Indian Journal of Entomology 85(1): 229-233 (2023) DoI. No.: 10.55446/IJE.2022.800

INSECTICIDAL ACTIVITY OF ESSENTIAL OILS FROM MINT AND AJWAIN AGAINST PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* (L)

Grishma Pipariya, Pankaj Sharma, Nishi Pandya and Pragna Parikh*

Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, Gujarat, India
*Email: Php59@yahoo.co.in (corresponding author): ORCID ID 0000-0001-7990-3517

ABSTRACT

The present study evaluates the insecticidal activity of two essential Oil (EOs) mint (*Mentha arvensis*), and ajwain (*Carum capyicum*) against pulse beetle (*Callosobruchus chinensis)* (L). Contact toxicities of these were evaluated using parameters of lifecycle like total development period, numbers of eggs laid, adult emergence and adult longevity. Along with these detoxification enzyme inhibition activities of acetyl cholinesterase (AChE), alkaline phosphatase (ALP), transaminases enzymes- aspartate aminotransferases (AST) and alanine aminotransferases (ALT) and total protein were estimated. EOs were observed showing toxicity (mint LC_{50} = 5.9 µl/ ml and ajwain LC_{50}= 7.02 µl/ ml). Exposure of EOs altered the lifecycle parameters significantly ($p<0.01$). The detoxification enzyme inhibition activities were also significant ($p<0.01$). Thus, it is concluded that these EOs can be recommended as safe and ecofriendly alternatives.

Key words: *Callosobruchus chinensis*, essential oils, *Mentha arvensis*, *Carum capyicum*, lifecycle, acetyl cholinesterase, alkaline phosphatase, transaminases enzymes, inhibition

India is one of the leading producers of food in the world and it produces more than a billion tonnes of agricultural product. 58% of India's population is dependent on agriculture as its primary source of livelihood. In India, advancement of technology has increased the production of grains; however, improper storage has resulted in huge loss and has been reported to be around INR 926 billion loss annually (Singh and Khanna, 2019; Sirohi et al., 2021). Infestation of stored grain by many insects, mite and fungi degrade the quality and quantity of grains (Lal et al., 2017; Jerbi et al., 2021). The total productivity of agricultural crops of India is 3 tonnes/ha; out of which loss due to insect pest is about 26 % (Lal et al., 2017), like the lesser grain borer, *R. dominica*'s larva and adult infests the grains and declines its quality (Jerbi et al., 2021). Rice pest *S. oryzae*, causes qualitative and quantitative loss (Saad et al., 2018). *C. chinensis* a major pest of stored pulses and is reported to cause 32-64% loss under storage condition (Femeena et al., 2018). After discovery of DDT, Insect pests are mainly controlled by synthetic pesticides (Lal et al., 2017; Demeter et al., 2021). WHO has reported that every year two lakhs people die due to pesticide poisoning owing to its carcinogenic and teratogenic properties (Sarwar, 2016). Use of synthetic pesticide is a easy and quick solution for controlling insect pests but pose a potential risk not only to humans but also to the environment as their residues have been reported to be present in soil, air and water (Said and Pashte., 2015; Lal et al., 2017). The repeated uses of synthetic insecticide for decades has disrupted biological control by natural enemies and has led to outbreaks of other insect species and at times have resulted in resistance of pesticides in insect pest (Hill et al., 2017; Hawkins et al., 2019). Hence, there is need for alternative solution which environment friendly does not harm other non-target species. Plants and their derivatives have been proved to be a viable alternative as more than 2000 plant species have been recorded to possess insecticidal properties and possess low health risks (Pavela, 2016; Jerbi et al., 2021). EOs are naturally produced by plants as secondary compounds which are volatile, but as natural products protects the stored grains from pest attack (Omar, 2020). EOs has multiple components mixture and causes toxicity by interfering with various aspects of insect's physiology and biochemistry (Kiran et al., 2017). Present work evaluates the insecticidal potential of the two EOs *M. arvensis* and *C. capyicum* against *C. chinensis* (pulse beetle) adults.

MATERIAL AND METHODS

The adult insects were collected from the infested grains from the granary and were reared on 500 g green gram (variety - Sabarmati PS 16) maintained in laboratory at Department of Zoology, The Maharaja Sayajirao University of Baroda. A culture of *C. chinensis*

was maintained on green grams, the legume seeds were washed with hot water to remove any pesticide residues, dried under sun to eliminate any infestation. These seeds were stored in air tight plastic container until required for experiments, which were carried out at a constant temperature of 28± 2ºC, relative humidity 70± 5% at 16:8 light: dark photoperiod (Bhumi et al., 2017). Morphological Identification of insect pest was done by using standard keys (Raina, 1970).

C. chinensis were categorized into two groups: Group I: Control (Acetone treated). Group II: EOs exposed test group, which were again subdivided into Low dose (LD) and High dose (HD) following the standard toxicological guidelines of OECD. To determine the lethal concentration (LC_{50}) value of EOs, serial dilutions (0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 1, 1.5, 2, and 2.5) µl/ml in acetone as solvent were prepared. 1 ml of each concentration was uniformly applied a glass petridish of diameter 15cm x 2cm; after evaporation of acetone (10 minutes), 10 pairs of adult were placed in each petridish. Same procedure was used for the control treated with acetone. Mortality was recorded after interval of every 24 hours till 96 hours. (Bhumi et al., 2017). After obtaining LC_{50} value, 10 pairs of adult *C. chinensis* in 50gms of green grams were exposed to the LD and HD of mint and ajwain for seven days on and on the eight day they were sacrificed and tissue were pooled for the further analysis. The green grams with the eggs were separated and on every 24hrs interval eggs were observed for the egg incubation period, larval development period, pupation period. Day of adult emergence was recorded followed by total number of adult emerges and longevity of the emerged adults.

For performing biochemical assays, homogenate was prepared for which individual adult insects were separately weight & homogenized in 10 volumes (w/v) of ice cold 0.1 M phosphate buffer (pH 7.2 - AChE, AST and ALT and total protein) and for ALP the homogenate was made at 9.8 pH using glass homogenizer. Homogenates were centrifuged at 5000 rpm for 30 min at 4ºC. The obtained supernants were divided into small portion & stored at -20ºC. Ache Activity was determined according to Ellman et al. (1961), specific activity was expressed as mmol/ml/min. Alkaline Phosphatase Activity was determined according to Klin (1972), specific activity was expressed as IU/ L. Transaminases Activity was determined according to Reitman and Frankel (1957), specific activities were expressed as IU/ L. Total soluble protein was determined calorimetrically according to the method of Lowry et al. (1951) by using bovine serum albumin (BSA) as a standard. Total protein expressed as g/ dl. The difference in the life cycle parameters and detoxification enzyme activities of control and test groups were determined by One-Way ANOVA using Graph pad Prism software v.8.

RESULTS AND DISCUSSION

The results revealed that the LC_{50} value of the EOs of mint is 5.9 µl/ ml and that of ajwain is 7.02 µl/ ml; $1/5^{th}$ and $1/ 20^{th}$ concentration were selected as low dose and high dose from the obtained LC_{50} with the pulse beetle *Callosbruchus chinensis*; other values were- (M) LD (0.29 µl/ ml) and (M) HD (1.18 µl/ ml) while (A) LD (0.35 µl/ ml) and (A) HD (1.40 µl/ ml). A dose dependent variation was observed in the parameters of lifecycle- significant reduction in eggs laid, adult emergence and adult longevity was observed ($p<0.01$). High dose of ajwain gave more significant alteration compared to mint (Fig.1). Campolo et al. (2018) suggested that application of EOs act as oviposition deterrent, and impacts the overall lifecycle. Perez et al. (2010) reported a dose dependent mortality on exposure of *A. inulifolium* oil to *S. granarius.* A dose dependent alteration in the enzyme activities of AChE, ALP, AST and ALT was also observed. Both the EOs significantly inhibited the enzyme activities compared to control ($p<0.01$) (Fig. 2). Similar effect was also noticed with total protein. Thymol, a terpenoid is major constituent of ajwain oil (67.4%) and is reported to have an insecticidal effect due to its aromatic ring and hydroxyl group (Vitali et al., 2016). Singla et al. (2018), opined that the conversion of thymol to thymyl acetate and thymyl phenylether inhibits the enzyme activity. Fumigant action of thymol results into interdicting plasma membrane lipids altering the membrane potential leading to loss of metabolites and ions from the cells (Chaudhari et al., 2021). Main constituents of mint oil have been well explored (Vendan et al., 2017), and menthol, a volatile molecule had been shown to possess fumigant and repellency properties against *C. maculatus* and *Sitophilus oryzae* (Vendan et al., 2017; Saeidi et al., 2017)

In inhibition of the AChE activities observed now in in accordance with previous work on *M. piperita* EOs compounds, against *S. oryzae* and *T. castaneum* (Rajkumar et al*.,* 2019). EOs from cumin and basil have been reported to inhibit the ALP activity in *S. oryzae* (Omar*,* 2020). In the present study, a significant reduction in the ALP at high dose of ajwain was

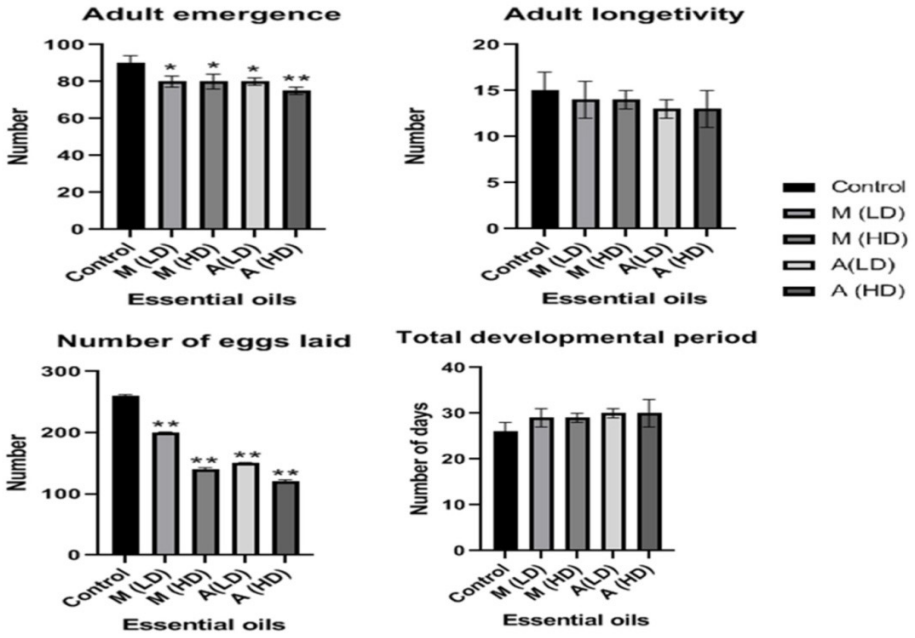

Fig. 1. Effect of EOs on adult emergence, longevity, eggs laid and development period *(p<0.05); ** (p<0.01)

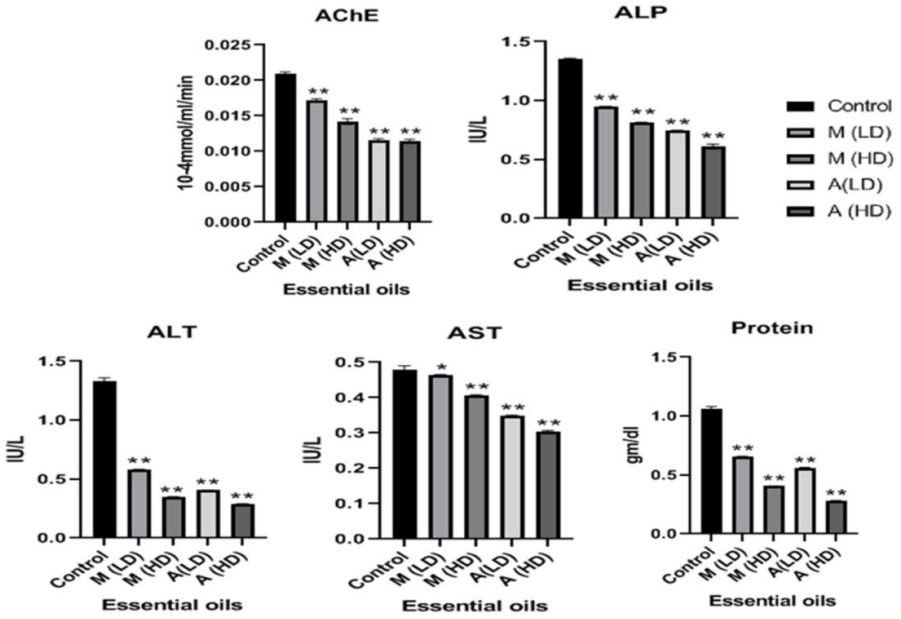

Fig. 2. Inhibitory effects of EOs on the enzymes- AChE, ALP, AST, ALT and total protein *(p<0.05); ** (p<0.01)

observed. It is probably due to reduction of phosphorous liberated for energy metabolism, decreased rate of metabolism, as well as decreased rate of transport of metabolites (El-Gizawy et al., 2019; Omar, 2020). ALT as well as AST both serves as a strategic link between the carbohydrates and protein metabolism known to be altered during various physiological and pathological conditions (Tawfeek et al., 2021) Various EOs have been reported to alter AST and ALT activities (Shahriari et al., 2017; Kisa et al., 2018; Hashem et al., 2020). The intrinsic properties of EOs interfere with basic metabolic, biochemical, and physiological functions of insect pests. The two EOs evaluated cause significant decrease in total protein content which may be attributed to reduced protein synthesis and low uptake of amino acids (Omar et al., 2020). Ayalew (2020) and Tawfeek et al. (2021) also observed a decrease in total protein content in *S. oryzae*. Thus, it can be concluded that mint

and ajwain EOs can be recommended as ecofriendly alternatives.

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara for providing the laboratory facilities. Dr Parth Pandya from Navrachana University is acknowledged for help in improving the manuscript.

AUTHOR CONTRIBUTION STATEMENT

GP, PS, NP, PP designed and conceptualized the study. GP performed the experimental work. GP and PS analyzed the data. GP, PS and NP, wrote the draft manuscript. PS, NP, PP reviewed and revised the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Ayalew A. 2020. Insecticidal activity of *Lantana camara* extracts oil on controlling maize grain weevils. Toxicology Research and Application 4: 1-10

Bhumi T, Urvi C, Pragna P. 2017. Biopesticidal potential of some plant derived essential oils against the stored grain pests. International Journal of Zoological Investigations 3: 188-197.

Campolo O, Giunti G, Russo A, Palmeri V, Zappalà L. 2018. Essential oils in stored product insect pest control. Journal of Food Quality 2018: 1-18

Chaudhari A K, Singh V K, Kedia A, Das S, Dubey N. K. 2021. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. Environmental Science and Pollution Research 28(15): 18918-18940.

Demeter S, Lebbe O, Hecq F, Nicolis S C, Kenne K T, Martin H, Hance, T. 2021. Insecticidal activity of 25 essential oils on the stored product pest, *Sitophilus granarius*. Foods 10(2): 200.

Ellman G L, Courtney K D, Andres V, Featherstone R M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7(2): 88-95.

El-Gizawy K, Halawa S M I, Mehany A L, Mohamed S A. 2019. Toxicity of some essential oils and its biochemical effect against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Egyptian Academic Journal of Biological Sciences, F. Toxicology and Pest Control 11(1): 27-38.

Femeena P V, Sudheer K P, Cibin R, Chaubey I. 2018. Spatial optimization of cropping pattern for sustainable food and biofuel production with minimal downstream pollution. Journal of Environmental Management 212: 198-209.

Hashem A S, Ramadan M M, Abdel-Hady A A, Sut S, Maggi F, Dall'Acqua S. 2020. *Pimpinella anisum* essential oil nanoemulsions toxicity against *Tribolium castaneum* shedding light on its interactions with aspartate aminotransferase and alanine aminotransferase by molecular docking. Molecules 25(20): 4841.

Hawkins N J, Bass C, Dixon A, Neve P. 2019. The evolutionary origins of pesticide resistance. Biological Reviews 94(1): 135-155.

Hill M P, Macfadyen S, Nash M A. 2017. Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. PeerJ 5: e4179.

Jerbi M, Hamouda A B, Hamdi F, Laarif A, Chaieb I. 2021. Evaluation of repellent activity and toxicity of three essential oils against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) using a mixture design. International Journal of Entomology Research 3: 69.

Kiran S, Kujur A, Patel L, Ramalakshmi K, Prakash B. 2017. Assessment of toxicity and biochemical mechanisms underlying the insecticidal activity of chemically characterized *Boswellia carterii* essential oil against insect pest of legume seeds. Pesticide Biochemistry and Physiology 139: 17-23.

Kisa A, Akyüz M, Çoğun, H Y, Kordali Ş, Bozhüyük A U, Tezel B, Çakır A. 2018. Effects of *Olea europaea* L. leaf metabolites on the tilapia (*Oreochromis niloticus*) and three stored pests, *Sitophilus granarius, Tribolium confusum* and *Acanthoscelides obtectus*. Records of Natural Products 12(3): 201.

Klin Z. 1972. Recommendations of the German Society for Clinical Chemistry. Standardization of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions. Zeitschrift für Klinische Chemie und Klinische Biochemie 10(6): 281-291.

Lal M, Ram B, Tiwari P. 2017. Botanicals to cope stored grain insect pests: a review. International Journal of Current Microbiology and Applied Sciences 6(6): 1583-1594.

Lowry O H, Rosebrough N J, Farr A L, Randall R J. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Omar A F, El-Ebiary M E, Nasr G M, Hassan H M. 2021. Toxicity and biochemical effects of cumin and basil essential oils. Scientia Agriculturae Bohemica 52(3): 39-48.

Pavela R. 2016. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects- a review. Plant Protection Science 52(4): 229-241.

Pérez S G, Ramos-López, M A, Zavala-Sánchez M A, Cárdenas-Ortega N C. 2010. Activity of essential oils as a biorational alternative to control coleopteran insects in stored grains. Journal of Medicinal Plants Research 4(25): 2827-2835.

Raina A K. 1970. *Callosobruchus* spp. infesting stored pulses (grain legumes) in India and comparative study of their biology. Indian Journal of Entomology 32(4): 303-310.

Rajkumar V, Gunasekaran C, Christy I K, Dharmaraj J, Chinnaraj P, Paul C A. 2019. Toxicity, antifeedant and biochemical efficacy of *Mentha piperita* L. essential oil and their major constituents against stored grain pest. Pesticide Biochemistry and Physiology 156: 138-144.

Reitman S, Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28(1): 56-63.

Saad A S, Tayeb E H. M, El-Shazli M M, Baheeg S. A. 2018. Susceptibility of certain Egyptian and imported wheat cultivars to infestation by *Sitophilus oryzae* and *Rhyzopertha dominica*. Archives of Phytopathology and Plant Protection 51(1-2): 14-29.

Saeidi K, Mirfakhraie S. 2017. Chemical composition and insecticidal activity *Mentha piperita* L. essential oil against the cowpea seed beetle

Callosobruchus maculatus F. (Coleoptera: Bruchidae). Journal of Entomological and Acarological Research 49: 127-134.

Said P P, Pashte V V. 2015. Botanicals: The protectants of stored grains pests. Trends in Biosciences, 8(15): 3750-3755.

Sarwar M. 2016. Indoor risks of pesticide uses are significantly linked to hazards of the family members. Cogent Medicine 3(1): 1155373.

Shahriari M, Sahbzadeh N, Zibaee A, Khani A, Senthil-Nathan S. (2017). Metabolic response of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) to essential oil of ajwain and thymol. Toxin reviews 36(3): 204-209.

Singh R, Khanna V. 2019. Agribusiness as a tool to mitigate the risks of post-harvest losses, Trends and prospects in post harvest management of horticultural crops, Today and Tomorrow Printers and Publishers, New Delhi. pp.103-133

Singla N, Chahal K K, Kumar A, Bhardwaj U, Kang B K. 2018. Ajwain, *Trachyspermum ammi* (L.) constituents/some derivatives as stored grain protectant against *Sitophilus oryzae*. Pesticide Research Journal 30(2): 188-199.

Sirohi R, Tarafdar A, Gaur V K, Singh S, Sindhu R, Rajasekharan R, Pandey A. 2021. Technologies for disinfection of food grains: Advances and way forward. Food Research International 145: 110396.

Tawfeek M E, Ali H M, Akrami M, Salem Z M. 2021. Potential insecticidal activity of four essential oils against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). BioResources 16(4): 7767-7783.

Vendan S E, Manivannan S, Sunny A M, Murugesan R. 2017. Phytochemical residue profiles in rice grains fumigated with essential oils for the control of rice weevil. PloS One 12(10): e0186020.

Vitali L A, Beghelli D, Nya P C B, Bistoni O, Cappellacci L, Damiano S, Petrelli, D. 2015. Diverse biological effects of the essential oil from Iranian *Trachyspermum ammi*. Arabian Journal of Chemistry 9(6): 775-778.

(Manuscript Received: June, 2022; Revised: September, 2022;
Accepted: October, 2022; Online Published: October, 2022)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e22543