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Evaluation of insecticidal activity of *Nardostachys jatamansi* essential oil against some grain storage insects

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Abstract

Water-distilled essential oil from *Nardostachys jatamansi* (Caprifoliaceae) roots was analyzed by gas chromatography-mass spectrometry (GC-MS). Twenty-nine compounds, accounting for 98.1% of the total oil, were identified. The main components of the essential oil of *N. jatamansi* were calerene (25.9%), patchouol (10.6%), α -gurjunene (7.5%), aristolone (7.1%) and β -maaliene (6.5%) followed by spathulenol (4.3%). The essential oil had higher sesquiterpenoids content (85.4%) than monoterpenoids (8.5%). It exhibited contact toxicity with LC₅₀ value of 277.05 $\mu\text{g}/\text{cm}^2$ and fumigant toxicity with LC₅₀ value of 0.74 mg/l against *Liposcelis bostrychophila*. The oil exhibited contact toxicity against *Sitophilus zeamais* and *Tribolium castaneum* adults with LC₅₀ values of 70.83 and 45.38 $\mu\text{g}/\text{adult}$, respectively. This oil possessed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC₅₀ values of 82.13 and 53.29 mg/l. The results indicate that the essential oil of *N. jatamansi* has potential for development into natural insecticides or fumigants for control of insects in stored grains.

Keywords: *Nardostachys jatamansi*; *Liposcelis bostrychophila*; *Sitophilus zeamais*; *Tribolium castaneum*; Fumigant; Contact Toxicity

1. Introduction

Maize weevils (*Sitophilus zeamais* Motsch.) and red flour beetles (*Tribolium castaneum* Herbst) are two of the major pests of stored grains and grain products in the tropics and subtropics [1]. Infestation not only causes significant loss due to the consumption of grains; It also results in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species [2]. Booklice (*Liposcelis bostrychophila* Badonnel) were regarded as secondary pests, often overlooked due to their small size and the existence of other more damaging post-harvest primary pests e.g. *S. zeamais*, rice weevils (*S. oryzae*) and lesser grain borer (*Rhyzopertha dominica*) in cereal grains [3, 4]. However, new evidence indicates that psocids are perhaps the most important emerging pests in stored grains and related commodities due to their small size, and resistance to chemicals [4, 5]. Currently, control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as environmental disturbances, increasing costs of application, pest resurgence, pest resistance and lethal effects on non-target organisms in addition to direct toxicity to users [6-8]. Thus, there is an urgent need to develop safer, environmentally friendlier and efficient alternative with potential to replace synthetic insecticides and fumigants. Evaluation of local plants especially medicinal plants (cultivated, low toxicity to human and well-studied chemical composition) as sources of protectants is very desirable to help farmers use locally available and environmental friendly products to limit post-harvest losses of their produce [9]. Many plant essential oils and their constituent compounds have been evaluated for repellency and insecticidal activity against stored products insects and some of them are quite promising in the development of natural repellents/insecticides [10-19].

Nardostachys jatamansi (D. Don) DC. (syn. *N. chinensis* Batalin, Family: Caprifoliaceae) is a perennial herb that grows in West and Northwest China (Gansu, Qinghai, Sichuan, Yunnan and Tibet) as well as Bhutan, India, Nepal [20]. In traditional Chinese medicine, the roots and rhizomes of *N. jatamansi* are used for their stomachic and sedative effects [20]. It is also used as

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a stimulant, antiseptic, insect repellent and for the treatment of epilepsy, hysteria, convulsive affections, stomachache, constipation and cholera in Ayurvedic and Unani systems of Medicine [21]. Previous phytochemical investigations of *N. jatamansi* revealed the presence of phenolic compounds, caffeoylquinic acid derivatives, lignans, neolignans, monoterpenoids, sesquiterpenoids, diterpenoids, and iridoids [22-31]. Chemical composition of essential oils derived from the three species has been analyzed previously [25, 32-37]. The methanol extract of *N. jatamansi* possessed strong repellency against female blood-starved *Aedes aegypti* [38] while the essential oil of *N. jatamansi* also exhibited repellency against *L. bostrychophila* and *T. castaneum* [18]. Moreover, the methanol extract of *N. jatamansi* showed weak acute toxicity against *Lasioderma serricorne* adults using the filter paper diffusion method and only 22% mortality was observed after 4-day exposed to 3.5 mg/cm² [39]. However, insecticidal activities of the essential oil against three grain storage insects, *L. bostrychophila*, *S. zeamais* and *T. castaneum* have not been determined so far. In this paper, the essential oil of *N. jatamansi* roots was evaluated for contact and fumigant toxicity against these three grain storage insects.

2. Materials and Methods

2.1. Insects

The booklice (*L. bostrychophila*) were obtained from laboratory cultures in the dark in incubators at 28-30 °C and 70-80% relative humidity (RH) and was reared on a 1: 1: 1 mixture, by mass, of milk powder, active yeast, and flour. All the containers housing insects and the Petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon®, Blades Biological, Edenbridge, UK). Laboratory bioassays (August-October, 2013) were done within one week after adult collections.

The maize weevils (*S. zeamais*) and red flour beetles (*T. castaneum*) were obtained from laboratory cultures maintained in the dark in incubators at 29-30 °C and 70-80% RH. The red flour beetles were reared on wheat flour mixed with yeast (10:1, w/w) while maize weevils were reared on whole wheat at 12-13% moisture content. Unsexed adult weevils/beetles used in all the experiments were about 2 weeks old.

2.2. Chinese medicinal herb and essential oil extraction

Dried roots (5 Kg) of *N. jatamansi* were purchased from Anguo Chinese Medicinal Herbs Market (Anguo 071200, Hebei Province, China). A voucher specimen was deposited at the Department of Entomology, China Agricultural University (CMH-GanSong-Hebei-2011-10). The sample was ground to powder using a grinding mill (Retsch Muhle, Haan, Germany). It was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4 °C for subsequent experiments. Pyrethrum extract (25% pyrethrin I and pyrethrin II) was purchased from Fluka Chemie (Buchs, Switzerland). Dichlorvos (99.9%) was purchased from Aladdin-reagent Company (Shanghai, China).

2.3. GC-MS analysis

Analyses of volatile constituents were determined using an Agilent 5973 GC-MS system operating in the EI mode at 70 eV [equipped with a 30m HP-5MS column (0.25mm ×30m × 0.25 μm) and coated with 5% phenyl-methylpolysiloxane using a HP-5MS (df = 0.25 μm) (Agilent J&W Scientific,

USA)]. The temperature program used for the analysis was as follows: initial temperature at 60 °C, held for 1 min, ramped at 4 °C /min to 290 °C and held for 0.5 min. Helium was the carrier gas at 1.0 ml/min; the sample (1 μl, diluted to 1/100, v/v, in hexane) was injected in the split mode (1:5). The injector and detector temperatures were preformed at 230 °C and 300 °C, respectively. The Kovats retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C₈-C₂₄. Quantification was performed using percentage peak area calculations and the identification of individual compartments was done using the Wiley/ NBS Registry of Mass Spectral Database and NIST MS Search, literature [40] and several authentic compounds. The relative concentration of each compound in essential oil was quantified based on the peak area integrated by the analysis program.

2.4. Contact toxicity with treated filter paper against the booklice

Contact toxicity of the essential oil of *N. jatamansi* against the booklice was measured as described by Zhao *et al.* [41]. Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. The essential oils were diluted in acetone. The filter paper with 3.5 cm in diameter (Whatman) was treated with 150 μl of the solution. The treated filter paper after treated with solid glue (Glue Stick, Jong Ie Nara Co., Ltd. Hong Kong) was placed in a petri dish (3.5 cm in diameter) and 10 booklice were put on the filter paper. The plastic cover with holes was put and all the petri dishes were kept in incubators at 27-29 °C, 70-80% RH for 24 h and mortality of insects was observed. Acetone was used as control and pyrethrum extract was used as a positive control. Six concentrations (2.0%, 2.4%, 2.9%, 3.5%, 4.2%, and 5.0%) and five replicates of each concentration were used in all treatments and controls.

2.5. Fumigant toxicity against the booklice

Fumigant toxicity of the essential oil of *N. jatamansi* against the booklice was determined as described by Zhao *et al.* [41]. Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. A filter paper strip (3.5 cm ×1.5 cm) was treated with 10 μl of the respective concentration of test essential oils in acetone. The impregnated filter paper was then placed in the bottom cover of glass bottle of 250 ml. The insects, 10 adults with undefined sex in a small glass bottle (8 ml), were exposed for 24 h and each concentration with five replicates. Six concentrations (3.1%, 3.3%, 3.5%, 3.6%, 3.8%, 4.0%) were used in all treatments and controls. All the treatments were replicated five times. Acetone was used as controls and dichlorvos was used as a positive control. Mortality of insects was observed.

2.6. Contact toxicity using topical application

The contact toxicity of the essential oil of *N. jatamansi* against *S. zeamais* and *T. castaneum* adults was measured as described by Liu and Ho [1]. Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. A serial dilution of the essential oil was prepared in *n*-hexane. Aliquots of 0.5 μl of the dilutions (six concentrations: 15.0%, 11.5%, 8.9%, 6.8%, 5.3%, and 4.0% for *S. zeamais*; 2.0%, 3.0%, 4.5%, 6.8%, 10.0%, and 15.0% for *T. castaneum*) were applied topically to the dorsal thorax of the insects, using an Arnold Automatic Micro-applicator (Burkard, Ricksmanworth, England). Controls were determined using *n*-hexane. Both treated and control insects were then transferred to glass vials

(10 insects/vial) with culture media and kept in incubators. Mortality of insects was observed at 24 h after treatment.

2.7. Fumigant toxicity against *S. zeamais* and *T. castaneum*

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. A Whatman filter paper (diameter 2.0 cm, CAT No. 1001020) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 ml). Ten microliters of the dilutions of essential oils (6 concentrations: 2.0%, 3.0%, 4.4%, 6.7%, 10.0%, and 15.0% for *S. zeamais*; 14.8%, 19.3%, 25.0%, 32.5%, 42.0%, and 55.0% for *T. castaneum*) was added to the filter paper. The solvent was allowed to evaporate for 15 s before the cap was placed tightly on the glass vial (with 10 unsexed insects) to form a sealed chamber. Fluon (ICI America Inc, Parkersburg, West Virginia) was used inside glass vial to prevent insects from the treated filter paper. *n*-Hexane was used as a control. Six replicates were used in all treatments and control and they were incubated at 29-30 °C and 70-80% RH for 24 h. Mortality of insects was observed.

2.8. Statistical analysis

The observed mortality data were corrected for control mortality using Abbott's formula. The results from all replicates in fumigant and contact toxicity were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LC₅₀ values with their fiducial limits [42].

3. Results and Discussion

3.1. Chemical composition of the essential oil

The water distillation for 6 h of *N. jatamansi* roots afforded essential oil (yellow) with a yield of 1.07% (v/w, based on dry weight) and the density of the concentrated oil was 0.83 g/ml. A total of 29 components of the essential oil of *N. jatamansi* were identified, accounting for 98.1% of the total oil (Table 1). Monoterpenoids only represented 8 of the 29 compounds, corresponding to 8.5% of the whole oil, while 18 of the 27 constituents were sesquiterpenoids (85.4% of the crude essential oil) (Table 1). The principal compounds in the essential oil of *N. jatamansi* were calerene (25.9%), patchouol (10.6%), α -gurjunene (7.5%), aristolone (7.1%) and β -maaliene (6.5%) followed by spathulenol (4.3%). The chemical profiles of the essential oil of *N. jatamansi* in this study were similar to those (collected from China) of the previous reports [32-35]. For example, major compounds have been identified in the essential oil of *N. jatamansi* as calerene (29.44%), δ -1(10)-aristolone-2 (16.57%) and jatamansinol (8.80%) [32] while Deng et al. [33] determined that the essential oil of *N. jatamansi* contained calerene (35.39%), β -maaliene (10.20%), α -gurjunene (9.12%), and aristolone (6.28%). In some other reports, calerene (37.7%), β -maaliene (7.9%), valerana-4,7(11)-diene (6.6%), 9-aristolene (4.7%) and patchouol (4.5%) were identified as the major volatile constituents of *N. jatamansi* [34] while calerene (25.31%), aristolone (13.35%), α -selinene (7.32%) and β -maaliene (6.70%) were the major compounds of the 23 identified components which accounted for 92.76% of the total oil of *N. jatamansi* [35]. However, the chemical profiles of the essential oil of *N. jatamansi* in this study were quite different from those (not from China) of the previous reports [21, 36, 37]. For example, the essential oil of *N. jatamansi* harvested from Pakistan mainly contained ledene oxide [II] (13.02%) and patchouli alcohol (9.58%) [21]. Moreover, the predominant constituents in the essential oil of *N. jatamansi* harvested from

Indian Himalayas were nardol (10.1%), and α -selinene (9.2%) [36]. In another report, jatamansone or valeranone (36.71%), and α -cadinol (22.67%) were found to be the major constituents in the essential oil of *N. jatamansi* rhizomes collected from India [37].

Table 1: Chemical constituents of the essential oil of *Nardostachys jatamansi*

No.	Compounds	RI ^a	Composition (%)
1	Isovaleric acid ^b	875	1.5
2	3-Methylvaleric acid ^b	902	2.3
3	Valeric acid ^b	912	0.4
4	α -Pinene ^b	931	0.6
5	Camphene	945	0.5
6	β -Pinene ^b	981	0.9
7	1,8-Cineole ^b	1032	3.6
8	γ -Terpinene	1057	0.8
9	Linalool ^b	1094	0.5
10	4-Terpinenol ^b	1179	1.1
11	α -Terpineol ^b	1191	0.5
12	β -Patchoulene	1382	0.6
13	β -Elemene	1393	0.1
14	α -Gurjunene	1406	7.5
15	β -Maaliene	1418	6.5
16	Aristolene	1427	3.1
17	Calarene	1432	25.9
18	Aromadendrene	1440	3.8
19	β -Ionone	1487	1.0
20	α -Selinene	1492	3.7
21	α -Bulnesene	1503	1.4
22	Alloaromadendrene	1508	2.3
23	δ -Cadinene	1520	0.5
24	α -Panasinsen	1526	0.5
25	Spatulenol	1578	4.3
26	Viridiflorol	1592	2.8
27	Patchoulol ^b	1660	10.6
28	Valeranone	1679	3.7
29	Aristolone	1762	7.1
	Total identified		98.1
	Monoterpenoids		8.5
	Sesquiterpenoids		85.4
	Others		4.2

^a RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons; ^b Identification based on comparison of LRI and spectra with authentic standards.

3.2. Insecticidal activities against *S. zeamais* and *T. castaneum*

The essential oil of *N. jatamansi* roots exhibited contact toxicity against *S. zeamais* and *T. castaneum* adults with LC₅₀ values of 70.83 μ g/adult and 45.38 μ g/adult, respectively (Table 2). Pyrethrum extract showed contact toxicity against *S. zeamais* and *T. castaneum* adults with LC₅₀ values of 5.15 μ g/adult and 1.24 μ g/adult, respectively. Thus the essential oil of *N. jatamansi* roots possessed only 14 and 36 times less toxicity to *S. zeamais* and *T. castaneum* adults, respectively. When compared with the other essential oils in the previous studies, the essential oil of *N. jatamansi* exhibited stronger acute toxicity against the maize weevils, e.g. essential oils of *A. sieversiana* (LD₅₀ = 112.7 μ g/adult) [10], *A. capillaris* (LD₅₀ = 105.95 μ g/adult) and *A. mongolica* (LD₅₀ = 87.92 μ g/adult) [43], *Illicium simonsii* (LD₅₀ = 112.74 μ g/adult) [44], and *Caryopteris incana* (122.65) [45]. *T. castaneum* (LC₅₀ = 53.29 mg/l) proved to be more susceptible (on overlap in 95% fiducial limits) to the essential

oil of *N. jatamansi* in fumigant toxicity bioassay than *S. zeamais* ($LC_{50} = 82.13$ mg/l) (Table 2). The commercial grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 0.67 mg/l and 1.75 mg/l, respectively [1], thus the essential oil of *N. jatamansi* was 122 and 30 times less toxic to *S. zeamais* and *T. castaneum*, respectively. However, fumigant activity of the essential oil of *N. jatamansi* against *S. zeamais* and *T. castaneum* adults is quite promising because the most effective fumigants (e.g. phosphine and MeBr) are highly toxic to humans and other non-target organisms.

3.3. Insecticidal activities against *L. bostrychophila*

The essential oil of *N. jatamansi* exhibited strong contact toxicity against *L. bostrychophila* with an LC_{50} value of 277.05 $\mu\text{g}/\text{cm}^2$ (Table 2). However, compared with the positive control, pyrethrum extract ($LC_{50} = 18.99$ $\mu\text{g}/\text{cm}^2$), the essential oil of *N. jatamansi* showed 15 times less toxic to *L. bostrychophila*. The essential oil of *N. jatamansi* roots possessed strong fumigant toxicity with an LC_{50} value of 0.74 mg/l against the booklice (Table 2). The positive control, dichlorvos had LC_{50} value of 1.35 $\mu\text{g}/\text{l}$. Thus the essential oil of *N. jatamansi* showed only 500 times less toxic against *L. bostrychophila* when compared with the positive control. However, the essential oil of *N. jatamansi* possessed stronger fumigant toxicity against the booklice than essential oils of *Artemisia rupestris* ($LC_{50} = 6.67$ mg/l) and *A. frigida* ($LC_{50} = 1.25$ mg/l) [46, 47], *Curcuma wenyujin* ($LC_{50} = 2.76$ mg/l) [48], *Valeriana jatamansi* ($LC_{50} = 5.98$ mg/l) [49] and *Ageratum houstonianum* ($LC_{50} > 50$ mg/l) [50]. But less toxic than the essential oils of *Acorus calamus* ($LC_{50} = 0.39$ mg/l) [51] and *Foeniculum vulgare* ($LC_{50} = 0.03$ mg/l) [41]. However, considering the commercial fumigants are synthetic

insecticides and the most effective fumigants (e.g. phosphine and MeBr) are also highly toxic to humans and other non-target organisms, fumigant activity of the essential oil of *N. jatamansi* is quite promising. The essential oils of *N. jatamansi* roots showed potential to be developed as possible natural fumigants/insecticides for the control of grain storage insects.

There is no report on insecticidal activity of the major constituents, calarene, α -gurjunene, aristolone and β -maaliene. The other constituent, patchoulol exhibited strong acute toxicity to the booklice *L. bostrychophila* with a LC_{50} value of 61.35 $\mu\text{g}/\text{cm}^2$, but no fumigant toxicity was observed [49]. Patchoulol was also demonstrated to exhibit toxic and repellency against Formosan subterranean termites (*Coptotermes formosanus*) [52] and exhibited pupicidal and repellent activities against three important vector mosquitoes (*Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*) [53]. Weak larval activity of patchoulol against *Culex pipiens pallens* was also observed [54]. The roots of *N. jatamansi* have been commonly used in traditional Chinese medicines for their stomachic and sedative effects [20] and seem to be safe to human consumption. However, for the practical application of the essential oil of *N. jatamansi* as novel insecticide/fumigant, further studies on the safety of the essential oil to humans and plants are needed. A further study is also necessary to determine the toxicity of the essential oil on other economically important pests and their natural enemies in greenhouse conditions where pest management depends on chemical applications. Further studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce cost.

Table 2: Contact and fumigant toxicity of the essential oil of *Nardostachys jatamansi* against *Liposcelis bostrychophila*, *Sitophilus zeamais* and *Tribolium castaneum*

Insects	Treatment	Extracts	LC_{50}	95% FL	Slope \pm SE	Chi square (χ^2)
<i>Liposcelis bostrychophila</i>	Contact ($\mu\text{g}/\text{cm}^2$)	<i>N. jatamansi</i>	277.05	249.82-304.16	6.57 ± 0.58	9.24
		Pyrethrum extract	18.99	17.56-20.06	7.64 ± 0.75	-
	Fumigant (mg/l)	<i>N. jatamansi</i>	0.74	1.95-3.67	5.25 ± 0.43	11.20
		Dichlorvos	1.35×10^{-3}	$(1.25-1.47) \times 10^{-3}$	6.87 ± 0.77	-
<i>Sitophilus zeamais</i>	Contact ($\mu\text{g}/\text{adult}$)	<i>N. jatamansi</i>	70.83	66.01-76.28	5.63 ± 0.55	21.00
		Pyrethrum extract	5.15	4.66-6.18	4.14 ± 0.33	6.78
	Fumigant (mg/l)	<i>N. jatamansi</i>	82.13	75.01-89.55	3.46 ± 0.35	14.00
		MeBr*	0.67	-	-	-
<i>Tribolium castaneum</i>	Contact ($\mu\text{g}/\text{adult}$)	<i>N. jatamansi</i>	45.38	41.82-49.16	5.43 ± 0.47	7.56
		Pyrethrum extract	1.24	1.05-1.28	4.64 ± 0.35	13.43
	Fumigant (mg/l)	<i>N. jatamansi</i>	53.29	49.35-58.49	5.22 ± 0.52	10.36
		MeBr*	1.75	-	-	-

* from Liu and Ho [1]

4. Conclusion

The essential oil of *N. jatamansi* roots demonstrated contact and fumigant toxicity against three grain storage insects. They showed potential to be developed as possible natural insecticide and fumigant for control of the stored product insects but need to be further evaluated for safety to humans.

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investigated medicinal herb.

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