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The Toxic Spectrum of *Ricinus Communisand Zingiber Officinalis* Essential Oils against *Musca Domestica* L.

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Abstract: The housefly, *Musca domestica* L., is a cosmopolitan insect, associated with various diseases and is one of the species with the greatest ability to develop resistance to insecticides. The study designed to evaluate the larvicidal, pupicidal, adulticidal and ovicidal effect of lethal concentrations LC50 of *R. communis* and *Z. officinalis* essential oils against the housefly *M. domestica* L. The larvicidal mortality (LC50) values of *R. communis* and *Z. officinalis* essential oils against *M. domestica* were 349.40 ppm and 132.60 ppm, with mortality percentage 46.67±2.89 and 50.00±0.00, respectively. The LC50 doses showed pupation mortality percentage (31.21 and 46.67) and adult mortality percentage (36.90 and 63.33), respectively. As compared to control, the treated 2nd larval instar with either *R. communis* or *Z. officinalis* essential oils showed significant decline in the fecundity of *M. domestica* females through significant decrease in the total eggs number as compared to control (72.67% and 62.00%) with Effective Repellency (ER%) (51.34 and 58.48) and the Oviposition Activity (OAI) (-0.35 and -0.41), respectively. The hatchability of eggs recorded significant reduction as compared to control (86.38% and 73.66%) for *R. communis* and *Z. officinalis*, respectively. The present study revealed toxic properties of the tested plant extracts of *R. communis* and *Z. officinalis* against *M. domestica* L. and pave the way for its use as a measure of the eco-friendly housefly control.

Keywords: *Musca Domestica* L., *Ricinus Communis*, *Zingiber Officinalis*, Essential Oils, Larvicidal, Pupicidal, Adulticidal, Oviposition

Introduction

The housefly, *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae) considered one of the most hazardous pest on health in the environment transmitting many infectious diseases to human and livestock. (Cirillo, 2006; Barin *et al*., 2010; Farag *et al*., 2013; Hung *et al*., 2015). The larvae of the fly can also be myiasis-producing agents in human and animals leading to huge economic loses particularly in livestock industry (Stevens and Wallman, 2006; Singh and Singh, 2015).

The use of chemical insecticides for housefly control induced insect resistance, affected the environment through water and soil contamination and became toxic to vertebrates (Yoke and Sudderuddin, 1975; do Prado, 2003; Abbas *et al*., 2016; Kasai *et al*., 2017).

The use of plant extracts, as alternatives for housefly control could be very promising since these are eco-friendly, biodegradable as well as cost effective. A large number of plants have shown the remarkable insecticidal activities (Seo and Park, 2012; Singh and Kaur, 2016). Essential oils showed relatively non-toxic to fish, birds and mammals and easily biodegrade in the environment, turning them into good bio pesticides (Kumar *et al*., 2012). Many studies revealed satisfactory results from the use of several essential oils for the house fly *M. domestica* management (Abdel Halim and Morsy, 2005; Sinthusiri and Soonwera, 2013; Pinto *et al*., 2015). In addition, the synergistic action of essential oils with conventional chemical pesticide previously studied (Mansour and Mohamed, 2013).

The study designed to evaluate the larvicidal, pupicidal, adulticidal and ovicidal effect of lethal concentrations (LC50) of either *R. communis* or *Z. officinalis* essential oils against the housefly *M. domestica* L.

Materials and Methods

Collection of Flies

Adults of *M. domestica* were collected in June 2015 from the garbage site of the Abu Arish area, (Eastern Jazan, 16°58'N to 42°47'E), southern Saudi Arabia, by using a sweeping net and transported into a small cage to

the Department of Biology, Faculty of Science, Jazan University for identification and reared in laboratory for four generations before experiment.

Maintenance of Flies

Adult flies maintained in cages (30 × 30 × 30 cm) and reared at 29±1°C and 65±5 percentage RH and provided with granulated sugar petri dishes containing cotton pads soaked in milk powder dissolved in water (10% w/v) and jars (500 ml) containing larval media for egg laying. The larval media consisted of yeast, dry milk powder, wheat bran and water according to the method previously described (Pavela, 2006). The jars removed from cages after 2-3 days when eggs visible and provided with wood dust for pupation and kept in separate cages for the fly emergence.

Essential Oils

The essential oils purchased from Sigma-Aldrich Company, Castor oil, *R. communis* of 99% purity, 0.961 g/ml density and *n*20/D 1.478 refractive index and *Z. officinalis* of 99% purity, 0.871 g/ml density and *n*20/D 1.49 refractive index. Four concentrations (4, 3, 2.5 and 1%) prepared for the two essential oils dissolved in acetone and stored in glass bottles at 4°C until used.

Lethal Concentration (LC50) Bioassay

The bioassay used the second instar larvae (3-days-old) hatched from the same egg batch. Larval treatment carried out in petri dishes according to method previously explained (Brady, 1966), where the interior of each petri dish treated with 1ml from each of the four aforementioned concentrations of the tested essential oils. Each experiment conducted in four replicates (20 larvae/replicate) along with the control group. After treatment, the larvae transferred to the rearing jar and the mortality assessed by touching each one larva with a paintbrush (no. 0) and those not responding considered dead. The LC50 for larval mortalities determined based on mortality data at 24 h (Hanan, 2013).

The calculated LC50 concentration values for either *R. communis* or *Z. officinalis* essential oils prepared using acetone solvent and stored in glass bottles at 4°C until they used for each one as a dose in the next bioassays.

Pupicidal and Adulticidal Bioassays

The larval mortality recorded as previously mentioned and the survived pupa used for the determination of the pupal mortality at 7 days. Then the survived adults used for the adulticidal bioassay, which determined following WHO susceptibility test guidelines (Sinthusiri and Soonwera, 2013).

Oviposition Deterrent and Ovicidal Bioassay

The oviposition deterrent and ovicidal bioassay according to the method of Morey and Khandagle (2012). Ten of house fly females and males aged 4 days old introduced in a screened cage where two oviposition boxes lined with cotton pad, size 3X10X0.25cm. The first box filled with 1ml of 10%w/v milk solution and 1 ml of each test solution (LC50 of each essential oil), while the second box, which served as control, filled with only 1 ml of 10% w/v milk. The boxes switched every day to avoid the position effects. The eggs laid into each box collected separately until no further eggs laid for at least 48 h. Four replicates carried out with each test solution (LC50). The percentage of Effective Repellency (ER %) and Oviposition Activity Index (OAI) were calculated using the next formula (Siriporn and Mayura, 2012)**:**

$$ER\ (\%) = NC\text{-}NT/NC \times 100$$

Where:
ER = Effective repellency
NC = The total number of eggs in the control solution
NT = The total number of eggs in each test solution:

$$OAI = NT\text{-}NC/NT+NC$$

Where:
NC =The total number of eggs in the control solution
NT =The total number of eggs in each test solution

The OAI ranges from -1.0 to +1.0, with o indicating neutral response. The positive index values indicate that more eggs deposited in the test boxes than in the control boxes and that the test solutions attractive, on the other side, more eggs in the control boxes than on the test boxes results in negative index values and the test solution deterrent.

Statistical Analysis

The observed mortality corrected by Abbott's formula (Abbott, 1987). Data analyses were performed using a one-way ANOVA (Least Significant Difference (LSD) and significant differences were determined at $P<0.05$. Statistical Package for Social Science "SPSS" for Windows software, Release 20.0 (SPSS, Chicago, IL) was used.

Results

Results of present study represented in Tables (1) and (2) declared the effect of both essential oils, *R. communis* and *Z. officinalis* as regard to larvicidal, pupicidal, adulticidal, total number of eggs and hatching percentage against *M. domestica* as compared with the analogous control. The assessment of *R. Communis* and *Z. officinalis* toxicity against *M. domestica* larvae revealed LC50 values 349.40 ppm and 132.60 ppm, respectively, recording 46.67±2.89 and 50.00±0.00 mortality percentage that are significantly different at 0.05 level as compared with control value (11.67±2.89).

Table 1: Insecticidal effect of *Ricinus communis* and *Zingiber officinalis* essential oils against larvae, pupae and adult of housefly

Treatment	Larvicidal activity		Pupicidal activity	Adulticidal activity
	Mortality (%) ± SD	LC_{50} (ppm)	Mortality (%) ± SD	Mortality (%) ± SD
Control	11.67±2.89[a]	-	15.14±3.53[a]	8.75±3.98[a]
R. communis	46.67±2.89[b]	349.40	31.21±4.67[b]	36.90±10.31[b]
Z. officinalis	50.00±0.00[b]	132.60	46.67±5.77[c]	63.33±15.28[c]

Table 2: The total number of eggs laid, oviposition deterrent activity and hatching percent of *Ricinus communis* and *Zingiber officinalis* essential oils against housefly

Treatment	Total number of eggs ± SD	ER%	OAI	Hatching% ± SD
Control	149.33±1.15[a]	-	-	95.53±1.05[a]
R. communis	72.67±3.06[b]	51.34	-0.35	86.38±5.82[b]
Z. officinalis	62.00±2.65[c]	58.48	-0.41	73.66±1.44[c]

As regard to the survived pupae the recorded percentage mortality after 7 days (pupicidal activity) showed significant increase in mortality rate (31.21%±4.67) and (46.67%±5.77) for *R. communis* and *Z. officinalis* essential oils groups, respectively as compared to that of the control (15.14%±3.53). The adulticidal activities recorded in this study showed significant increase in mortality rate(36.90%±10.31) and (63.33%±15.28)for *R. communis* and *Z. officinalis* essential oils groups, respectively as compared to that of the control (8.75%±3.98) (Table 1).

The effect of the LC50 of both essential oils on fecundity and eggs hatchability represented in Table (2). Data showed significant decrease in the total number of eggs laid in *Z. officinalis* group (62.00±2.65) with ER (58.48%) and OAI (-0.41) as compared to that recorded number for *R. communis* group (72.67±3.06) with ER (51.34%) and OAI (-0.35) and both groups showed significant decrease as compared to the control group recorded number of eggs laid (149.33±1.15).

Results in Table (2) showed that the hatching percentage of eggs in in *Z. officinalis* group (73.66 ± 1.44) significantly decreased as compared to that recorded for *R. communis* group (86.38 ± 5.82) and both groups showed significant decrease as compared to that recorded for the control group (95.53 ± 1.05).

Discussion

The toxicity recorded in this study support different previous studies represented toxicity on larvae and adults as well as on fertility of plant origin essential oils against *M. domestica* (Pavela, 2008; Morey and Khandagle, 2012; Singh and Kaur, 2016; Walia *et al*., 2017). A previous study using *R. communis* extract recorded LC50 value of 353.4 ppm after 72 h of exposure to the extract and reported that the extract contained the ribosome inactivating protein ricin (Alvarez Montes de Oca *et al*., 1996). A study using the chloroform leaves extract of *R. communis* predict larval and pupal mortality as well as reduction of pupations and non-emergence of adults of *M. domestica* as regard to the potent insecticidal component of *R. communis* (ricinine) confirming the effect on the housefly life cycle (Singh and Kaur, 2016) because ricinine has maximum solubility in chloroform (Rao, 1945). The gas chromatographic analysis with mass spectrometry (GC-MS) detected the main constituents of the *R. communis* essential oil, α-thujone, 1,8-cineole, α-pinene, camphor and camphene (Kadri *et al*., 2011; Zarai *et al*., 2012). The toxicity of these monoterpenoids against the housefly assessed (Kumar *et al*., 2012; 2013; Zhang *et al*., 2017). The mode of action of these constituents may elucidate the mechanism of the essential oil insecticidal activity, which had multiple targets. One of these targets is the effect on the nervous system receptors like γ-aminobutyric acid (GABA) receptors that predicted previously for the aforementioned monoterpenoids on the fly head (Tong and Coats, 2012). Another mechanism of the insecticidal activity for *R. communis* proposed due to inhibition of certain vital enzymatic pathways, in which flavonoids block hydroxylase enzyme by action of cytochrome- P450 which is involved in regulation of moulting process of insects (Upasani *et al*., 2003). In addition, flavonoids of *R. communis* reported to affect the insect ecdysone-20-monooxygenase, which is responsible for the synthesis of 20- hydroxyecdysone the precursor of insect growth hormone- ecdysone responsible for regulating the insects life cycle by initiating molting to grow into adults. Hence, the hindrance in the hormone synthesis affects the duration of prepupation period and adult emergence rates (Singh and Kaur, 2016).

Both essential oils significantly affect the oviposition deterrent and ovicidal activities as well as hatching rate but the advantage in toxicity recorded for *Z. officinalis* as compared to *R. communis*. The essential oil of *Z. officinalis* evaluated larvicidal activity and recorded LC50 value of 259 ppm against *M. Domestica* (Morey and Khandagle, 2012). Products isolated from *Z. officinalis* found to be effective as insect antifeedant and insect growth regulators (Agarwal *et al*., 2001). The essential oil of *Z. officinalis* caused significant larvicidal and repellency activity against *M. domestica* and found effective in repellent and oviposition deterrence assay (Morey and

Khandagle, 2012; Krishna, 2013). A study recorded that *Z. officinalis* not only has a larvicidal action against *M. domestica* larvae but also affected the adult fecundity (Abdel, 2008). Data demonstrated that minimum pupal survival was shown in use of *Z. officinalis* essential oil which in line with previous study (Morey and Khandagle, 2012) where the essential oil of *Z. officinalis* exhibited significant bioactivities against *M. domestica* with larvicidal activity with Lethal Concentration (LC50) (137 ppm), repellency (84.9) and (98.1%) oviposition deterrence both at 1% concentration. A study to evaluate Z. *cussumunar* essential oil as well as six other oils against *M. domestica* showed that the high concentration (10%) of the essential oil revealed high percent effective repellency and showed the hatching rate decreased with oviposition deterrence (Sinthusiri and Soonwera, 2013). The analysis of *Z. officinalis* essential oil by GC-MS detected relatively large amounts of the monoterpenoids 1,8-cineole, linalool, borneol, alpha-terpineol, neral, geraniol, geranial, trans-dimethoxy citral and geranyl acetate (Gupta *et al*., 2011) which may related to its advantage of toxicity as compared to that recorded of *R. communis* against the fly (Kumar *et al*., 2013). In addition, these monoterpenoids existence in essential oil support the insect repellant activity (Showler, 2017).

Conclusion

The present study suggest that the essential oils of Z. officinalis *and R. communis*can be effectively used as in controlling fly populations of *M. domestica* as the efficient, ecofriendly and economic alternative to synthetic insecticidal agents.

Data expressed as mean of three replicates One Way analysis performed between groups with LSD post hoc test with significance level 0.05. Significance between different letters within the same column.

Data expressed as mean of three replicates One Way analysis performed between groups with LSD post hoc test with significance level 0.05. Significance between different letters within column. ER% is the percentage of effective repellency.OAI is the oviposition activity index. Hatching rate is the hatching percent. Hatching % calculated by dividing the total hatch from the total number of eggs laid in each group.

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Ethics

The authors confirmed that this manuscript is an original work and do not contain any conflict of interest.

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