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Synergistic effect of juniper oil with phosphine gas on pulse beetle, *Callosobruchus Chinensis* L. (Coleoptera: Bruchidae)

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Abstract
In this study, the toxic effect of locally available volatile oil i.e. juniper oil in comparison with an organophosphorus insecticide / fumigant, phosphine gas was tested against pulse beetle, *Callosobruchus chinensis* L. 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. The effect of juniper oil doses (5, 10 and 15 per cent) and phosphine 4 ppm were studied alone and in combination against pulse beetle, *C. chinensis* L. Per cent mortality was observed at 20 minutes and 24 hours after exposure. It was observed that per cent mortality increased with increase in exposure period. Maximum mortality (100 per cent) was observed in the treatment, juniper oil 15 per cent + phosphine 4 ppm after 24 hours of exposure. While minimum mortality (13.33 per cent) was observed in the treatment, juniper oil 5 per cent after 20 minutes of exposure and it was 56.67 per cent after 24 hours of exposure to the treatment.

Keywords: Juniper oil, phosphine gas, test insect, per cent mortality, *Callosobruchus chinensis*

Introduction

Callosobruchus chinensis L. commonly known as pulse beetle or adzuki beetle is a major pest of stored lentils. Infestation of the pest can start in the field before harvest, and thus gain entry into storage bins. Pulse beetle may cause over 80% losses in weight of seeds and in germination rates. Infested seeds are less nutritious and unfit for human consumption. [1]. From the records, it is found that *Callosobruchus chinensis* was first observed and described in China, because of that the name of species is *chinensis* [2]. Pulse beetle attacks different species of stored legumes and it is distributed across the tropical and subtropical regions of the world. From the studies, it was observed that *C. chinensis* caused maximum seed damage in cowpea followed by green gram and then in moth bean while highest weight loss was observed in green gram followed by moth bean and cowpea [3].

Hence, it is impossible to store the grains without insect infestation. Different insecticides like malathion, pyrethrins, cyfluthrin, etc. and fumigants like phosphine, carbon dioxide, methyl bromide are used for management of stored grain pests in warehouses [4].

As methyl bromide is banned, phosphine is the only fumigant used to control insect pests in warehouses to protect stored grains and stored products [5]. Phosphine leaves minimum residues on treated products and is economically viable. Because continuous use of phosphine in stored products, the resistance has been recorded in different insect species of stored products across many countries [6].

Juniper oil from berries of *Juniperus communis* act as antirheumatic, antiseptic, antispasmodic, antitoxin, diuretic, emmenogogue, rubifacient, sedative agent. For culinary purposes, it is used as flavouring agent [7]. In Hindi, juniper is also called as Aaraar, Haubera, Abhal. In Marathi, it is called Hosha, in Bengali, Hausha and in Punjabi, Abhal. This oil from berries may have the potential to act as control agent against *Rhyzopertha dominica* and *Tribolium castaneum* [8].

Keeping in view, all of this, the present work was carried out to determine the fumigant toxicity of essential oil obtained from the berries of *Juniperus communis* and also the synergistic effect of juniper oil with phosphine against pulse beetle, *Callosobruchus chinensis* L.

2. Materials and Methods

The present experiment was carried out at the Regional Research Centre, Amaravati (M.S.) during 2017-18. The materials were comprised of adults of pulse beetle, *Callosobruchus chinensis* L., juniper oil 5 ml bottle, aluminium phosphide 10 gms sachet, filter papers, jars, glass gas chambers 125 ml volume, injection syringe, falcon tubes, pipette, 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, 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. The jars were covered with the muslin cloth so that aeration took place. The jars were kept on iron racks for mating and oviposition.

After 22 days of release, adults started emerging out from the infested grains/ seeds. Then again they were further reared for multiplication up to eight generations. The adults from ninth susceptible generation were taken for conducting the experiment.

2.2 Test material

Crude juniper oil was obtained from local market to carry out the experiment. Juniper oil was diluted with acetone to derive the required doses of 5, 10 and 15 per cent concentrations for evaluation.

2.3 Generation of phosphine gas

The procedure of phosphine gas generation was followed as per the DETIA DEGESCH GMBH [9]. The printed copy of instructions for handling the phosphine gas/ aluminium phosphide was adhered in the laboratory where work was going to be done. The personal protective equipments like mask and goggles were used while handling the 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.

As in 10 gms sachet of celphos, aluminium phosphide is 56 per cent i.e. 5.6 gms. Hence, in 1050 ml canister, 5300 ppm phosphine was prepared. 5300 ppm phosphine was further diluted to 500 ppm and then to 4 ppm using injection syringe and 125 ml glass bottles to conduct the experiment.

2.4 Toxicological impact of volatile oil against *Callosobruchus chinensis* L.

The insecticidal activity of juniper oil (*Juniperus communis*) against pulse beetle *Callosobruchus chinensis* L. was evaluated by direct contact and fumigation application. This essential oil was prepared in acetone having different concentrations i.e. 5%, 10% and 15%. To prepare different concentrations, first 10 ml acetone was taken in falcon tube. Then 2 ml juniper oil was added in that to make 20 per cent stock solution.

Through this stock solution, some quantity was taken out and diluted in the acetone to form further concentrations by the formula [10]:

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

Where,

V = Volume of phosphine gas
C = Required concentration/ppm of Phosphine gas
A = Volume of glass gas chamber
a.i. = Active ingredient in aluminium phosphide/stock solution By this formula, 15 per cent juniper oil was prepared by taking 3.75 ml juniper oil from 20 per cent stock solution and then 1.25 ml acetone was added in that to make the volume 5 ml. Likewise 10 per cent juniper oil was prepared by taking 2.50 ml juniper oil from 20 per cent stock solution and then 2.50 ml acetone was added in that to make the volume 5 ml. And for 5 per cent juniper oil, 1.25 ml was taken out from stock solution and then 3.75 ml acetone was added. The filter papers were cut in circular shape according to the diameter of bottom of the glass canister.

The experiment was consisted of eight treatments and three replications. There were total 10 adult bruchids used as test insects 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. Then each paper was placed at the bottom of a glass canister and covered with rubber cork. The inner side of the cork was coated with vasaline to prevent insect staying on the lid.

Different three combinations of phosphine 4 ppm and juniper oil with concentrations 5, 10 and 15 per cent were used as treatments and ten adults were released in each canister as discussed earlier. One treatment was phosphine 4 ppm alone and another was untreated control with acetone applied on filter paper only. Number of dead insects was observed 20 minutes after exposure to oils and phosphine gas and thereafter 24 hours. The treatment details are as follows.

Table 1: Treatment details in this study.

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

2.5 Statistical analysis

The data were analysed statistically by Completely Randomized Design test.

3. Results and Discussion

The fumigant action of juniper oil and phosphine was tested on adults of *C. chinensis*. The results of the present studies are given in Table 2. It was observed that there was a difference in mortality of the insects as the concentration of oil was increased. It was also observed that there was significant combined or synergistic effect of essential oil and phosphine on the adult mortality of *C. chinensis*. Table 2 shows that 20 minutes after exposure to fumigants, the treatment T6 (Juniper oil 15 % + phosphine 4 ppm) was found significantly effective showing the synergistic effect having mortality 43.33 per cent. Treatment T6 was followed by treatment T5 (Juniper oil 10 % + phosphine 4 ppm) with mortality 30.00 per cent which was followed by treatment T4 (Juniper oil 5 % + phosphine 4 ppm). No mortality was observed in untreated control i.e. acetone only. Very less mortality was observed in treatment T1 (Juniper oil 5 %), 20 minutes after exposure (13.33 per cent).

Table 2 also shows that 24 hours after exposure to juniper oil and phosphine gas, 100 per cent adult mortality of *C. chinensis* was observed in treatment T6 (Juniper oil 15 % + phosphine 4 ppm) and treatment T7 (phosphine 4 ppm). These two treatments were found at par with treatment T5 (Juniper oil 10 % + phosphine 4 ppm) (93.33 per cent) and T4 (Juniper oil 5 % + phosphine 4 ppm) (86.67 per cent). Treatment T1 (Juniper oil 5 %) was found less effective even after 24 hours of the treatment (56.67 per cent).

These research findings showed that the per cent mortality in pulse beetle, *C. chinensis* increased with the increase in concentration of juniper oil in combination with phosphine and the exposure period. These findings showed same results with the findings of Hasan, M., *et.al.*[11] 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 μ L). The Per cent mortality was observed for different exposure periods of 3, 5 and 7 days. The results showed that per cent mortality increased with increase in phosphine concentration in combination with *Acorus calamus* oil and with increase in exposure period.

The present findings are in accordance with the findings of Hanif, M.S.[12] 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 present research findings showed that as the concentration of juniper oil increased with increase in exposure period, the per cent mortality of adult pulse beetle also increased. These findings also showed similar results with the findings of Hashemi, S.M. and Rostaefar, A.[8] who evaluated insecticidal activity of essential oil from fruits of *Juniperus communis* L. against *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) by fumigation at 24, 48, and 72 h exposure times. Results showed that *R. dominica* was more susceptible than *T. castaneum* for all exposure times. LC_{50} values at 24 h were estimated 36.96 μl/l air for *R. dominica*, and 107.96 μl/l air for *T. castaneum*.

Similar results were observed in the research findings by Hamza, A.F., *et.al.*[13] who studied the effect of pure plant volatile oils of Thuja, Eucalyptus and Peppermint on one-week-old adults of *Sitophilus granarius* (L.) reared on wheat. The results showed that the mortality increased with increases in concentration and exposure periods. The per cent mortality of *S. granarius* reached 91.2, 95.0 and 91.2% when 1-w-old adult exposed to higher concentration of Thuja, Eucalyptus and Peppermint oils, respectively, comparing to 0% in the control after 24 h. After 72 h the per cent mortality was 100% at the higher concentration of the three volatile oils.

These present research findings showed similarity with the findings of El-Sayed, K.K., *et.al.*[14] who evaluated the toxic and biological effects of some locally available essential oils i.e. basil (*Ocimum basilicum* L.), cassia (*Cinnamomum cassia* L.), caraway (*Carum carvi* L.), spearmint (*Mentha spicata* L.) and fennel (*Foeniculum vulgare*) in comparison with an organophosphorus insecticide, malathion, against *C. maculatus*. Results showed that basil and cassia were the most effective oils after malathion against *C. maculatus* in both contact and residual toxicity studies. Caraway and spearmint were the weakest oils in the efficacy, while fennel exhibited middle effect.

In the present research, the synergistic effect of juniper oil in combination 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 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 adult of three pests and results showed that Acetone have synergistic effect on fumigant toxicity of *A. annua*.

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

Treatments	Per cent Adult mortality after exposure period of	
	20 min	24 hours
T1 – Juniper oil 5 %	13.33 (4.10)	56.67 (7.98)
T2 - Juniper oil 10 %	16.67 (4.54)	66.67 (8.63)
T3 - Juniper oil 15 %	16.67 (4.54)	76.67 (9.25)
T4 - Juniper oil 5 % + phosphine 4ppm	23.33 (5.31)	86.67 (9.81)
T5 - Juniper oil 10 % + phosphine 4ppm	30.00 (5.92)	93.33 (10.16)
T6 - Juniper oil 15 % + phosphine 4ppm	43.33 (7.07)	100.00 (10.50)
T7 - Phosphine 4ppm	20.00 (4.97)	100.00 (10.50)
T8 - Untreated control/ Acetone only	0.00 (0.50)	3.33 (1.55)
SE +	0.29	0.40
C.D. at 5 %	0.60	0.81

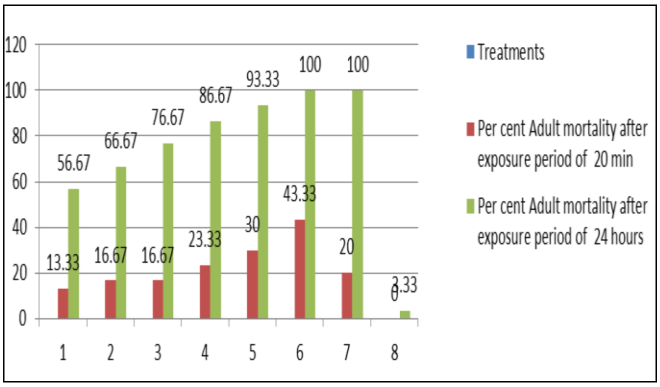

Fig 1: Graph showing the per cent adult mortality after exposure period of 20 min. and 24 hours

4. Conclusion

From this research, it is concluded that juniper oil when applied with phosphine against pulse beetle gives synergist effect. It is also concluded that juniper oil alone could be used to manage the storage pest without phosphine. The results of bioassay and toxicological impact of the tested volatile oil i.e. juniper oil agree with those mentioned references, showing the importance of insecticides of plant origin in the management of *C. chinensis* as alternatives to synthetic insecticides. Phosphine resistant population could be controlled by juniper oil as an organic alternative.

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