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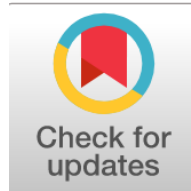
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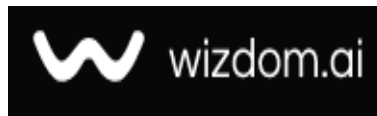
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Evaluation of Insecticidal Activity of Tanacetum parthenium Plant against Housefly (Musca domestica) in Bojnord, Iran: Evaluasi Aktivitas Insektisida Tanaman Tanacetum parthenium terhadap Lalat Rumah (Musca domestica) di Bojnord, Iran

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Abstract

General Background Botanical insecticides are increasingly explored as alternatives to synthetic chemicals due to rising resistance in medically important pests. **Specific Background** Tanacetum parthenium is a medicinal plant rich in pyrethrins and traditionally known for multiple pharmacological properties, yet its bioactivity against Musca domestica had not been tested. **Knowledge Gap** No previous experimental data existed on the toxicity of feverfew extracts against larval and adult houseflies from North Khorasan, Iran. **Aims** This study aimed to extract active ingredients from wild T. parthenium and evaluate their insecticidal activity against larvae and adults of M. domestica. **Results** Bioassays showed dose- and time-dependent mortality in both life stages, with LC50 and LC90 values of 5135 and 8840 ppm for adults at 48 h, and 329 and 551 ppm for larvae at 24 h, indicating higher susceptibility in larvae. **Novelty** This is the first report demonstrating the insecticidal properties of T. parthenium against houseflies under controlled laboratory conditions. **Implications** The findings indicate that native feverfew extracts could serve as a botanical bio-insecticide for vector and pest control, supporting environmentally friendly alternatives to synthetic insecticides.

Keywords:

Tanacetum Parthenium, Musca Domestica, Botanical Insecticide, Pyrethrins, Bioassay

Key Findings Highlights:

1. Few extracts caused strong dose-dependent mortality in both developmental stages.

2. Juvenile forms required much lower concentrations than mature forms.

3. Pyrethrin-containing fractions were responsible for toxic action.

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Introduction

Feverfew or *Tanacetum parthenium* L. (syn. *Matricaria parthenium*, *Chrysanthemum parthenium* (L.) Bernh., *Leucanthemum parthenium* (L.) Gren. Et Godron, *Pyrethrum parthenium* L. sm. *Chrysanthemum persicum*, *Pyrethrum persicum*) is a perennial herbaceous plant belonging to the Asteraceae family. Current taxonomic classification has transferred this species from the *Chrysanthemum* genus to *Tanacetum* [1].

The *Tanacetum* genus naturally grows in the northern and southern hemispheres and includes 160 species reported from Europe, Asia, Northern Africa, and North America. At least 35 species of this genus are distributed in various regions of North, West, East, and Central Iran [2, 3].

The major constituent of the essential oil of *T. parthenium* is camphor [4]. The active ingredients of this herb are produced in all aerial organs and vegetative parts of the plant, especially in leaves and flowers. The leaves are used in the pharmaceutical, cosmetics, and healthcare industries. Some parts of the plant have been used to treat asthma, arthritis, fever, migraine, stomach-ache, toothache, and insect bites. [1, 4].

Musca domestica L. is a well-known cosmopolitan pest of health importance. It can transmit many human pathogens, including viruses, bacteria, fungi, protozoa, and nematodes, and is one of the most difficult pests to control. Resistance of this species against various kinds of chemical insecticides, including organochlorines, organophosphates, carbamates, pyrethroids, insect growth regulators (IGR), neonicotinoids, phenyl pyrazoles, macrocyclic lactones, and spinosyns, has been reported from different parts of the world [5-7].

Insecticides derived from some kinds of plants showed efficacy against houseflies and are regarded as alternatives to synthetic chemicals [5, 7-9]. Presence of *T. parthenium* has been reported from North Khorasan Province previously [10] The purpose of this study was to evaluate the toxicity of the essential oils of *T. parthenium* collected from its natural habitats in North Khorasan against the housefly, *M. domestica*.

Materials and Methods

Plant collection and extraction

The flower, leaves, and stem of *T. parthenium* (Fig.1) were collected from the highlands of Goloul Dam of Shirvan County in the North Khorasan Province (north-east of Iran; N57° 19' 54.12" E37° 28' 33.96") (Fig. 2). The plant material was extracted using Chloroform solvent. Plant extraction, pyrethrin separation, and detection were carried out according to a previous study [10].



Figure 1. Fig.1. Different parts of *Tanacetum parthenium* (https://species.wikimedia.org/wiki/Tanacetum_parthenium)



Figure 2. Fig.2. Map of study area, North Khorasan Province, Iran

Rearing of *M. domestica* culture

The adult houseflies were sampled from wild populations inhabiting livestock sites and dairy farms at the outskirts of Bojnord City, the capital of the North Khorasan Province. These houseflies have been extensively exposed to insecticides through several applications per annum. The adult flies were captured using a net and kept in a small plastic cage (30 x 30 x 30 cm) covered with white muslin cloth equipped with a sleeve at room temperature (25 ± 2 °C) under a 12:12 light-dark cycle and 60% Relative humidity. They were fed with a solution of sugar (66%) and milk (33%) via soaked cotton pads. A mixture of wheat bran (30 g), hay powder (10 g), peanut powder (10g), and water was provided in plastic beakers (15 x 10 x 5 cm³) as ovipositional media [10]. The medium provided a suitable bed for egg laying and hatching. The hatched larvae were fed the same medium until the second instar stage, when some cohort batches were used for dose-mortality analysis. The remaining larvae were then reared until the adult stage, and the newly emerged adults were used for bioassay experiments. Synchronized larval and adult batches were obtained by placing beakers containing fresh media in adult cages for egg laying and removing them at specified time intervals.

Bioassay experiments

Effect of extracts of *T. parthenium* against the housefly larvae

The second instar larvae (3 days old) used in these experiments were obtained from the same egg batches. A total of 120 larvae were randomly selected and distributed across 12 Petri dishes (10 larvae per dish), which were then assigned to 4 treatments, including a control (3 replicates per treatment). The tested larvae were fed 2 g of dried larval media impregnated with 2 mL of one of three concentrations of *T. parthenium* extract (167, 333, or 667 ppm). The applied concentrations were prepared from a stock concentration of 30 mg/mL of dried extracts dissolved in ethanol and diluted with distilled water. The dried extract contained 0.5 percent pure pyrethrin; therefore, to obtain a concentration of 667 ppm, 4 mL of the stock solution was diluted with distilled water to a total volume of 10 mL. The other concentrations were prepared by diluting 1 to 2 in a serial order. The control larvae were fed with larval media prepared with distilled water. Larval mortality was recorded at 12-hour intervals until 48 hours of treatment. The larvae, which did not respond to touches with a camel brush, were considered dead.

Effect of extracts of *T. parthenium* against the housefly adults

Newly hatched adult flies were distributed at 10 adults per 500 cc jar and covered with mesh or cheesecloth. A total of 12 jars were used to accommodate bioassay and control adults in this set of experiments. Three concentrations of *T. parthenium* extract were mixed with adult medium (sugar + milk) to make 2500, 5000, and 7500 ppm and supplied to the test adults, preceded by a 12-hour starvation period. Adults of the control group were fed adult medium diluted with distilled water void of *T. parthenium* extract. Each experiment was conducted in three replicates (30 adults/replicate) along with the control group. Adult mortality was recorded at 12-hour intervals until 48 hours post-treatment.

Statistical analysis

The percentage mortality was calculated for each extract concentration, and correlation equations were obtained using Excel software version 16.0. The mortality data were subjected to probit analysis using SPSS 16.0 probit procedure. The LC50 and LC90 values, and their 95% confidence intervals for each concentration, were also calculated (IBM-SPSS Software) and tabulated.

Results

The lowest and the highest mortality rates of adults of *M. domestica* after treatment (16% and 76%) were recorded upon application of 2500 and 7500 ppm extracts of *T. parthenium*, respectively (Fig. 3).

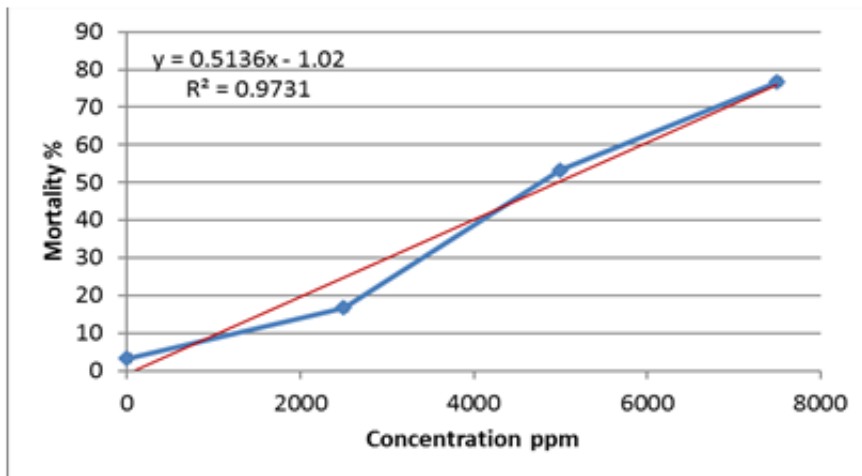


Figure 3. Fig. 3. Adult mortality of *M. domestica* after treatment with different concentrations of *T. parthenium* extract

The larval mortality followed the same pattern, although 100% mortality was achieved upon application of a less concentrated extract equal to 667 ppm (Fig 4).

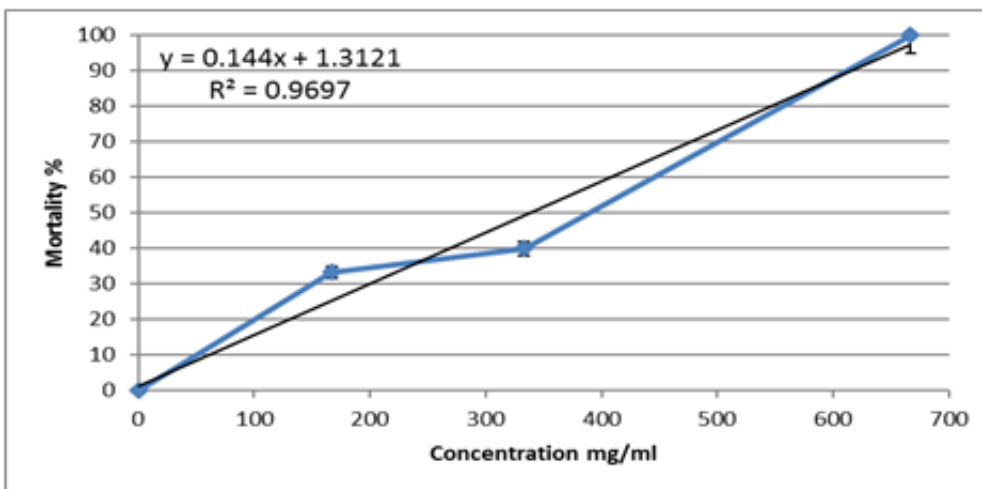


Figure 4. Fig. 4. Percentage of larval mortality of *M. domestica* after treatment with different concentrations of *T. parthenium* extract.

Calculation of LC50 and LC90 values of *T. parthenium* extracts for adult houseflies upon 48 h exposure to various feverfew extracts showed to be 5135 and 8840 ppm, respectively. However, LC50 and LC90 values of feverfew extracts for larval stage upon 24 h exposure were 329 and 551 ppm, respectively (Table 1).

Housefly stage	Probit	95% Confidence interval
Adults *	LC ₅₀	5135 (4360-6005)
	LC ₉₀	8840 (7590-11090)
Larvae**	LC ₅₀	329 (277-395)
	LC ₉₀	551 (469-697)

* After 48 h exposure time. ** After 24 h exposure time.

Figure 5. Table. 1. Lethal concentrations of *T. parthenium* extracts against adults and larvae of housefly (ppm)

Time-dependent mortality of adults of *M. domestica* upon application of feverfew extracts at different time intervals is presented in Fig. 5. As depicted, the mortality increases with increasing time, and this was more evident at higher concentrations than at lower concentrations. On the other hand, the highest rate of larval mortality occurred at the first 12 h post treatment, more significantly upon application of 667 ppm feverfew concentration (Fig. 6).

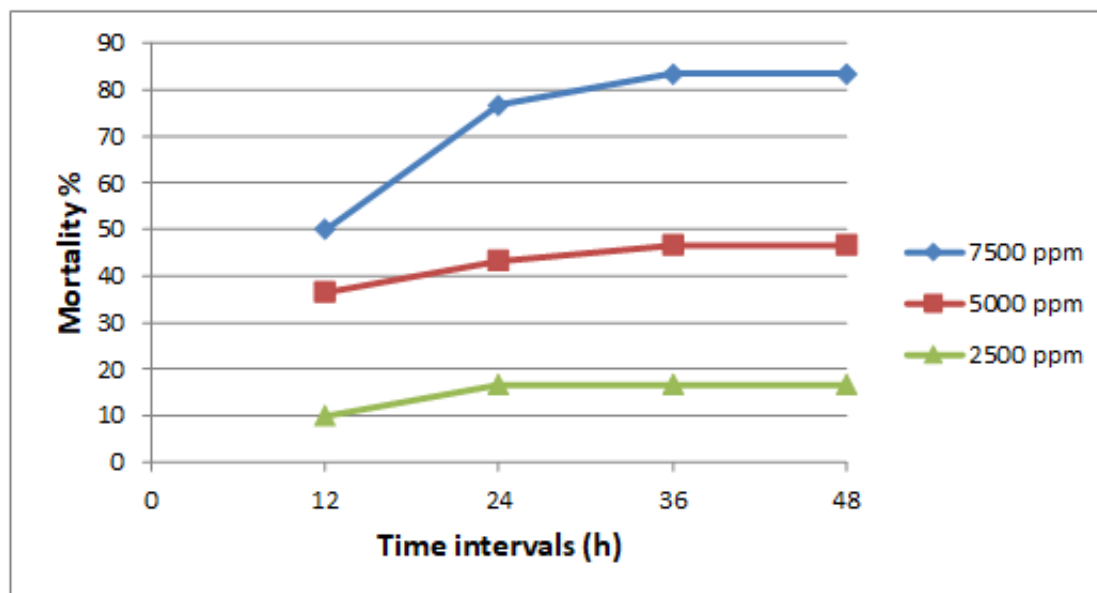


Figure 6. Fig. 5. Time-dependent mortality of adult *M. domestica* upon treatment with various concentrations of *T. parthenium* extract at different time intervals

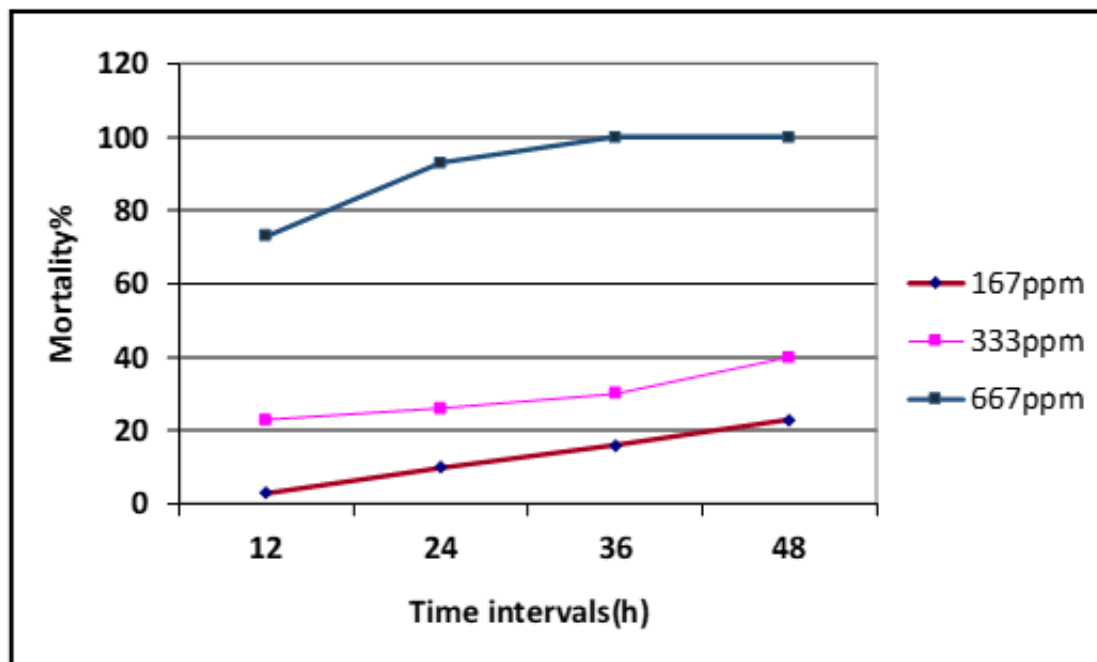


Figure 7. Fig. 6. Time-dependent mortality of larval *M. domestica* upon treatment with various concentrations of *T. parthenium* extract at different time intervals

Discussion

Numerous studies have been conducted on the insecticidal activity of medicinal plant extracts against insect pests such as

M. domestica, including *Origanum vulgare*, *Citrus sinensis*, *Mentha cervina*, *Ocimum basilicum*, *Coriandrum sativum*, *Eucalyptus globules*, etc. [11-20]. However, to the best of our knowledge, this is the first study describing the insecticidal activity of *T. parthenium* against *M. domestica*.

The bioassays revealed that the mortality of the adult fly and larval stages was dose-dependent. The mortality increases with increasing dose of *T. parthenium* extract. The LC₅₀ and LC₉₀ values of *T. parthenium* extracts for adult houseflies were much higher than the larval stage. This finding indicates that *T. parthenium* is more effective against the larval stage than against the adult stage.

Using technical grade pyrethrum (50%) for topical application against susceptible and multi-resistant strains of housefly adults, Joffe et al reported LC₅₀ values equal to 213 and 1930 ppm for the fly strains, respectively [21]. Given that, in our experiments, the active pyrethrins accounted for only 5.5% of the feverfew extracts, the efficacy of the extract against the wild population of houseflies in Bojnord (LC₅₀ = 5135 ppm) falls within a workable range.

Pyrethrins are contact insecticides that quickly penetrate the nervous systems of insects, causing paralysis in minutes and killing the insects in hours, particularly when combined [22]. However, pyrethrin II is easily metabolized by insects within a few hours, particularly when applied at sub-lethal dosages [23]. This may explain the results presented in Fig. 5. As shown, application of pyrethrins at lethal dosages caused acute mortality within the first 12 hours, while sub-lethal dosages lost effects at successive time intervals, probably due to metabolism. However, if applied at lethal dosages, pyrethrins may produce the highest mortality rates within the first 12 h post applications, followed by less steep rates at the following intervals, as depicted in Fig. 6. Nonetheless, both adult and larval stages of houseflies exposed to in-diet dosages of feverfew extract produced dose-dependent responses, as depicted in Fig. 3 and Fig. 4.

Conclusion

According to the WHO guideline, the following insecticides have been recommended for housefly control: Bendiocarb, Azamethiphos, Chlorpyrifos-methyl, Diazinon, Dimethoate, Fenitrothion, Malathion, Naled, Pirimphos-methyl, α -Cypermethrin, β -Cypermethrin, Betacyfluthrin, Bifenthrin, Cyfluthrin, Cypermethrin, Cyphenothrin, Deltamethrin, Esfenvalerate, Etofenprox, Fenvalerate, Lambdacyhalothrin, Permethrin, D-Phenothrin. The following larvicides also recommended: Diflubenzuron, Cyromazine, Pyriproxifen, Triflumuron. As environmentally friendly insecticides, the usage of natural products for the control of houseflies will decrease the resistance to synthetic insecticides [24].

Given the efficacy and high cytotoxic effects of *T. parthenium* extract on houseflies, we may recommend its derivatives as readily available bio-insecticides of broad spectrum to insect pests but with a high degree of safety to humans and the environment. We may also emphasize promotion of its industrialized cultivation and exploitation, as well as preservation of wild populations in natural habitats. This study may contribute to highlighting the importance of the country's rich flora, which needs to be explored for more environmentally friendly biopesticides [25].

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Conflict of Interest

The authors declare that there is no conflict of interest.

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