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Bio Extract- The Green-Derived Essential Oils with Bio-Active Derivatives Of Sphaeranthus Amaranthoides

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Abstract:

The present investigation was highlighted the green-derived Essential oils with bio-active derivatives of Sphaeranthus amaranthoides (Sa-EO) can be employed in larvicidal and enzyme inhibitory effects against threatening tobacco cutworm S. litura and their detrimental effects against soil indicator earthworms. Chemical screening of EO-Sa using GC-MS exposed thoroughly five major derivatives with major peak area in D-Carvone (89.7%). The larvicidal activity of both third and fourth instar were improved steadily with higher dosage of treatments (400, 600, 800 and 1000 ppm). The lethal concentration (LC50) for third and fourth instar larvae was observed at 2.71 and 2.74 ppm (log dosage) respectively. Correspondingly, the sub-lethal dosage of EO-Sa (500 ppm) reduced the enzyme activity of Acid Phosphatase (ACP), Alkaline Phosphatase (ALP) and Adenosine triphosphate (ATP) significantly in both third and fourth instars. Further, the gut-histological activity of EOSa (500 ppm) against fourth instar showed damaged and disintegrated epithelial cells (EL) and columnar cells (CC), gut lumen (GL) and brush border membrane (BBM). Finally, the non-target screening against E. euginae showed that the EO-Sa was found to be harmless even at the maximum dosage of 1000 and 1500 ppm as compared to synthetic chemical monocrotophos in both filter paper test (FPT) and artificial soil test (AST). Thus the present investigation evidenced that active phyto-chemistries of EO-Sa delivers a sturdy toxicological response against S. litura and harmless against non-target earthworm.

Keywords: EO-Sa, Lepidopteran, D-Carvone, Digestive enzymes, Gut-histology, Non-targets

1. Introduction

Synthetic chemicals deeds on wide range of host species and they are suitable for their active role in pest management of agriculture importance (Edwin et al., 2016; Vasantha-Srinivasan et al., 2019). The continuous accumulation of chemical fertilizers and pesticides in the environment harms the ecosystem, causes pollution, and inflicting diseases at alarming levels (Gerhardson, 2002). Synthetic chemicals counting pyrethroids and organophospates can be connected to many unsolicited side-effects, in other organisms (Abudulai et al., 2001). Due to this unselective practice diversified research has been rooted to search for new plant based alternatives which are economically valuable and bio-safety agent's holds wide range of active compounds (Senthil-Nathan, 2013a,b, 2015; Selin-Rani et al., 2016a; Ayil-Gutièerrez et al., 2018). Due to complexity of chemical factories (Phenolics, alkaloids, sterols, flavonoids, and steroids) in green derivatives agriculture pests are found difficulty to generate resistance as compare to synthetic chemicals (Regnault-Roger, 2002). More importantly, green drugs

derived from major families of Asteraceae, Rutaceae, Labiatae, Piperaceae, Annonaceae and Canellaceae which are traditionally utilized to treat several maladies have been reported as insecticidal agents. However research focused on the environmental risks linked with plant borne derivatives including crude extracts, essential oils (EO's) and plant derivative toxicity that are cast-off as green pesticides is desperately required (Siegwart et al., 2015; Ayil-Gutièerrez et al., 2018). The genus Spodoptera (Lepidoptera: Noctuidae) comprises species that root damage to a diverse significant crops including cotton, maize,soybean and rice among others (Huang et al., 2014; Khedr et al., 2015; Ayil-Gutièerrez et al., 2018). Among them, Spodoptera litura Fab. is an sparingly significant polyphagous insect pest is lively all through the year on a diversified crops in developed and developing countries causing key damage to vegetables and important crops (Wan et al., 2014). It is estimated Spodoptera sp. causes highest damage and monetary losses of USD $ 11,590 (Aprox.) per year in the 12 countries including Africa (Vasantha-Srinivasan et al., 2016). There are different systems, platforms and strategies were widely used such as chemical control, spatial and temporal analysis and more importantly biological control for the effective management of Spodoptera sp. (Magarey et al., 2011; Pavela, 2016). Despite, clear, inexpensive and biologically safe technologies are needed to uplift to control the progressive invasion of Spodoptera litura. The herb Sphaeranthus sp. (Family: Asteraceae) has been cast-off in many regions in India for different traditional medicines systems for treating human disease and widely premeditated for its different biological activities (Chellappandian et al., 2017). Among them, Sphaeranthus amaranthoides Burm is a petite procumbent herb, with steam rooting and widely found in the paddy field of Southern India (Thanigaivelan et al., 2012; De et al., 2013). Moreover, chemical characterization of S. amaranthoides crude extracts revealed enriched bio-actives of alkaloids, phenolics, steroids, saponins, sugars, flavonoids and tannins (De et al., 2013). Earthworm is an indispensable soil invertebrate, which rots organic matter and apposite bio-indicators connected with fauna diversity and soil fertility (Ravindran et al., 2015; Vasantha-Srinivasan et al., 2017). Changes in earthworm behavior largely depend in the degree of exposure to contaminants in the soil and considered as a sensitive biological indicators in eco-toxicological investigation (Gao et al., 2015). Thus the present investigation was highlighted the green-derived Essential oils with bio-active derivatives of Sphaeranthus amaranthoides (Sa-EO) can be employed in larvicidal and enzyme inhibitory effects against threatening tobacco cutworm S. litura and their detrimental effects against soil indicator earthworms.

2. Methodology

2.1. Plant harvesting

The fresh leaves of the S. amaranthoides Burm. were collected near from the rice field near southern Western Ghats, Alwarkurichi, Tirunelveli District, Tamil Nadu, India (Fig. 1). Identification of plant species were valid with voucher specimen number by taxonomical researcher and deposited in the herbarium of SPKCES, Manonmaniam Sundaranar University, Tirunelveli, TamilNadu, India.

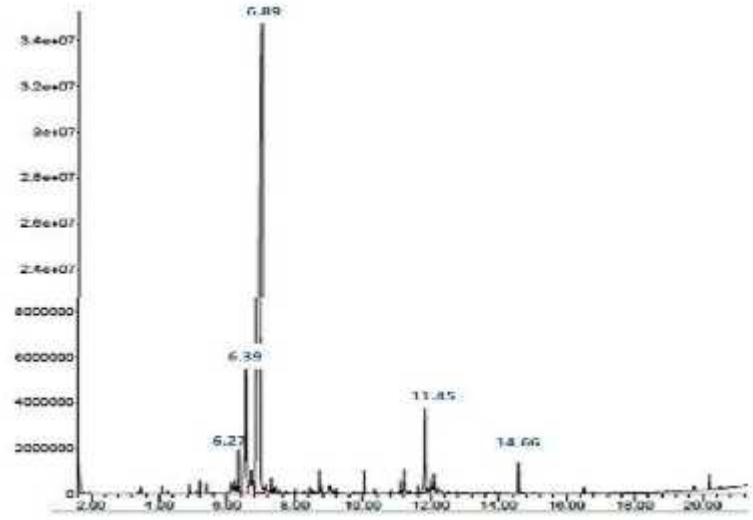

Figure 1:- GC-MS chromatogram of active fraction of Sa-EO.

2.2. Sa-Eo preparation and chemical characterization

The S. amaranthoides leaves were finely chopped and open to rotary evaporation steam distillation unit for EO extraction. The EO-Sa were separated and collected in clean glass containers. Water droplets were separated using anhydrous sodiumsulphate and the EO were kept at 5°C for the future experiments. Total of 5.7 mL of oil was extracted from one kg of leaves (% of yield varied seasonally). The isolated EO-Sa were dissolved with ethyl alcohol at 1:1 ratio. Further, 2 µl of prepared solutions were dissolved in methanol (HPLC grade) and subjected to JEOL GC-MATE II GC-MS (Agilent-Technologie(6890N) GC system) furnished with secondary electron multiplier. Further experiments were adapted on our previous experiments (Dinesh-Kumar et al., 2018). The molecular weight, empirical formula and chemical structure of the characterized compounds of EO-Sa were ascertained by interpretation using the National Institute Standard and Technology (NIST) database.

2.3. Larvicidal activity

Larvicidal toxicity were performed with third and fourth using lethal dosage of 400 ppm, 600 ppm, 800 ppm and 1000 ppm of Eo-Sa. Sterile castor leaves (75–125 cm2) were sprayed with diverse dosage of Eo-Sa and air dried for 10 min to remove the excess moisture content. In parallel, control leaves were treated with sterile distilled water containing 0.05% of DMSO at the ratio of (1:1). The treated leaves were kept in the bioassay chamber (9 ×5 × 4 cm3) damped with wetted cotton and tissue paper which deliver humidity and water supply for the leaves, which do not permit them to dry during the investigational period. The bioassay unit was incubated at 28 ± 1°C with 95% humidity and 15:9 (L: D) photoperiod. A minimum of 20 larvae/dosage were used for all the treatments and these treatments were replicated five times (n ¼ 100). The dried leaves were shifted every 24 h, and exchanged with sterile castor leaves. The rate of mortality was pragmatic from the fourth day post treatment to day 10. The mortality percentage was deliberated by using formula (1) and corrections for natural mortality when necessary were done by using Abbott's formula. (2) and subjected to Probit analysis (Finney, 1971) to calculate the treatment concentrations for biological studies.

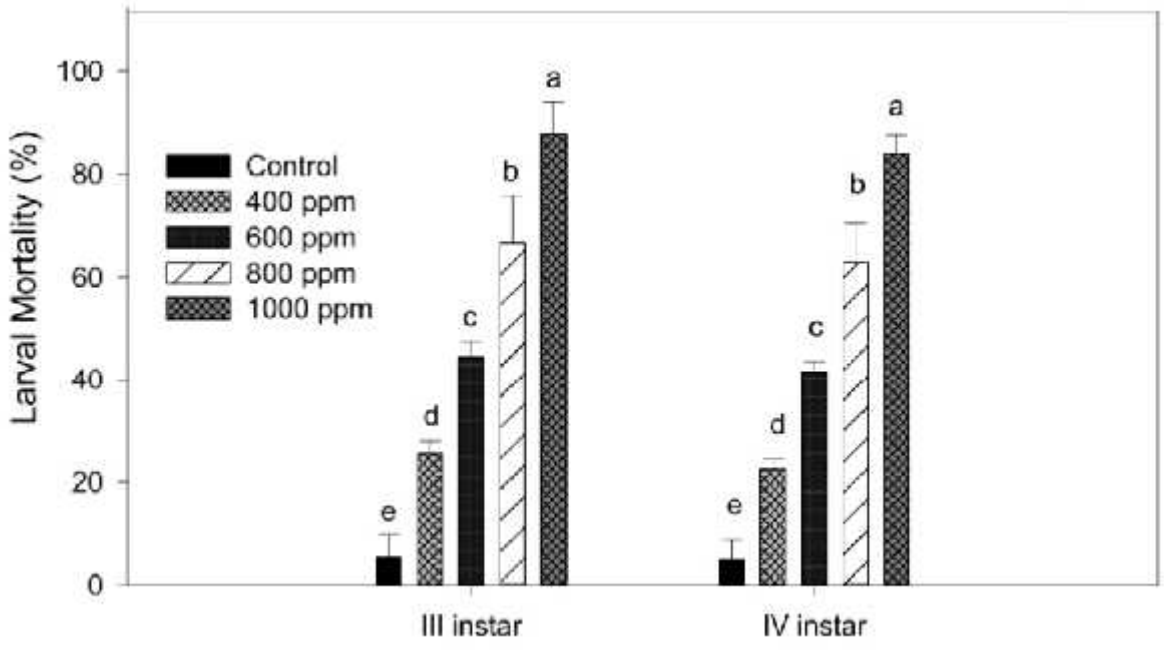

Figure2:- Larvicidal activity of SO-Ea against III and IV instar larvae of S. litura. Means (SEM±) followed by the same letters above bars indicate no significant difference (P ≤ 0.05) by using Probit analysis.

2.4. Enzyme assay

2.4.1. Enzyme extract preparation

Enzyme activity were performed using third and fourth instar larvae. The extraction procedure were adapted using the previous procedure (Edwin et al., 2016). Larvae were sedated and the whole digestive system were alienated out in ice-cold Ringer's solvent. The Malpighian tubules, gut contents and adhering tissues were completely removed. The gut regions were spited, weighed (accuracy in mg) and standardized in ice-cold citrate-phosphate buffer (pH 6.8) using a tissue chopper for 4 min at 4°C. Further the whole homogenates were suspended in to ice-cold buffer and the final homogenates were makeup to 1 ml and centrifuged at 500 rpm for 15 'g' force min and the ensuing supernatant were used for the further enzyme assays.

2.4.2. Estimation of acid phosphatase and alkaline phosphatase

Enzyme assays of acid phosphatase and alkaline phosphatase was performed using our previous procedure (Edwin et al., 2016). On converting the p-nitrophenolate into p-nitrophenol by acidification, the absorption maximum is shifted to about 320 nm with no detectable absorption at 400 nm.

2.4.3. Estimation of adenosine triphosphatase

Estimation of adenosine triphosphatase was performed using the adapted procedure (Selin-Rani et al., 2016b). The protein is precipitated with trichloroacetic acid, then the protein-free filtrate is treated with acid molybdate solution and the phosphoric acid formed is reduced by the addition of 1-amino-2-naphthol-4-sulfonic acid (ANSA) reagent to produce blue color. The intensity of the color is proportional to the amount of phosphorous present.

2.4.4. Gut-histology

The effects of the EO-Sa intake was studied on the fourth instar larvae of S. litura. Sub-lethal dosage treatment (500 ppm) and control larval gut-tissue was fixed overnight in Bouins reagent. Further, the blocks were cooled about 27°C for 2 h and cut into 1.5 μm pieces with an ultracryo-microtome (Made: Cryocut 1800; Leica, Germany). The cut slices were further stained with Delafield's hematoxylin and counter-stained with eosin, and mounted after the slides were dried. Finally the sectioned slides were detected and photographed under Optika, Flow series HBO light microscope (model: B-600 TiFl-Italy).

2.5. Earthworm toxicity

2.5.1. Earthworm culture

The earthworm species used E. eugeniae stock was maintained at Biopesticides and Environmental toxicology lab, M.S. University, Tirunelveli, Tamil Nadu. The earthworm was cultured at an average ambient temperature of 28.9 ± 0.36°C with our adapted methodology (Vasantha-Srinivasan et al., 2017).

2.5.2. Contact filter paper assay

Contact filter paper test (FPT) was performed with slight modification of OECD (1984). A piece of filter paper was placed in an 8 cm petri-plate and treated with the EO-Sa and Monocrotophos. The filter paper treated was re-moistened with 2.5 ml distilled water, and single earthworm was placed on it. The petri-plates were raised in the dark at 25 ± 1°C for 48 h and mortality rate was observed. A preliminary bioassay was carried out to determine the mortality concentration range for each chemical. Three different concentrations of Monocrotophos (10, 20 μg/cm), EO-Sa (1000 and 1500 ppm/cm) with a control were included. Five replication was performed for each treatment. Treated earthworms were transferred into the plates and held at 20 ± 1°C under 85–90% relative humidity (RH) in the dark, for 48hrs.

2.5.3. Artificial soil assay

Artificial soil comprised of 10% ground sphagnum peat, 20% kaolinite clay, 70% fine sand was used for artificial soil tests with slight modifications (Ponsankar et al., 2016a). A minute amount of calcium carbonate was added to control the pH to 6.0 ±0.5. The content of water was adjusted to 30% of the dry weight in toxicity test. The soil was formulated by providing diverse dosage of Monocrotophos (Sigma-Aldrich, PESTANAL®, analytical standard) (10 ppm/kg; 20 ppm/kg) and EO-Sa (1000 and 1500 ppm/kg) in dry weight basis. 10 adult earthworms were kept in glass containers (1 L) packed with test substrate and test containers were enclosed with a polythene sheet with integrated gauze (þ1 mm) to avert the worms from escaping and to ensure optimal ventilation. After 3, 7 and 14 d

of incubation, living earthworms were sorted by hand and the test endpoint was mortality. Four replicates were applied for the acute tests. In parallel, control treatment (free from pesticides or leaf extracts) was also tested with four replicates.

2.5.4. Statistical analysis

Mortality was corrected using Abbott (1925) formula. The treatment dosage was calculated using Probit analysis (Finney, 1971) and values were expressed as means ± standard error of five replicates. Data from biology and mortality experiments were subjected to analysis of variance (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range tests ($P \leq 0.05$) (Snedecor and Cochran, 1989).

3. Results

3.1. Chemical characterization through GC-MS

Chemical screening of EO-Sa using GC-MS exposed thoroughly five major derivatives (Fig. 1A). Chemical formula, empirical weight (g/ mol), retention time (RT), peak area (%), and chemical structures were displayed (Table 1). The peak area percentage was found to be prominent at D-Carvone (89.7%) with retention time (RT) of 6.89. Apart from that, trans-2-Caren-4-ol (3.50%), 2-Cyclohexen-1-ol (6.50%), Copane (6.42%) and (þ)-epi-Bicyclosesquiphellandrene (3.30%) were displayed in the peak area with RT of 3.50, 6.50, 6.42 and 3.30 respectively.

3.2. Larvicidal activity

The active metabolites of EO-Sa had distinct toxicity against the different instars of S. litura larvae. Mortality rates of both third and fourth instar were improved steadily with higher dosage of treatments (400, 600, 800 and 1000 ppm). Among, third instar larvae displayed higher mortality rate. The significant mortality rate between the treatments was observed against third and fourth instars were recorded (Fig. 2). Mortality rate was statistically different between 1000 ppm EOSa (92%-$F_{4,20}$ ¼ 34.11, $p \leq 0.001$) as compared to 800 ppm (73%-$F_{4,20}$ ¼ 34.11, $p \leq 0.001$), 600 ppm (56%-$F_{4,20}$ ¼ 34.11, $p \leq 0.001$) and 400 ppm (35%-$F_{4,20}$ ¼ 34.11, $p \leq 0.001$) and control (8%-$F_{4,20}$ ¼ 34.11, $p \leq 0.001$) in third instar larvae. Similarly, fourth instar also showed highest mortality rate at the maximum dosage of 1000 ppm EO-Sa (88%- $F_{4,20}$ ¼ 62.11, $p \leq 0.001$) and it was significant to 800 ppm (67%-$F_{4,20}$ ¼ 62.11, $p \leq 0.001$), 600 ppm (49%-$F_{4,20}$ ¼ 62.11, $p \leq 0.001$), 400 ppm (27%-$F_{4,20}$ ¼ 62.11, $p \leq 0.001$)

Peak No.	R. Time	Peak Area%	Compound Name	Molecular formula	Molecular weight (g/mol)	Structure
1	6.27	3.50	trans-2-Caren-4-ol	$C_{10}H_{16}O$	152.23	
2	6.39	6.50	2-Cyclohexen-1-ol	$C_6H_{10}O$	98.145	
3	6.89	80.20	D-Carvone	$C_{10}H_{14}O$	150.22	
4	11.45	6.42	Copaene	$C_{15}H_{24}$	204.36	
5	14.66	3.30	(+)-epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$	204.34	

and control (10%-$F_{4,20}$ ¼ 62.11, $p \leq 0.001$in fourth instar respectively. Besides, the mortality rate was slightly higher in third instars as compared to fourth instars respectively across all the treatment dosage. Moreover, the mortality rate was less in the minimal dosage of 400 ppm in both third and fourth instars. Minimal dosage were sufficient enough to give prominent LC50 and LC90 values for both the instars. Lethal concentration (LC50) for third and fourth instar larvae was observed at 2.71 and 2.74 ppm (log dosage) respectively. Correspondingly, lethal concentration (LC90) was observed at 3.36 and 3.42 ppm (log dosage) for third and fourth instars respectively (Fig. 3A&B).

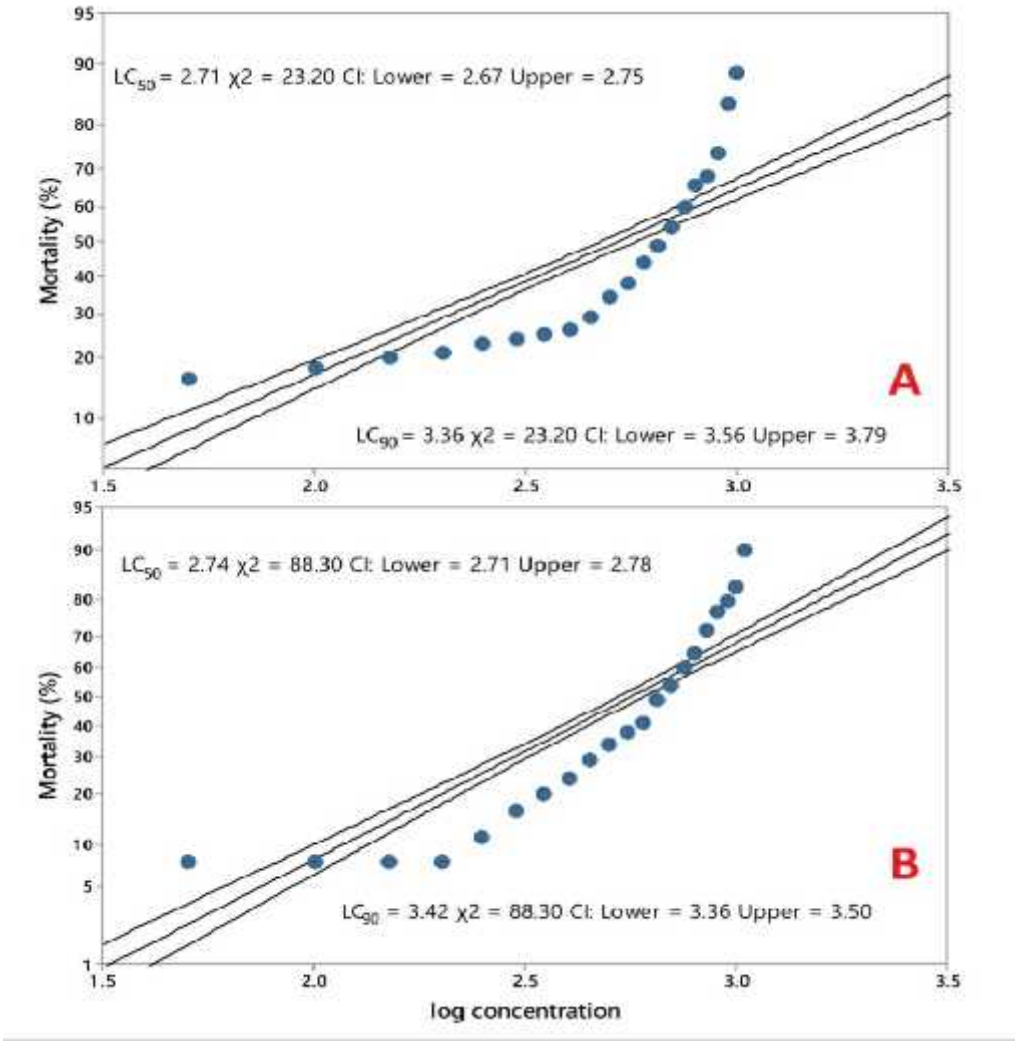

Figure 3:- Lethal concentrations (LC50 and LC90) of Sa-EO against (A) third and (B) fourth instar larvae of S. litura.

3.3. Enzyme activity

3.3.1. Acid phosphatase (ACP) activity

The active biomolecules present in the EO-Sa reduced the enzyme activity of both third and fourth instars of S. litura. The activity of acid phosphatase (ACP) reduced significantly in the third instar larvae even at the maximum sub-lethal dosage of 500 ppm (4.1032 µmol-F 4,20 ¼ 23.12, p ≤ 0.001), and it was statistically significant with 400 ppm (4.432 µmol), 300 ppm (4.542 µmol), 250 ppm (4.701 µmol), 200 ppm (4.802 µmol), 150 ppm (4.953 µmol), 100 ppm (5.184 µmol) and control (5.789 µmol) respectively (Fig. 4A). Similar trends were observed in the fourth instar larvae, with maximum decline in the ACP level displayed at the sub-lethal dosage of 500 ppm (4.01 µmol-F 4,20 ¼ 31.75, p ≤ 0.001) and it was significantly different with other treatment dosage and control (Fig. 4A).

3.3.2. Alkaline phosphatase (ALP) activity

ALP level also gets downregulated in steady manner when the sublethal dosage gets increased. The ALP was significantly reduced at the maximum dosage of 500 ppm (3.901 µmol-F 4,20 ¼ 11.32, p ≤ 0.001) and it was significant with other treatment dosage of 400 ppm (4.052 µmol-F 4,20 ¼ 23.12, p ≤ 0.001), 300 ppm (4.362 µmol-F 4,20 ¼ 23.12, p ≤ 0.001), 250 ppm (4.590 µmol-F 4,20 ¼ 23.12, p ≤ 0.001), 200 ppm (4.820 µmol-F 4,20 ¼ 23.12, p ≤ 0.001), 150 ppm (5.1023 µmol-F 4,20 ¼ 23.12, p ≤ 0.001), 100 ppm (5.332 µmol-F 4,20 ¼ 23.12, p ≤ 0.001) and control (5.670 µmol-F 4,20 ¼ 23.12, p ≤ 0.001) in the third instar larvae. Similarly, The ALP level was decreased significantly in the fourth instar larvae when treated with the maximum sub-lethal dosage of 500 ppm (3.870 µmol-

F 4,20 ¼ 17.12, p ≤ 0.001) and the level was normal in the control (5.74 µmol-F 4,20 ¼ 23.12, p ≤ 0.001) (Fig. 4B).

3.3.3. Adenosine triphosphate (ATPase) activity

The ATPase level gets declined significantly in all the sub-lethal dosage as compared to control in both the instars (Fig. 4C). Though, there is no statistical difference between the maximum dosage of 500 ppm (4.390 µmol-F 4,20 ¼ 15.89, p ≤ 0.001) and 400 ppm (4.590 µmol-F 4,20 ¼ 15.89, p ≤ 0.001). Besides, the level of ATPase reduced steadily in dose dependent manner. Correspondingly, the ATPase gets downregulated in the fourth instar in all the sub-lethal dosage and the level was found to be maximum at 500 ppm (4.490 µmol-F 4,20 ¼ 27.12, p ≤ 0.001) and maximum in control (5.970 µmol-F 4,20 ¼ 27.12, p ≤ 0.001) respectively. However, the level of downregulation is slightly higher in third instars as compare to fourth instar larvae.

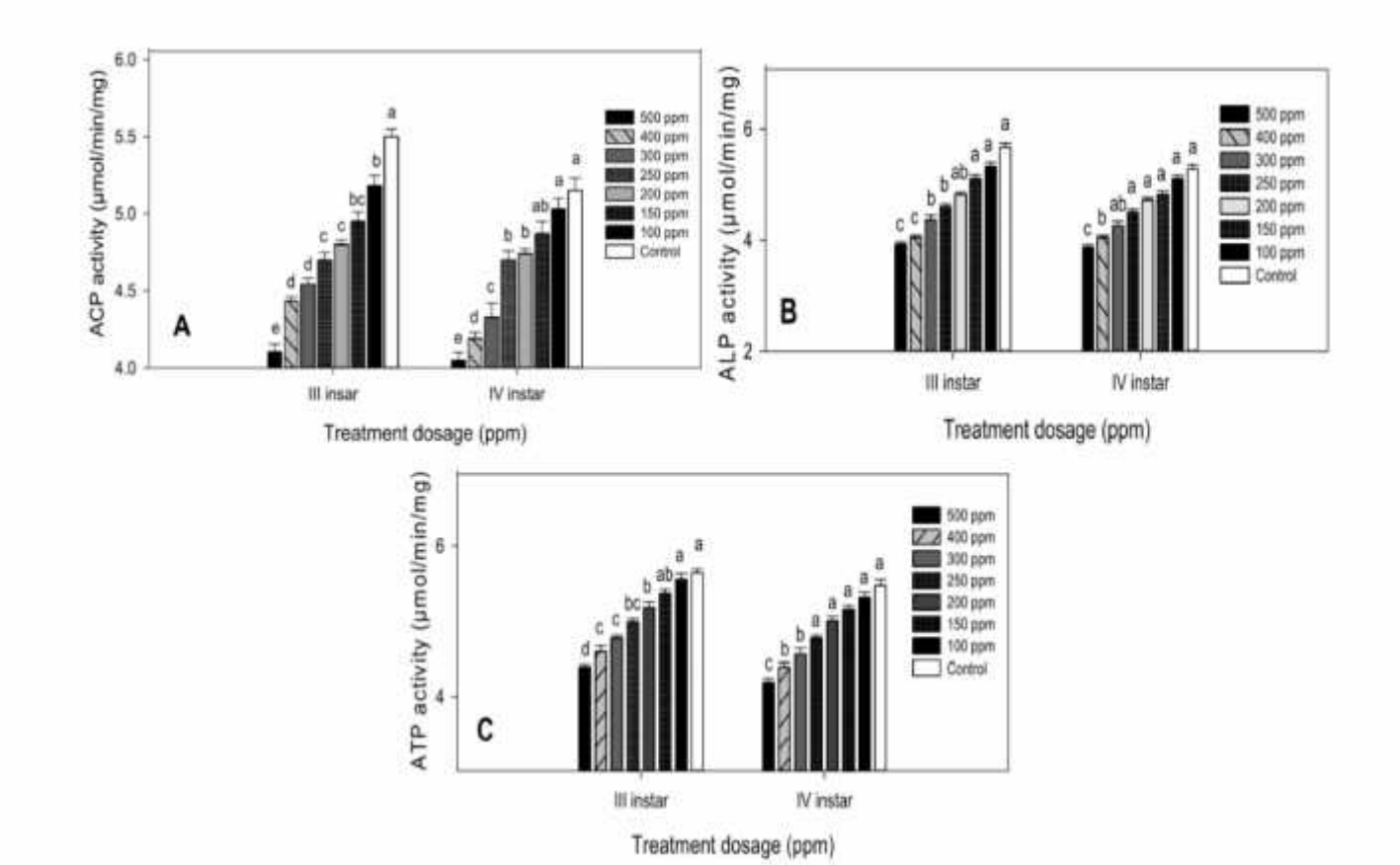

Figure4:-(A) ACP (B) ALP and (C) ATP activity of enzyme activity of third and fourth instar larvae of S. litura after treatment with Sa-EO. The data were fitted on polynomial (regression) model, whereas vertical bars indicate standard error (±SEM)

3.3.4. Gut-histological activity

The gut-histological changes were observed in the sub-lethal dosage treated (500 ppm) fourth instar larvae as compared to control. As compared to treated gut samples, control larvae had fine distinguished epithelial cells (EL) and columnar cells (CC), gut lumen (GL) and brush border membrane (BBM) (Fig. 5A). Besides, the gut cells displayed damage to gut cells, brush boarder membrane being partially disintegrated, columnar cells (CC) becoming cracked and no clear identification of cell organelles (Fig. 5B).

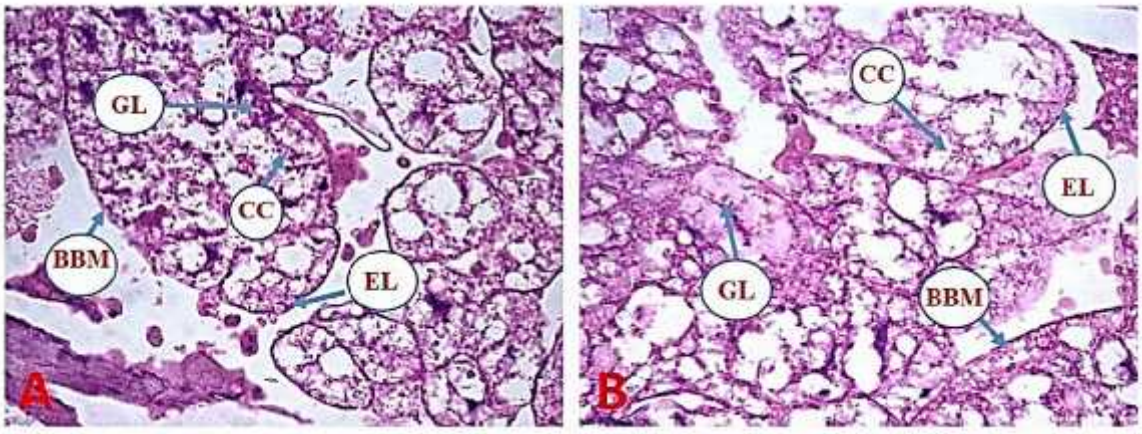

Figure 5. Histological section of the mid-gut region of fourth instar larvae of S. litura (A) Control (CC-columnar cells; EL- epithelial layer; BBM- brush border membrane; GL- gut lumen). (B) Treated concentration Sa-EO (CC-columnar cells; EL- epithelial layer; BBM- brush border membrane; GL- gut lumen).

3.4. Earthworm toxicity

3.4.1. Filter paper test (FPT)

Filter paper toxicity assay on E. euginae displayed a significant differences to both the monocrotophos and EO-Sa. In all the treatment concentrations monocrotophos at 20 µg produced higher mortality rate (81%) at 24 h ($F_{4,20}$ ¼ 31.11, $P \leq 0.001$) compared to other treatments of monocrotophos and EO-Sa (Fig. 6). However, there is no significant differences between the EO-Sa (1000 and 1500 ppm) and control.

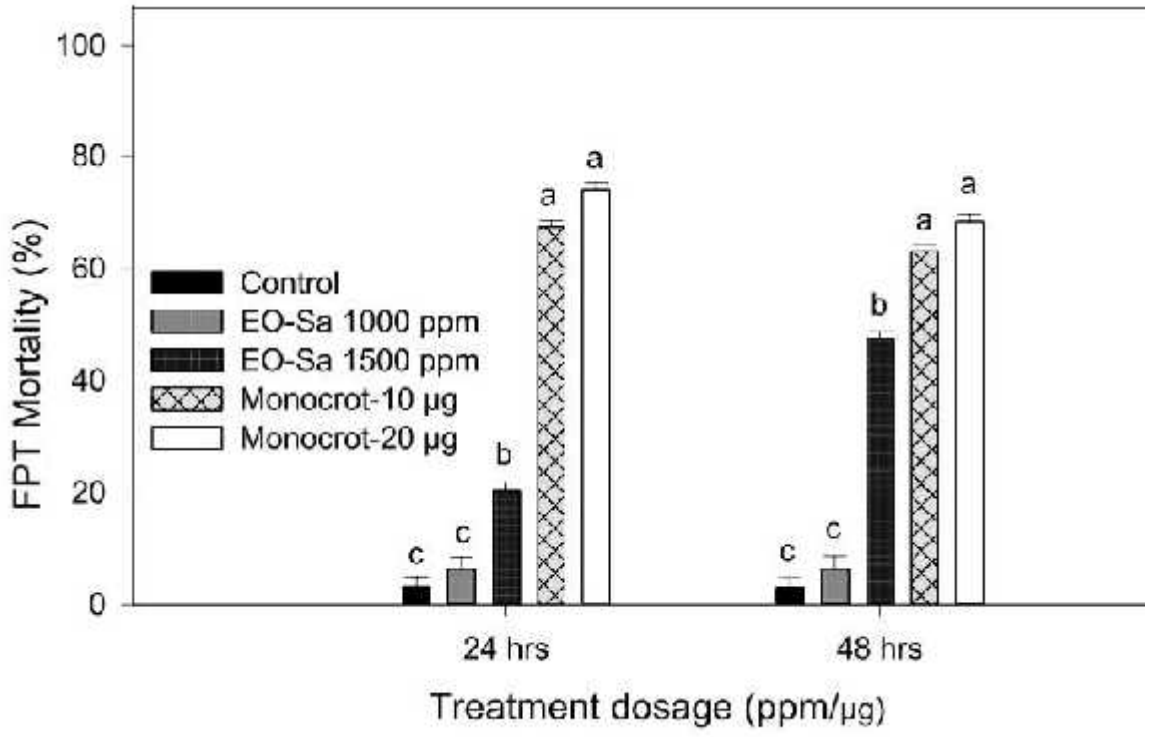

Figure 6:- Percentage mortality of earthworm in filter paper test (FPT) after treatment with chemical pesticides and Sa-EO. Means (± (SEM) standard error) indicate no significant difference ($P \leq 0.05$) according to a Tukey's test.

3.4.2. Artificial soil test (AST)

In artificial soil test (AST), the mean mortality percentage of E. euginae was significantly different between the treatments. The result demonstrated that the percentage of mortality was significantly higher at monocrotophos 20 ppm/kg at 7 ($F_{4,20}$ ¼ 27.21, $P \leq 0.001$) and 14 d ($F_{4,20}$ ¼ 43.85, $P \leq 0.001$) and it was statistically different with EO-Sa (1000 ppm/kg and 1500 ppm/kg) and control. In all the treatments EO-Sa displayed less mortality rate and it was not-significant with EO-Sa treatments (Fig. 7).

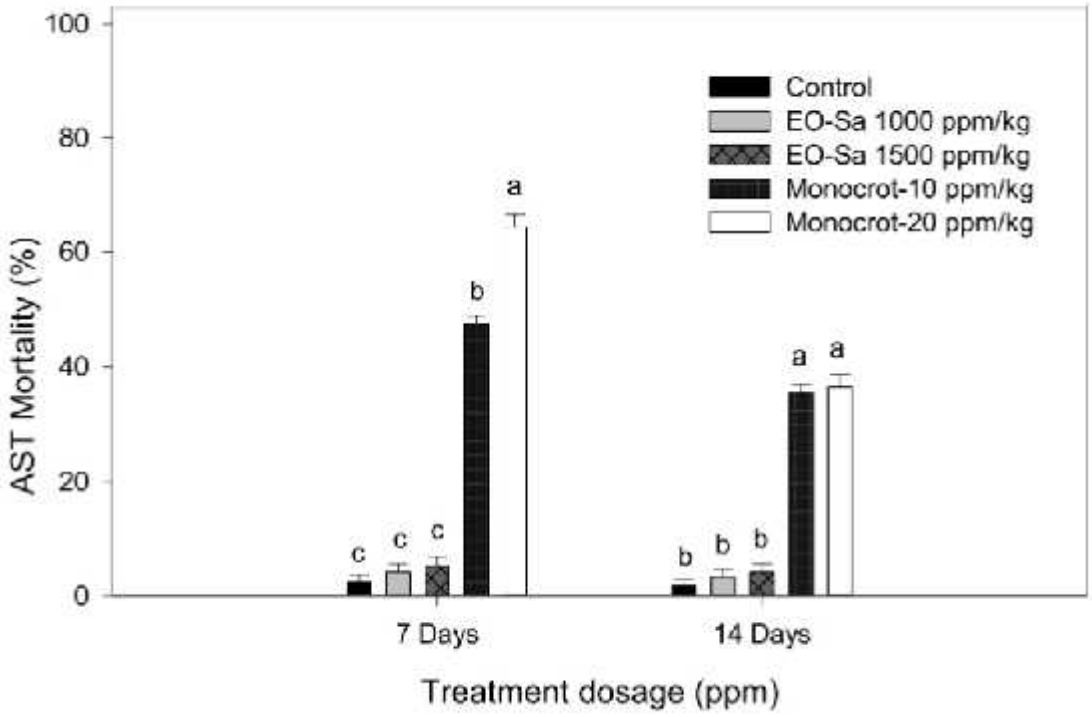

Figure 7:- Percentage mortality of earthworm in Artificial Soil Test (AST) after treatment with chemical pesticides and Sa-EO. Means (± (SEM) standard error) indicate no significant difference ($P \leq 0.05$) according to a Tukey's test.

4. Discussion

Plant derived chemicals are mainly secondary metabolites that aid as means of defensive actions of the plants to resist the incessant selection pressure from predators and other biological factors (Ponsankar et al., 2018; Chellappandian et al., 2018). Diverse groups of compounds such as, alkaloids, steroids phenolics, terpenoids and volatile oils from more than two thousand species of plants have been identified earlier for their pesticidal activities (Gbolade and Lockwood, 2008; Senthil-Nathan et al., 2008a,b; Giatropoulos et al., 2013; Soonwera, 2015; Govindarajan et al., 2016a). At present, phytochemicals make up to 1% of world's pesticide market (Anupam et al., 2012). Preceding green chemistry research revealed that the majority of the plant families have exposed potential insecticidal responses which includes Asteraceae, Solanaceae, Lamiaceae, Meliaceae, Rutaceae, Oocystaceae, Poaceae, Piperaceae and Zingeberaceae (Bakkali et al., 2008; Kalaivani et al., 2012; Pavela, 2015; Govindarajan et al., 2016b; Baskar et al., 2016). Characterization of novel phytochemitry's delivers an emergent basis which may generate chemistries with rapid degradation into benign chemicals, while as long as active pest and pathogen suppression. Similar to the above statement, Our present result also delivers five major derivatives with peak area maximum obtained in the prominent at D-Carvone (89.7%) these compounds may play a pivotal role in causing insecticidal activity. Plant derivatives are mainly aid as defensive mechanism against pests, predators and other herbivores. They are comparatively less toxic to synthetic chemicals (Tong and Bloomquist, 2013).

The larvicidal activity of EO-Sa displayed significant mortality rate across different treatment dosage and control.

The mortality rate was prominent in 1000 ppm in both third and fourth instar larvae of S. litura. Similarly, Pb-CVO (crude volatile oil derived from Piper betle) exhibited dose dependent mortality rate against different instars of S. litura (Vasantha-Srinivasan et al., 2016). Even though, the dose responsible for larval mortality seems to be prominent as linked to synthetic chemicals, plant derivatives provide an effectively harmless and more eco-friendly agents compared to broad spectrum and persistent pesticides (Koul et al., 2013; Vasantha-Srinivasan et al., 2016). The sub-lethal dosage of EO-Sa significantly reduced the gut enzyme such as ACP, ALP and ATPase activity. Down regulation of ACP level treated with EO-Sa delivers that the phytochemicals had declined the phosphorous release for energy metabolism, this grounds the reduced rate of metabolism (Senthil-Nathan, 2013a,b). Similar trends of decline in the rate of ALP and ATPase were observed in the EO-Sa treatment. This is mainly due to the fluxes in the physiological balance and nutritional metabolic rates. Our results were well supported by the previous findings of Selin-Rani et al. (2016a) stated that the hydrolytic enzymes such as acid phosphatase (ACP), alkaline phosphatase (ALP) inhibited in the Alangium salvifolium (L.f.) active fractions treated larvae compared with controls. Similar results were observed while Cnaphalocrocis medinalis (rice leafroller) treated with extracts of Vitex negundo (horseshoe vitex) and Azadirachta indica (neem) (Senthil-Nathan, 2006). Mid-gut is commonly considered as the site for digestion and absorption of nutrients from the food (Selin-Rani et al., 2016a). Gut-histological examination supports that the effective compounds bound in the EO-Sa was severely infected the insect gut cells. This is mainly due to the strong binding capability of phytochemicals to the tissues and display the oxidative damage to the midgut cells (Barbehenn et al., 2005; Selin-Rani et al., 2016b). Similar, results were found in the other plant extracts or essential oil treatments against the gut of different insect pests (Vasantha-Srinivasan et al., 2016, 2017; Dinesh-Kumar et al., 2018). Toxicological assessment of bio-indicators earthworm has been measured as a fruitful end-point in eco-toxicological research (Ponsankar et al., 2016, 2018). Earthworm plays a pivotal part for the nutrient value and structure maintenance of the soil (Benelli et al., 2018). The FPT and AST is a primary screening technique to measure the chemical toxicity (Lankadurai et al., 2015). The present non-target screening using FPT and AST exposes that the E. euginae showed noticeable mortality rates when treated with different dosage of Monocrotophos and it was significantly less and non-toxic while treated with plant derived EO-Sa. Our present research were well braced with the previous findings illustrates that the green derivatives from Foeniculum vulgare (Mill) commonly known as fenne had a lethal effects against Eisenia fetida (older spelling: foetida) and Harmonia axyridis (Multicolored Asian lady beetle) (Pavela, 2018). Thus the present investigation evidenced that active phytochemistries of EO-Sa delivers a sturdy toxicological response against S. litura and significantly alters the detoxifying and digestive enzymes of gut tissues at the sub-lethal dosage and also notably harmless activity against the beneficial earthworm. Hence, as an endnote, the present study will boost to develop prominent plant-borne EO's as an effective product formulations as targeted pesticides against the threatening polyphagous pests and less-toxic against the beneficial insects and species sharing the same ecological niche.

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