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RESEARCH ARTICLE

# Assessment of Repellency and Acaricidal Potential of *Nigella sativa* Essential Oil Using *Rhipicephalus microplus* Ticks

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**A B S T R A C T**

Owing to the development of resistance in ticks, the presence of drug residues in food products and the non-target toxicity associated with synthetic acaricides, the scientists are forced to discover some other effective tick control alternatives like botanicals. Hence, in this perspective, the current study was focused on the investigation of repellent and acaricidal potential of *Nigella (N.) sativa* essential oil against the *Rhipicephalus (R.) microplus* ticks. Moreover, this research also included the phytochemical analysis of *N. sativa* essential oil through GC-FID procedure which indicated nerol to be its major constituent. Both the repellent and the acaricidal experiments were conducted using the *N. sativa* essential oil at 1, 2.5, 5, 10 and 20% v/v dilutions. The results of these experiments indicated the *N. sativa* essential oil to exert repellent, acaricidal and reproductive effects in terms of various parameters with dose-dependent responses. Thus, the *N. sativa* essential oil may serve as an effective alternative for the control of *R. microplus* tick infestation.

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## INTRODUCTION

Ticks and ticks-borne diseases in animals, particularly the large ruminants, proves to be the most important limiting factor for the livestock economy in developing countries (Basit *et al.,* 2021; Atif *et al.,* 2022; Masih *et al.,* 2022). Various estimates put 80% population of the cattle worldwide to be at risk of being affected with ticks and ticks-borne diseases (Burrow *et al.,* 2019). Ticks as the hematophagous ectoparasites are found globally causing huge financial losses. Among the various genera affecting the ruminants, *Rhipicephalus* is the most important (Ceylan *et al.,* 2021; Rooman *et al.,* 2021). The species of this genus inflict heavy losses in the form of reduced production either through direct means or indirectly through transmission of various diseases (Hussain *et al.,* 2021; Salman *et al.,* 2023). It can be understood from the fact that *Rhipicephalus (R.) microplus* alone causes 22-30 billion dollars loss globally per annum (Lew-Tabor and Valle, 2016). Moreover, an engorged female tick of *R. microplus* is estimated to reduce 0.6g weight in beef calves (Zaman *et al.,* 2012).

Synthetic drugs have long served as the epicentre for all the efforts aimed at the prophylactic control and eradication of these ticks through the provision of a quick and an effective response (Selles *et al.,* 2021). However, owing to the long-term irrational use, the ticks have become resistant to most of the available acaricidal drugs (Abbas *et al.,* 2014a; 2014b; Sindhu *et al.,* 2022). Additionally, the detection of residues of these drugs in food products and their non-target toxicity have created serious concerns (Nath *et al.,* 2018; Goswami *et al.,* 2022; Salman *et al.,* 2022). Hence, the vaccination is developed as a preventive strategy against tick infestation, but it also has limited effectiveness. This is because of the presence of different species and the antigenic variations between different strains as a single vaccine cannot protect against infestation of all the species. Moreover, vaccine development is also an

expensive process which lowers its practical significance (de La Fuente and Estrada-Pena, 2019).

Hence, in the light of above discussion, other alternatives are needed which can provide an effective control of parasites including ticks (Abbas *et al.,* 2014; Zaheer *et al.,* 2021; Bajwa *et al.,* 2022; Jamil *et al.,* 2022; Zaheer *et al.,* 2022a; 2022b). These alternatives may be the botanicals as almost 2000 species of plants are reported to have pest control properties (Abbas *et al.,* 2018). These properties may be exhibited by the plants as whole or their components (Mamun and Ahmed, 2011; Salman *et al.,* 2020). Many experiments conducted on essential oils extracted from several plants for the investigation of their acaricidal and repellent effects against various genera of ticks like *Rhipicephalus*, *Amblyomma*, *Hyalomma* and *Dermacentor* have shown promising results (Benelli and Pavela, 2018). Similarly, the *N. sativa* essential oil is also reported to have insecticidal, acaricidal and repellent properties against a number of arthropods. It has shown its effectiveness even against the *R. annulatus* ticks (Aboelhadid *et al.,* 2016; Carroll *et al.,* 2016; Faheem and Abduraheem, 2019; Ndirangu *et al.,* 2020). However, the repellent and acaricidal effects of *N. sativa* essential oil against the *R. microplus* ticks have not yet been researched. Therefore, the current experiment was aimed at the investigation of both the acaricidal and the repellent effects of *N. sativa* essential oil using the *R. microplus* ticks from large ruminants.

## MATERIALS AND METHODS

**Ticks collection and identification:** Ticks were collected from the naturally-infested cattle and buffaloes in the Faisalabad district of Punjab, Pakistan during the summer season. The collections were made from those animals which were not administered any acaricidal drug during the last 30 days. These ticks were collected in plastic jars having minute holes in their lids and water-soaked cotton swabs placed inside for the provision of proper aeration and humidity. After collection, these ticks were brought to the Chemotherapy Laboratory, Department of Parasitology, University of Agriculture Faisalabad, Pakistan. After washing and drying, they were subjected to the identification process under the 10X magnification of stereomicroscope using the reference guide of Walker (2003).

**Essential oil extraction and analysis:** For the extraction of essential oil, the *N. sativa* seeds were ground and subjected to the hydro-distillation process using Clevenger apparatus. The essential oil, thus obtained, was processed through the GC-FID (Gas Chromatography-Flame Ionization Detection) procedure at Central Hi-Tech Laboratory, University of Agriculture Faisalabad for the determination of its chemical composition. The Shimadzu Gas Chromatograph (GC-17A) apparatus coupled with a flame ionization detector having DB WEX column (30m×0.25) with flow rate of 20 ml/min for its nitrogen mobile phase was used for this purpose. While processing, the oven temperature adjustments were followed as 90, 180 and 240°C for 2, 2 and 3 minutes respectively whereas the injector and the detector temperatures were respectively set at 250 and 270°C. During this procedure, the constituent components of the *N. sativa* essential oils were identified by comparing the retention times of the standards and those of the sample (Belhachemi *et al.,* 2022).

**Preparation of dilutions:** The essential oil of *N. sativa* was tested at 1, 2.5, 5, 10 and 20% v/v dilutions of the acetone solvent. The obtained results were then compared to those of the positive and negative control groups. The positive control groups comprised of DEET (diethyltoluamide) and cypermethrin for the repellency and the acaricidal treatments respectively. However, acetone acted as negative control treatment for both the experiments.

**Repellency experiment**

**Climbing test:** Tick climbing test was conducted following the protocol described by Ndungu *et al.* (1995). For this experiment, 10 ticks were observed for the estimation of repellency caused by *N. sativa* essential oil and the control treatments. The number of ticks above the filter paper for each treatment used in this experiment were used for the calculation of percent repellency. During the experiment, a temperature of 27°C and a relative humidity of 80% was maintained. The following formula was used the calculation of percent repellency:

$$\text{Repellency} = \frac{C - T}{C + T} \times 100$$

Where C and T represented the number of counted ticks above the filter paper for the control and the sample treatment respectively.

**Acaricidal experiment**

**Dipping test:** Following the procedure of Koc *et al.* (2013), ten adult *R. microplus* ticks were dipped for 5 minutes in each dilution. Then they were put into the jars and placed inside the biological oxygen demand (BOD) incubator at 90% humidity and 27°C for 24 hours. After this, the ticks were examined and the mortality was calculated. During this experiment, each dilution was tested in three replications. Percent mortality was determined using the following formula (Sousa *et al.,* 2022):

$$\text{Mortality} = \frac{\text{Dead ticks count}}{\text{Total ticks count}} \times 100$$

**Adult immersion test:** For this test, the protocols of Drummond *et al.* (1973) were observed and ten engorged female *R. microplus* ticks were immersed in each of the test dilutions for 30 seconds. After this immersion, the ticks were gently dried with the help of tissue paper. Then, they were placed inside the BOD incubator at 27°C and 90% humidity for 20 days till the ovipositing. The eggs, thus collected, were then weighed and again put into the incubator 27°C and 90% humidity for 30 days. This experiment was replicated thrice and the required parameters were calculated using the given formulas (Castro *et al.,* 2018).

$$\text{Index of Fecundity (IF)} = \frac{\text{Weight of oviposited eggs}}{\text{Weight of ticks}}$$

$$\text{Hatchability (H)} = \frac{\text{No. of hatched larvae}}{\text{No. of eggs incubated}} \times 100$$

$$\text{Reduction in Oviposition} = \frac{(\text{IF Control} - \text{IF Treatment})}{\text{IF Control}} \times 100$$

$$\text{Estimated Reproduction (ER)} = \frac{\text{Weight of Oviposited eggs}}{\text{No. of ticks}} \times \text{H} \times 20000$$

$$\text{Effectiveness of Product (EP)} = \frac{(\text{Control ER} - \text{Treatment ER})}{\text{Control ER}} \times 100$$

**Syringe test:** Following the procedure of Sindhu *et al.* (2012), this test was conducted for the calculation of larval mortality (LM). This test started with taking a weighed sample of eggs into specially designed syringes and keeping it in the BOD incubator for hatching. The hatched larvae (14 days old) were used and the mortality was recorded at 24 hours post-treatment with the specified dilutions. While counting, only the walking larvae were considered alive. Following formula was used for the calculation of larval mortality (FAO, 2004):

$$\text{Corrected Larval Mortality} = \frac{(\text{LM in Treatment} - \text{LM in Control})}{(100 - \text{LM in Control})} \times 100$$

**Statistical analysis:** The results of various experiments were statistically analysed through ANOVA, Tukey's Means Comparison Test and Probit Analysis with the help of IBM SPSS software using 95% confidence level and considering the results to be significant when $P<0.05$ (Barrios *et al.*, 2022; Park *et al.*, 2022).

## RESULTS AND DISCUSSION

Infestation with ticks, especially the *R. microplus*, is responsible for huge economic damage across the world (Jabeen *et al.*, 2022). Mainly, these tick infestations are kept under control through the use of various synthetic acaricides but the emergence of serious threats have led to the discovery of new means of ticks control such as the essential oils. These essential oils are the secondary byproducts of plants' metabolism which act through different modes of action for the provision of effective tick control (Salman *et al.*, 2020; Sharmeen *et al.*, 2021).

The extraction of essential oils from the plants can be achieved through different techniques yet the *N. sativa* essential oil was obtained with the help of hydro-distillation. The extracted *N. sativa* essential oil upon GC-FID analysis was found to contain various components. Among these detected components, nerol was found to be the major component of this essential oil with the highest concentration of 24.2%. This finding resembles the results of a previous study (Marichali *et al.*, 2016) but the difference in the concentration of the detected nerol may be due to the various factors like extraction technique used, soil nature, age of the plant and the type of cultivar (Moghaddam and Mehdizadeh, 2017; Ayub *et al.*, 2023). The components detected in the *N. sativa* essential oil are listed in Table 1 corresponding to their respective retention times and percent concentrations.

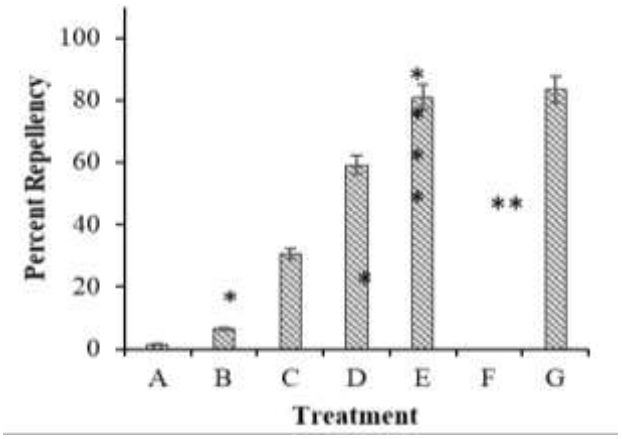


**Fig. 1:** Repellency for *Nigella sativa* essential oil. A: N. sativa oil 1%; B: N. sativa oil 2.5%; C: N. sativa oil 5%; D: N. sativa oil 10%; E: N. sativa oil 20%; F: Negative control; G: Positive control Bars with same superscript symbols differ non-significantly from each other (P>0.05)

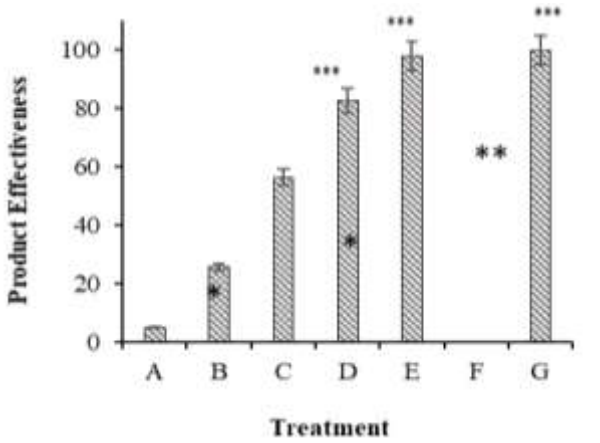


**Fig. 2:** Product Effectiveness Exhibited for Essential Oil of Nigella sativa. A: N. sativa oil 1%; B: N. sativa oil 2.5%; C: N. sativa oil 5%; D: N. sativa oil 10%; E: N. sativa oil 20%; F: Negative control; G: Positive control; Bars with same superscript symbols differ non-significantly from each other (P>0.05).

**Table 1:** Composition of *Nigella sativa* essential oil

| Name of the Component | Retention Time (min) | Concentration (%) |
|---|---|---|
| Acetaldehyde | 2.320 | 9.3 |
| Geraniol | 5.160 | 6.0 |
| Gamma-undecalactone | 7.800 | 7.5 |
| Isopropyl acetate | 13.040 | 6.8 |
| Octanal | 16.560 | 6.8 |
| Gamma-terpinene | 19.360 | 8.3 |
| Benzaldehyde | 22.560 | 6.5 |
| Furfuryl alcohol | 25.680 | 4.4 |
| Linalool | 28.760 | 3.8 |
| Limonin | 31.080 | 3.1 |
| Unknown | 33.000 | 6.1 |
| Citral | 37.280 | 6.6 |
| Nerol | 40.520 | 24.2 |

As the repellency of *R. microplus* ticks, towards *N. sativa* essential oil, is concerned, varying dose-dependent responses were observed for different treatments. However, only the 20% dilution of the essential oil was capable of eliciting such response that differed non-significantly (P>0.05) from that of the DEET treatment as shown in Fig. 1. Moreover, the $EC_{50}$ and the $EC_{90}$ values as calculated through the Probit Analysis were 8.435 and 27.456% respectively.

**Table 2:** Mortalities for *Nigella sativa* essential oil against larvae and adult ticks.

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G |
| Larvae | $3.00\pm1.73^{A}$ | $13.00\pm7.55^{A}$ | $46.33\pm7.50^{B}$ | $78.67\pm13.50^{C}$ | $93.67\pm7.09^{C}$ | $4.67\pm2.52^{A}$ | $98.00\pm3.46^{C}$ |
| Adults | $10.00\pm10.00^{AB}$ | $13.33\pm5.77^{AB}$ | $36.67\pm11.55^{BC}$ | $63.33\pm11.55^{CD}$ | $86.67\pm15.28^{DE}$ | $6.67\pm5.77^{A}$ | $96.67\pm5.77^{E}$ |

A: *N. sativa* oil 1%; B: *N. sativa* oil 2.5%; C: *N. sativa* oil 5%; D: *N. sativa* oil 10%; E: *N. sativa* oil 20%; F: Negative control; G: Positive control; Mean values (±SD) with same superscript letters within the row differ non-significantly from each other (P>0.05).

**Table 3:** Effect of different treatments on fecundity index and oviposition reduction of adult female ticks.

| Treatment | Fecundity Index | Oviposition Reduction (%) | Egg Hatchability (%) | Reproductive Estimation (×20000) |
|---|---|---|---|---|
| A | $52.15\pm8.44^{A}$ | $2.07\pm15.85^{A}$ | $80.2\pm7.2^{A}$ | $42.19\pm10.44^{A}$ |
| B | $43.68\pm4.57^{AB}$ | $17.96\pm8.58^{AB}$ | $76.0\pm5.3^{A}$ | $33.05\pm1.21^{A}$ |
| C | $32.56\pm2.59^{BC}$ | $38.85\pm4.87^{BC}$ | $59.7\pm4.9^{B}$ | $19.44\pm2.28^{B}$ |
| D | $19.73\pm3.89^{C}$ | $62.94\pm7.30^{C}$ | $38.5\pm6.2^{C}$ | $7.76\pm2.75^{BC}$ |
| E | $5.36\pm0.91^{D}$ | $89.94\pm1.70^{D}$ | $17.8\pm2.8^{D}$ | $0.97\pm0.29^{C}$ |
| F | $53.25\pm7.84^{A}$ | $0.00\pm14.73^{A}$ | $84.1\pm6.1^{A}$ | $44.50\pm3.75^{A}$ |
| G | $1.14\pm0.83^{D}$ | $97.86\pm1.56^{D}$ | $11.2\pm5.7^{D}$ | $0.10\pm0.02^{C}$ |

A: *N. sativa* oil 1%; B: *N. sativa* oil 2.5%; C: *N. sativa* oil 5%; D: *N. sativa* oil 10%; E: *N. sativa* oil 20%; F: Negative control; G: Positive control; Mean values (±SD) with same superscript letters within the column differ non-significantly from each other (P>0.05).

This repellent response of *N. sativa* essential oil may be ascribed to the nerol and the synergistic action of all the components (Susurluk, 2023). Nerol, being the major component of this essential oil, has proved its repellent efficacy against ticks and mosquitoes in a previous experiment (Wong *et al.,* 2022). These volatile components produce a vapour barrier which imparts them a repellent action and drives these arthropods away from this odorous source. However, this repellent barrier diminishes relatively quickly due to the volatility of these substances (Salman *et al.,* 2020).

Similar to the repellent experiment, a dose-dependent acaricidal response was observed for the *N. sativa* essential oil. From these results, the observation of Ellse and Wall (2014) was confirmed which advocated the higher susceptibility of larvae to the essential oils as compared to adult ticks (Table 2). This acaricidal potential of the *N. sativa* essential oil may have been exerted by the synergistic action of all its components. Moreover, the extract of *N. sativa* has already proven its acaricidal efficiency (Aboelhadid *et al.,* 2016; Soares *et al.,* 2016).

Furthermore, the *N. sativa* essential oil not only exerted its acaricidal effect in terms of mortality but also influenced the reproductive capability of the *R. microplus* ticks. This reproductive effect was manifested as the decline in several parameters like oviposition, egg hatchability and overall reproductive performance (Table 3). During the current experiment, the 10% concentration was indicated to have non-significantly different (P>0.05) results from those of the cypermethrin-treated positive control group as shown in Fig. 2.

The essential oils exert their reproductive and acaricidal impacts owing to their contact toxicity. They cause break down of cuticular waxes and clog the ticks' respiratory spiracles resulting in water stress and suffocation (Agwunobi *et al.,* 2020). Moreover, these oils penetrate the cuticle and diffuse into the haemolymph which ultimately transports them to internal organs like salivary glands and ovaries, thus, causing impairment of the digestive and reproductive systems (Remedio *et al.,* 2016; Wang *et al.,* 2020). To add further, the neurotoxic impact of the essential oils may also influence the survival of ticks (Selles *et al.,* 2021).

**Conclusions:** The results of this research indicate *N. sativa* essential oil to be an effective choice for the control of *R. microplus* ticks infestation by either repelling or killing them. However, these findings need to be validated through field experiments before recommending its commercial use. Moreover, means of extending the residual life of essential oils as well as the extraction techniques for obtaining maximum oil yield need to be refined.

**Conflict of Interest:** The authors have no conflict of interest.

**Authors Contribution:** NA-H, MAZ, KMAS and MS designed the experiment. MS conducted the research trial. NA-H, KMAS, TUR and ASO provided advisory services throughout the experiment. NA-H, KMAS, TUR and ASO helped in statistical analysis. All authors contributed in writing and approving this manuscript.

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