

## EXPERIMENTAL PAPER

# Biological activities of *Allium sativum* essential oil against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae)

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Summary

Essential oil from *Allium sativum* was isolated and investigated for its repellent, insecticidal, ovipositional and egg hatching inhibition activities against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *A. sativum* essential oil repelled bruchid adults at a very low concentration in choice oviposition assay. *A. sativum* essential oil caused both fumigant and contact toxicity in *C. chinensis* adults in a concentration dependent manner. Oviposition potency of *C. chinensis* adults was reduced significantly when sublethal concentrations of *A. sativum* essential oil were applied by fumigation and contact method. In chronic toxicity assay, *A. sativum* essential oil reduced F<sub>1</sub> progeny emergence, damage and weight loss in seeds. Findings of the present study suggest that *A. sativum* essential oil can be useful as promising agent in insect pest management programme.

Key words: Allium sativum, oviposition deterrence index, hatching inhibition rate, essential oil

## **INTRODUCTION**

With the beginning of agricultural practices, storage of food grains started as a safeguard against poor harvests and famine. Simultaneously, insect pests also started damaging stored grains, both quantitatively and qualitatively. At present, this damage accounts for 10–40% loss in countries depending on traditional storage technologies and has created a major problem in grain storage. In India, this damage approaches 10% of total production at farm level [1, 2]. Pulse beetle *Callosobruchus chinensis* (Order: Coleoptera, Family: Bruchidae) is one of the serious stored grain insect pests infesting gram, cowpea, beans, lentil and other pulses. Its grubs are the infective stages and cause 32-64% loss under storage conditions during April to October [3]. These make holes in grains and consume inner part leaving empty kernel. Damaged grains become unpalatable for human, incapable of producing sprout and thus, lose its market value.

In the pest management programmes, application of different synthetic chemicals as fumigants, sprays and dusts are in practice. Excessive and continuous uses of these chemicals have developed resistance in insects causing loss of several billion dollars each year [4-6]. In humans, more than twenty thousands accidental deaths and three million cases of pesticide poisoning are reported annually [7]. These chemicals increase chances of chromosomal aberrations [8], DNA damage [9], and formation of DNA adducts [10]. Besides, synthetic insecticides cause ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenic effects in non-target animals and cross- and multi-resistance in target insects [11-14]. These problems have diverted scientific interest towards use of plant products especially essential oils in storedgrain insect pest management programme. Essential oils produced as secondary metabolites in different plant parts have strong odour, volatility and lower density [15]. Due to volatility, essential oils are environmentally nonpersistent and 'generally recognized as safe' by United States Food and Drug Administration [16]. Essential oils are produced in different members of plant families like Alliaceae, Apiaceae, Asteraceae, Cupressaceae, Myrtaceae, Lamiaceae, Lauraceae, Piperaceae, Poaceae, Rutaceae and Zinziberaceae. The chemical constituents and biological activities of essential oils vary with plant parts used for extraction, extraction method, plant phenology, harvesting season, plant age, soil and environmental conditions of habitat [17,18]. The biological activities of essential oils depend on its major constituents.

Garlic, *Allium sativum* belonging to family *Alliaceae (Garlic)* is one of the most important ingredients of human food and Ayurvedic medicines since ancient time. Allicin, a key component of garlic reduces blood pressure by inhibiting angiotensin II and vasodilating effects [19]. Its various preparations have antidiabetic properties [20]. Its consumption protects human from cancer [21]. Garlic inhibits proliferation of atherosclerotic cells and other cell types as well as collagen synthesis and accumulation in the aorta [22]. Garlic preparations having allyl sulfides show antibacterial activity against both gram-negative and gram-positive bacteria like *Bacillus, Clostridium, Escherichia, Klebsiella, Proteus, Salmonella, Staphylococcus* and *Streptococcus,* and antifungal activities against *Candida albicans* [23, 24]. Diallyl sulfide and diallyl disulfide act as free radical scavangers by activating antioxidant enzymes like glutathione-s-transferase and catalase [25]. Alcoholic extract of garlic shows anthelmintic activity against *Ascaris lumbricoldes.* Garlic oil also protects intestine from injurious helminth parasites [26].

Garlic bulbs contain a number of active compounds, especially sulphur containing compounds which are responsible for the pharmacological activities. Steam distillation of garlic bulb produces essential oil containing diallyl, allyl methyl and dimethyl mono to hexa sulfide [27]. Sihem et al., have reported that A. sativum essential oil extracted by steam distillation method have allyl methyl trisulfide (34.61%) and diallyl disulfide (31.65%) as major components [28]. Other components of low percentage are allyl methyl disulfide, diallyl sulfide, diallyl trisulfide and diallyl tetrasulfide. Douiri et al. have reported that principal groups of components present in A. sativum essential oil are sulfur componunds represented mainly by trisulfides (57.4%) and disulfides (23.16) [28]. Douiri et al. have reported that A. sativum essential oil contains 1,3 dithiane, di-2-propenyl, 1-propene,3,3'-thiobis, methyl 2-propenyl, 3-vinyl-1,3-dithiin, 2-vinyl-1,3-dithiin, di-2-propenyl, 3-vinyl-1,2 dithiin1-chloro-4-(1ethoxy)-2-methylbut-2-ene, methyl 2-propenyl, diallyl disulfide, 3-vinyl-1,2 dithiin, methyl1-methyl-2-butenyl sulphide, octane 4-brom [29]. These components contribute to acaricidal [30], antibacterial [31], fungicidal [32], insecticidal [33], molluscicidal [34], nematicidal [35] and antiparasitic [36] properties of garlic. In the present study, essential oil of A. sativum bulbs has been evaluated for their repellent insecticidal, antiovipositional and egg hatching inhibitory activities against pulse beetle, C. chinensis.

## MATERIALS AND METHODS

## **Essential oil**

Dried *A. sativum* bulbs were purchased from the local market of Gorakhpur. Essential oil was isolated by crushing the bulb and hydrodistilation of crushed bulbs for 4 hours in Clevenger apparatus. Essential oil was kept in Eppendorff tubes at 4°C till further use.

## Insect

Pulse beetle, C. *chinensis* were used to investigate the insecticidal activities of *A. sativum* essential oil. The insects were reared on cowpea seeds in laboratory at  $30\pm2^{\circ}$ C,  $75\pm5^{\circ}$  RH in a photoperiod of 12:12 (L:D) h.

## **Repellency assay**

In a plastic box (10 cm in diameter and 13 cm in height), two transparent glass vials (3 cm in diameter and 10 cm in height) with screw cap interconnected horizontally by a plastic tube (2 cm long and 1.5 cm in diameter) 2 cm above the base were taken. One vial of the pair was supposed as treated and other as untreated. A filter paper

disc (2.5 cm in diameter) treated with 0.5 ml aliquot of essential oil solution (prepared by dissolving *A. sativum* essential oil in acetone) was pasted under the cover of vial (treated). In the untreated vial of the box, filter paper treated with acetone only was applied as in the treated. Solvent was allowed to evaporate from filter paper disc for 5 minutes before its use. Neck of vial was blocked by a piece of plastic mesh. In each vial, twenty cowpea seeds were taken and introduced 0-24 h old ten adults of mixed sex. After 96 h, the number of eggs laid on cowpea seeds was counted in treated and untreated vial. For each concentration, six replicates were set. Percent Repellency (PR) was measured by comparing number of eggs laid on cowpeas in treated vial against number of eggs laid on cowpea seeds in untreated using the formula:

$$PR = \frac{N_{UT} - N_T}{N_{UT} + N_T} \times 100$$

 $N_{\mu\tau}$  – number of eggs in untreated vial, and  $N_{\tau}$  – number of eggs in treated vial.

#### **Toxicity assays**

#### Fumigant toxicity assay

Fumigant toxicity of *A. sativum* essential oil was determined against 2–4 days old bruchid adults using glass vials (3 cm diameter and 10 cm in height) with screw cap. Test solutions of different concentrations were prepared by diluting *A. sativum* essential oil with acetone. For fumigation, filter paper strip (2.5 cm in diameter) impregnated with 100  $\mu$ l aliquot of test solution was pasted on inner side of cap and solvent was allowed to evaporate for 5 min. Neck of vial was blocked with a piece of plastic mesh to avoid contact effect of test solution. Twenty cowpea seeds were taken in each vial and ten adults were introduced into it. Open end of vial was closed by screw cap so that treated filter paper remained inside vial. All vials were kept in conditions maintained for insect culture. Mortality in adults was recorded after 24h, 48h, 72h and 96h of treatment. In control, filter paper impregnated with solvent only was used. Four different concentrations of essential oil were used and for each concentration of essential oil and control six replicates were set.

#### Contact toxicity assay

Contact toxicity of *A. sativum* essential oil was determined against 2-4 days old bruchid adults using glass vials (3 cm in diameter and 10 cm in height) with screw cap. Test solutions of different concentrations were prepared by diluting *A. sativum* essential oil with acetone. A 2 ml aliquot of test solution was applied on whole inner surface of vials and under surface of screw cap by rolling it. The treated vial was kept open for 5 min to evaporate solvent. Twenty cowpea seeds were

taken in each vial and ten adults were introduced into it. Vials were closed and kept in conditions maintained for insect culture. Mortality in adults was recorded after 24h, 48h, 72h and 96h of treatment. In control, filter paper impregnated with solvent only was used. Four different concentrations and for each concentration of essential oil and control six replicates were set.

## **Oviposition inhibition assay**

## By fumigation method

In this assay, ten 0–24 h old bruchid adults were fumigated with two sublethal concentration (40 and 80% of 96h- $LC_{50}$  determined in fumigation toxicity assay) of *A. sativum* essential oil solutions as was done in fumigation toxicity assay. After 96h of fumigation, number of eggs laid over the cowpea seeds was counted. For each concentration of essential oil as well as control group, six replicates were set. In control group only solvent was used.

## **Contact method**

In this assay, ten 0–24h old bruchid adults were treated with two sublethal concentrations (40 and 80% of 96h- $LC_{50}$  determined in contact toxicity assay) of *A. sativum* essential oil solutions as was done in contact toxicity assay. After 96h of treatment, number of eggs laid over the cowpea seeds was counted. For each concentration of essential oil as well as for control group, six replicates were set. In control group only solvent was used.

Percent Oviposition Deterrence Index (%ODI) was calculated as:

$$\%\text{ODI} = \frac{\text{C} - \text{T}}{\text{C} + \text{T}} \times 100$$

C – number of eggs in control, and T – number of eggs in test.

## **Ovicidal assay**

In ovicidal assay, 25 eggs were fumigated with test solutions prepared by diluting *A. sativum* essential oil with acetone. A 100  $\mu$ l aliquot of test solution was applied on filter paper strip (2.5 cm diameter) and solvent was allowed to evaporate for 5 min. The filter paper was pasted to undersurface of screw cap of vial (3 cm in diameter and 10 cm in height). Cap of vial was screwed and incubated for 96 h in conditions maintained for insect culture. After fumigation, eggs were allowed to hatch and number of eggs hatched was recorded after 14 days of treatment. Four different concentrations of essential oil were used and for each concentration and control six replicates were set. Percent Hatching Inhibition Rate (% HIR) was calculated as:

$$\% \text{HIR} = \frac{\text{C}_{\text{n}} - \text{T}_{\text{n}}}{\text{C}_{\text{n}}} \times 100$$

 $C_n$  – number of adults in control, and  $T_n$  – number of adults in test.

#### Chronic toxicity assay

In chronic toxicity assay, 100 gm of cowpea seeds was taken into a plastic box (7 cm diameter and 11 cm height), and mixed well with 2 ml aliquot of test solution prepared by diluting *A. sativum* essential oil in acetone. Twenty 0–24 h old bruchid adults were introduced into the box. Number of  $F_1$  progeny emerged was counted after 24 days of initiation of the experiment and removed. The counting and removal of  $F_1$  progeny emerged was continued for 5 days more. The potency of *A. sativum* essential oil was estimated as percent protection (PP) using formula:

$$PP = \frac{N_{UT} - N_T}{N_T} \times 100$$

 $N_{UT}$  – number of  $F_1$  progeny in untreated, and  $N_T$  – number of  $F_1$  progeny in treated.

After 90 days, weight loss in cowpea was estimated and represented as percent weight loss. The damaged and undamaged cowpea seeds were counted and represented as percent grain damage.

#### Data analysis

Median lethal concentration  $(LC_{50})$  was calculated by POLO programme [37]. Regression analysis and one-way analysis of variance (ANOVA) was performed to test the significancy of data [38].

#### RESULTS

#### Repellency assay

*A. sativum* essential oil inhibited oviposition in repellency assay in a concentration-dependent manner. Oviposition was reduced to 51.69, 40.35, 31.37 and 17.13% at 0.056, 0.085, 0.113 and 0.169  $\mu$ lcm<sup>-3</sup> concentration of *A. sativum* essential oil (fig. 1). This reduction in oviposition was estimated in terms of percent repellency (PR). PR was found 31.84, 42.5, 52.23 and 70.74 at 0.056, 0.085, 0.113 and 0.169  $\mu$ lcm<sup>-3</sup> concentration of *A. sativum* essential oil (Fig. 1).



#### Figure 1.

Effect of *A. sativum* essential oil at different concentration (0.056, 0.085, 0.113 and 0.169  $\mu$ l cm<sup>-3</sup>) on percent oviposition and percent repellency of *Callosobruchus chinensis* adults in repellency assay

## **Toxicity assay**

*A. sativum* essential oil caused both fumigant and contact toxicity in *C. chinensis* adults. In fumigation toxicity assay, median lethal concentrations  $(LC_{50})$  were determined 0.094, 0.077, 0.07 and 0.056  $\mu$ l cm<sup>-3</sup> air after 24, 48, 72 and 96 hours respectively (Table 1). In contact toxicity assay, median lethal concentrations  $(LC_{50})$  were determined 0.527, 0.443, 0.373 and 0.275  $\mu$ l cm<sup>-2</sup> after 24, 48, 72 and 96 hours respectively (tab. 1). Regression analysis showed concentration dependent correlation of *A. sativum* essential oil and mortality in bruchid adults (tab. 1).

Table 1.

Regression parameters of toxicity assay to study the effect of *A. sativum* essential oil against *C. chinensis* adults

Toxicity	Exposure period	LC <sub>50</sub>	Exposure period	Intercept	Slope	Regression equation	Regression coefficient
Fumigant	24 h	0.094 μl cm <sup>-3</sup>	24 h	2.88	8.07	Y = -2.88 + 8.07X	0.991
	48 h	0.077 μl cm <sup>-3</sup>	48 h	5.69	11.09	Y = -5.69 + 11.09X	0.977
	72 h	0.07 μl cm <sup>-3</sup>	72 h	4.86	10.84	Y = -4.86 + 10.84X	0.987
	96 h	0.056 μl cm <sup>-3</sup>	96 h	6.21	15.31	Y = -6.21 + 15.31X	0.976
Contact	24 h	0.527 μl cm <sup>-2</sup>	24 h	4.28	7.67	Y = -4.28 + 7.67X	0.990
	48 h	0.443 μl cm <sup>-2</sup>	48 h	4.20	10.4	Y = -4.20 + 10.4X	0.924
	72 h	0.373 μl cm <sup>-2</sup>	72 h	2.97	10.94	Y = -2.97 + 10.94X	0.995
	96 h	0.275 μl cm <sup>-2</sup>	96 h	5.40	12.05	Y = -5.40 + 12.05X	0.975

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## Oviposition inhibition assay

*A. sativum* essential oil significantly (p < 0.01) reduced oviposition potency of bruchid adults when exposed. In oviposition inhibition assay carried out by fumigation method, mean numbers of eggs laid per insect was 13.07 and 3.16 when bruchid adults were fumigated with 40 and 80% of 96h-LC<sub>50</sub> of *A. sativum* essential oil as compared to 19.12 eggs laid per insect in control (fig. 2). In another oviposition inhibition assay carried out by contact method, mean numbers of eggs laid per insect were 9.13 and 2.56 when bruchid adults were treated with 40 and 80% of 96h-LC<sub>50</sub> of *A. sativum* essential oil as compared to 19.12 eggs laid per insect to 19.12 eggs laid per insect in control (fig. 2). NODI was calculated 18.79 and 71.58 when adults were fumigated with 40 and 80% of 96h-LC<sub>50</sub> *A. sativum* essential oil (fig. 2). Similarly, %ODI was calculated for 35.34 and 76.33 when adults were treated with 40 and 80% of 96h-LC<sub>50</sub> of *A. sativum* essential oil respectively (fig. 2).



Fumigation method: F = 303.68 (df = 2.15)Contact method: F = 173.33 (df = 2.15)

#### Figure 2.

Effect on percent eggs laid per insect and oviposition deterrence index (%ODI) of *Callosobruchus chinensis* adults when treated with 40% and 80% of 96h-LC<sub>50</sub> of *A. sativum* essential oil for 96 hours by fumigation and contact method

## **Ovicidal assay**

*A. sativum* essential oil significantly (p < 0.01) reduced hatching rate in *C. chinensis* eggs when fumigated. Mean number of eggs hatched per 25 eggs was reduced to 20.33, 18.66, 16.16, 14.0 and 5.16 when fumigated with 0.141, 0.212, 0.282, 0.353 and 0.423  $\mu$ lcm<sup>3</sup> air of *A. sativum* essential oil respectively as compared to

22.33 eggs hatched in control (fig. 3). Increase in %HIR was 8.95, 16.45, 27.63, 37.30 and 76.89 when fumigated with 0.141, 0.212, 0.282, 0.353 and 0.423  $\mu$ lcm<sup>-3</sup> air of *A. sativum* essential oil respectively (fig. 3).



F = 173.33 (df = 4,25)

Figure 3.

Effect of different concentrations (0.141, 0.212, 0.282 and 0.423  $\mu$ l cm<sup>-3</sup>) of *A. sativum* essential oil on hatching rate and percent hatching inhibition rate (%HIR) when eggs of *Callosobruchus chinensis* 

## Chronic toxicity assay

A. sativum essential oil significantly (p<0.01) reduced grain damage, weight loss and F<sub>1</sub> progeny production during chronic exposure of *C. chinensis* adults in comparison to untreated group. When *C. chinensis* adults were exposed to *A. sativum* essential oil at a concentration of 0.5, 1.0, 1.5 and 2.0 µl gm<sup>-1</sup> of cowpea seeds in chronic toxicity assay, grain damage was reduced to 12.95%, 8.91%, 5.57% and 1.63% respectively in comparison to untreated where grain damage was reported to be 18.97% (fig. 4). In chronic toxicity assay, weight loss in treated cowpea seeds was recorded 6.46%, 4.5%, 1.93% and 0.62% when *C. chinensis* adults were exposed to *A. sativum* essential oil at concentrations of 0.5, 1.0, 1.5 and 2.0 µl gm<sup>-1</sup> (fig. 4). This grain damage and weight loss in cow pea seeds were due to the reduction in F<sub>1</sub> progeny. F<sub>1</sub> progeny was reduced to 58.63, 30.35, 10.29 and 2.31 at concentration of 0.5, 1.0, 1.5 and 2.0 µl gm<sup>-1</sup> (fig. 4). Percent protection was increased to 41.35%, 69.64%, 89.7% and 97.69% when *C. chinensis* adults were exposed to *A. sativum* essential oil at concentration of 0.5, 1.0, 1.5 and 2.0 µl gm<sup>-1</sup> (fig. 4).





F = 113.71 (%Grain damage), 164.86 (%Weight loss)F = 171.67 (%F1 progeny emergence), 120.34 (%Protection)

#### Figure 4.

Effect of different concentrations (0.5, 1.0, 1.5 and 2.0  $\mu$ l gm<sup>-1</sup>) of *A. sativum* essential oil on % grain damage, % weight loss and % protection in cowpea, and % F<sub>1</sub> progeny emergence during chronic exposure of *Callosobruchus chinensis* adults

## DISCUSSION

Use of natural products, especially essential oils and its components as pesticides is gaining importance in integrated pest management programme due to environmental problems and health hazard created by synthetic insecticides [39,40]. The volatile components of essential oils can be classified into four main groups: terpenes, benzene derivatives, hydrocarbons and other miscellaneous compounds [41]. Terpenes and terpenoids are the most representative molecules constituting 90% of essential oils allowing the diversity in functions [15]. Many of the volatile components of various chemical groups have been also evaluated for their role in insect pest management programme. Anethole, thymol, 1,8-cineole, carvacrol, terpineol and linalool have been evaluated as fumigants against *Tribolium castaneum*, however, only anethole showed significant effect against this insect species [42].

Earlier attempts to explore the toxicity of essential oils against *C. chinensis* have been made and proved that essential oils affect insects by antifeedant, repellent, oviposition inhibitory, ovicidal and progeny production inhibitory activities by disrupting metabolic pathways [43, 47]. In the present study, *A. sativum* essential oil significantly repelled the bruchid adults at a very low concentration as the oviposition capacity decreased in choice oviposition assay. This volatile oil caused

fumigant and contact toxicity in bruchid adults in a concentration-dependent manner. *A. sativum* essential oil reduced egg laying capacity in *C. chinensis* adults in oviposition inhibition assay performed either by fumigation or contact method. *A. sativum* essential oil reduced hatching rate in *C. chinensis* eggs when fumigated. In fumigant toxicity assay, *Anethum sowa* and *Artemissia annua* essential oils have been reported to show ovicidal and oviposition-deterrence in *C. maculatus* [48]. Elhag (2000) have shown oviposition inhibition activity of several essential oils against *C. maculatus* [49]. The exposure of the cowpea seeds to the vapour of tridecanone is very effective to control their infestation by *C. maculatus* since adult emergence was reduced as compared to untreated seeds [50]. The number of eggs laid and fecundity was reduced when *C. maculates* were on seeds of chickpea has been reduced when fumigated with garlic essential oils [29].

In chronic toxicity assay, numbers of F, progenies were reduced. In general, higher the concentration of essential oil, the higher the reduction in adult emergence. The reduction in adult emergence could either be due to the reduction in egg hatching rate or death of larva. Jilani et al. have reported that larvae hatched must penetrate seeds to ensure survival [51]. However, larvae are unable to do so unless eggs are firmly attached to the seeds. Amount of seed damage and grain weight losses caused by C. chinensis was reduced in chronic toxicity assay when exposed to A. sativum essential oil as compared to untreated group. This grain damage and weight loss in cow pea seeds was due to the reduction in F, progenv. Similarly, tridecanone exhibits fumigant toxicity and its efficacy in protecting the cowpea seeds against C. maculatus which is mainly due to its ovicidal activity [52]. Since adult emergence is based on the proportion of hatched eggs that develop into adults inside the seeds, the results suggest that A. sativum essential oil vapours cross the seed coat and therefore, interfere with the larvae development [50]. Mode of action of essential oil constituents has not been known yet, although, it may be due to the suffocation and inhibition of various biosynthetic processes of insect [52]. Toxicity of menthol, methonene, limonene,  $\alpha$ -pipene,  $\beta$ -pipene and linalool against S. oryzae is proved to its effect on acetylcholinesterase enzyme activity [53]. Findings of the present study indicate that A. sativum essential oil can be a promising tool in insect pest management. However, before its application, it must be kept in mind that essential oil should be toxic to target insects and but not toxic to non-target organisms such as other beneficial insects and other animals such as fish, birds and humans. There are several other factors that must be considered during the evaluation of insecticides like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity to be used effective for control of stored-product insect populations.

## CONCLUSION

Use of essential oils as an alternative in insect pest management programmes is a sustainable alternative as they can be obtained from nature. Essential oils

cause contact toxicity, fumigant toxicity, repellent, oviposition inhibitory and developmental inhibitory activities and act on various levels in the insects, so that possibility of generating resistance is low. Thus, essential oils can be considered as a natural alternative in the control of stored grains insects.

#### REFERENCES

- 1. Lal S. Saving grain after harvest. In: The Hindu Survey of Indian Agriculture. National Press. Madras, India, 1988:246-248
- Shaaya E, Kostjukovski M, Eilberg J, Sukprakarn C. Plant oils as fumigants and contact insecticides for the control of stored-product insects. J Stored Prod Res 1997; 33:7-15
- 3. Weaver DK, Phillips TW, Dunkel FV, Weaver T, Grubb RT, Nance EL. Dried leaves from the rocky mountain plants decrease infestation by stored product beetles. J Chem Ecol 1995; 21:127-142
- 4. Elzen GW, Hardee DD. United State Department of Agricultural-Agricultural Research on managing insect resistance to insecticides. Pest Manag Sci 2003; 59:770-776
- 5. Benhalima H, Chaudhry MQ, Mills KA, Price NR. Phosphine resistance in stored-products insects collected from various grain storage facilities in Morocco. J Stored Prod Res 2004; 40:241-249
- 6. Islam MS, Talukdar FA. Toxic and residual effects of *Azadirachta indica*, *Tagetes erecta* and *Cynodon dactylon* extracts against *Tribolium castaneum*. J Plant Disease Protect 2005; 112:594-601
- 7. Pan Africa, 2008.
- Muniz JF, Mc Cauley L, Scherer J, Lasarev M, Koshy M, Kow YW, Nazar-Stewart V, Kisby GE. Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. Toxicol Appl Pharmacol 2008; 227(1):97-107
- 9. Simoniello MF, Kleinsorge EC, Scagnetti JA, Grigolato RA, Poletta GL, Carballo MA. DNA damage in workers occupationally exposed to pesticide mixtures. J Appl Toxicol 2008; 28(8):957-65
- Le Goff J, Andre V, Lebailly P, Pottier D, Perin F, Perin O, Gauduchon P. Seasonal variations of DNA-adduct patterns in open field farmers handling pesticides. Mutat Res 2005; 587(1-2):90-102
- 11. WMO. Scientific assessment of ozone depletion: World Meteorological Organization Report No. 25, World Meteorological Organization of the United Nations, Geneva, 1991
- 12. Lu FC. A review of the acceptable daily intakes of pesticides assessed by the World Health Organization. Reg Toxicol Pharmacol 1995; 21:351-364
- 13. UNEP. The Montreal Protocol on substances that deplete the ozone layer. Nairobi (Kenya): United Nations Environment Programme, 2000
- 14. Beckel H, Lorini I, Lazzari SMN. Resistência de Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) a inseticidas piretróides e organofosforados usados em trigo armazenado. In: Resumos e Atas do III Seminário Técnico do Trigo/XVII Reunião da Comissão Centro-sul Brasileira de Pesquisa de Trigo 2002; 44
- Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food Chem Toxicol 2008; 46: 446-475
- Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya P.R. A review on prospects of essential oils as biopesticides in insect pest management. J Pharmacog Phytother 2009; 1:52-63
- Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. Chemical composition, seasonal variability and antifungal activity of *Lavandula stoechas* L. spp. *stoechas* essential oils from stem/ leaves and flowers. J Agric Food Chem 2006; 54: 64-70
- Isman MB, Machial C, Miresmailli S, Bainard L. Essential oil based pesticides: new insights from old chemistry. In: Ohkawa H, Miyagawa H, Lee P Eds. Pesticide Chemistry. Wiley-VCH, Weinheim, Germany, 2007; 201-209
- 19. Ried K, Frank OR, Stocks NP, Fakler P, Sullivan T. Effect of garlic on blood pressure: A systematic review and meta-analysis. BMC Card Dis 2008; 1-12
- 20. Khayatnouri M, Bahari K, Safarmashaei S. Study of the effect of gliclazide and garlic extract on blood sugar level in stz–induced diabetic male mice. Adv Environ Biol 2011; 5(7):1751-1755

- 21. Islam MS, Kusumoto Y, Al-Mamun MA. Cytotoxicity and cancer (HeLa) cell killing efficacy of aqueous garlic (*Allium sativum*) extract. J Sci Res 2011; 3(2):375-382
- 22. Ashraf MZ, Hussain ME, Fahim M. Antiatherosclerotic effects of dietary supplementations of garlic and turmeric: Restoration of endothelial function in rats. Life Sci 2005; 77:837–857
- Benkeblia N et al. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Food Science Tech 2004; 37:263–268
- 24. Motsei ML, Lindsey KL, Staden JV, Jager AK. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. J Ethnopharma 2003; 86:235–241
- 25. Tsai TH, Tsai PJ, Ho SC. Antioxidant and anti-inflammatory activities of several commonly used spices. J Food Sci 2005; 70(1):C93-C97
- Worku M, Franco R, Baldwin K. Efficacy of garlic as an anthelmintic in adult Boer goats. Arch Biol Sci Belgrade 2009; 61(1):135-140
- Lawson LD. Bioactive organosulphur compound of garlic and garlic products role in reducing blood lipids. ACS Symposium Series 534. Human medicinal agents from plants (Eds. Kinghorn, A.D., Balandrin M.F.) American Chemical Society 1993; Washington D.C. 3-6-330
- 28. Khadri S, Boutefnouchet N, Dekhil M. Evaluation of the antibacterial activity of essential oil of *Allium* sativum of eastern Algeria compared to different strains of *Pseudomonas aeruginosa. St. Cerc. St.* CICBIA 2010; 11(4):421-428.
- 29. Douiri LF, Boughdad A., Assobhei O., Moumni M. Chemical composition and biological activity of *Allium* sativum essential oils against *Callosobruchus maculatus*. J Env Sci Toxicol Food Tech 2013; 3(1):30-36
- El-Zemity S, Hussien R, Saher F, Ahmed Z. Acaricidal activities of some essential oils and their monoterpenoidal constituents against house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J Zhejiang Univ Sci B. 2006; 7(12):957–962
- 31. Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl Environ Microbiol 2001; 67(1)475-480
- 32. Ledezma E, Apitz-Castro R. Ajoene the main active compound of garlic (*Allium sativum*): a new antifungal agent. Rev. Iberoam. Micol. 2006. 23(2):75-80
- 33. Hasan M, Sagheer M, Saleem S, Hanif S, Akhter S, Hanif CMS. Evaluation of insecticidal potential of powders of *Azadirachta indica*, *Momordica charentia* and *Allium sativum* against *Callosobruchus chinensis* (Coleoptera: Bruchidae). Pak Entomol 2012; 34(2):71-73
- 34. Singh DK, Singh A. Allium sativum (Garlic), A Potent New Molluscicide. Biol Agri Hort 1993; 9(2):121-124
- 35. Abbas S, Dawar S, Tariq M, Zaki MJ. Nematicidal activities of spices against *Meloidogyne javanica* (Treub) Chitwood. Pak J. Bot 2009; 41(5):2625-2632
- 36. Ayaz E, Türel I, Gül A, Yilmaz O. Evaluation of the anthelmentic activity of garlic (*Allium sativum*) in mice naturally infected with *Aspiculuris tetraptera*. Recent Pat Antiinfect Drug Discov 2008; 3(2):149-52
- Russel RM, Robertson JL, Savin SA. POLO: A new computer programme for probit analysis. Bull Entomol Res 1977; 23:209-213
- Sokal RR, Rohlf F.J. Introduction to biostatistics. W.H. Freeman and Co, San Francisco, CA, USA, 1973:185-207
- 39. Agrawal KK, Tripathi AK, Prajapati V, Kumar S. Toxicity of 1,8- Cineole against three species of stored product coleopterans. Insect Sci Appl 2001; 21:155-160
- 40. Zapata N, Smagghe G. Repellency and toxicity of essential oils from the leaves and bark of *Laurelia sempervirens* and *Drimys winteri* against *Tribolium castaneum*. Indust Crop Prot 2010; 32:405-410
- Ngoh SP, Choo LEW, Pang GFY, Huang Y, Kini MR, Ho SH. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach *Periplanata americana* (L.). Pest Sci 1998; 54:261-268
- 42. Koul O, Singh G, Singh R, Singh J. Mortality and reproductive performance of *Tribolium castaneum* exposed to Anethol vapours at high temperature. Biopes Int 2007; 3:126-137
- 43. Chaubey MK. Fumigant toxicity of essential oils against rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). J Biol Sci 2011; 11:411-416
- 44. Islam R, Khan RI, Al-Reza SM, Jeong YT, Song CH, Khalequzzaman M. Chemical composition and insecticidal properties of *Cinnamomum aromaticum* (Nees) essential oil against the stored-product beetle *Callosobruchus maculatus* (F.). J Sci Food Agri 2009; 89:1241-1246

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- 45. Krishnappa K, Laksmanan S, Elumalai K, Jayakumar S. Insecticidal action of certain essential oils against *Callosobruchus maculatus* (Coleoptera: Bruchidae). Int J Cur Agri Sci 2011; 1:10-14
- 46. Jiang CH, Liu QZ, Du SS, Deng ZW, Liu ZL. Essential oil composition and insecticidal activity of *Evodia lepta* (Spreng) Merr. Root barks from China against two grain storage insects. J. Med. Plants Res. 2012; 6:3464-3469
- Chaubey MK. Biological activities of *Zingiber officinale* (Zingiberaceae) and *Piper cubeba* (Piperaceae) essential oils against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Pak J Biol Sci 2013; 16(11):517-523
- Tripathi AK, Prajapati V, Khanuja SPS, Kumar S. Toxicity, feeding deterrence and effect of activity of 1,8-cineole from *Artemisia annua* on progeny production of *Tribolium castaneum* (Coleoptera: Tenebrionidae). J Econ Entomol 2001; 94:979-983
- 49. Elhag EA. Deterrent effects of some botanical products on oviposition of the cowpea bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Int J Pest Manag 2000; 46:109-113
- Braga YFB, Graneiro TB, Freire EA, Lopes HL, Bezerra JNS, Mano. Insecticidal activity of 2-tridecanone against the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). Anais da Academia Brasileira de Ciências 2007; 79(1):35-39
- 51. Jilani G, Saxena RC. Evaluation of neem oil, combination of neem oil and fumigation, and Actellic as paddy/ rice protectants against storage insects. Proc. Final Workshop on Botanical Pest Control in Ricebased Cropping Systems. Int Rice Res Inst Manila, 1988; pp.28
- 52. Don-Perdo KM. Investigation of single and joint fumigant insecticidal action of citrus peel oil components. Pest Sci 1996; 46:79-84
- 53. Lee BH, Choi WS, Lee SE, Park BS. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Prot 2001; 20:317-320.

## AKTYWNOŚĆ BIOLOGICZNA OLEJKU ETERYCZNEGO Z ALLIUM SATIVUM PRZECIWKO CALLOSOBRUCHUS CHINENSIS (COLEOPTERA: BRUCHIDAE)

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#### Streszczenie

Wyizolowano olejek eteryczny z *Allium sativum* i badano go na działanie odstraszające, owadobójcze, hamujące składanie jaj i wylęganie jaj przez *Callosobruchus chinensis* (Coleoptera: Bruchidae). Olejek eteryczny z *A. sativum* odstraszał dorosłe chrząszcze w bardzo niskich stężeniach w badaniu wyboru miejsca złożenia jaj. Zależnie od stężenia, olejek eteryczny z *A. sativum* powodował toksyczność wziewną i kontaktową w stosunku do dorosłych osobników *C. chinensis*. Potencjał składania jaj dorosłych osobników *C. chinensis* został istotnie zredukowany przy podaniu subletalnych dawek olejku eterycznego metodą wziewną i kontaktową. W badaniu przewlekłej toksyczności olejek eteryczny *A. sativum* zredukował rozród  $F_1$ , a także powodował zniszczenie i obniżenie masy nasion. Wyniki przedstawionego badania sugerują, że olejek eteryczny z *A. sativum* może być przydatny jako obiecujący środek w przeciwdziałaniu szkodnikom.

**Słowa kluczowe:** *A. sativum, wskaźnik zahamowania składania jaj, hamowanie wylęgania, olejek eteryczny* 

