Evaluation of Aqueous and ethanol extracts of *Alepidea amatymbica* for bio-active constituents and insecticidal activity against *Tenebrio molitor*

**Abstract**

*Alepidea amatymbica* (Apiaceae) is a good source of bioactive compounds with folkloric use for the management of different ailments by the indigenous people of African descent. Despite the acclaimed traditional use of the plant, there is a dearth of scientific information on its use as a botanical insecticide. This study evaluated the impacts of aqueous and ethanol solvents on bio-active constituents yield of *Alepidea amatymbica* (*A. amatymbica*) and the insecticidal activities of the plant against *Tenebrio molitor* (*T. molitor*). The laboratory study was laid out in a completely randomized design of five treatments and a control. The treatment included; 1%, 0.75%, 0.50%, 0.25% and 0.125% concentrations of both aqueous and ethanol extracts of the plant. Larvae of *T. molitor* were subjected to the different concentration levels for mortality test. Data on the yield of bioactive compounds and insect mortality were subjected to analysis of variance, and mean differences were separated using Tukey’s Honestly Significant Test. Ethanol extraction of bioactive compounds gave significantly higher yield (14.48%) compared to the aqueous extraction (8.12%). Mortality of *T. molitor* was dependent upon the concentration levels of the aqueous and ethanol extracts of *A. amatymbica*. The larvae of *T.molitor* were significantly more susceptible to lower concentrations of ethanol extract than aqueous extract. However, both the aqueous and ethanol extracts of *A. amatymbica* proved to be toxic to the insect at relatively low concentrations between 0.125 and 0.50%. This study suggests that *A. amatymbica* could be explored for its insecticidal activity in the control of stored pests and other insect pests.

**INTRODUCTION**

*Tenebrio molitor* L. (Coleoptera: Tenebrionidae), also known as yellow mealworm, is a cosmopolitan secondary pest and scavenger with a high reproductive capacity [1]. The larvae and adult of *T. molitor* feed voraciously on several classes of stored grains, milled cereals, animal feeds, meat scraps, dead insects, flour, tobacco and other crops [2]. The infestation of stored grains by *T. molitor* contaminates grains with fragments of the body, faecal matter, and indirectly by the development of saprophytic microorganisms, causing loss of food quality [3]. A loss of about 15% in grain and flour products throughout the world has been attributed to *T. molitor* [3], [4]. Moreover, *T. molitor* has been documented as a laboratory test organism for studying microbial infections [5], [6]. Several groups of insecticides, including synthetic pesticides, fungal substances and plant-derived substances are used against insect pests in food storage [7]. With the ban of most synthetic pesticides (Plata-Rhueda et al., 2017; Spochacz et al., 2018) [4], [7] in the management of agricultural produce pests, attention has shifted to the use of eco-friendly approaches. Use of botanical pesticides is one such eco-friendly approach with potency often based on feeding or contact toxicity. Many plants have been documented with insecticidal properties against beetles infesting grains [8] tested the essential oils from *Tanacetun vulgare* and *Thuja occidentalis* on the lesser mealworm, *Alphutobius diaperinus* (Tenebrionidae). These oils inhibited insect growth and increased their mortality. The insecticidal activity of essential oils from *Artemisia dracunculus* L. and *Origanum vulgare* L. spp. *hirtum* have been shown to improve sanitary conditions and control the lesser mealworm inhabiting poultry houses [9]. Longe and Oso (2017) [10] tested the ash from bulbs of garlic (*Allium sativum* L) and onion (*Allium cepa* L) for fumigant action against adult emergence of *Callosobruchus maculatus*; the ash from garlic bulb was more effective in the control of *C. maculatus* compared with the ash from the onion bulb. It is noteworthy that few studies presented on the management of *T. molitor* have been centred on plants’ essential oils with toxicological properties. The working hypothesis of this study was that extracts from *A. amatymbica* have insecticidal activity against *T. molitor*. Hence, *A. amatymbica* could be a promising alternative for the control of insect pests affecting essential grains.

**MATERIALS AND METHODS**

1. *Plant Collection*

Fresh corms of *A. amatymbica* (Larger tinsel plant) were collected at Makeneng around Qwaqwa, eastern Free State province, South Africa. The identity of the plant was confirmed and authenticated by Prof. A.O.T. Ashafa of the Department of Plant Sciences, University of the Free State, Qwaqwa campus. Voucher specimen, OsoMed/01/2019/QwHB, was prepared and deposited in the department’s Herbarium. The fresh corms were washed under running tap water, air-dried and ground using an electric blender (Nanning Mainline Food Machinery Company Ltd, China). The ground material was homogenized into a fine powder and stored in air-tight bottles.

1. *Aqueous and ethanol extraction of A. amatymbica*

The *A. amatymbica* extract was prepared according to the protocol of Handa et al. (2008) [11] with little modifications. Ten grams of the powdered plant material was immersed in aqueous and ethanol solvent separately at the solvent ratio of 1:8 (w/v) and placed on a rotary shaker for 24 hours at a speed of 100rpm. After that, it was filtered using Whatman No.: 1 filter paper (https://www.sigmaaldrich.com). The filtrate from ethanol was concentrated at 40oC using rotary evaporator while that of aqueous was evaporated in a water bath at 45oC for two days. All experiments were repeated three times to prepare different batches for the bioassay; the extracts were weighed and kept in the refrigerator at 4oC until when needed for efficacy studies.

1. *Determination of extraction yield*

The extraction yield was calculated as follows [12]:

Extraction yield (%) = weight of extract & container – weight of the container x 100

weight of the initial dried sample

1. Source and rearing of *T. molotor*

*T. molitor* was reared in a colony at Agricultural Research Centre Small Grain, Bethlehem, Free State province, South Africa. They were reared in glass clear plastic containers with perforated lids to allow for air circulation. The containers covered were filled with bran to about 2-3 inches deep, and two to three potato halves were added per container as a source of moisture for the larvae. The last instars were collected and used in the trials.

1. *Mortality against T. molitor*

The efficacy of the extracts was determined by calculating the lethal concentration (LC50) values under laboratory conditions. Each of the extracts was reconstituted in their original solvent on weight/ volume basis (g/ml). Five descending concentrations of aqueous and ethanol extracts besides the control were diluted based on volume/volume (1.0, 0.75, 0.50, 0.25, 0.13%) aliquots taken from the stock solution and mixed with water in 2.5 ml glass vials to make a total volume of 1 ml. Control treatments were treated with sterile distilled water only. Different concentrations of the extracts solution (1uL) were applied on the thorax of the larvae of *T. molitor* using a micropipette. Ten larvae were treated per concentration, and they were placed in 90 mm Petri dishes lined with filter paper disc and maintained in the dark at 250C. The number of dead larvae in each dish was counted at 24, 48 and 72 h after exposure to the extract.

1. *Statistical Analyses*

Lethal concentrations (LC50) and their confidence limits for *A. amatymbica* were determined by logarithm equation in dose-dependent treatments based on concentration probit mortality using ExcelSTAT- Program for Windows. Chi-square Statistic analysis was used for pairwise comparisons regarding lethal time effects in *T. molitor*.All data were subjected to analysis of variances (ANOVA) and mean values were compared using Tukey’s Honestly Significant test (HSD) at P<0.05.

**RESULTS**

*A. Effect of aqueous and ethanol solvents on theextraction yield*

Aqueous and ethanol solvents were examined for their impact on the extraction yield of *A. amatymbica*. Significant differences were recorded in the extraction yield between the two solvents. The mean highest yield (14.48%) was recorded in ethanol solvent while the aqueous solvent had the mean yield of 8.12% (Fig. 1).

Figure 1. The effect of Aqueous and ethanol solvents on extraction yield of *Alepidea amatymbica*. Vertical bars represent standard error of the mean (n=3). Different lower-case characters represent significant difference at p<0.05 by Duncan’s multiple range tests.

*B. Impacts of A. amatymbica on T. molitor*

Different rates of mortality for *T. molitor* were obtained at the different concentration levels of the aqueous and ethanol extracts of *A. amatymbica*. Lethal concentration level at 50% mortality of the *T. molitor* was estimated using treadline equation (Figure 2). The LC50 values for aqueous extract indicated that half of the sampled population of *T. molitor* would be killed at 0.50% level of concentration (X2 = 3.6, P<0.05) at 24, 48 and 72 hours after exposure. For ethanol extract, it was observed that half of the sampled population of *T. molitor* would be killed between 0.125 and 0.50% levels of concentrations (X2 = 1.6 and 3.6, P<0.05) at 24, 48 and 72 hours after exposure (Table 1). Control mortalities were zero.

Figure 2: Lethal concentrations of aqueous and ethanol solvents based on logarithm scale of P<0.05.

Table 2: Lethal concentrations of aqueous and ethanol extracts of *Alepida amatymbica* against *Tenebrio molitor* at 24, 48 and 72 hours of exposure.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Aqueous** | | | | **Ethanol** | | |
| **Exposure** | LC50 | IC | X2 | LC50 | IC | X2 |
| **24 HR** | 0.41 | 3.52-0.48 | 3.6 | 0.21 | 2.88-1.12 | 3.6 |
| **48 HR** | 0.30 | 5.48-1.32 | 3.6 | 0.26 | 5.28-1.52 | 3.6 |
| **72 HR** | 0.37 | 8.57-1.03 | 3.6 | 0.19 | 5.42-2.18 | 1.6 |

LC5 – lethal concentration causing 50% mortality (estimated value)

IC – Confidential interval

X2  - Chi-squared value for lethal concentrations based on a log scale with significance level at P<0.05.

**DISCUSSION**

The use of bioactive compounds from natural plant products in agriculture for the management of insect pests has been increasingly attracting considerable attention [13], [14]. In this study, *A. amatymbica* was used as a natural source of secondary metabolites compounds for insecticidal activity against *T. molitor*. Among several steps taken to obtain bioactive compounds from plants, extraction using different solvents is an important step which helps to recover and isolate bioactive compounds from the plant materials [15]. The present study used distilled water and ethanol solvents to extract bioactive compounds from *A. amatymbica.* The results showed that significantly higher yield was recorded in ethanol when compared to distilled water. This result is inconsistent with other documented reports that the higher the polarity of a solvent, the greater its extraction efficiency [16], [7]. The higher yield observed in ethanol solvent could be registed because of the unique chemical constituents in *A. amatymbica*. Okwute (2010) [18] earlier noted that certain plants contain active compounds that are lipophilic and are therefore more readily extracted into an organic solvent than an aqueous solvent.

Documented reports have proven the potency of plant-derived compounds against pests of stored grains. The essential garlic oil and their compounds were reported for their lethal and sub-lethal effects on *T. molitor* [4]. Bett et al., (2016) [19] reported on the toxicity of essential oils containing from *Cupressus lusitanica* and *Eucalyptus saligna* on adults *Tribolium castaneum, Acanthoscelides obtectus,* and *Sitophilus zeamais* (Curculionidae). Similarly, Taban et al. (2017) [20] administered essential oils from *Satureja rechingeri, Satureja bachtiarica,* and *Satureja khuzestanica* directly on the cuticle of the red flour beetle, *T. castaneum*. They reported that the oils exhibited rapid insecticidal action against the beetle.

Furthermore, the insecticidal potential of *A. amatymbica* have been implicated in the control of aphids and cutworms of cabbage [21] and stalk borers of maize [22] among the smallholding farming communities in the Eastern Cape of South Africa. Comparing contact toxicity of aqueous and ethanol extracts of *A. amatymbica* on *T.molitor*, the larva was significantly more susceptible to lower concentrations of ethanol extract than aqueous extract, showing that . The LC50 values of ethanol extract (0.21, 0.26, 0.19%) at different time exposures (24, 48, 72 hrs.) indicate that concentrations as low as 0.125% of *A. amatymbica* are toxic to *T. molitor*. For aqueous extract, the LC50 values (0.41, 0.30, and 0.37%) showed that *A. amatymbica* could only be toxic to *T. molitor* at concentrations above 0.25%. Our result also corroborated the report of Parekh et al., (2005) [23] who found that plant extracts in organic solvents provided more consistent antimicrobial activity compared to those extracted in aqueous solvents. These observations are justified by the polarity of compounds obtained by each of the solvent and their inherent bioactivity.

**CONCLUSION**

*A. amatymbica* has been proven for its potency against *T. molitor*. Hence, to achieve pest control with minimal negative impacts on the environment and reduced resistance of insects to insecticides, the plant could be explored in the control of stored pests attacking grains.

**REFERENCES**

[1] Morales-Ramos, J. A., Rojas, M. G., Shapiro-Ilan, D. I., Tedders, W. L. (2011). Nutrient regulation in *Tenebrio molitor* (Coleoptera: Tenebrionidae): self-selection of two diet components by larvae and impact on fitness. *Environmental Entomology*, 40: 1285- 1294.

[2] Nur Hasyimah, R., Nor Atikah, A. R., Halim, M., Muhaimin, A. M. D., Nizam, M. S., Hanafiah, M. M., and Yaakop, S. (2018). CO2 effects on larval development and genetics of mealworm beetle, tenebrio molitor l. (coleoptera: tenebrionidae) in two different CO2 systems. *Applied Ecology and Environmental Research,* 16(2):1749-1766.

[3] Martynov, V. O., Hladkyi, O. Y., Kolombar, T. M., & Brygadyrenko, V. V. (2019). Impact of essential oil from plants on migrato-ry activity of Sitophilus granarius and Tenebrio molitor. *Regulatory Mechanisms in Biosystems*, 10(4), 359–371. doi:10.15421/021955.

[4]Plata-Rhueda, A., Martínez, L.C., Dos Santos, M.H., Fernandes, F.L., Wilcken, C.F., Soares, M.A., Serrăo, J.E., and Zanuncio, J.C. **(**2017**)**. Insecticidal activity of garlic essential oils and their constituents against the mealworms beetle, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). *Sci. Rep.* 7, 46406; doi:10:1038/srep46406 (2017).

[5] Tindwa, H., Patnaik, B.B., Kim, D.H., Mun, S., Jo, Y.H., Lee, B.L., Lee, Y.S., Kim, N.J., and Han, Y.S. (2013). Cloning characterization and effect of TmPGRP-LE gene silencing on survival of *Tenebrio molitor* against *Listeria monocytogenes* infection. *Int. J. Mol.Sci.*, 14:22462-22482.

[6] De Souza, P.C., Morey, A.T., Castanheira, G.M., Bocate, K.P., Panagio, L.A., Augustolto, F., Furlaneto, M.C., Yamada-Ogatta, S.F., Costa, I.N., Mora-Montes, H.M., and Almeida, R.S. (2015). *Tenebrio molitor* (Coleoptera: Tenebrionidae) as an alternative host to study fungal infections. *Journal of Microbiology Methods*, 118:182-186.

[7] Spochaz, M., Chowanski, S., Walkowiak-Nowicka, Szymczak, M., and Adamski, Z. (2018). Plant-derived substances used against beetles- Pests of stored crops and food- and their mode of action: A review. *Comprehensive Reviews in Food Science and Food Safety*, 17(5): 1339-1366.

[8] Szołyga, B., Gniłka, R., Szczepanik, M., and Szumny, A. (2014). Chemical composition and insecticidal activity of *Thuja occidentalis* and *Tanacetum vulgare* essential oils against larvae of the lesser mealworm, *Alphitobius diaperinus*. *Entomologia Experimentalis et Applicata*, 151(1):1–10.

[9] Szczepanik, M., Walczak, M., Zawitowska, B., Michalska-Sionkowska, M., Szumny, A., Wawrzé, C. and Brzezinska, M.S. (2017). Chemical composition, antimicrobial activity against lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) of *Origanum vulgare* L. ssp. Hirtum (Link) and *Artemisia dracunculus* L. essential oils. *Journal of the Science of Food and Agriculture* 98(2). DOI: 10.1002/jsfa.8524.

[10] Longe, O.O. and Oso, A.A. (2017). Fumigant effect of ash from two *Allium* species (*Allium Cepa* and *Allium sativum*) on the survival of *Callosobruchus maculatus* (Fabricius). *International Journal of Current Agricultural Research*, 5(6):217-220.

[11] Handa, S.S., Khanuja, S.P.S., Longo, G., & Rakesh, D.D. (2008). Extraction technologies for medicinal and aromatic plants (First Eds.), United Nations Industrial Development Organization and the International Centre for Science and High Technology, Italy: No. 66.

[12] Anokwuru, C.P., Anyasori, G.N., Ajibade, O., Fakoya, O., and Okebugwu, P. (2011). Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. *Nature and Science*, 9(7): 53-61.

[13] Nyirenda S.P.N. (2013). Botanical pesticides: optimized pest management with botanical pesticides on legume cropping systems in Malawiand Tanzania. McKnight Collaborative Crop ResearchProgramme. <http://mcknight.ccrp.cornell.edu/projects/saf_cop/SAF_09-297/09-297_project.html> Accessed August 2013.

[14] Oso, A.A., and Okunoye, J.A. (2019). Efficacy of turmeric (*Curcuma longa*) and lambdacyhalothrin on insect pest infestation and performance of okra in Ado-Ekiti, Nigeria. *Asian Journal of Advances in Agricultural Research*, 11(4), 1-6.

[15] Truong, D-H, Nguyen, Nguyen, D.H. Nhat, T.A.T., Anh, V.B., Tuong, H.D., and Hoang, C.N. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants and in vitro anti-inflammatory activities of *Severinia buxifolia*. *Hindawi Journal of Food Quality*. <https://doi.org/10.1155/2019/8178294>.

[16] Kuppusamy, P., Yusuff, M.M., Parine, N.R. and Govindan, N. (2015). Evaluation of in vitro antioxidant and antibacterial properties of *Commelina nudiflora* L. extracts prepared by different polar solvents. *Saudi Journal of Biological Sciences*, 24(3):293-301.

[17] Ngo, T.V., Scarlett, C.J., Bowyer, M.C., Ngo, P.D. and Vuong, Q.V. (2017). Impacts of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. https//doi.org/10.1155/2017/9305047.

[18] Okwute, S. (2012). Plants as potential sources of pesticidal agents: A review.*Pestic.Adv.Chem.Bot.Pestic*. doi: 10.5772/46225.

[19] Bett, P. K., Deng, A. L., Ogendo, J. O., Kariuki, S. T., Kamatenesi‐Mugisha, M., Mihale, J. M., and Torto, B. (2016). Chemical composition of *Cupressus lusitanica* and *Eucalyptus saligna* leaf essential oils and bioactivity against major insect pests of stored food grains. *Industrial Crops and Products*, 82, 51– 62.

[20] Taban, A., Saharkhiz, M. J., and Hooshmandi, M. (2017). Insecticidal and repellent activity of three *Satureja* species against adult red flour beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Acta Ecologica Sinica*, 37(3):201– 206.

[21] Skenjana, N.L. and Poswal, M.A. (2018). A survey of plants used by rural small-scale farmers to control pests of cabbage in the Eastern Cape Province, South Africa. *Journal of Medicinal Plants for Economic Development.*2(1), a57. <https://doi.org/10.4102/jumped.v2i1.57>.

[22] Skenjana, N.L. (2018). Identification and documentation of ethnobiological methods used by rural farmers to control stalk borers on maize in the Eastern Cape Province, South Africa. MSc. Thesis. Department of Botany, University of Fort Hare.

[23] Parekh, J., Jadeja, D. and Chanda, S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk J. Biol*, 29:203-210.