



RESEARCH ARTICLE

Evaluation of Insecticidal Properties of Plant Essential Oils against
Musca Domestica L.

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ABSTRACT

Musca domestica L. commonly known as housefly is a pest of public importance. It also vectors several pathogens causing human diseases like amoebic dysentery, shigellosis, salmonellosis, cholera and helminthic infections as well as viral and rickettsial infections throughout the tropical, subtropical parts of the world. Chemical control method is the most commonly practiced method to control housefly population. Though effective this method has significant disadvantages like development of insect resistance, mammalian toxicity and bioaccumulation. To avoid these disadvantages development of alternative user and environment friendly strategy must be developed and used. Presently, natural products, especially those derived from plant origin, have been progressively assessed in controlling pest/vectors of medical importance. In order to search for effective and user friendly control strategy, the essential oils of *Withania somnifera*, *Cymbopogon nardus* and *Asparagus racemosus* were evaluated for their larvicidal, attractant/repellent, and oviposition attractant/deterrent activity by standard methods against *M. domestica*. The highest larvicidal activity, i.e., $LC_{50}=99$ ppm was shown by *Withania somnifera*. This oil also exhibited 92.16 % repellency at the concentration of 1%. The highest oviposition deterrence activity of 94.22 % was exhibited by *Asparagus racemosus* oil at the concentration of 1%. These plants can find a place in the fly control program.

Key words: *Musca domestica*, *Withania somnifera*, *Cymbopogon nardus*, *Asparagus racemosus* Larvicide, repellency, oviposition deterrence

INTRODUCTION

Insects are one of the most economically important groups of organisms. Their association with human is known since long time. Amongst the various insect pest/vectors the housefly, *Musca domestica* L., is one of the most common insects, intimately associated with human settlements, and belongings. The food and breeding places of house fly is decaying matter, waste, and eatables, and are therefore considered to be mechanical vectors of pathogenic groups such as bacteria, protozoa, and viruses. House fly (*Musca domestica* L.: Diptera: Muscidae) is an important medical and veterinary insect pests that causes irritation, spoils food and acts as a vector for more than 100 human and animal pathogenic organisms such as enteropathogenic bacteria, enterovirus and protozoa cysts (Hanan 2013; Morey and Khandagle 2012). Several chemicals such as organochlorines and organophosphates, and more recently pyrethroid and spinosad, have been used against housefly. However, houseflies can develop resistance to these pesticides and health and environmental risks are associated with these compounds; thus, investigators continue to search for alternative methods of fly management. In this sense, essential oils and natural terpenes are potential alternatives and environmental friendly insecticides (Pohlit, et al. 2006).

Chemical control strategy is widely used presently throughout the affected areas. They have adverse effects on environment and health, threat of persistence & biomagnifications and Secondary pest resurgence. Control measure against this insect in the short-term is the use of conventional insecticides (Malik, et al. 2007). The flies are tarnished for their capacity to change

behavioral and metabolic strategies to avoid and detox the chemical insecticides. Moreover house fly have developed resistance to conventional chemicals (Khan, et al 2013) With discovery of new molecules and its test the flies still developed resistance and the flies responded by developing resistance to compound like organophosphate, carbamate, and pyrethroid insecticides (Kozaki, *et al.*, 2009; Memmi, 2010) in addition to chemicals like diflubenzuron and cyromazine (Bloomcamp, *et al.*, 1987). Spinosad, imidacloprid and nithiazine, were highly effective at the time of their introduction to the market however resistance to these products was documented within a short period (within a couple of year) of their introduction (Kaufman, *et al.*, 2010). In view of the disadvantages of chemicals there is an urgent need to find out an ecofriendly alternative for fly control program. The present study deals with the evaluation of essential oils of *Withania somnifera*, *Cymbopogon nardus* and *Asparagus racemosus* against house fly.

Withania somnifera, is a common plant which is also known as *ashwagandha*, It is a plant in the Solanaceae family. *Withania somnifera* is an erect, evergreen, branching, tomentose shrub of 30 to 150 cm in height. Leaves are of simple type, with petiole and blade of varying shape from elliptic to ovate, entire along margins, acute to obtuse at apex, oblique at base, clothed with a persistent grayish tomentum on sides, 4-10 cm long and 2-7cm broad. Leaves on vegetative shoots are alternate and large and those on floral branches are opposite, arranged somewhat laterally in pairs of one small leaf and one large leaf, with green monoceous flowers. It produces flowers during summer (Mirjalili, *et al.* 2009). Ashwagandha, is commonly used in Ayurvedic medicine, the traditional medical system of India. It is used in musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health (Chatterjee and Prakash 1995; Bone 1996). The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins and withanolides It is also rich in iron (Rastogi and Meherotra, 1998). Currently, "around more than 10 alkaloids, over 35 withanolides and sitoindosides; have been studied from *Withania* species. The major chemical constituents of these plants, withanolides, are mainly localized in leaves, (Mirjalili, *et al.* 2009).

Cymbopogon nardus is a tall tufted perennial grass with narrow leaf-blades. Panicle narrow, 15-30 cm long with racemes 8-10 mm long, often covered with long soft hairs; spikelets without stalks, flat or concave on the back with winged keels 5-6 cm long. *Cymbopogon nardus* is commonly grown for citronella, an essential oil (Harrington and Pratchett, 1974). *C. nardus* essential oil, contain compounds like citronellal, or 6-octenal, 3, 7-dimethyl-2,6-octadienal, 3,7-dimethyl-, cis-2,6-dimethyl-2,6-octadiene and propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester (Wei and Wee, 2013).

A. racemosus, climber growing upto 1-1.5 m in height. The leaves are like pine needles, small and uniform and flowers are white and have small spikes. The plant is included in genus *Asparagus* in the family Liliaceae to a newly created family Asparagaceae. (Shashi Alok, *et al.* 2013), The chemical composition includes Steroidal saponins, known as shatvarins; Oligospirostanoside referred to as Immunoside; Polycyclic alkaloid- Aspargamine A: Isoflavones-8-methoxy-5,6,4-trihydroxy isoflavone-7-O-beta-D-glucopyranoside; Cyclic hydrocarbon- racemosol, dihydrophenantherene; Furan compound- Racemofuran; Carbohydrates- Polysacharides, mucilage; Flavanoids, Sterols, Kaepfrol-Kaepfrol along with Sarsapogenin Miscellaneous-Essential fatty acids-Gamma linoleinic acids, vitamin A, diosgenin, quercetin 3-glucourbnides (Gaitonde and Jetmalani, 1969; Ahmad, *et al* 1991; Subramanian and Nair, 1968). In recent times, there were considerable efforts made to promote the use of environmental friendly and biodegradable natural insecticides and repellents, particularly from natural products. An attempt to explore the bioactive potential of the selected plants against house fly was made in this study.

MATERIALS AND METHODS

The collected plant material (Roots of *Withania somnifera*, leaves of *Cymbopogon nardus* and roots of *Asparagus racemosus*) was washed with distilled water and dried in shade at room temperature. The dried material was then powdered separately and subjected to steam

distillation. The extracted essential oils were collected in small bottles and stored at 4 °C until further use. All extracted oils were dissolved in AR grade acetone, and serial dilutions were prepared as per requirement for the experimental purpose.

REARING OF HOUSEFLY COLONY

The nucleus culture of *M. domestica* was obtained from Entomology Section, National Chemical Laboratory, Pune. The colony was maintained at 28±2°C and 70–75% relative humidity. Adults were reared in 30 cm ×30 cm ×30 cm metal frame cages. Aply wood floor was fixed at the base of each cage. A muslin sleeve was fitted on the side to serve as an access for the rearing activities. A cotton swab soaked in 5 g of milkpowder; 2 g of yeast dissolved in 30 ml of water was offered to these adults as food. The cotton swab served as substrate for oviposition. The eggs were transferred to a plastic jar, 25×15 cm, on fly rearing medium. The eggs were allowed to develop in this medium only up to pupal stage. The pupae were collected and kept in another container for adult emergence. Fresh emergence was transferred to separate containers to know the exact age of the adults.

LARVICIDAL BIOASSAY

Uniform residual film with desired concentration of the test plant oils in acetone was prepared on the petri dish (4" diameter on both lower and upper sides). Ten prepupal larvae were introduced in each filmed petri dish. In case of control only carrier solvent i.e. acetone was added. Food was provided and mortality was observed after 24 hours. For each experiment three replicates were used and each experiment was repeated five times. LC₅₀ value was calculated using log probit analysis (Finney 1971). Data obtained was subjected to statistical analysis.

OVIPOSITION ATTRACTION/DETERRENCE

Five males and five females (1–2 days old) were confined in a cage (size 18×24×24 in.). Cotton swab soaked with 1 % test oil and milk was offered to these flies. For control, cotton swab soaked with carrier solvent and milk was offered. After 24 h, egg count was taken. For this experiment, three replicates were taken, and the experiment was repeated three times. Data obtained were subjected to statistical analysis. The following formula was used to calculate percentage of oviposition deterrence (Tare 1995):

$$\text{Oviposition deterrence} = \{T - E/T\} \times 100$$

Where,

T= total number of eggs laid in both control and treated and

E= number of eggs laid in treated.

ATTRACTION AND REPELLENT ASSAY

Twenty newly emerged adults of mixed sexes were released in a cage (size 18×24×18 in.) containing two conical flasks. One flask contained 1 % test oil in 5 ml of milk, while the other contained solvent and 5 ml of milk to serve as control. Funnels (4-in. diameter) were introduced in each flask to trap insects in these flasks and count was taken after 24 h. The results were expressed in terms of percentage attraction/repulsion. The percentage repellency (R percent) was calculated by the following formula (Campbell 1983):

$$R\% = \{100(C-T)/C\} \text{ where}$$

C=the number of flies trapped in the control flask and

T=the number of flies trapped in the treated flask.

For this experiment, five replicates were taken, and each experiment was repeated three times.

RESULT AND DISCUSSION

The disadvantages of the indiscriminate use of chemicals have made it evident to search for alternatives to chemical control strategies used in insect control. Insecticides derived from biological origin, especially botanicals, have been increasingly evaluated in controlling the insect population of medical importance (Siriwattananurungsee, *et. al.*2008). Plants and plant derivatives

viz. essential oils are alternative agents for insect control as they are rich source of bioactive chemicals (Abdel Fattah, et al. 2009). Many studies have drawn attention of the toxic effects of plant extracts and dipterans (Dhar, et al. 1996). Plants are rich sources of alkaloids, flavanoids, terpenes, saponins and several bioactive compounds that can be used to develop environmentally safe vector and pest-managing agents. The botanical extracts from the plant leaves, roots, seeds, flowers, and bark in their crude form have been used as conventional insecticides for centuries (Bagavan, et al. 2009).The result of the present work revealed that among the three essential oils tested, the essential oil of *Withania somnifera* leaves has significant larvicidal and repellent activities against *M. domestica*. While the essential oil of *Asparagus racemosus* was highly effective in deterring the oviposition of *M. domestica*. The larvicidal activity as depicted in table 1 reveal that *Withania somnifera* has highest larvicidal activity i.e., LC_{50} = 99 ppm followed by *Asparagus racemosus* and *Cymbopogon nardus* with LC_{50} = 112 and 128 ppm respectively. These results of the selected plant oils are more significant than those obtained by Islam and Akthar,2013 with LC_{50} concentrations at 72 hours of exposure against *M.domestica* second instar larvae of the extracts of the plant parts were 5399.93, 7276 and 8149.33ppm, respectively for the root of *C. procera*, leaf of *P. hydropiper* and the root of *P. longum*.Begum et al. (2011) reported larvicidal activity of crude ethanolic extracts of *Calotropis procera* and *Annona squamosa*against *M. domestica* (LC_{50} =282.5 and 550 ppm,respectively). 98 % mortality of houseflylarvae is reported with dried *Libocedrus bidwillii* leaf extract at100 ppm concentration (Russell, et al. 1976). Seo and Park (2012) studiedthe larvicidal activity of medicinal plant extract from 27plant species against *M. domestica*, and their result reveals that *Phryma leptostachya* shows 100 % larvicidal activity while larvicidal activities of *Atractylodes japonica*, *Saussurea lappa*, *Asiasarum sieboldi*, and *Gleditsia japonica* were89.3, 85.3, 93.3, and 96.6 % at 10 mg/g concentration against *M. domestica* at 10 mg/g concentration, The *Withania somnifera* oil also exhibited 92.16 % (Table 2) repellency at the concentration of 1% while *Asparagus racemosus* exhibited lowest repellency i.e. 79.92 % at the same concentration and *Cymbopogon nardus* showed intermediate repellency with 82.51 % (Table 2).The essential oils obtained from *Ocimum gratissimum* L.,*T. serpyllum* L., *Illicium verum* Hooks, f., *Myristica fragrans* Houtt, and *Curcuma amanda* Roxb showed 100 % repellentactivity at 2 % concentration (Singh and Singh 1990). Kumar, et al. (2011) reported *M. piperita* essential oil to be mosteffective in repellency bioassay at 61.0 g cm⁻², followedby *E. globulus* 214.5 g cm⁻² and *Cymbopogon citratus*289.2 g cm⁻², while formulated *M. piperita* and *E. globulus*resulted in larval mortality at LC_{50} =5.12 and 6.09 g cm⁻²,respectively. Pavela et al. (2008) reported 50 % mortality offourth instar larvae of *M. domestica* at the concentration of193, 256, >500, and 256 mg/ml for the extracts of *Satureja hortensis*, *Thymus vulgaris*, *Salvia officinalis*, and *M. piperita*,respectively. The highest oviposition deterrence activity (Table 3) of 94.22 % was exhibited by *Asparagus racemosus* oil followed by 86.91% and 74.96 % *Withania somnifera* and *Cymbopogon nardus* essential oils at the concentration of 1%.

Table 1: Larvicidal assay of plant essential oils against house fly

| Essential oils | $LC_{50} \pm SE$ (ppm) | 95 % confidential limits | | Regression equation | LC_{90} (ppm) |
|----------------------------|------------------------|--------------------------|--------|---------------------|-----------------|
| | | LCL | UCL | | |
| <i>Withania somnifera</i> | 99±0.61 | 62.11 | 159.72 | Y=3.61X-5.22 | 144 |
| <i>Cymbopogon nardus</i> | 128 ±0.72 | 96.64 | 191.51 | Y = 4.63X-5.72 | 190 |
| <i>Asparagus racemosus</i> | 112 ±0.13 | 86.53 | 173.67 | Y = 3.41X-3.61 | 172 |

LC₅₀ lethal concentration that kills 50 % of exposed larvae, *LC₉₀* lethal concentration that kills 90 % of exposed larvae, LCL lower confidence limit, UCL upper confidence limit, SE standard error,

Table 2: Repellency assay of plant essential oils against house fly.

| Essential Oils | Repellency (%) |
|----------------------------|------------------|
| <i>Withania somnifera</i> | 92.16 (± 0.288) |
| <i>Cymbopogon nardus</i> | 82.51 ((± 0.313) |
| <i>Asparagus racemosus</i> | 79.92 ((± 0.335) |

Table 3: Oviposition deterrence assay of plant essential oils against house fly

| Essential Oils | Oviposition deterrence (%) |
|----------------------------|----------------------------|
| <i>Withania somnifera</i> | 86.91 (\pm 0.326) |
| <i>Cymbopogon nardus</i> | 74.96 (\pm 0.157) |
| <i>Asparagus racemosus</i> | 94.22 (\pm 0.221) |

The findings of the present study reveal that, the essential oil of *Withania somnifera* exhibited multifarious activity against *M. domestica*, while the results obtained with essential oil of *Asparagus racemosus* are also significant with respect to Oviposition deterrence and larvicidal activity. Essential oils from plants may be alternative sources of vector control since they constitute a rich source of bioactive compounds that are biodegradable (Zahir, *et al.* 2009).

Ensuing upon the findings of the selected plant oils in the present study it can be concluded that these oils with further field trials and toxicological studies can be a good addition to the fly control program. They can also be a valuable component in the IPM practices.

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