks of domestic animals in Africa: a guide to identification of species* (pp. 3-210). Edinburgh: Bioscience reports.
30. Weil, A. T. (1965). Nutmeg as a narcotic. Journal of *Economic Botany*, *19*(3), 194-217
31. Reddy, P.J., Krishna, D., Murthy, U.S., Jamil, K. 1992. A microcomputer FORTAN program for rapid determination of lethal concentration of biocides in mosquito control. *CAMBIOS*, **8**: 209-13.