Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(5): 94-97

Received: 19-07-2018
Accepted: 20-08-2018

Vaishali P Sawant
Ph.D. Scholar, Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Shyamkant S Munje
Assistant Professor, Regional Research Centre, Amravati, Maharashtra, India

Yugal K Yadu
Principal Scientist, Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur Chhattisgarh, India

Correspondence
Vaishali P Sawant
Ph.D. Scholar, Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur Chhattisgarh, India

Interactive effect of rosemary oil and phosphine gas on pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Chysomelidae)

Vaishali P Sawant, Shyamkant S Munje and Yugal K Yadu

Abstract
In this study, the toxic effect of locally available volatile oil of Rosemary, *Rosemarinus officinalis* L. (Lamiales: Lamiaceae) doses (5, 10 and 15 percent) alone and in combination with phosphine gas 4 ppm has been investigated against pulse beetle, *Callosobruchus chinensis* L. under laboratory conditions. The research work was carried out during September, 2017 to March, 2018 at the Regional Research Centre, Amravati (M.S.). Ninth homogenous susceptible generation of pulse beetle was taken as test insect. Percent mortality was observed at 20 minutes and 24 hours after exposure to fumigants. It was observed that percent mortality increased with increase in exposure period. Maximum mortality (100 percent) was observed in all the treatments of rosemary oil + phosphine 4 ppm after 24 hours of exposure. Minimum mortality of pulse beetle (26.67 percent) was observed in the treatment, rosemary oil 5 percent after 20 minutes of exposure and it was increased up to 70.00 percent after 24 hours of exposure to the treatment.

Keywords: Rosemary oil, phosphine gas, test insect, percent mortality, *Callosobruchus chinensis*

1. Introduction
The pulses are considered to be an important source of protein which fulfils the protein demand of vegetarian and low income groups of the population. The production of pulses in India during 2015-16 was 16.47 million tonnes which was lowered by 0.68 million tonnes as compared to the production of previous year 2014-15 i.e. 17.15 million tonnes [1]. It is essential to store this produce in good conditions for use in further seasons. But in storage, pulses are mostly damaged by pulse beetles. Pulse beetle, *Callosobruchus chinensis* L. is the major pest of legumes. *C. chinensis* L. attack green gram, chickpea, lentils, cowpea seeds in storage [2, 3]. It is not possible to store legumes and other grains/ seeds without insect infestation. Hence to manage these stored grain pests in warehouses or storage containers or silo, various insecticides like malathion, pirimiphos methyl, chlorpyrifos methyl, dichlorvos, pyrethrin/piperonyl butoxide, etc. and fumigants like phosphine, methyl bromide are used [4].

Methyl bromide reduces the ozone layer in the atmosphere which causes increase in ultraviolet radiation reaching the earth's surface. Hence, the Montreal amendment, 1997 included the phase out of methyl bromide in developed and developing countries in 2005 and 2015, respectively [5]. Hence, phosphine is the only fumigant used to control insect pests in stored grains and stored products. Phosphine kills insects at comparatively low cost and leave minimum residues on treated products. But because of continuous use of phosphine in stored products, it is under threat as resistance has been recorded in different insect species of stored products across many countries [6].

Some plant and plant products are also in use from several years to control insects in stored grains/ seeds but generally used when stored in small scale. Various essential oils *viz.* juniper oil, rosemary oil, mint oil, eucalyptus oil, citronella oil, clove oil etc. are used for controlling the stored insect pests [7-10].

Rosemary oil is obtained from flowering tops of *Rosemarinus officinalis* L. (Lamiales: Lamiaceae).The oil is effective as analgesic, antibacterial, anticancer, anticatarrhal, antifungal, anti-infection, anti-inflammatory, antioxidant, and expectorant [11]. The essential oil of Rosemary, *Rosemarinus officinalis* was found to be effective against *Tribolium castaneum* and *Callasobruchus maculatus* [8]. It was also observed that the combination of gamma radiation and *R. officinalis* oil fumigation had a potential for application in integrated management of *C. maculatus* [12].

The present work is carried out to determine the fumigant toxicity of essential oil of the flowering tops of *Rosemarinus officinalis* and also the combined effect of rosemary oil with phosphine against pulse beetle, *Callosobruchus chinensis* L.

2. Materials and Methods

The experiment was carried out at the Regional Research Centre, Amaravati (M.S.) during 2017- 2018. The materials were comprised of adults of pulse beetle, *Callosobruchus chinensis* L., crude rosemary oil 5 ml, aluminium phosphide sachet 10 gms, plastic canister of 1050 volume, filter papers, jars, glass gas chambers 125 ml volume, injection syringe, falcon tubes, pipette, rubber bands, muslin cloth, healthy, uninfested green gram, etc.

2.1 Collection and rearing of homogenous insect culture:-

Nucleus culture of Pulse beetle, *Callosobruchus chinensis* L. was collected from the Department of Post Harvest Technology, College of Agril. Engineering Technology, Dr. Panjabrao Deshmukh Krishi Viyapeeth, Akola (M.S.). The insect culture was maintained in sterilized plastic jars at 29$\pm$2^{0}C temperature and 80$\pm$5% relative humidity. The culture medium comprise of green gram seeds. Initially 1-2 days old 25 adults were released on 250 grams of healthy, uninfested green gram seeds in two plastic jars each. For proper aeration, jars were covered with the muslin cloth. The jars were then kept on iron racks for mating and oviposition.

Adult emergence started from the infested grains/ seeds at 22 days after release. These adults were further reared for multiplication up to ninth generations. The adults from ninth susceptible generation were taken for conducting the experiment.

2.2 Test material

Crude rosemary oil was obtained from the local market to carry out the experiment. Rosemary oil was diluted with acetone to obtain the required doses of 5, 10 and 15 percent concentrations for evaluation.

2.3 Generation of phosphine gas

The procedure of phosphine gas generation was followed as per the procedure given by DETIA DEGESCH GMBH [13]. The personal protective equipment like mask and goggles were used while handling aluminium phosphide and generating phosphine gas. First the plastic canister with rubber cork of 1 litre having total air volume 1050 ml was taken. 10 ml of water was added in it. One sachet of 10 gm Celphos i.e. aluminium phosphide was put in the canister and sealed the canister immediately with rubber cork. The canister was shaked carefully and kept as it was for 60 minutes and allowed the phosphine gas to release.

Celphos in 10 gms sachet contains 56 percent aluminium phosphide i.e. 5.6 gms. Hence, in the canister having air volume 1050 ml, 5300 ppm phosphine was prepared. This was refered as ‘Stock A’. From stock A, phosphine was collected for further dilution to 500 ppm. We refered this as ‘Stock B’. From stock B, phosphine gas was collected with the help of injection syringe to dilute up to 4 ppm in the gas glass chamber having Air volume 125 ml to conduct the experiment.

2.4 Toxic effect of essential oil alone and in combination with phosphine on *Callosobruchus chinensis* L.:-

The insecticidal activity of rosemary oil (*Rosemarinus officinalis*) against pulse beetle *Callosobruchus chinensis* L. was evaluated by direct contact application assay. This essential oil prepared in acetone at different concentrations i.e. 5%, 10% and 15%. To prepare different concentrations, first 10 ml acetone was taken in falcon tube. Then 2 ml rosemary oil was added in it to make 20 percent stock solution. Through this stock solution, some amount of solution was taken out and diluted in the acetone to form further concentrations by following formula [14]:

$$V = \frac{C \times A}{a.i.}$$

Where,

V = Quantity of essential oil to be collected from stock solution

C = Required concentration of essential oil

A = Total volume of the solution

a.i. = Stock solution i.e. 20 percent

By using this formula, 15 percent rosemary oil was prepared by taking 3.75 ml rosemary oil from 20 percent stock solution and then 1.25 ml acetone was added in that to prepare 5 ml solution of 15 percent rosemary oil. Similarly 10 percent and 5 percent rosemary oil solution was prepared. The filter papers were cut in circular shape according to the shape of bottom of the glass canister.

There were eight treatments and three replications in this experiment. As test insects, 10 adult bruchids were used per replication. The oil of different concentrations was applied on filter papers. Solvent i.e. acetone was allowed to evaporate for 10-15 min. prior to introduction of insects. Each filter paper was then placed at the bottom of a glass gas chamber. Test insects were released in each replicated glass gas chamber and then covered with rubber cork. As the test insects should not stay on the lid, the inner side of the cork was coated with vasaline. In the treatments from T4 to T6, 4 ppm phosphine gas was released in the glass gas chamber in addition to the essential oils. In treatment T7, only phosphine gas was released to observe the efficacy of poisonous gas. Number of dead insects were observed 20 minutes after exposure to oils and phosphine gas and thereafter 24 hours. The data thus obtained were analysed statistically by Completely Randomized Design test. The treatment details are as follows.

Table 1: Treatment details in this study

Sr. No.	Treatments	Treatment details
1.	T1	Rosemary oil 5%
2.	T2	Rosemary oil 10%
3.	T3	Rosemary oil 15%
4.	T4	Rosemary oil 5% + phosphine 4ppm
5.	T5	Rosemary oil 10% + phosphine 4ppm
6	T6	Rosemary oil 15% + phosphine 4ppm
7.	T7	Phosphine 4 ppm
8.	T8	Untreated control / acetone only

3. Results

In this experiment, the fumigant action of rosemary oil alone and in combination with phosphine gas was tested against adults of *C. chinensis* L. The results are presented in Table 2. It was observed that, as the concentration of oil was increased, there was increase in the mortality of the test insects. The significant combined effect of essential oil and phosphine was also observed on the adult mortality of *C. chinensis*. Table 2 showed that 20 minutes after exposure to fumigants, the treatment T6 (Rosemary oil 15% + phosphine 4 ppm) was found significantly superior over all other treatments (except treatment T5) showing the synergistic effect with mortality 63.33 percent. Treatment T6 was at par with the treatment T5 (Rosemary oil 10% + phosphine 4 ppm) having mortality 60.00 percent which was followed by treatment T4 (Rosemary oil 5% + phosphine 4 ppm) (50.00 percent). No mortality was observed in untreated control i.e. acetone only. 20 minutes after exposure, minimum mortality was observed in treatment T1 (Rosemary oil 5%) i.e. 26.67 percent.

From table 2, it was also revealed that 24 hours after exposure to rosemary oil and phosphine gas, 100 percent adult mortality of *C. chinensis* was observed in treatment T6 (Rosemary oil 15% + phosphine 4 ppm), T7 (phosphine 4 ppm), T5 (Rosemary oil 10% + phosphine 4ppm) and T4 (Rosemary oil 5% + phosphine 4ppm). These treatments were followed by the treatment T3 (Rosemary oil 15%) (86.67 percent) which was at par with the treatment T2 (Rosemary oil 10%) i.e. 83.33 percent.

Table 2: Efficacy of rosemary oil with phosphine against pulse beetle, *Callosobruchus chinensis*

Treatments	Percent Adult mortality after exposure period of	
	20 min	24 hours
T1 - Rosemary oil 5%	26.67 (5.64)*	70.00 (8.85)
T2 - Rosemary oil 10%	43.33 (7.07)	83.33 (9.63)
T3 - Rosemary oil 15%	46.67 (7.32)	86.67 (9.81)
T4 - Rosemary oil 5% + phosphine 4ppm	50.00 (7.55)	100.00 (10.50)
T5 - Rosemary oil 10% + phosphine 4ppm	60.00 (8.23)	100.00 (10.50)
T6 - Rosemary oil 15% + phosphine 4ppm	63.33 (8.45)	100.00 (10.50)
T7 - Phosphine 4ppm	30.00 (5.92)	100.00 (10.50)
T8 - Untreated control/ Acetone only	0.00 (0.50)	3.33 (1.55)
SE +	0.27	0.33
C.D. at 5%	0.55	0.68

*Figures in parantheses are square root values

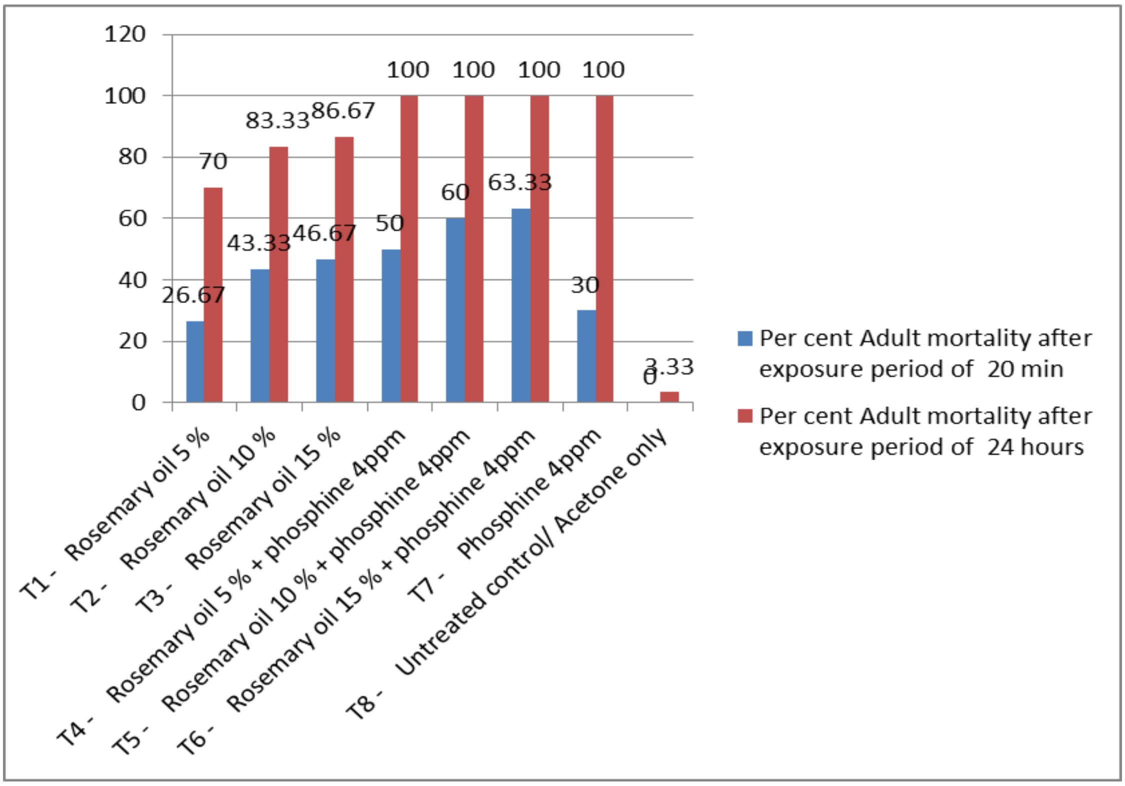

Fig 1: Graph showing the percent adult mortality after exposure period of 20 min. and 24 hours.

4. Discussion

These research findings showed that rosemary oil was very effective in controlling *C. chinensis* and when it was applied with phosphine gas, it showed synergistic effect causing 100 percent mortality in *C. chinensis* at 24 hours after exposure. These research findings showed similarity with the finding by Ahmadi and Saeid [12]. They determined the combined effects of essential oil from *Rosmarinus officinalis* L. and gamma radiation on mortality of *Callosobruchus maculatus* F. They observed that integration of gamma radiation and *R. officinalis* oil enhanced the mortality of *C. maculatus* compared with control treatments of either irradiation or fumigation alone.

In the present research, the synergistic effect of rosemary oil along with phosphine on pulse beetle, *C. chinensis* was observed. Similar synergistic effect was also observed in the research findings by Foruzan, M., *et al.* [15]. They determined the insecticidal activity of essential oil of *Artemisia annua* L. against adult of *Tribolium castaneum* Herbst., *Sitophilus granarius* L. and *Callosobruchus maculatus* F. They observed that the fumigant toxicity of essential oil and acetone against these pests and found that the oil and acetone separately were effective causing mortality in all the three pests. They also tested LC_{25} value of Acetone with a combination of LC_{25} value of Artemisia essential oil against adults of three pests and results showed that acetone had synergistic effect on fumigant toxicity of *A. annua*.

In these findings, we observed that rosemary oil alone was also effective in controlling *C. chinensis*. Similar findings were shown by Singh, V. [8]. She has investigated the toxic effect of *Rosemarinus officinalis* on *Tribolium castaneum* and *Callosobruchus maculatus* and observed that the essential oil has significant and good toxicity against *Callasobruchus maculatus* as compared to *Tribolium castaneum.*

Our findings also showed that as the exposure time to fumigants increased, there was increase in the mortality of *C. chinensis*. Similar results are shown by Ahmady A. *et al.* [10]. They tested the insecticidal activity of eight botanical oils, Citronella, Clove, Eucalyptus, Jojoba, Lemon, Orange, Rosemary, Spearmint against the adults of *C. maculatus* in the laboratory. The results showed that all used oils were effective in controlling *C. maculatus.* The highest mortality was observed on clove and jojoba followed by rosemary, eucalyptus and citronella. The mortality was increased with increase of concentration levels and the exposure time.

The present findings are in accordance with the findings of Hanif, M.S. [16] who evaluated the effect of essential oils *viz. Melia azadarach, Datura stramonium* and *Azadirachta indica* and phosphine fumigation alone and in combination for their repellent and mortality effect against three stored grain insect pests at various concentrations viz., 5%, 10% and 15% for plant oils, while 100 ppm, 200 ppm and 300 ppm for phosphine fumigation and found maximum mortality in combination at higher oil and phosphine concentration.

These research findings showed that the percent mortality in pulse beetle, *C. chinensis* increased with the increase in concentration of rosemary oil in combination with phosphine and the exposure period. These findings showed same results with the findings of Hasan, M., *et al.* [17] who studied the response of *Trogoderma granarium* to different combinations of phosphine concentrations (100 ppm, 200 ppm and 300 ppm) and *Acorus calamus* oil doses (30, 50 and 70 μL).The Percent mortality was observed for different exposure periods of 3, 5 and 7 days. The results showed that percent mortality increased with increase in phosphine concentration in combination with *Acorus calamus* oil and with increase in exposure period.

5. Conclusion

Overall, our results demonstrate a strong detrimental effect of rosemary oil when applied alone and in combination with phosphine against pulse beetle. This experiment added to a growing corpus of research showing synergistic impact of rosemary oil in combination with phosphine against *C. chinensis*. Application of rosemary oil alone and with very less concentration of phosphine gas against *C. chinensis* is effective having complete mortality showed some similarity with those mentioned references, showing the importance of insecticides of plant origin in the management of *C. chinensis* as alternatives to synthetic insecticides. In future, phosphine resistant population could be controlled by rosemary oil as an organic alternative. Further studies should investigate the synergistic effect on phosphine resistant population on large scale.

6. Acknowledgements

The present research has received infrastructure facilities and valuable guidance from Regional Research Centre, Amravati, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.) and Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) under Ph.D. study programme.

7. References

1. http://agricoop.nic.in/sites/default/files/Annual_rpt_2016 17_E.pdf. 12/07/2018.
2. Chakraborty S, Mondal P, Senapati SK. Studies on biology of the pest beetle, *Callosobruchus chinensis* Linn. infesting green gram. Current Biotica. 2015; 9(1):93-97.
3. Muhammad A. Pest Status of Stored Chickpea Beetle, *Callosobruchus chinensis* Linnaeus on Chickpea. Journal of Entomology. 2004; 1(1):28-33.
4. http://www.fao.org/docrep/t1838e/T1838E1g.htm. 20/07/2018.
5. https://www.epa.gov/ozone-layer-protection/international-treaties-and-cooperation. 21/07/2018.
6. Schlipalius DI, Ebert PR, Collins PJ. An emerging international picture of phosphine resistance: opportunities for global cooperation. Proceedings of the 11th International Working Conference on Stored Product Protection. 24-28 November 2014 Chiang Mai, Thailand, 2014, 11-14.
7. Hashemi SM, Rostaefar A. Insecticidal Activity of Essential Oil from *Juniperus communis* L. subsp. *hemisphaerica* (Presl) Nyman against two stored product beetles. Ecologia Balkanica. 2014; 6(1):87-93.
8. Singh V. Phytotoxic Efficacy of Rosemary Oil Against *Tribolium Castaneum* and *Callasobruchus Maculatus.* Global Journal of Science Frontier Research: C Biological Science. 2016; 16(3):59-63.
9. Kumar P, Mishra S, Malik A, Satya S. Insecticidal properties of Mentha species: A review. Industrial Crops and Products. 2011; 34:802-817.
10. Ahmady A, Mousa MAA, Zaitoun AA. Efficacy of Some Botanical Oils against Stored-product Pest Cowpea Beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). International Journal of Zoology Studies. 2017; 2(1):05-09.
11. https://articles.mercola.com/herbal-oils/rosemary-oil.aspx. 24/07/2018.
12. Ahmadi M and Moharramipour S. Interaction between essential oil of *Rosmarinus officinalis* and gamma radiation against *Callosobruchus maculatus.* Journal of Crop Protection. 2016; 5(4):519-527.
13. Detia Degesch GmbH. Phosphine Resistance Test Kit. Directions for use, 2011, 1-15.
14. Munje SS. Molecular studies on resistance mechanism to chlorantraniliprole in *Leucinodes orbonalis* (Guen.). Doctoral thesis, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.), 2015.
15. Forouzan M, Hosseinzadeh A, Aramideh S, Ghassemi-Kahrizeh A, Mirfakhraie S, Mahinfar S. Fumigant toxicity of essential oils from *Artemisia annua* L. and the synergistic effect of acetone against three most important stored pests. Journal of Entomology and Zoology Studies. 2016; 4(6):117-120.
16. Hanif MS. Response of Three Stored Grain Insect Pests to Different Combinations of Phosphine and Essential Oils of Different Plants. Doctoral thesis, University Of Agriculture, Faisalabad, 2013.
17. Hasan M, Sagheer M, Ahmad F. Response of *Trogoderma granarium* (Everts) to different combinations of phosphine and *Acorus calamus* oil. Proceedings of the 8th International Conference on Controlled Atmosphere and Fumigation in Stored Products, 2008, 538-541.