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Insecticidal activity of the essential oil of *Thymus transcaspicus* against *Anopheles stephensi*

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ABSTRACT

Objective: To investigate the insecticidal activity of the essential oil of *Thymus transcaspicus* (*T. transcaspicus*) against *Anopheles stephensi* (*An. stephensi*).
Methods: *An. stephensi* were exposed to 31, 63, 125 and 250 μg/L of essential oil of *T. transcaspicus* for 24 h.
Results: The most toxicity was observed at 250 μg/L of essential oil with the LC_{50} values of 134.1 μg/L after 24 h.
Conclusions: The essential oil of *T. transcaspicus* exhibited strong insecticidal activity against *An. stephensi* which can be attributed to its constituent especially carvacrol and thymol phenols.

1. Introduction

Control of the insects usually necessitates the use of synthetic insecticides, whereas these chemical-based insecticides lead to serious concerns. Expensiveness, being environmentally hazardous, growing incidence of insect resistance, bioaccumulation through food chains and toxic effects on human health, encouraged researchers to find naturally occurring, reasonable and more environmentally friendly agents[1,2]. Therefore, several plants have been studied as insecticide, larvicidal and repellent agents[3-6].

Thymus is a genus of plant which contains different species. About 350 species have been reported from this genus, over 14 of which are identified in Iranian flora[7]. The antibacterial, insecticidal and antifungal properties of *Thymus* species have been reported in various studies[8]. *Thymus transcaspicus* (*T. transcaspicus*) (Khorasan thyme) is an aromatic plant which is native to northern regions of Iran[9]. It has been illustrated that carvacrol and thymol are the major constituents of this plant[10].

The insects play an important role as vectors for transmission of a variety of diseases including malaria, filariasis, yellow fever, dengue fever and Japanese encephalitis between human populations[11]. More than 300 million people are infected with malaria every year worldwide[3]. *Anopheles stephensi* (*An. stephensi*) is the main vector of human malaria in the Middle East and in the Persian Gulf area[12,13]. It has been also demonstrated that *An. stephensi* is the most prevalent anopheline species in the southern areas of Iran[13].

In light of the above knowledge, the present study has been designed to investigate the insecticidal activity of the essential oil of *T. transcaspicus* against *An. stephensi*.

2. Material and methods

2.1. Preparation of essential oil

T. transcaspicus was collected from the Tandoureh National Park (Khorasan Province, Iran), identified by the

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herbarium of School of Pharmacy, Mashhad University of Medical Sciences and a voucher sample was deposited (Voucher No. 153-2020-18).

The plants were cleaned; the leaves, flowers and stems were separated and dried in the dark. The dried plants were then powdered with a mechanical grinder. Batches of 75 g powder were subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus[14].

2.2. An. stephensi cultures

An. stephensi was obtained from insectarium of the School of Public Health, Tehran University of Medical Sciences. The larvae were fed with tetramin and yeast powder.

2.3. Insecticidal activity of the essential oil on An. stephensi

The larvicidal activity of essential oil was examined according to the standard method suggested by World Health Organization[15]. Different concentrations of essential oil of *T. transcaspicus* (31, 63, 125, 250 μg/L) in acetone were prepared. Dechlorinated tap water was used for all the experimental groups. Twenty five early fourth instar larvae were exposed to different concentrations in a working volume of 250 mL in 400-mL glass beaker. Two replicates for each concentration and controls (acetone) were included for larval bioefficacy. The larval mortality for each concentration was recorded after 24 h of exposure.

2.4. Data analysis

LC_{50} (lethal concentration to cause 50% mortality in the population) and LC_{90} (lethal concentration to cause 90% mortality in the population) values were calculated by probit analyses using SPSS (2001). The percentage mortality was corrected using Abbott's formula.

3. Results

The final yield of the hydrodistillation of *T. transcaspicus* was 0.7% (v/w). The influence of different concentrations of *T. transcaspicus* essential oil against *An. stephensi* after 24 is shown in Figure 1.

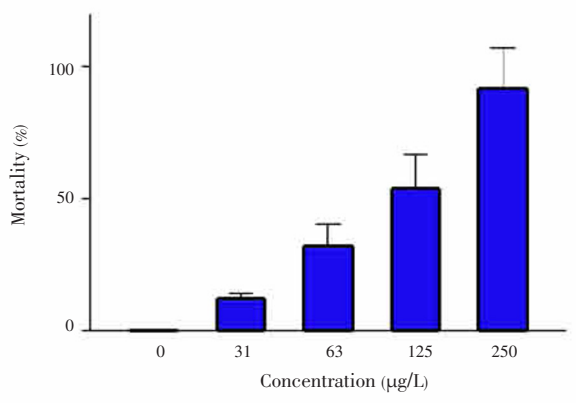

Figure 1. Percentage mortality response of *An. stephensi* exposed to different concentrations (μg/L) of *T. transcaspicus* for 24 h.

The concentration-dependent lethal toxicity was occurred due to essential oil treatment. The most toxicity was observed at 250 μg/L. Probit analysis indicated that LC_{50} and LC_{90} values of the essential oils against *An. stephensi* were 134.1 (52.8-328.1) and 248.0 (159.0-457.4) μg/L.

4. Discussion

Insects are responsible for considerable damage to human environment and health. About 15% of the world's crop losses are occurred due to insects[16]. Moreover, insects play an important role as vectors in transmission of different diseases among human. These diseases include malaria, yellow fever, dengue fever, Japanese encephalitis and filariasis[11]. It has been suggested that the best way for protecting human health against the vector-borne diseases is vector control[17]. The extensive use of synthetic insecticides have led to disturbing consequences such as environmental hazards, bioaccumulation of toxic agents in food, incidence of insect resistance and many human health problems[2]. Botanical insecticides have been applied in control of insects for at least two thousand years in Asia[18]. The considerable interest for new botanical insecticides is due to their physiological activity, biodegradability and bioefficiency[19]. The genus *Thymus* L. (Lamiaceae) comprises more than 250 herbaceous perennial and sub-shrubs throughout the world. Over 14 species of this genus such as *T. transcaspicus* are represented in the flora of Iran[20]. It has been illustrated that *Thymus* L. possesses various biological properties such as antifungal, antimicrobial and insecticidal[8,21].

In the present study, the insecticidal potential of *T. transcaspicus* (Khorasan thyme) against malaria vector (*An. stephensi*) was investigated. The essential oil of *T. transcaspicus* was prepared by hydrodistillation using a Clevenger-type apparatus.

The insecticidal activity of the *T. transcaspicus* essential oil is significant when considering that more than 90% of *An. stephensi* larvae were killed due to the exposure to 250 μg/L of the essential oil (LC_{50}: 134.1 μg/L). *An. stephensi* is the major vector of malaria in the southern area of Iran, with an expansion through the Middle East into the Indian subcontinent[22]. The widespread resistance from this species has been reported against synthetic insecticides including malathion, benzene hexachloride and DDT[23].

It has been shown that *Thymus* species have strong insecticidal activity against various insects such as *Aedes aegypti* (L)[24], *Tribolium castaneum* and *Callosobruchus maculatus*[25], *Aedes albopictus*[26] and *Sitophilus oryzae*[21]. The insecticidal activity of *T. transcaspicus* could be attributed to its chemical composition. Thymol (56.4%), γ-terpinene (7.7%), carvacrol (7.6%) and *p*-cymene (6.3%) are the main compounds of *T. transcaspicus* essential oil[10]. Carvacrol and thymol are the monoterpenoid phenols which have broad toxicity against insects. Carvacrol [2-methyl-5-(1-methylethyl) phenol], is a constituent of essential oils produced by various aromatic plants and species such as *Thymus* species[27]. It has been proved that carvacrol is effective against different insect pests like *Leucania separate*[28]. Thymol which is another major monoterpenoid phenol was reported to have insecticidal activity against

An. stephensi[29].

The results obtained indicate that the essential oil of *T. transcaspicus* has a significant insecticidal activity against malaria vector (*An. stephensi*). This toxicity against insects can be attributed to its constituent especially carvacrol and thymol phenols.

Conflict of interest statement

We declare that we have no conflict of interest.

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