Potential of plant products as protectants of stored maize against *Sitophilus zeamais* Motschulsky (Coleoptera:Curculionidae)

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Abstract

Laboratory studies were carried out to evaluate the effects of some formulations from *Mentha pulegium*, *Lonchocarpus sericeus*, *Daphne gnidium*, *Laurus nobilis*, *Momordica charantia*, *Nerium oleander* and *Ptaeroxylon obliquum* as protectants against adult insects of *Sitophilus zeamais* on stored maize. The dusts from leaves of *L. nobilis* at 30% w/w caused 86% mortality and reduced F1 progeny emergence up to 57%. At the same concentration, dusts of pink flowers from *N. oleander* and leaves from *L. sericeus* reduced the F1 progeny up to 68% and 70%, reduced the developmental index and prolonged the developmental period by 4 and 6 d, respectively.

The suspensions (2% v/v) from *M. charantia*, *N. oleander* and *P. obliquum* reduced the F1 progeny emergence up to 58, 91 and 94% and the number of holes in grains by 75, 91 and 97%, respectively. The methanol extracts were more effective than n-hexane extracts and affected the F1 progeny emergence and the developmental index.

Keywords: *Sitophilus zeamais*, Botanical insecticides, Repellence, Insect control agents.

1. Introduction

The use of natural products from plant origin as insecticides for defence against phytophagous insects can be traced back over many centuries to written documents of the early civilizations in China, India, Near East and over 150 years in Europe and North America (Matos, 2004; Isman, 2006). Nevertheless, in the early 19th century, emphasis shifted away from insecticides of plant origin with the tremendous development of synthetic insecticides industry after the Second World War. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic point of view during that period. Over the last two decades, however, interest in substances of plant origin and their potential in the pest management as environmentally friendly insecticides has been growing steadily. Since pyrethrum and rotenone, two of the first commercial natural insecticides, hundreds of new natural substances, including the volatile oils, are isolated and identified every year and their potential as plant protection agents is now of particular interest (Amaro, 2003; Isman, 2006), Such is the case of the triterpenoid azadiractin, extracted from the neem tree *Azadiracta indica* (A. Juss.), that is useful for pest control due to its properties; antifeedant, ovipositional deterrence, repellent, growth disruption, reduction of fitness and sterility.

The present study was inspired from the use of plant products as protectants of stored maize at the farm level in some rural communities in Angola. The main objective was to investigate the effects of *Laurus nobilis* L., *Nerium oleander* L., *Daphne gnidium* L. and *Mentha pulegium* L. from Portugal, species of tropical origin, *Ptaeroxylon obliquum* (Thumb.) from Angola, *Lonchocarpus sericeus* (Poir.) Khunt. and *Leonotis nepetifolia* (L.) R. Br. from São Tomé and Principe, against the maize weevil, *Sitophilus zeamais* Motschulsky.

2. Materials and methods

2.1. Maize, insects and plant products

The maize grain was yellow and was acquired in local trade with an average moisture content of 14 ± 0.5%. The unsexed adult insects aged from 1 to 7 d. The stock cultures of insects and all the biological tests were carried out in a single incubator at 27ºC and 75 ± 5% r.h. During the total period of the

bioassays it was necessary to collect the plant material several times from the same locations and during flowering. The harvested plant material was washed and dried out at room temperature in the darkness for two weeks, to reduce the water content of the structures and to optimize the concentration of bioactive substances. Then the plant material (leaves and flowers) was lyophilised, grounded and the dusts were kept in a desiccator protected from light. Due to shortage of plant material, the methanol suspensions were prepared only from dusts of *L. nobilis*, *M. charantia*, *N. oleander* and *P. obliquum* at a single concentration of 2% w/v (Table 1). From the lyophilised dusts methanol and n-hexane extracts were also produced (macerating 0.4 g of plant in 20 mL of solvent, for 24 h at room temperature, followed by filtration under vacuum).

Table 1 Plants, vegetal structures, type of formulation and dosage rates used in the bioassays with *Sitophilus zeamais*.

Plant species	Structure	Formulation	Concentrations of the formulation(% w/v)	Dosage rate
D. gnidium	Leaves	Extract	2.0. 3.5. 5.0 6.5. 8.0	15 mL/300 g maize (5% v/w)
L. nobilis	Leaves	Dust	—	15 and 30% w/w
		Suspension	2.0	20 mL/80 g maize
		Extract	2.0. 3.5. 5.0 6.5. 8.0	15 mL/300 g maize (5% v/w)
L. sericeus	Leaves	Dust	—	15 and 30% w/w
M. pulegium	Leaves	Dust	—	15% w/w and 30% w/w
		Extract	2.0. 3.5. 5.0 6.5. 8.0	15 mL/300 g maize (5% v/w)
M.charantia	Leaves	Dust	—	15 and 30% w/w
	Leaves	Suspension	2.0	20 mL/80 g maize
N. oleander.	Leaves and flowers	Dust	—	15 and 30%w/w
		Suspension	2.0	
		Extract	2.0. 3.5. 5.0 6.5. 8.0	15 mL/300 g maize (5% v/w)
P. obliquum	Leaves	Dust	—	15 and 30%w/w
		Suspension	2.0	20 mL/80g maize
		Extract	2.0. 3.5. 5.0 6.5. 8.0	15 mL/300 g maize (5% v/w)

2.2. Bioassays

The maize and the dusts were mixed in glass jars for 10 min with a mechanical mixer. After mixing, samples of 30 g of treated maize were transferred into glass jars (4 replicates per dosage rate) and 10 adult insects were used per replicate. After 168 h of exposure the parent adult insects were removed for observation of mortality, and the grains with the immature stages were incubated for the F1 emergence. The reduction in emergence (RE) of progeny (F1) was evaluated by using the formula RE (%) = [(Cn-Tn) / Tn] x100, where Cn is the number of insects present in control samples and Tn is the number of insects present in the treated samples (Tapondjou et al., 2005). The development index was calculated based on the emergency (F1) and average duration of development (ADD), which reflects the average number of days between the middle of the laying period and the emergence of 50% of F1 (Dobie, 1974).

The criterion from Haryadi and Rahayu (2002) was adopted for calculating the development index, using the following formula: DI = (ln F1/ADD) x 100, where DI = development index of *S. zeamais*, F1 = number of adult insects emerged from F1, ADD = average duration of development.

To evaluate the effect of the suspensions of *L. nobilis, M. charantia, N. oleander* and *P. obliquum*, a multiple-choice device was used (Fig. 1) with some modifications, adapted from Wesolowska and Ignatowicz (1994).

In the center of the Petri dishes (9 cm in diameter) was placed a central ring with 14 mm high and 20 mm diameter, to which were engaged in three equidistant points, three tabs of cardboard hydraulic of 32 mm long and 10 mm height (Fig. 1). The central ring and the tabs did not entirely touch on the bottom of the Petri dish. The ring had in the bottom three cuts of 4 mm height where the insects could pass. The tabs were stuck just above the bottom of the ring, also allowing the movement of insects between the sections. With the aim of providing ventilation inside the Petri dishes, a circular portion of 75 mm was removed from the lid and replaced by screening, which ensured the retention of insects and allowed the desired ventilation and observation.

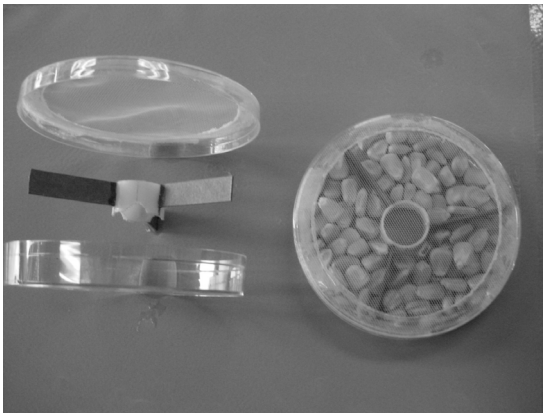

Figure 1 Multiple choice device for testing suspensions; a - lid with screening, b – central ring and cardboard tabs; c - base of the Petri dish; d - Petri dish with maize grain.

Ten adult insects were placed in the central ring. Mortality was assessed 24 h, 48 h and 168 h after exposure to treatments and repellency after 1, 24, 48 and 168 h. The parent adults were removed from the maize after 168 h. Maize seeds infested with eggs and immature stages were transferred to 60 mL glass jars, which were placed in the incubator for 35 d for observation and counting of progeny (F1). The count of insect damaged grains as an indicator of the feeding-inhibition was carried out 7 d after exposure of insects to treated maize. In the tests performed with extracts the species *D. gnidium, L. nobilis, M. pulegium, N. oleander* and *P. obliquum* were used at concentrations of 2% w/v, 3.5% w/v (0.7 g/20 mL), 5% w/v (1 g/20 mL), 6.5% w/v (1.3 g/20 mL) and 8 % w/v (1.6 g/20 mL) (Table 2).

Table 2 Mortality, average duration of development (ADD), emergency (F1), development index (DI), and life cycle of *Sitophilus zeamais* in maize treated with lyophilised dusts, n=4.

Plant	Dose (%w/w)	Mortality after 168 h (%)	*ADD* (d)	Mean F1	DI (d)	F1 reduction (%)	Life cycle (d)
L. nobilis	0	8	34.1 ± 1.0a	47.3 a	10.8 a		31.5 ± 0.9 b
	15	31	38.3 ± 5.5 a	25.5 ab	8.4 ab	46	33.8 ± 2.6 ab
	30	86	37.3 ± 5.6 a	15.3 b	6.8 b	68	35.5 ± 2.5 a
L. sericeus	0	3	32.4 ± 1.5b	41.5 a	10.6 a		31.8 ± 1.5 b
	15	8	36.2 ± 1.8 b	16.0 ab	7.1 a	65	36.0 ± 2.3 a
	30	8	38.1 ± 4.3 a	12.5 b	5.4 a	70	37.8 ± 2.1 a
M. pulegium	0	0	32.2 ± 1.1 a	47.8 ab	10.9 a		30.3 ± 0.5 a
	15	3	33.9 ± 2.7 a	54.0 a	11.6 a	-29	30.3 ± 0.5 a
	30	5	32.2 ± 7.4 a	22.0 b	8.8 a	14	30.0 ± 0.0 a
N. oleander	0	5	36.8 ± 4.1 a	28.3 a	8.9 a		33.5 ± 2.9 a
flowers	15	8	37.3 ± 4.4 a	30.5 a	8.2 a	-14	35.8 ± 6.6 a
	30	3	37.2 ± 5.9 a	16.3 a	6.1 a	57	33.3 ± 2.1 a
N. oleander	0	0	32.2 ± 1.3 a	48.3 a	11.5 a		30.3 ± 0.5 a
leaves	15	3	33.3 ± 2.6 a	26.3 a	9.8 a	46	31.0 ± 0.8 a
	30	5	37.2 ± 4.1 a	35 a	9.6 a	28	31.3 ± 1.0 a
P. obliquum	0	0	35.6 ± 1.4 a	48.3 a	11.6 a		30.3 ± 0.5 a
	15	5	36.8 ± 2.6 a	39.3 a	10.8 a	16	30.5 ± 0.6 a
	30	8	40.6 ± 4.1 a	32.8 a	9.3 a	8	32.0 ± 2.3 a

For a given plant, values followed by same letter do not differ significantly at $P \leq 0.05$.

The extracts were mixed with maize in the jars by manual shaking and then using a mechanical mixer for 10 min. The treated maize was placed in Petri dishes and / or glass jars and kept at room temperature in a laminar air flow for one hour to remove excess solvent, leaving the active substances of the extract impregnated maize. There was ten replicates for each concentration and 10 adult insects in each replicate. Mortality was observed after 24, 48 and 168 h of exposure of adult insects to treated maize. After 168 h exposure the adult insects were removed and the treated maize containing the immature stages was kept in the incubator over 3 wk and the emerging progeny were counted. The Development Index (DI) and the life cycle (LC) were also evaluated. The results were analyzed by Statistica ® 6.0 and SPSS 11.5 for Windows.

3. Results and discussion

The dusts of natural products require, in general, the use of high concentrations, sometimes exceeding 20%, which have to be applied to submerge the grain kernel in order to have protective effects (Golob, 1997; Golob et al., 1999). For that reason the concentrations of 15 and 30%, were used in the present tests. The most effective treatment of lyophilised dusts was *L. nobilis* at concentrations of 15% and 30% with mortalities of 31 and 86%, respectively (Table 2). There was a large reduction in the F1 progeny when treated with 30% dust; 68% reduction with *L. nobilis*, 70% reduction with *L. sericeus* and 57% reduction with flowers of *N. oleander* (Table 2). The average duration of development (ADD) and life cycle of insects were affected at a concentration of 30% of *L. nobilis* and *L. sericeus*. However, the difference between 15 and 30% in each plant is not significant. Boeke et al. (2001) showed that most of the effects of formulations of dust plants applied against adult insects are revealed in the reproduction and, consequently, on the embryonic development of insect larvae. In this study, the progeny production and the development index (DI) were affected in all plants, although the differences are significant only in *L. nobilis* and *L. sericeus* (Table 2).

Table 3 Repellency, damage and F1 production of seed treated with just methanol or methanol suspensions of plant dusts treated maize and exposed to *Sitophilus zeamais.*

		Multiple choice test (Mean number of insects/section)								
		Duration (h)				**Damage**				
Plant	**Treatment**	**1**	**24**	**48**	**168**	**Grain with holes (%)**	**Mean number of holes**	**Reduction of holes (%)**	**F1**	**F1 reduction (%)**
L. nobilis	Untreated	5a	5a	3a	4a	50a	26.0a		17a	
	Methanol	2a	3b	2a	2b	56a	32.1a		19a	
	Methanol Suspension	3a	2b	5b	4a	29b	13.5b	48	15a	13
M. charantia						47a	25.2a		21a	
	Untreated	1a	3a	2a	2a					
	Methanol	5b	4b	4a	4b	47a	24.6a		19a	
	Methanol Suspension	4b	3a	4a	4b	14b	6.2b	75	9a	58
N. oleander	Untreated	5a	2b	3ab	2b	48a	29.5a		17a	
	Methanol	3ab	3b	3b	4ab	48a	20.2a		19a	
	Methanol Suspension	2b	5a	4a	4a	4b	0.9b	91	2a	91
P. obliquum	Untreated	3a	4a	5a	3b	53a	21.6a		18a	
	Methanol	4a	3b	1b	4a	49a	23.9a		20a	
	Methanol Suspension	3a	3b	4a	3a	2b	1.9 b	97	1a	94

Values with the same letter for the species in the same column do not differ significantly ($P \leq 0.05$), n=8.

The methanol suspensions were highly variable in their repellency. In the case of *M. charantia* the effect of repellency is not clear, although the number of holes and the F1 progeny has been reduced, the number of insects present in treated maize was higher than in the control (Table 3). However, the mortality of *S. zeamais* with methanol suspensions was below 15% for all mixtures tested (results not shown), the percentage of perforated grains reveals the intensity of attack of *S. zeamais*. There were

significant differences in perforated grains and F1 production. The maize seed treated with *M. charantia*, *N. oleander* and *P. obliquum* had reduced the number of perforations in 75, 91 and 97%, respectively. For the same treatments the reduction of the F1 progeny was 58, 91 and 94%.

Table 4 Mortality of *Sitophilus zeamais* adults after 24, 48 or 168 h of exposure to maize treated with plant extracts of methanol and hexane, n=10.

		Methanol			**Hexane**		
		Mortality (%)			**Mortality (%)**		
		Duration (h)			**Duration (h)**		
Plant	**Treatment**	**24**	**48**	**168**	**24**	**48**	**168**
D. gnidium	Untreated	0	0	0	0	0	0
	Solvent	33	33	36	0	0	0
	2.0	-	-	-	0	0	2
	3.5	61	62	66	0	0	1
	5.0	63	67	72	0	0	2
	6.5	68	75	81	-	-	-
	8.0	73	79	85	-	-	-
L. nobilis	Untreated	0	0	0	0	0	1
	Solvent	48	50	62	0	0	2
	2.0	-	-	-	0	0	0
	3.5	72	72	76	0	0	0
	5.0	78	80	89	1	1	1
	6.5	93	94	96	0	0	0
	8.0	94	95	96	0	0	4
M. pulegium	Untreated	0	2	2	2	2	2
	Solvent	43	43	48	0	0	6
	2.0	2	8	17	0	2	8
	3.5	17	18	24	0	5	8
	5.0	20	24	30	-	-	-
	6.5	26	39	43	-	-	-
	8.0	-	-	-	-	-	-
N. oleander	Untreated	0	0	1	0	0	0
	Solvent	39	39	40	0	0	3
	2.0	27	37	39	0	0	0
	3.5	45	50	55	0	0	0
	5.0	47	53	61	-	-	-
	6.5	49	67	69	-	-	-
	8.0	50	69	73	-	-	-
P. obliquum	Untreated	0	0	0	0	0	0
	Solvent	48	48	60	0	0	4
	2.0	-	-	-	-	-	-
	3.5	78	78	79	0	0	5
	5.0	83	83	87	0	0	11
	6.5	56	64	71	0	0	25
	8.0	-	-	-	-	-	-

- Not available

Methanol suspensions of *L. nobilis, M. pulegium, M. charantia, N. oleander* and *P. obliquum* probably contained various substances dissolved in the solvent, for example, linalool, geraniol, cineol and ethanol in the case of *L. nobilis* (Saim and Meloan, 1986, Fiorini et al., 1997), psitosterol and several proteins in *M. charantia* (Golob et al., 1999), glycosides and proteins in *N. oleander* (Golob et al., 1999) or coumarins and diterpenoids in *P. obliquum* (Mulholland et al., 1999). However, the identification of active substances was not the objective of the present study.

The results obtained by counting the number of insect damaged grains and holes showed that, on average, the suspensions of *P. obliquum, N. oleander* and *M. charantia* have inhibited the feeding

activity and the reproduction of *S. zeamais* . Throne (1990) and Baker et al. (1991) demonstrated that non-lethal inhibition of insect populations can result in significant reductions in populations of stored product insects. We found that the suspensions of *M. charantia, N. oleander and P. obliquum* at 2% w/v reduced the F1 progeny in 58, 91 and 94%, respectively.

Table 5 Effects of methanol and hexane plant extracts on the F1 progeny, average duration of development (ADD), developmental index (DI) and life cycle (LC) of *Sitophilus zeamais* n=10.

		Methanol [a]					Hexane				
Plant	**Treatment**	**F1**	**F1 Reduction (%)**	**ADD (d)**	**DI**	**LC (d)**	**F1**	**F1 Reduction (%)**	**ADD (d)**	**DI**	**LC (d)**
D. gnidium	Control	42.3 a	-	36.3	10.2 a	33 b	59.0 a	-	37.6	10.7 a	33.9 a
	Solvent	10.0 b	76	39.3	7.3 ab	37 ab	49.8 ab	16	38.5	10.1a	35.1 a
	2.0	-	-	-	-	-	-	-	-	-	-
	3.5	3.7 b	91	37.5	5.8 b	35 ab	31.5 b	47	41.2	8.1 a	34.2 a
	5.0	11.1 b	74	39.4	7.3 ab	34 ab	28.5 b	52	40.4	7.9 a	35.6 a
	6.5	4.6 b	89	36.4	6.0 b	35 ab	-	-	-	-	-
	8.0	7.2 b	83	38.4	4.4 b	38 a	-	-	-	-	-
L. nobilis	Control	32.7 a	-	37	8.7 a	35 c	59.0 a	-	34.6	11.6 a	33.9 b
	Solvent	11.1 b	66	39.4	5.0 b	40 b	49.8 ab	16	33.9	11.4 a	35.1 b
	2.0	-	-	-	-	-	33.1 b	44	35.5	8.8 ab	35.7 b
	3.5	10.5 b	68	41	4.1 b	42 ab	23.7 bc	60	35.5	9.6 ab	33.2 b
	5.0	1.3 b	96	41.1	2.1 bc	44 ab	17.4 bc	71	35.5	7.5 b	33.9 b
	6.5	0.4 b	99	41.5	0.5 c	45 a	32.9 bc	44	41.1	7.1 bc	36.2 ab
	8.0	1.8 b	94	43.1	3.5 bc	45 ab	11.6 c	80	43.1	5.0 c	39.3 a
M. pulegium	Control	46.7 ab	-	33.9	10.8 a	33 b	47.9 a	-	34.7	11.1 a	28.6 b
	Solvent	26.1 bc	44	36.6	7.5 b	37 a	28.7 ab	40	33.9	9.1 a	31.7 a
	2.0	59.0 a	126	33.2	11.4 a	32 b	26.5 ab	45	36.1	8.8 ab	32.6 a
	3.5	32.6 ab	30	41	8.9 ab	36 ab	19. 6 b	59	35	7.9 b	32.4 a
	5.0	31.7 b	32	36.4	9.6 ab	34 ab	-	-	-	-	-
	6.5	19.2 b	59	38	8.3 ab	37 a	-	-	-	-	-
	8.0	-	-	-	-	-	-	-	-	-	-
N. oleander	Control	33.5 a	-	32.9	10.1 a	33 b	59.0 a	-	37.6	10.7 a	33.9 a
	Solvent	4.2 b	87	36.5	4.2 b	39 ab	49.8 a	16	38.5	10.1 a	35.1 a
	2.0	9.2 b	73	34.5	5.8 b	37 b	44.6 a	24	40.7	11.4 a	34.1 a
	3.5	7.2 b	79	32.9	7.7 ab	35 b	40.0 a	32	38.1	11.3a	34.7 a
	5.0	3.7 b	89	32.6	5.4 b	34 b	-	-	-	-	-
	6.5	2.6 b	92	39.8	3.7 b	45 a	-	-	-	-	-
	8.0	5.9 b	82	38.9	5.9 b	37 b	-	-	-	-	-
P.obliquum	Control	32.7 a	-	38	8.7 a	35 c	41.4 a	-	34.7	9.8 a	35.7 a
	Solvent	12.3 b	62	37	5.1 b	40 ab	40.3 a	3	33.9	9.6 a	35.6 a
	2.0	-	-	-	-	-	-	-	-	-	-
	3.5	2.8 b	91	41.4	2.4 b	43 a	41.5 a	0	33.3	11.0 a	35.1 a
	5.0	3.4 b	90	39.5	4.0 b	40 ab	25.1 a	39	-	-	35.7 a
	6.5	4.5 b	86	36.9	4.0 b	38 bc	22.7 a	45	-	-	37.1 a

Values followed by same letter do not differ significantly; - not available

The maize treated with methanol alone caused mortalities ranged from 33% to 60% (Table 4), probably due to vapour effect caused by methanol inside the closed glass jars. However, the results obtained with most concentrations are higher than those of methanol alone. At the concentration of 3.5% plant extract after 168 h the order of effectiveness was *P. obliquum* (79%) > *L. nobilis* (76%) > *D. gnidium* (66%) > *N. oleander* (55%) > *M. pulegium* (24%), at 5% plant extract the mortality was; *L. nobilis* (89%) > *P. obliquum* (87%) > *D. gnidium* (72%) > *N. oleander* (61%) > *M. pulegium* (30%) and at 6,5% plant extract the mortality was *L. nobilis* (96%) > *D. gnidium* (81%) > *P. obliquum* (71%) > *N. oleander* (69%) > *M. pulegium* (30%).

For the methanol extracts the best results on the reduction of the F1 progeny were obtained with *D. gnidium* (89%), *L. nobilis* (99%), *M. pulegium* (59%) and *N. oleander* (92%) at 6.5% and *P. obliquum* (91%) at 3.5%. The average duration of development (ADD) of the insect increased with the concentration of both methanol and hexane extracts (Table 5).

The results of the development (ADD) and the life cycle on maize treated with both solvents alone have shown higher values when compared to the untreated maize grains. There were significant differences in the life cycle between the extract treatments. The life cycle was delayed from 3 to 10 d and from 2 up to 6 d for methanol and hexane extracts, respectively, when compared to the untreated control maize. There was also a decrease in DI with the increase in concentration. The DI values obtained for concentrations above 3.5% in extracts of both solvents of *L. nobilis, N. oleander* and *P. obliquum* are significantly lower than those of the untreated control samples.

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