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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_1 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 stored-grain 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-(1-ethoxy)-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±2°C, 75±5% 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_{UT} – number of eggs in untreated vial, and N_T – 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 μl 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:

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

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 μl 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:

$$\%HIR = \frac{C_n - T_n}{C_n} \times 100$$

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 μlcm^{-3} 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 μlcm^{-3} concentration of *A. sativum* essential oil respectively (fig. 1).

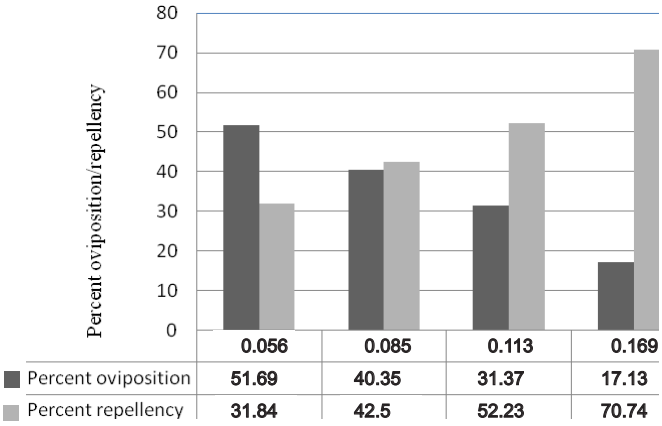


Figure 1.

Effect of *A. sativum* essential oil at different concentration (0.056, 0.085, 0.113 and 0.169 μl cm^{-3}) 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 μl cm^{-3} 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 μl cm^{-2} 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).

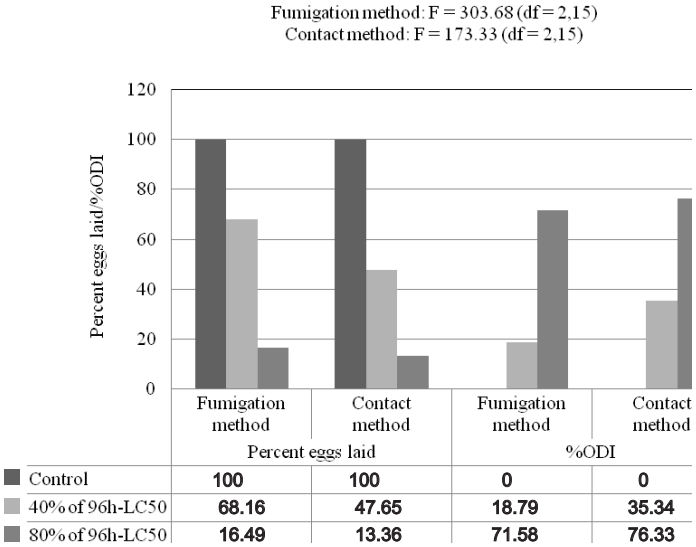
Table 1.

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

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

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_{50} 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_{50} of *A. sativum* essential oil as compared to 19.12 eggs laid per insect in control (fig. 2). %ODI was calculated 18.79 and 71.58 when adults were fumigated with 40 and 80% of 96h-LC_{50} *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_{50} of *A. sativum* essential oil respectively (fig. 2).



	Percent eggs laid		%ODI	
Control	100	100	0	0
40% of 96h-LC50	68.16	47.65	18.79	35.34
80% of 96h-LC50	16.49	13.36	71.58	76.33

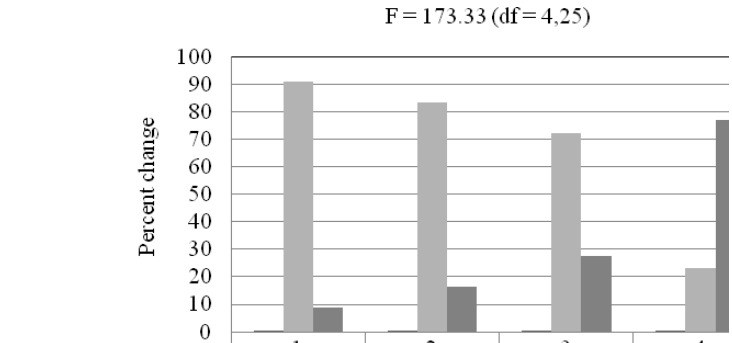
Figure 2.

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_{50} 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 μlcm^{-3} 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 μlcm^{-3} air of *A. sativum* essential oil respectively (fig. 3).



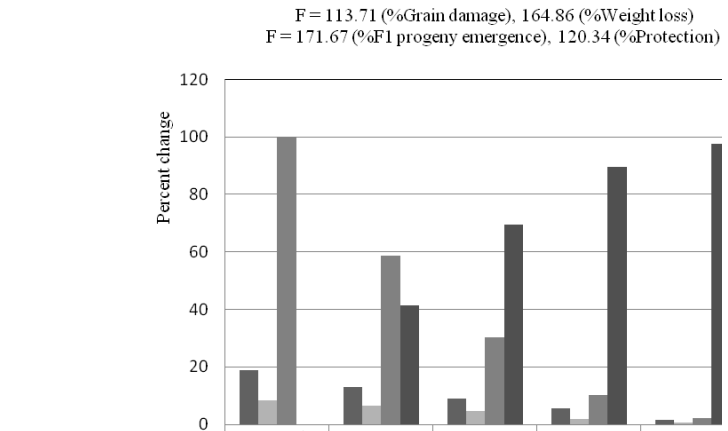
	1	2	3	4
Concentration	0.141	0.212	0.282	0.423
Percent eggs hatched	91.04	83.56	72.37	23.11
%HIR	8.95	16.45	27.63	76.89

Figure 3.

Effect of different concentrations (0.141, 0.212, 0.282 and 0.423 μl cm^{-3}) 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_1 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^{-1} 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^{-1} (fig. 4). This grain damage and weight loss in cow pea seeds were due to the reduction in F_1 progeny. F_1 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^{-1} (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^{-1} (fig. 4).



	Control	0.5	1	1.5	2
% Grain damage	18.97	12.95	8.91	5.57	1.63
% Weight loss	8.25	6.46	4.5	1.93	0.62
% F1 progeny emergence	100	58.63	30.35	10.29	2.31
% Protection	0	41.35	69.64	89.7	97.69

Figure 4.

Effect of different concentrations (0.5, 1.0, 1.5 and 2.0 μl gm^{-1}) of *A. sativum* essential oil on % grain damage, % weight loss and % protection in cowpea, and % F_1 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_1 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_1 progeny. 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, α-pipene, β-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.

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

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S t r e s z c z e n i e

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*