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ORIGINAL ARTICLE

Bioactive Potential of Three 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, Cymbopogan 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, Cymbopogan nardus, Asparagus racemosua, Larvicide, repellency, oviposition deterrence*

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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. Flies live on and near decaying matter, human waste, and eatables, and are therefore considered to be mechanical vectors of pathogenic 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 sand 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). House flies are known for their notorious capacity to develop mechanisms to avoid and detoxify chemical insecticides.Moreover house fly have developed resistance to most of chemical insecticides (Khan, *et al.* 2013) New chemistries were introduced over the years, and the flies responded by developing resistance to organophosphate, carbamate, and pyrethroid insecticides (Kozaki, *et al.,* 2009; Memmi, 2010) as well as to metabolic regulators 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*, *Cymbopogan nardus* and *Asparagus racemosus* against house fly. *Withania somnifera*, commonly known as *ashwagandha*, Indianginseng, poison gooseberry, or winter cherry is a plant in the Solanaceae family. *Withania somnifera* is an erect, evergreen, branching, tomentose shrub of 30 to 150 cm in height. Leafs are simple, petiolate with the leaf blade varying in shape from elliptic-ovate to broadly ovate, entire along margins, acute to obtuse at apex, oblique at base, clothed with a persistent grayish tomentum on sides, 4-10cm 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, bearing in axially cymose group of 4-25 inconspicuous pale green monoceous flowers. It produces flowers indeterminately round the year with a peak of flowering between March and July (Mirjalili, *et al.* 2009). *Withania somnifera* Dunal (ashwagandha) is known for its use in Ayurvedic medicine, the traditional medical system of India. It is an constituent in many preparations approved for a range of musculoskeletal ailments (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and endurance, and prevent disease in youth, the elderly, and during gestation. (Chatterjee and Prakashi, 1995; Bone, 1996) The bioactive constituents are chemicals like alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins and withanolides. It is also rich in iron (Rastogi and Meherotra, 1998). At present, "more than twelve alkaloids, forty withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27)" have been isolated and reported from aerial parts, roots and berries of *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 withracemes 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 is a woody climber growing to 1-2 m in height. The leaves are like pine needles, small and uniform and flowers are white and have small spikes. This plant belongs to the genus *Asparagus* which has recently moved from the sub family Asparagae in the family Liliaceae to a newly created family Asparagaceae (Shashi, *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-7-0-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 *Cymbopogan nardus* and roots of *Asparagus racemosus*) was washed with distilled waterand dried in shade at room temperature. The dried materialwas 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 fromEntomology Section, National Chemical Laboratory, Pune. The colony was maintained at 28±2°C and 70–75% relativehumidity. Adults were reared in 30cm×30cm×30cm metal frame cages. Aplywood floor was fixed at the base of each cage. A muslin sleeve was fitted on the side to serve as an access forthe rearing activities. A cotton swab soaked in 5 g of milkpowder; 2 g of yeast dissolved in 30 ml of water was offeredto 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 wereallowed to develop in this medium only up to pupal stage.The pupae were collected and kept in another container foradult 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 hrs. For each experiment three replicates were used and each experiment was repeated five times. LC_{50} value was calculated using log probitanalysis. (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)-

Oviposition deterrence = {T – E/T} x 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 5ml 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 24h. For this experiment, five replicates were taken, and each experiment was repeated three times. The results were expressed in terms of percentage attraction/repulsion. The percentage repellency (R %) was calculated by the following formula (Campbell 1983)-

R%= {100(C-T)/C}
Where,
C=the number of flies trapped in the control flask and
T=the number of flies trapped in the treated flask.

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 (Siriwattanarungsee, *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 repellentactivities 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}= 99ppm followed by *Asparagus racemosus* and *Cymbopogan nardus* with LC_{50}= 112 and 128ppm 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 27 plant species against *M. domestica*, and their result revealsthat *Phryma leptostachya* shows 100% larvicidal activity while larvicidal activities of *Atractylodes japonica*, *Saussurea lappa*, *Asiasarum sieboldi*, and *Gleditsia japonica* were 89.3, 85.3, 93.3, and 96.6% at 10mg/g concentration against *M. domestica* at 10mg/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 *Cymbopogan 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 citrates* 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 of 193, 256, >500 and

256mg/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 *Cymbopogan nardus* essential oils at the concentration of 1%.

Table1: Larvicidal assay of plant essential oils against house fly

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

LC_{50} lethal concentration that kills 50 % of exposed larvae, LC_{90} 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 (%)
Withania somnifera	92.16 (± 0.288)
Cymbopogan nardus	82.51 ((± 0.313)
Asparagus racemosus	79.92 ((± 0.335)

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

Essential Oils	Oviposition deterrence (%)
Withania somnifera	86.91 (± 0.326)
Cymbopogan nardus	74.96 (± 0.157)
Asparagus racemosus	94.22 (± 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 larvicadal activity. Essential oils from plantsmay be alternative sources of vector control since they constitutea 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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