Research Article

doi:10.25081/jpc.2022.v50.i2.7983
Journal of Plantation Crops, 2022, 50(2): 94-99

Phytochemical profile and toxicity of thyme-derived (*Thymus vulgaris*) essential oil against the exotic rugose spiraling whitefly (*Aleurodicus rugioperculatus* Martin) infesting coconut

E. K. Saneera*, S. Raguraman, A. Suganthi and K. Venkatesan[1]

Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India

[1]Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India

(Manuscript Received: 20-07-2022, Revised: 15-08-2022, Accepted: 18-08-2022)

Abstract

Microwave-assisted heat extraction of thyme (*Thymus vulgaris*) yielded 1.64 per cent w/v of yellow to amber coloured and less viscous oil with a peculiar aroma. GC-MS analysis revealed the presence of 20 compounds, out of which thymol (51.94%), p-cymene (14.5%), γ-terpinene (10.09%), linalool (3.48%), and endo-borneol (3.95%) were the major compounds. Eggs, second instar nymphs and pupae of rugose spiraling whitefly (RSW) were subjected to contact toxicity assessment by complete immersion (dip method) in various concentrations of thyme oil. The results showed that essential oils at 0.35 per cent concentration exhibited 100 per cent mortality in the second instar nymphs. Thyme oil (0.5%) inhibited egg hatching and adult emergence to the tune of 100 per cent when the eggs and pupal stages were treated. Probit analysis indicated that the median lethal concentration (LC_{50}) of thyme oil to eggs, second instar nymph sand pupal stages were 0.19, 0.13 and 0.21 per cent, respectively. Thyme oil proved to be an excellent toxicant to different developmental life stages of RSW; hence it can be successfully incorporated into the integrated pest management (IPM) programme for whitefly management in the coconut ecosystem.

Keywords: Botanical insecticide, invasive pest, metabolite profile, thyme oil, whitefly

Introduction

Coconut palm is considered a versatile crop because of its multi-faceted utility. In India, coconut plays a vital role in the agrarian economy by providing food and livelihood comforts to more than 12 million people (Raghavim *et al.,* 2019). The rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin, is an invasive pest first reported from Pollachi, Tamil Nadu; Palakkad and Kottayam, Kerala, in coconut gardens in 2016 (Sundararaj and Selvaraj 2017; Srinivasan *et al*., 2016; Shanas *et al*., 2016). Since then, its distribution has expanded to various coconut-growing tracts, including the northeastern states (Mohan *et al.,* 2018). Recently, three more neotropical whitefly species, namely *Paraleyrodes bondari*, *Paraleyrodes minei* and *Aleurotrachelus atratus,* were also found to be infesting coconut in India (Josephrajkumar *et al.,* 2020).

RSW infestation depletes phloem sap nutrients and water. Also, the insect excretes wax and sticky honeydew on the infested leaves, leading to copious growth of black sooty mould, which reduces the photosynthetic efficiency in palms. RSW has now become a regular pest of coconut, warranting control measures to avoid crop losses. As the coconut system harbours an enormous population of natural enemies associated with the whitefly complex, the pesticide holiday approach is recommended to conserve them (Josephrajkumar *et al*., 2019). Hence,

**Corresponding Author: saneeraedayakkal@gmail.com*

eco-friendly interventions for whitefly management are warranted to tackle this invasive pest efficiently.

Attention is increasingly paid to using natural compounds as a promising alternative to agrochemicals in agricultural pest control. Of several options, plant oils have been used for a long time, while essential oils are the latest addition. Thyme (*Thymus vulgaris*) is an aromatic herb primarily used in various culinary preparations. Thyme oil has a myriad of other applications because of its unique chemical composition. In addition, it has also been used as an antihelminthic, carminative and anti-broncholitic agent in Romanian folk medicine (Boruga *et al*., 2014; Youdim *et al*., 2002). As this essential oil is a component of the plant's defense system, it confers toxicity to a wide array of microorganisms and several arthropod pests. Essential oil acts on multiple targets in the insect body; hence the chances for the development of resistance are less, making them ideal candidates for pest management. Hence, the present study was undertaken to evaluate the toxicity of thyme oil against rugose spiraling whitefly.

Materials and methods

Plant material and microwave-assisted heat extraction of essential oil

T. vulgaris plants were collected from a commercial farm in Kotagiri (11^{0}25'14.59"N; 760 51' 37.26" E) Nilgiri district, Tamil Nadu, India. Aerial parts, including fresh leaves and tender twigs, were chopped into 5-7 cm pieces and subjected to hydro distillation using the microwave-assisted heat extractor following Golmakani *et al*. (2008). The collected oil was kept in a glass bottle, tightly wrapped in aluminium foil and stored at 4 ^{o}C to prevent loss and degradation. The recovery of oil was calculated using the following formula.

$$\text{Essential oil yield (\%)} = \frac{\text{Quantity of essential oil obtained (g)}}{\text{Quantity of herbage used for extraction (g)}} \times 100$$

Phytochemical profiling through GC-MS analysis

The *T. vulgaris* sample was analysed through the Thermo Scientific Trace GC Ultra chromatograph system (Thermo Fischer Scientific, Austria) coupled with Thermo Scientific DSQ II quadruple mass spectrometer equipped with a 5 per cent phenyl-methyl silicone fused capillary column (TG- SQC, 30 mm in length, 0.25 mm ID, and 0.25 µm film thickness) following the procedures of Golmakani *et al*. (2008). Helium was used as a carrier. The oven temperature was held at 50 ^{o}C for 5 min and increased to 240 ^{o}C for 3 min, followed by a further increase to 300 ^{o}C (5 ^{o}C min^{-1}). Sample (1 µL) was injected at 290 ^{o}C in split mode (1:10). Electron ionisation detector was used in the analysis. From the chromatogram, the percentage of peak area was worked for each compound compared to the total peak area for all the detected compounds.

Culturing of *A. rugioperculatus*

The culture of rugose spiraling whitefly was maintained over 1-1.5 years old potted coconut seedlings (Chowghat Orange Dwarf) kept under a shade net. Twenty adult whiteflies of mixed sex from naturally infested coconut plantations were collected and introduced into several self-fabricated clip cages (Gill and Rataul, 1994) attached to the lower surface of coconut leaves. After 48 h, the adults were removed, and the eggs deposited on leaves were allowed to grow into different developmental stages, later used in the bioassays.

Bioassay to evaluate contact toxicity

Five-day-old eggs, two-day-old second-instar nymphs and two-day-old pupae were used for evaluation in the contact toxicity bioassays. The leaflets containing respective life stages from the stock culture were cut into bits (5-7 cm), leaving the petiole exposed for 2-3 cm at the base of the leaf. Live stages of the pest, along with leaf bits, were immersed in test solutions for 30 s. The dried leaf bits were maintained in the plastic jars as described in IRAC 008 methodology with slight modifications.

A preliminary range finding test was conducted for the above life stages with different serial dilutions of thyme oil prepared in distilled water and Triton-X 100 (0.05 per cent) as surfactant. The thyme oil concentrations used include 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35 and 0.5 per cent. Six concentrations in the range of 20 to 80 per cent mortality under each category were further selected to determine the median lethal concentration (LC_{50}). Thirty life stages under each category were exposed to different concentrations. Un-hatched and collapsed eggs were considered dead (Khederi *et al.,* 2019). Mortality of the nymphal stage was noted by changes in the colour, appearance and further moulting into successive instars (Yang *et al*., 2010; Pradhan *et al*., 2020). Adults failing to emerge were considered the mortality index in the treated pupal stage.

Statistical analysis

The laboratory bioassay trials were conducted in a completely randomised design (CRD) with three replicates. The per cent data on mortality were subjected to arc sine transformation followed by one-way ANOVA, and means were compared with Tukey's HSD test. To determine LC_{50}, probit analysis was performed (Finney, 1971). All the statistical analyses were carried out using IBM SPSS version 20.

Results and discussion

Yield and phytochemical profile of thyme oil

Plants have been considered a great repository for several bioactive compounds; most of these compounds possess immense insecticidal properties. Presently, there is great demand for eco-friendly and green pesticides. Hence these compounds are promising candidates to be exploited in pest management. In the present study, microwave-assisted heat extraction of aerial parts of thyme grown in Nilgiris yielded 1.64 per cent w/v of yellow to amber coloured and less viscous oil with a peculiar aroma.

GC-MS analysis of essential oil derived from thyme cultivated in the Nilgiris revealed the presence of 20 compounds (Table 1), of which thymol was the major compound, accounting for 51.43 per cent. The other compounds identified include p-cymene (14.5%), α myrcene (1.42%), α pinene (0.71%), linalool (3.48%), camphor (2.33%) carvacrol (0.10%), caryophyllene (1.97%) *etc*. The above compounds were also recorded by Ahmed *et al.* (2014) when the thyme oil in South Africa was subjected to phytochemical profiling, wherein thymol accounted for 60 per cent, the difference being attributed to the difference in climate and plant genotype. Thyme oil exhibit chemotypic variation in the relative proportion of monoterpenes (Keefover-Ring *et al*., 2003; Thompson *et al*., 2003). Factors such as genetics, plant age, extraction method, geographical origin and prevailing climate play vital roles in determining essential oil yield and their composition (Assami *et al*., 2013).

Table 1. Phytochemical profile of essential oil derived from *T. vulgaris* analyzed by GC/MS

Sl. No.	Compound	Retention time (min)	Area (%)
1.	α pinene	7.31	0.71
2.	Camphene	7.67	0.33
3.	α myrcene	8.67	1.42
4.	p-Cymene	9.52	14.50
5.	ϒ terpinene	10.30	10.09
6.	Linalool	11.24	3.48
7.	Camphor	12.30	2.33
8.	Endo-borneol	12.75	3.95
9.	α- terpineol	13.34	0.62
10.	Thymol methyl ether	14.10	2.82
11.	Thymol	15.43	51.94
12.	Carvacrol	16.50	0.10
13.	Cardinene	16.95	0.18
14.	Caryophyllene	17.88	1.97
15.	α caryophyllene	18.61	0.14
16.	Cis γ cardine	19.09	0.33
17.	γ delta cardinine	20.31	0.78
18.	Caryophyllenene oxide	21.14	0.02
19.	Cardinol	23.15	0.44
20.	Limonen-6-ol, pivalate	29.49	0.19

Essential oils are a blend of several bioactive compounds, and their mode of action might be due to the individual effect as well as the interaction of several individual compounds. The mode of action of thymol in the insect nervous system is attributed to their role as positive allosteric modulators of the insect GABA receptors (Priestley *et al*., 2003).

Contact toxicity of thyme oil to life stages of *A. rugioperculatus*

The present study demonstrated the potential of thyme oil to be used as a biopesticide against *A. rugioperculatus*. The mortality of egg, nymph and pupa of RSW increased in a concentration-dependent manner when they were treated with thyme oil (Fig. 1).

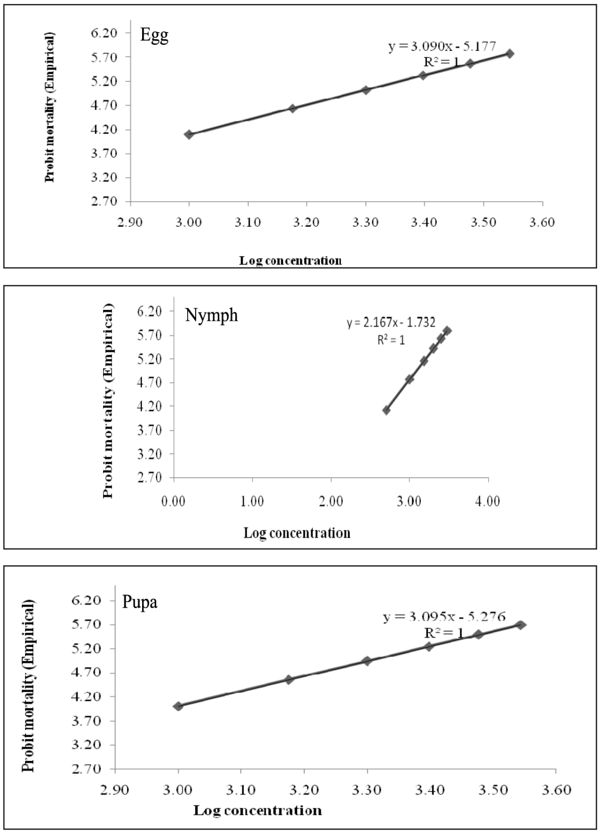

Fig. 1 Lethal concentration probit mortality (LCPM) regression lines for life stages of *A. rugioperculatus* to thyme oil

Different concentrations of thyme oil significantly suppressed egg hatching, nymphal survival and adult emergence as compared to the control (Table 2). Thyme oil displayed exceptional ovicidal properties to the RSW eggs; a hundred per cent inhibition in the hatching was documented when the eggs were immersed in 0.5 per cent concentration. The eggs were severely collapsed and shrunken when they were subjected to treatment with concentrations above 0.25 per cent (Fig. 2 A). However, the eggs failed to hatch at lower concentrations (below 0.25%) without any remarkable morphological change.

Table 2. Per cent mortality in the different developmental stages of *A. rugioperculatus* with thyme oil treatment

Concentration* (%)	Cumulative mortality (%) Eggs	Second instar nymphs	Pupa
0.01	0.00	6.67	0.00
	(4.05)^{g}	(14.97)^{e}	(4.05)^{g}
0.05	10.00	26.67	6.67
	(18.43)^{f}	(31.09)^{d}	(14.97)^{f}
0.1	23.33	33.33	20.00
	(28.88)^{e}	(35.26)^{d}	(26.57)^{e}
0.15	33.33	46.67	33.33
	(35.26)^{de}	(43.09)^{cd}	(35.26)^{de}
0.2	43.33	63.33	40.00
	(41.17)^{d}	(52.73)^{bc}	(39.23)^{cd}
0.25	60.00	76.67	53.33
	(50.77)^{c}	(61.12)^{b}	(46.91)^{c}
0.3	73.33	86.67	73.33
	(58.91)^{b}	(68.59)^{b}	(58.91)^{b}
0.35	83.33	100.00	80.00
	(65.90)^{b}	(90.00)^{a}	(63.43)^{b}
0.5	100.00	-	100.00
	(90.00)^{a}		(90.00)^{a}
Control	0.00	6.66^{e}	0.00^{g}
	(4.05)^{g}	(14.97)	(4.05)
S Ed	2.36	4.95	2.87
F value	243.784	52.76	169.619
P value	0.000	0.000	0.000

*Mean of three replications. Figures in parentheses are arcsine transformed values. Means followed by common letter within column are not significantly different in Tukey's test at $p \leq 0.05$

The second instar nymphs of *A. rugioperculatus* showed a great change in appearance following thyme oil application. The body became flaccid; the colour changed to orange-brown, devoid of honeydew and wax secretion (Fig. 2B). At the highest tested concentration (0.35%), thyme oil resulted in 100 per cent mortality of second instar nymphs. When the pupal stages were treated with

Table 3. Median lethal concentration (LC_{50}) of thyme oil to *Aleurodicus rugioperculatus*

Stage	Slope ± SE	LC_{50} (%)	Confidence interval	(χ^2) Value (df= 4)	P value
Eggs	3.10 ± 0.563	0.196	0.167-0.228	1.93	0.749
Nymphal stage	2.17 ± 0.395	0.128	0.99-0.157	4.317	0.365
Pupal stage	3.09 ± 0.567	0.209	0.178-0.243	2.143	0.709

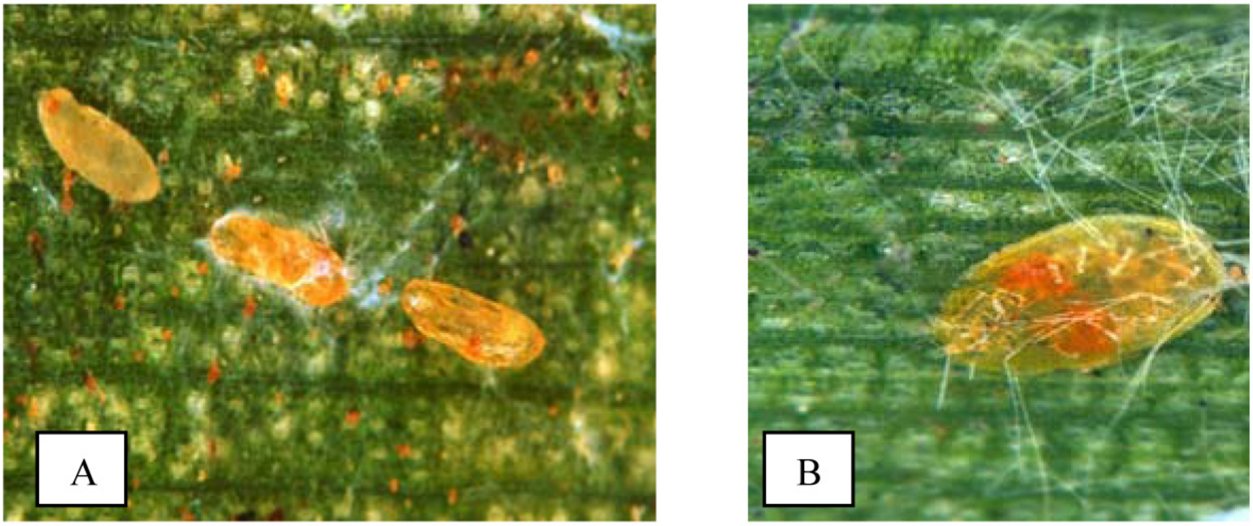

Fig. 2. Morphological changes in the life stages of *A. rugioperculatus* after thyme oil treatment A: Shrunken eggs; B: Flaccid second instar nymph

the highest concentration (0.5%) of thyme oil, there was complete inhibition in the emergence of adults (Table 2).

As the chi-square test was non-significant, which indicated the data is homogenous. The LC_{50} values for egg, second instar nymph and pupa stages were recorded as 0.196 per cent, 0.128 per cent and 0.209 per cent, respectively (Table 3). The difference in the LC_{50} value of thyme oil across life stages suggests a difference in their vulnerability, second instar nymph being most sensitive, followed by egg and pupa. In *Bemisia tabaci*, treatment of thyme oil at 1000 ppm caused a 45 to 60 per cent reduction in egg hatching, 76.42 per cent mortality in the nymphs and 58.93 per cent inhibition in adult emergence (Hussain*,* 2017).

Yang *et al*. (2010) reported that essential oils extracted from *T. vulgaris* (0.5%) possessed great contact toxicity, which reduced the survival rate of *B. tabaci* biotype B by 73.4 per cent, 79.0 per cent and 58.2 per cent in the eggs, nymphs and pupal stages, respectively, compared to control. Similarly, thyme oil (8 ppm) caused 100 per cent mortality of greenhouse whitefly, *Trialeurodes vaporariorum*, in melon when applied by mist spray under greenhouse conditions (Aroiee *et al.,* 2005). The essential oil derived from thyme decreased the survival rate of eggs, nymphs and pupae of *Aleuroclava jasmini* Takahashi to the tune of 64 per cent, 76 per cent and 50 per cent, respectively, as compared to the control (Khederi *et al*., 2019).

Conclusion

Thyme oil proved to be a better toxicant not only to *A. rugioperculatus* but also to many whiteflies in the horticultural ecosystem. This essential oil can be better exploited in the integrated pest management of *A. rugioperculatus,* especially in the context of the pesticide holiday approach.

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