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POTENTIAL OF POLYMER MATRIX IN DELIVERY OF LEMON GRASS *CYMBOPOGON CITRATUS* STAPF ESSENTIAL OIL AGAINST HOUSE FLY *MUSCA DOMESTICA* L.

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ABSTRACT

House fly *Musca domestica* L. is a pest of humans, poultry, and livestock across the world. Dependence on chemical insecticides to contain the flies provided varying results and their continued use has led to development of insecticide resistance. Bioactive compounds in plants are an alternative source to manage *M. domestica.* Lemon grass, *Cymbopogon citratus* Stapf essential oil caused fumigant toxicity to eggs (LC_{50} 1.299 mg/dm^3) and adults (16.56 mg/ dm^3). The *C. citratus* EO caused larval repellence. Polyvinylpyrrolidone when used as polymer matrix to load *C. citratus* at 1:1, 1:2, and 1:3 caused toxicity to flies for a longer period as compared to use of EO alone. The EO loaded in polymer matrix had a slower dissipation, EO+ PVP polymer mixed at 1:3 retained over 80% of EO after 72 hr when exposed to 60°C. EO, whilst EO alone without a dispenser dissipated in 3 hr. The biological effect of *C. citratus* EO on *M. domestica* can be enhanced for a longer period if loaded into a polymer matrix and this would be an effective strategy to manage *M. domestica.*

Key words: *Cymbopogon citratus*, essential oil, fumigant toxicity, *Musca domestica*, polyvinyl pyrrolidone, polymer matrix, slow delivery, repellence activity, ovicidal toxicity, GC-MS

The house fly *Musca domestica* (L.), is a pest of medical and veterinary importance (Wang et al., 2019) transmitting pathogens that cause enteric diarrheal diseases (Chauhan et al., 2016; EL Zayyat et al., 2015). Indiscriminate use of chemical insecticide to manage flies leads to development of insecticide resistance, ill effects on non-target organisms and environmental contamination (Prado, 2003; Pavela et al., 2009). Biopesticides derived from plant parts are an alternative, as they are effective on target pests and safe to natural enemies, pollinators (Liu et al., 2000) and fit into the IPM measures (Koul et al., 2008). Derivatives from natural products termed as green pesticides (Benelli et al., 2019) include essential oils (EOs) that possess desirable qualities as a good pest control method (Benelli and Beier, 2017; Chellappandian et al., 2018). Essential oils are widely used as flavours and fragrances (Bakkali et al., 2008), antimicrobials (Dorman and Deans, 2000) and as pest control agents (Isman and Machial, 2006). EOs in addition to being a good candidate as insecticides also prevent the insects from developing insecticide resistance due to their complex mixtures of monoterpenes, sesquiterpene, hydrocarbons and their oxygenated derivatives (alcohol, aldehyde, and ketones) (Saad et al., 2013; Isman, 2017).

Essential oil derived from *Cymbopogon citratus* Stapf. (Oyedele et al., 2002) is widely used as flavour, fragrance and in aromatherapy (Shah et al., 2011). The major constituents of *Cymbopogon* spp. EOs are citral, geranial, citronellol, citronellal, geranyl acetate, geranyl formate and piperitone (Bhatnagar, 2018; Devi et al., 2020). Sinthusiri and Soonwera (2013) reported cidal and repellent properties of *C. citratus* against *M. domestica. C. citratus* EO alone or in combination with other oils was toxic to mosquitoes and house fly *M. domestica* L. (Sritabutra et al., 2011). The complex mixture in essential oils makes them an ideal candidate to mitigate the development of insecticide resistance (Feng and Isman, 1995). Essential oils are proven to have desirable biological effect on insects, but the hurdle in field level use its quick physical loss due to evaporation (Isman, 2006; Koul et al., 2008) and chemical breakdown due to photo degradation and hydrolysis. This gap can be addressed by using a matrix that could prevent the loss of compounds. The plasticizers and stabilizers from polymers have been utilised in development of controlled release pesticide polymer formulations (Flemming et al., 2000; Roy et al., 2014: Ravindran et al., 2019b). Combining the essential oil and its constituents with polymeric matrix resulted in better spatio temporal release (Koul et al., 2018). The present study attempts to establish the fumigant toxic effect of *C. citratus* EO to eggs and adults and repellence to larvae of *M. domestica*. The efficacy of EO loaded in polymer matrix and its dissipation pattern are also discussed.

MATERIALS AND METHODS

The essential oil from *C. citratus* leaves (500 gm) was extracted by hydro-distillation for 8 h. The shade dried leaves were powdered and loaded into a round bottom flask with 500 ml of distilled water. The flask was placed over a heating mantle and the contents in the flask were raised to 100 ºC. The essential oil collected in the receiver tube and the aqueous portion was separated from oil using a separating funnel. It was passed over anhydrous sodium sulphate to remove the moisture trace and stored at 4 ºC until use. The *C. citratus*, EO was characterized using GC-MS as suggested by Ravindran et al. (2019a). Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FT-IR) was utilized for the identification of the functional groups present in polymer matrices. Perkin Elmer Premium HATR instrument with Germanium (Ge) crystal was used to acquire the FT-IR spectra. All the samples were dried prior to measurements. Lemon grass oil, Polyvinylpyrrolidone (PVP) and their physical mixture were placed over the sample plate. The IR spectra were obtained over a wave number region of 4000-400 cm^{-1} at room temperature. Functional groups possessed by each individual ingredient should be identical in their blend/mixture which confirms their compatibility.

The dissipation of EO loaded in PVP polymer matrix was assessed by loading 500 mg of EO along with 500 µl dichloromethane in Eppendorf tubes containing 500, 1000 and 1500 mg of PVP so as to achieve EO + polymer mix of 1:1, 1:2 and 1:3 ratio. The setup was placed on a heating platform maintained at 60ºC. Care was taken to check if there were temperature fluctuations. The entire setup was placed under fume hood and the weight loss of the contents in the tube was recorded by gravimetric method using a precision balance (Shimadzu) at 0.5, 1, 2, 3, 4, 6, 12, 18, 24, 36, 48, 60 and 72 hr after start of the assay. Three replicates were maintained for each concentration. The *M. domestica* were reared by the method suggested by Senthoorraja et al. (2020). The life stages of the flies collected from the rearing chamber were used in experiments.

The fumigant toxicity to *M. domestica* was done as suggested by Kumar et al. (2012). Briefly, 100 freshly laid *M. domestica* eggs were placed on a moist filter placed in a polypropylene container. The *C. citratus* EO was loaded on to 2x 2 cm filter paper at a concentration ranging from 0.3 - 36.61 mg/ l to assess ovicidal effect and concentration ranging from 7.32- 58.58 mg/ l to assess adult fumigant toxicity. The cap (with the filter paper) of the container was tightly sealed and placed in an incubator at 25± 2ºC and RH 65 ± 5%. Five replicates were maintained for each treatment. DDVP was used as positive control and acetone treated filter paper was maintained as negative control. Observations were made 3hrs after the start of the experiment to assess mortality of adults. The flies that ceased to move its appendages on a gentle prick with the pin were considered dead. In case of eggs exposed to EO and DDVP the per cent hatchability after 48 hrs were recorded to assess the ovicidal effect. Mortality if any in control was subjected abbot's correction prior to calculating the probit.

The toxicity of EO loaded in polymer matrix was assessed by mixing the EO (8 mg) with polymer Polyvinyl pyrrolidone at 1:1, 1:2 and 1:3 ratio (w/w basis). The constituents were mixed with 100 µl dichloromethane in an Eppendorf tube. This was mixture was then transferred to filter paper (2x 2 cm) and dried in fume hood for 10 min to facilitate the drying of the solvent from EO + polymer mix prior to use. These filter sheets loaded with the analyte and polymer were placed in the inner lid of the sample container as mentioned in previous section of fumigant toxicity. Batch of *M. domestica* eggs and adults were exposed to EO + polymer mix sheet 1 and 3 days after loading. Mortality of adults were recorded after 24 hr and the ovicidal effect after 48 hr. Filter paper loaded with EO alone (8mg) was used as positive control. In addition, the PVP treated filter and solvent treated paper were maintained as negative control.

The larval repellence to *C. citratus* EO was assessed by the method followed by Sinthusiri and Soonwera (2014). Insect breeding dishes (9x 4.5 cm) (Tarsons) base was divided to two halves and larval diet (2 g) treated with *C. citratus* oil at varied concentrations was placed in one half and diet treated with acetone was maintained as control. Neem oil treated diet was used as positive control. The III[rd] instar larvae, 20 numbers per replicate, were released in the midline. The number of larvae in treated and untreated diet was recorded after 30 min to calculate the repellence. Five replications were maintained for each concentration.

The repellency percent was calculated by using the formula: Repellency %= (NC−NT)/(NC+NT) x 100; here, NC represents no. of larvae at control region and for NT, no. of larvae at treated region. Fumigant toxicity of eggs and adults was subjected to probit analysis to calculate the dose response and Chi-square values. The effect of EO loaded in polymer matrix was subjected to one-way ANOVA and post hoc test to compare the means.

RESULTS AND DISCUSSION

The major components present in *C. citratus* EO were citronellol (64.73%), geraniol (10.51%), linalool (5.24%), phenyl ethyl alcohol (4.82%), isopulegol acetate (2.91%), neryl acetate (1.10%), citronellal and 1,8 cineole (1.08%), limonene (1.00%), lavandulyl acetate (0.44%), and menthone (0.34%). The ATR-IR spectrum of PVP revealed the absorption band located around 1644 cm^{-1} and it can be ascribed to the stretching vibration of the C=O bond in the pyrrolidone group. In addition, the CH stretching modes can be assigned at 2944 cm^{-1}. The peak at 1415 cm^{-1} corresponds to the CH deformation modes from the CH_2 group. The peak at 3435 cm^{-1} is due to the O-H stretching. The absorption bands at 1263 cm^{-1} is due to the C–N stretching vibration from the pyrrolidone structure. The absorption bands of amines at around 3400–3500 cm^{-1} were not observed because PVP is a bi-substituted amide.

The physical mixture of the lemon grass oil and PVP gave a sharp peak in the ATR spectrum at 2932 cm^{-1} and 2857 cm^{-1} for –CH stretching due to the presence of alkane group in the structure. The Peak at 1420 cm^{-1} is due to C–H scissoring for the mono-substituted alkane group. A sharp peak at was observed 1058 cm^{-1} for strong C-O stretching which indicates alkyl aryl ether in the structure (Fig. 1). A strong C=O stretching at 1657 cm^{-1} might be a confirmatory peak for the ester group that corresponds to lemon grass oil. The peak at 1284 cm^{-1} was observed for the CN stretching due to the presence of an imine/oxime group. The physical mixture showed intact characteristic peaks of lemon grass oil and PVP, thereby indicating no particular interactions of the herbal oils with any excipient in the physical mixture.

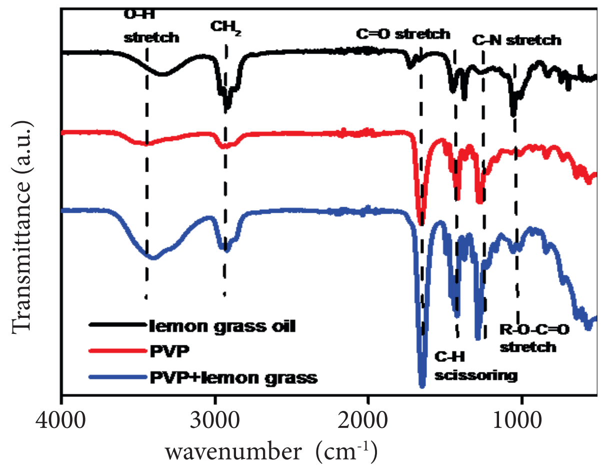


Fig. 1. FTIR spectra of EO, PVP and EO + PVP mix

ATR-IR spectrum of C. citratus EO oil exhibited a characteristic peak at 1710 cm−1 for the C=O stretching due to α, β-unsaturated ester. The broad peak at 3331 cm-1 is due to O-H stretching, sharp Peak at 2902 cm-1 and 2854 cm−1 is due to CH2 stretching, the sharp peak at 1441 cm−1, 1374 cm−1 is due to the C=C stretching. A peak at 1050 cm−1 for the R-O-C=O is observed.

Dissipation pattern of *C. citratus* loaded in PVP polymer matrix: *C. citratus* EO used as such without a carrier dissipates quickly as compared to EO loaded in a PVP polymer dispenser. More than 50 per cent of EO dissipated within 3 hrs from start of the run. The EO entrapped in PVP delivery matrix irrespective of the ratio in which they were blend caused controlled release of essential oil loaded in polymer matrix when exposed to temperature at 60 °C. Among the blends, EO + PVP polymer mixed at 1:3 was more effective in retaining over 80 % of EO even after 72 hr followed by 1:2 and 1:1 mix (Fig. 2). Higher quantum of PVP polymer in 1:3 ratio would have facilitated better cross linking of polymer with EO. This results in controlled release which would in turn facilitate in keeping its efficacy over a longer period

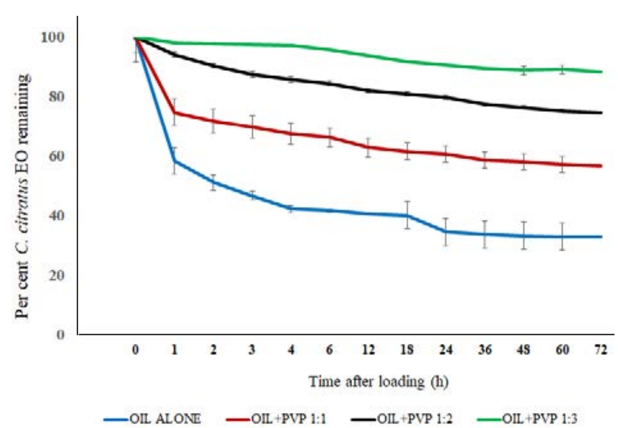


Fig. 2. Dissipation pattern of essential oil entrapped in PVP matrix

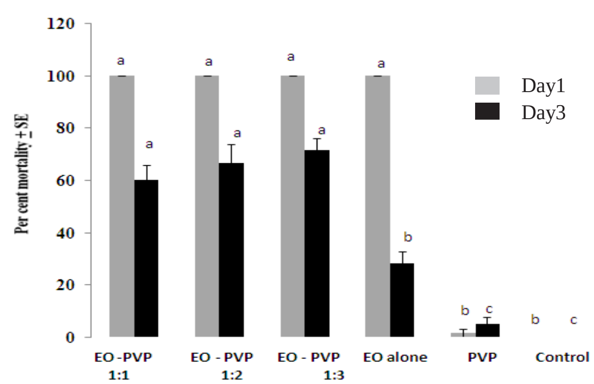
of time as compared to EO exposed as such without a delivery matrix. The dissipation pattern revealed that EO entrapped in PVP polymer matrix offers an appropriate dispensing mechanism for controlled delivery. Sweet basil oil and citridora oil loaded in poly vinyl alcohol sheets dissipated slowly as compared oil exposed as such without a dispenser (Ravindran et al., 2019a, b).

Fumigant toxicity: The quantum of *C. citratus* EO required to cause toxicity to adults (LC_{50} 16.56 mg/dm^3) was higher than to *M. domestica* eggs with LC_{50} at 1.299 mg/dm^3. The synthetic insecticide DDVP was highly toxic than *C. citratus* EO to both eggs and adults (Table 1). There are no earlier reports on fumigant toxicity of *C. citratus* EO on *M. domestica* eggs. Topical application of *C. citratus* caused less than 5% hatching inhibition (Sinthusiri and Soonwera, 2014). Pushpanathan et al. (2006) reported that *C. citratus* oil had ovicidal effect on *A. aegypti* and *C. quinquefasciatus*. A major constituent in *C. citratus* oil citral caused 96% inhibition of egg hatching in housefly (Rice and Coats, 1994). Targeting the non-motile stage like eggs is an easy method to eradicate the flies in poultry and livestock facilities.

These results on fumigant toxicity to adult *M. domestica* agree with those of fumigant toxicity of *C. citratus* to *M. domestica* (Kumar et al., 2012). Geraniol and 1,8 cineole present in *C. citratus* cause mortality of houseflies by inhibition effect of neuronal receptors with the symptoms ranging from hyperactivity, loss of balance and mortality (Chellappandian et al., 2018; Pavela, 2015; Pavela and Benelli, 2016). In our studies we observed that adults exposed to *C. citratus* EO caused uncoordinated movements and jitters prior to their death. The fumigant toxicity of *C. citratus* may be due to presence of 1, 8-cineole that is reported to be toxic to respiratory and digestive systems of adult mosquitoes (Pujiarti and Fentiyanti, 2017). The impact of *C. citratus* EO on intersegmental membranes of *M. domestica* and *An. stephesi* larvae was reported earlier (Chauhan et al., 2016; Mishra et al., 2017).

Though the chemical insecticide DDVP was effective against the biostages of flies (egg and adults) at an extremely low concentration as compared to *C. citratus*, they are highly toxic to non-targets and the applicators. Toxicity of EO loaded in PVP polymer matrix was assessed. Though *C. citratus* EO caused fumigant toxicity to eggs and adult of *M. domestica* there exists a hurdle in its use. EO when applied on surface is subjected physical and chemical degradation that reduces its field efficacy over a period. To tide over this gap, *C. citratus* EO was mixed with Polyvinyl pyrrolidone (PVP) polymer at 1:1,1:2 and 1:3 ratio and was compared with essential oil applied as such without any carrier. Exposure of eggs to filter paper treated with EO (LD_{90}) polymer mix showed that all the treatments on day 1 after exposure (essential oil alone and the EO polymer mix at various ratios) caused 100 per cent mortality. On day 3, the EO polymer mix at 1:3 caused higher mortality of eggs but was on par with other blends in causing ovicidal activity. EO when used without a carrier caused less than 40% egg mortality on day 3 which may be due to degradation of EO (Fig. 3). Similar trend was observed when EO polymer mix was used to assess the fumigant toxicity to adults. All the combination of EO+ PVP mix and EO alone caused 100% mortality on day 1. On day 3 all the EO Polymer mix at 1:1, 1:2 and 1:3 caused over 70% adult mortality, whilst the EO used without a carrier caused a lowest mortality of less than 40% (Fig. 4). PVP alone and control did not cause significant mortality of eggs and adults.

Larval repellence: Larval repellence on food treated



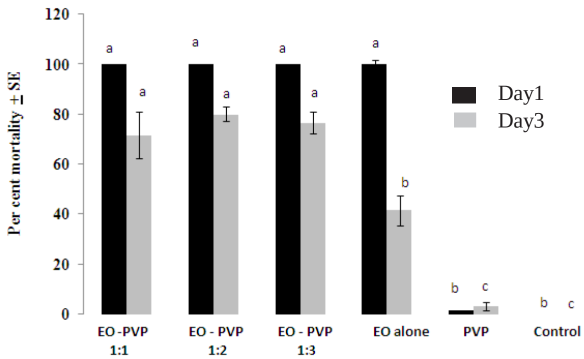
Means followed by same alphabet in a bar of given colour do not differ significantly by DMRT $p < 0.05$.

Fig. 3. EO + PVP mix toxicity on *M. domestica* eggs

Table 1. Fumigant toxicity of *C. citratus* against *M. domestica*

Bio stage	Treatment	LC_{50} (mg/ dm^3)	95% CI	LC_{90} (mg/ dm^3)	95% CI	df	Chi-square
Egg	*C. citratus* EO	1.299	0.810–1.891	13.23	8.354–25.281	5	8.64
	DDVP	0.215	0193 – 0.236	0.454	0.404 – 0.531	8	10.403
Adult	*C. citratus* EO	16.56	11.68-21.66	43.69	31.75-79.23	3	7.9644
	DDVP	0.125	0.094-0.157	0.36	0.27-0.56	6	6.143

CI- Confidence Interval; Df – Degree of freedom; p=0.05



Means followed by same alphabet in a bar of given colour do not differ significantly by DMRT $p < 0.05$

Fig. 4. EO + PVP mix toxicity on *M. domestica* adults

with *C. citratus* EO and neem oil revealed that EO at 0.5% caused maximum repellence of 74% but it was on par with EO at 0.3%. Across the doses tested EO caused higher repellence % as compared to neem oil tested at same dose. Both EO and neem at 0.3 and 0.5% caused more than 45% larval repellence (Fig. 5); *C. citratus* oil at 10% caused 87.93% oviposition repellency in house fly adults (Sinthusiri and Sonowera, 2014); *C. citratus* caused moderate repellence to house fly adults at 0.01µl/ $cm^{3.}$ Kumar et al. (2012) reported substantial house fly repellency of mentha oil (RC84, 61.0 µg/ cm^2) followed by eucalyptus (RC84, 214.5 µg/ cm^2) and lemongrass (RC84, 289.2 µg/ cm^2) against house fly. The present study observed 74% repellency at 0.5% concentration in house fly at larval stage. It indicates lemon grass to be the strong repellent and it can be formulated as promising repellent for house fly management. Lemongrass oil is considered safer for human health than chemical insecticides and this oil has been traditionally used as tonic and carminative medicine in Thailand (Sinthusiri and Soonwera, 2013), and used to repel mosquitoes in jungle regions of Bolivian Amazon (Nerio et al., 2010). Magierowicz et al. (2020) reported that *C. citratus* oil

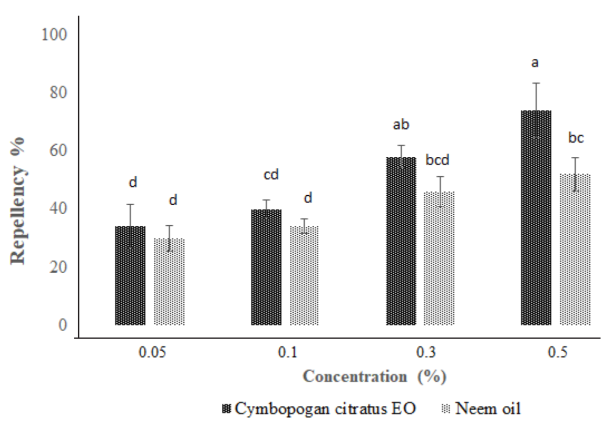


Fig. 5. Larval repellency of *C. citratus* on *M. domestica*

was non-effective as repellent on *Acrobasis advenella* (Zinck.), but from the current study it shows 74% repellent against house fly larva.

Citral and 1,8-cineole are major components of *Cymbopogon citratus* (lemongrass) which was responsible for the insecticidal activities against house fly have been reported in earlier studies (Kumar et al., 2012). The direct impact of essential oils and their components on mortality, secondary impacts are important in the development of fertility, repellency and anti-feedancy, as well. Lethal doses of citrus oils, applied to mature house flies, reduced the number of eggs delivered in a ratio of 50% per single female. In addition, repellency the secondary impacts may play a role in reduction of insect population (Pavela et al., 2009). *Cympopogon citratus* EO is an effective alternative to chemical insecticides in *M. domestica* management as it possesses fumigant toxicity, larval repellency and are safe to non-targets and environment. The pitfall in use of essential oil is its quick degradation. Loading them in a polymer matrix aided to overcome the problem as they caused sustained release of *C. citratus* EO. The polymer matrix loaded with EO is an ecofriendly technology for house fly management and can be adopted with ease.

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