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Effect of drying regime on the chemical constituents of *Plectranthus glandulosus* leaf powder and its efficacy against *Callosobruchus maculatus* and *Sitophilus zeamais*

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Key words: *Plectranthus glandulosus*; drying regime; chemical components; mortality; *Sitophilus zeamais*; *Callosobruchus maculatus*

Abstract

The objective of this study was to determine the chemical constituents and insecticidal efficacy of *Plectranthus glandulosus* Hook (Lamiaceae) leaf powders dried in shade or sunlight against *Callosubruchus maculatus* and *Sitophilus zeamais*. Leaf powder extracts were submitted to stir bar sorptive extraction (SBSE) and volatile compounds analyzed by GC and GC-MS. Fifty components were identified in both drying methods. α-pinene, limonene, 1,8-cineole, terpine and ocimene known for their insecticidal activity were found at higher levels in the shade-dried leaves while camphor, linalool and thymol were higher in the sun-dried leaves. Fenchone, β-pinene and eugenol were found in the same proportion, irrespective of drying regime. The drying regime had no effect (t = 0.34; P > 0.05) on the mortality caused by the leaf powders to *C. maculatus*. Within seven days of exposure, *S. zeamais* were more susceptible (t = -1.29; P < 0.001) to the powder from the sun-dried (LC_{50} = 14.04 g/kg) leaves compared to that from the shade-dried leaves (LC_{50} = 34.51 g/kg). Powders from the sun-dried leaves of *P. glandulosus* stand as a good candidate for protecting maize against the infestation of *S. zeamais* during storage.

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Introduction

Millions of people around the world depend on agriculture for their subsistence and the challenge is to feed 9 billion people by the year 2050 (Godfray *et al.,* 2010). Paradoxically, many smallholder farmers live on the margins of food insecurity in developing countries. This is because of climate change, absence of food-chain infrastructure and food losses (Beddington *et al.,* 2011, Gustavsson *et al.,* 2011). Food security could be achieved not only by increasing agricultural productivity but also by reducing pre- and post-harvest crop losses, particularly for smallholder farmers (Tschamtke *et al.,* 2012). In sub-Saharan African countries, where the wet season is short, crop production is done only within this period of the year, but the products are consumed and marketed all year round (Ngamo *et al.,* 2007a). Proper food storage becomes therefore a matter of survival. Maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* Walp.) are staple foods in many developing countries (Ndjouenkeu *et al*., 2010, Guèye *et al.,* 2011), where seventy-five percent of the harvest for both crops is stored by growers (Kumar, 1991). Unfortunately, during storage, the crops are heavily damaged by insect pests, especially the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae). Several methods are being employed in stored products protection, but the use of synthetic insecticides is dominant. According to Ofuya (2003), synthetic chemicals involve risks for human health and the environment, when they are not properly used. There is thus the need to find materials which combine broad spectrum action against stored product insect pests with low toxicity to non-targeted organisms, but at the same time also readily available and affordable to the small-scale grower (Talukder and Howse, 1995, Nukenine *et al.,* 2007). Plant materials are widely reported for their efficacy as insecticides, but with the dominance of essential oils, pure compounds or solvent extracts (Nukenine *et al.,* 2013) which are not practicable for small farm-families. Farmers often use the whole plant or plant parts like leaves, stem-bark, roots or seeds, and in a few cases they pulverize these plant parts into powders using traditionally-made wooden mortar and pestle to protect their crops (Tapondjou *et al.,* 2000, Arannilewa *et al.,* 2006, Khoshnoud *et al.,* 2008). The whole leaves of *P. glandulosus* are used by small-scale farmers to protect cereals and pulses during storage against insect infestation in northern Cameroon (Ngamo and Hance, 2007, Ngamo *et al.,* 2007b). The plant is also used locally to treat female infertility (Telefo *et al.,* 2008), colds and sore throat (Ngassoum *et al.,* 2001) and as a spice in some meals (Pele and Berre, 1966). Leaf powders and essential oils of *P. glandulosus* showed greater insecticidal efficacy against adult *S. zeamais* as compared with *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Tribolium castaneum* Du Val. (Coleoptera: Tenebrionidae) (Nukenine *et al.,* 2011, 2010, Goudoum *et al.,* 2012)**.** To date, there are no scientific publications reporting on *P. glandulosus* powder and *C. maculatus* on cowpea, although the essential oil of the leaves was effective against the beetle on filter paper (Ngamo *et al.,* 2007c). The efficacy of *P. glandulosus* against stored product insect pests is attributable to its richness in terpenoid compounds (Goudoum *et al.,* 2012). Drying methods could affect the chemical composition of leaves, leading to a decrease in the rate of or the transformation of some pure compounds (Najafian and Agah, 2012, Shahhoseini *et al.,* 2013).In turn, there may be differences in the diversity and quantity of pure compounds between the leaves dried in sunlight (sun-dried) and those dried in a room (shade-dried), independent of the plant species. The chemical composition of Lippia Citriodora and Ocimum basilicum plants from the same family are affected when the were sun-dried (Hassanpouraghdam *et al*., 2010, Najafian and Agah, 2012). Local farmers before using *P. glandulosus* dry the leaves in sun which may affect its efficacy against stored product insects. Therefore, it may be important, to evaluate the chemical composition and the insecticidal efficacy of the plant powder from sun-dried and shade-dried leaves. Such studies could decipher the better drying regime for botanicals, and thus help growers to obtain more efficient plant-

based insecticidal products for stored product protection. The present study was thus carried out to compare the trend of the chemical composition of the powders from the shade-dried and sun-dried leaves of *P. glandulosus*, as well as the susceptibility of *C. maculatus* and *S. zeamais* to the two types of powder. This is the first study reporting the effect of *P. glandulosus* powder on *C. maculatus*. It is also the first research work comparing the plant powder for its efficacy against *C. maculatus* and *S. zeamais*.

Materials and methods

Plant material

The leaves of *P. glandulosus* were collected in October (end of wet season) 2010 around Ngaoundere (latitude 7°22' North and longitude 13°34' East, altitude1.100 m.a.s.l.), located in the Adamawa region, Cameroon. The plants were less than one-year old and only the green leaves were collected from plants which were yet to reach the flowering stage. Half of the collected leaves were sun-dried and the other half was shade dried. The drying temperatures of leaves were recorded by data loggers (EL-USB-2$^+$, LASCAR, Chine) and amounted to 25 ± 1°C and 29 ± 2°C in shade and in sunlight, respectively. Dried leaves were hand crushed and kept in black plastic bags and kept for four months in a deep-freezer at -14 °C, after which they were transported to Berlin, Germany. In Berlin, crushed leaves of *P. glandulosus* were ground into powder using a Bosch Universal grinder (MUM 6012, Remscheid, Germany) until the particles passed through a 0.5 mm mesh sieve. The obtained powders were introduced into an opaque glass jar and stored at 4 °C in a refrigerator until a week to the bioassay time, when they were kept under the condition for which the experiments will be carried out. The moisture contents of the powders from the sun- and shade-dried leaves were determined with the aid of a moisture meter (Mettler LP16, Giessen, Switzerland) and were respectively 8.7 and 8.9% (t = 0.36; P > 0.05).The maize variety used in this study was yellow Ricardino (KWS) harvested in an experimental field of Julius Kühn-Institut (JKI), Braunschweig, Germany in 2012. The organic cowpea (Black-eyed bean, Perou variety) was purchased in a tropical foods store in Berlin, Germany.

Analysis of chemical constituents

The method of Ulrich and Olbricht (2013) was used for the extraction of powder volatiles by immersion stir bar sorptive extraction (imm-SBSE).100 µg of each powder were homogenized in 10 ml of a solution of 5 % ethanol by a household mixer for 1 min. The homogenate was centrifuged 4000 rpm for 30 min. One hundred milliliter of the supernatant were mixed with 10 µl internal standard (0.1 % (v/v) 2,6-dimethyl-5-hepten-2-ol dissolved in ethanol). An aliquot of 8 ml of the saturated homogenate but without the solid NaCl deposit was transferred in an empty glass vial for volatile isolation by SBSE. A stir bar with 0.5 mm film thickness and 10 mm length coated with polydimethysiloxan (PDMS) was placed in the liquid (Gerstel, Mülheim an der Ruhr, Germany). The stir bar was moved at 350 rpm at room temperature for 45 min. After removal from the extract, the stir bar was rinsed with purified water, gently dried with a lint-free tissue and then transferred into a glass tube for thermal desorption and subsequent GC analysis.

The Gas chromatography – mass spectrometry (GC-MS) was performed. The parameters for the thermal desorption unit (TDU, Gerstel) and the cold injection system (CIS4, Gerstel) were the following: thermal desorption at 250 °C, cryo trapping at -150 °C. The TDU-CIS4 unit was used in Gerstel-modus 3: TDU splitless and CIS4 with 15 ml/min split flow. The analyses were performed with an Agilent Technologies 6890N detector. Compounds were separated on a polar column ZB-Wax plus 30 m length × 0.25 mm ID × 0.5 µm film thickness. Helium was used as a carrier gas with a column flow rate of 1.1 ml/min. Temperature programme: 45 °C (3 min), temperature gradient 3 K/min to 210 °C (30 min). The mass spectrometer was used with electron ionization at 70 keV in the full scan mode. Compounds were identified by comparing major peak of chromatograph with those of mass spectra database generated from reference substances

Insect rearing

Parent adults *S. zeamais* and *C. maculatus* were obtained from colonies maintained at the Julius Kühn-Institute, Berlin, Germany since 1968 and 2011, respectively. *S. zeamais* were reared on disinfested maize in 2 l glass jars and *C. maculatus* on disinfested cowpea in 1 l glass jars, and the jars were kept under laboratory conditions (temperature: 25 ± 1° C and r.h.: 60 ± 5%). Insects aged 1 day for *C. maculatus* and between 7-14 days for *S. zeamais* were used for bioassay.

Bioassay

The application rates were 5, 10, 20, 30 and 40 g/kg of *P. glandulosus* leaf powder (Nukenine *et al.,* 2010). These rates were obtained by adding 0.25, 0.5, 1, 1.5 and 2 g powder to 50 g of cowpea or maize in a 250 ml glass jar which were placed on a bidimensional mixer (Gerhardt, Dreieich, Germany) for approximately 4 min to ensure uniform coating of powder to grains. Controls consisted of maize or cowpea without the test *P. glandulosus* powder. A group of 20 *S. zeamais* and *C. maculatus* of mixed sex were respectively added in each glass jar containing treated maize or cowpea. All treatments were arranged in a completely randomized design on shelves in the laboratory (temperature: 25 ± 1° C and r.h.: 60 ± 5) and each treatment had four replications. Mortality counts were recorded 1, 3, 7 and 14 days after treatment for *S. zeamais* and 1, 3 and 6 days for *C. maculatus*.

Statistical analyses of data

The mortality counts were corrected by using Abbott's formula Abbott, 1925). Data on % cumulative corrected mortality were transformed to arcsine [(square root(x/100)], then subjected to the ANOVA procedure of the Statistical Analysis System (SAS Version 9.2). Students'-*t*-test was used to compare the effect of drying regime on *P. glandulosus* insecticidal efficacy and Tukey's studentized (HSD) test was employed for mean separation with a significance of 95% (P = 0.05). The concentration required to kill 50% of insects (LC_{50}) was estimated using probit analysis Finney, 1971).

Results and discussion

Chemicals constituents of the powders

The trend of the semi-quantitative analysis of the chemical composition of *P. glandulosus* leaf powders showed that a total of the same 50 compounds with variable proportions were found in the sun-dried and shade-dried leaves, respectively (Table 1). Thus, the drying method had less effect on the diversity of the volatile compounds of the leaves. However, the overall tendency was lower rates of volatiles in the sun-dried compared to the shade-dried leaves. Eighteen compounds had similar rates (proportions) in the two drying-regime leaf powders, indicating that they were not significantly affected by the drying regime. Twenty four other compounds were higher in proportion in the shade-dried leaves compared to the sun-dried ones, with three (terpinolene, germacrene D and piperitone oxide) of them being particularly abundant in the shade-dried leaves. Only eight compounds were more abundant in the sun-dried than the shade-dried leaves. The compounds found in higher proportion in sun-dried leaves were oxygenated terpenes among which seven of them were oxygenated monoterpenes. All monoterpene hydrocarbons and more than half of sesquiterpene hydrocarbons were found abundantly in the shade-dried leaves. Previous studies (Hassanpouraghdam *et al.,* 2010, Najafian and Agah, 2012, Shahhoseini *et al.,* 2013) showed that drying plant parts in sunlight affected greatly the content of chemicals. Sellami *et al.* (2011) reported that the increase of temperature during drying process leads to the rapid release of monoterpenes which resulted in the loss of most monoterpene hydrocarbons (Pirbalouti *et al.*, 2013). Some compounds seem to have more affinity to the water fraction contained in the leaves and thereby were lost during drying (Pirbalouti *et al.,* 2013). Plants which belong to Lamiaceae family as *P. glandulousus* are known to keep their volatile compounds on or near the leaf surfaces and then easily lose such compounds when the temperature increases (Sellami *et al.,* 2011).This might explain the loss of hydrocarbon constituents of sun-dried leaves. Essential oils obtained from room dried leaves of *P. glandulousus* collected from the same location like

that in our present study, showed a higher percentage of piperitone oxide (Ngassoum *et al.,* 2001), when the leaves were shade-dried. This confirms the higher peak of piperitone oxide with the powder from the shade-dried leaves in the present study. Sun-drying effect is not only a consequence of the disappearance of some compounds but may also result in the appearance of others, which were absent or found in smaller quantities in the fresh or shade-dried leaves (Pirbalouti *et al.,* 2013).

Table 1. Comparison of the chemical constituents of the powders from shade- and sun-dried leaves of *Plectranthus glandulosus* collected at Ngaoundere, Cameroon.

Retention time	*Compound*	*shade*	*sun*
Monoterpene hydrocarbons			
7.21	α-Pinene	X	
8.77	Camphene	X	
10.26	β-Pinene	*	*
12.15	3-Carene	X	
12.91	Sabinene	X	
13.53	α-Terpinene	X	
14.40	Limonene	X	
16.16	R-α-Pinene	X	
16.57	γ-Terpinene	*	*
16.91	Ocimene (Z)-β or α	X	
17.68	Cymene	X	
18.31	Terpinolene	X XX	
24.62	bis(1-Methylethylidene)-cyclobutene	X	
	Oxygenated monoterpenes		
14.94	1,8-Cineole	X	
23.44	Fenchone	*	*
25.11	Dehydro-para-Cymene		X
26.55	(Z)-Sabinene hydrate	X	
28.42	Camphor		X
29.93	Linalool		X
31.38	(+)-Fenchol		X
32.68	β-Cyclocitral	*	*
36.83	Piperitone oxide	XXX	
37.63	(E)-Piperitol	X	
39.76	Diosphenol		X
41.27	p-Cymene-8-ol		X
41.35	Geranylacetone	*	*
43.73	Chrysanthenone	*	*
44.29	β-Ionone, (E)-	*	*
51.62	Eugenol	*	*
52.22	Thymol		X
58.78	(E)-Carveole	*	*
	Sesquiterpene hydrocarbons		
27.30	α-Cubebene	*	*
30.69	β-Cubebene	X	
33.25	γ -Elemene	X	
34.42	α-Caryophyllene	*	*
35.28	2-Carene	*	*
35.94	(E)-Germacrene D	XXX	
38.31	Δ-Cadinene	X	
53.65	γ -Gurjunene	*	*
	Oxygenated sesquiterpenes		
47.62	Nerolidol, (E) or (Z)	*	*
54.23	Ledol	*	*
61.71	Solavetivone	X	
	Table 1 Continue		
Retention time	Compound	shade	sun

57.36	Ledene oxide		X
Fatty acids			
54.08	Ethylpalmitate	X	
61.95	Ethyl linoleate	X	
64.30	Ethyl linolenate	X	
68.89	Myristic acid	*	*
82.78	Palmitic acid	X	
	Aromatic compounds		
48.75	7-Methoxy-2,2-dimethyl-3-chromene	*	*
61.30	1.2.3-Trimethylindene	*	*

XXX The peak height of the compound was far much higher in this drying regime than the other with an empty space; X The peak height of the compound was higher in this drying regime than the other with an empty space, * Equal peak height of the compound was observed in both drying regimes.

The increase of temperature may trigger oxidation processes and chemical reorganization, which leads to the appearance of some new molecules or the rapid release of others (Asekun *et al.,* 2007, Pirbalouti *et al.,* 2013). This might justify the higher proportion of oxygenated volatiles in the sun-dried leaves than in the shade-dried ones. Hassanpouraghdam *et al.* (2010) compared the effect of drying method on chemical composition of *Ocimum basilicum*, a plant in the same family of Lamiaceae like *P. glandulousus*. These authors observed that the concentration of compounds as linalool and camphor increased more in sun-dried than the shade-dried leaves. Our finding is close to their observation. There are also some volatiles which are not affected by drying regime. γ-terpinene, fenchone, β-pinene and eugenol were found in equal proportion in the sun- and shade-dried leaves. Díaz-Maroto *et al.* (2003) demonstrated that substances like sesquiterpenes with relatively low volatility are released more easily than the other compounds. Changes in the concentration of chemical during drying are affected by the drying regime/method (solar energy, oven temperatures, etc.) (Hassanpouraghdam *et al.,* 2010, Najafian and Agah, 2012, Shahhoseini *et al.,* 2013). Therefore, the drying regime/method determines the obtained compounds in plant materials.

Adult mortality

As expected, both the powders from the shade- and sun-dried leaves of *P. glandulosus* generally caused significant adult mortality to *S. zeamais* and *C. maculatus* (Tables 2 and 3) relative to the control, although the mortality in *C. maculatus* was rather low. The powders from the sun-dried leaves caused higher (t = -1.29; P < 0.001) mortality to *S. zeamais* than those from the shade-dried ones (Table 2), but the mortality with *C. maculatus* was similar (t = 0.34; P > 0.05) for the two drying regimes (Table 3). That the moisture contents of the powder from the sun- (8.7%) and shade-dried (8.9%) leaves were similar precludes any influence of the volume of the botanical on the mortality of *S. zeamais*. Percentage mortality increased with increasing powder contents and days post-exposure for both insect pests. Within 6 days of exposure and at the powder content of 40 g/kg ca. 50% mortality was recorded in *C. maculatus,* while *S. zeamais* registered 100% mortality within 7 days. The two insects showed similar susceptibility (P > 0.05; t-Test) to the shade-dried leaves. For the powders from the sun-dried leaves, the 6-d and 7-d LC_{50} values were 47.37 and 14.04 g/kg respectively for *S. zeamais* and *C. maculatus,* indicating that the former insect was more susceptible to the leaf powder than the later. This could be attributed to the fact that adult *S. zeamais* fed on the treated grains while *C. maculatus,* as all other bruchids, did not feed on the seeds, and thus did not ingest the plant powders. The intake of powder during feeding might act as stomach poison which led to the higher death rate of the adult insects in the case of *S. zeamais* (Mulungu *et al.,* 2007). Mulungu *et al.* (2010) reported that *S. zeamais* was more susceptible to botanicals than *P. truncatus*. These authors demonstrated that since adult *S. zeamais* spend more time feeding on the surface of grains while *P. truncatus* is found most of the time within the grain, the former insect usually ingest more surface insecticides than the latter. *P.*

glandulosus being an aromatic plant, might have released toxic volatiles from the powders which contributed to the death of the two insect species. Adult *C. maculatus* was more susceptible to the leaf powder of another aromatic plant (*Dracaena arborea*), than adult *S. zeamais* (Udo *et al.*, 2011), probably because the volatile compounds from the plant were more toxic to the former than the latter insect.

Table 2. Comparison of corrected cumulative mortality of *Sitophilus zeamais* exposed in grains treated with leaf powders of *Plectranthus glandulosus* obtained from sun-dried and shade-dried leaves.

Time exposure (days)	*Doses (g/kg)*	P. glandulosus *leaves*		t *value*
		Sun-dried	Shade-dried	
1				
	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	
	5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	20	0.00 ± 0.00^{a}	1.25 ± 1.25^{a}	1 ns
	30	0.00 ± 0.00^{a}	8.75 ± 4.27^{b}	2.05 ns
	40	2.50 ± 1.44^{a}	22.50 ± 4.33^{c}	4.38 ns
	F value	3.00 ns	15.01***	
3				
	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	
	5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	10	2.50 ± 2.50^{ab}	0.00 ± 0.00^{a}	-1 ns
	20	6.25 ± 1.25^{bc}	6.25 ± 2.30 ^{b}	0 ns
	30	13.75 ± 2.39^{cd}	17.50 ± 3.23^{c}	0.93 ns
	40	22.50 ± 1.44^{d}	33.75 ± 4.27^{d}	2.50 ns
	F value	30.15***	42.88***	
	LC_{50}†	88.84(62.16-189.22) ᵝ	50.87(43.76- 66.94) ᵝ	
7				
	0	0.00 ± 0.00^{a}	0.00 ± 0.00 ^{a}	
	5	13.75 ± 6.88^{ab}	17.50 ± 7.22^{b}	0.38 ns
	10	28.75 ± 4.27^{bc}	18.75 ± 3.75^{bc}	- 1.76 ns
	20	42.50 ± 6.61^{c}	41.25 ± 1.25^{cd}	- 0.19 *
	30	97.50 ± 2.50^{d}	45.00 ± 7.91^{cd}	- 6.63 ns
	40	100 ± 0.00^{d}	55.00 ± 7.91^{d}	5.69 ***
	F value	80.13***	16.91***	
	LC_{50}†	14.04(12.21-19.70)	34.51(27.10-49.62)	
14				
	0	0.00 ± 0.00^{a}	0.00 ± 0.00 ^{a}	
	5	37.04 ± 10.09^{b}	22.83 ± 7.28^{b}	-1.08 ns
	10	56.84 ± 8.75^{b}	40.26 ± 2.28^{c}	- 1.10 ns
	20	89.87 ± 3.54^{c}	69.47 ± 5.74^{cd}	- 3.02 *
	30	100 ± 0.00^{c}	79.80 ± 2.78^{d}	- 7.27 ***
	40	100 ± 0.00^{c}	83.43 ± 2.54^{d}	- 6.59 ns
	F value	69.17***	36.32***	
	LC_{50}†	7.28 (4.01-10.20)	12.12(10.35-13.94)	

Means ± SE in the same column followed by the same lowercase letter within the same group of treatments do not differ significantly at P = 0.05 (Tukey's test). ns non significant; * P<0.05; *** P<0.001; – *t*-test is impossible due to no mortality observed; ᵝ LC_{50} values were estimated by extrapolation; † Value in brackets are confident limits.

It is widely reported that the direct exposure of plants to sunlight or increasing temperatures has an effect on the sensitive compounds, leading to photodegradation or thermodegradation (Müller and Heindl, 2006, Ngamo *et al.,* 2007c). In addition, Plant materials for insect bioassays studies are generally dried under shade conditions (Arannilewa *et al.,* 2006, Ngamo *et al.,* 2007c, Goudoum *et al.,*

2012). Nukenine *et al.* (2013) revealed that the powder from the shade-dried leaves of *P. glandulosus,* with unknown chemical composition, was more effective against *S. zeamais* compared to sun-dried under fluctuating laboratory conditions. However, the present study indicated that under controlled laboratory conditions, mortality of *S. zeamais* was higher with the powders from the leaves of the same plant dried in sun light compared to those from shade-dried leaves. A mixture with higher levels of camphor with other phytochemicals like linalool was highly toxic to *S. zeamais* while phytochemical mixtures lacking camphor was more or less inactive against this insect (Bekele and Hassanali, 2001). In this line, the higher levels of camphor and thymol in the sun-dried leaves might have been responsible for the higher potency of the sun- compared to the shade-dried powders against adult *S. zeamais*. More so, the rate of linalool was higher in the sun- than the shade-dried leaves, and this compound was reported to act on the nervous system of insects, affecting ion transport and the release of acetylcholinesterase, which results in total breakdown of the nervous system (López and Pascual-Villabolos, 2010; Shukla *et al.,* 2011; Yeom *et al.,* 2012). As a corollary, as has been the practice, shade-drying of plant leaves may not improve their toxicity towards insects. Therefore, there is a need to intensify efforts towards studies involving different drying regimes of plant materials and bioactivity against several species of insects, since photodegradation and thermodegradation may not always correlate directly with insecticidal efficacy.

The results of our study showed that drying leaves of *P. glandulosus* in sunlight as compared to shade-drying could lead to a reduction in the rates of some volatile compounds found in the leaves and known for their insecticidal properties, such as, α-pinene, camphene, limonene 1, 8-cineole, cymene, ocimene, piperitone-1-oxide, terpinolene and Limonene. However, this photodegradation did not reduce the insecticidal efficacy of the sun-dried leaf powder, which instead showed better efficacy against *S. zeamais. C. maculatus* was less susceptible to *P. glandulosus* than *S. zeamais,* regardless of drying regime. Due to its pronounced efficacy on *S. zeamais*, the powder from sun-dried leaves of *P.glandulosus* stands as a potential candidate at the farmers' level for the control of *S. zeamais* in stored maize. *P. glandulosus* with modest efficacy against *C. maculatus* could be the object of further investigations, such as, its effects on the immature stages or ability to inhibit progeny production in the bruchid.

Table 3. Comparison of corrected cumulative mortality of *Callosobruchus maculatus* exposed in grains treated with leaf powders of *Plectranthus glandulosus* obtained from sun-dried and shade-dried leaves.

Time exposure (days)	*Doses (g/kg)*	P. glandulosus *leaves*		t *value*
		Sun-dried	Shade-dried	
1				
	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	
	5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	20	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	30	0.00 ± 0.00^{a}	1.25 ± 1.25^{a}	1 ns
	40	2.50 ± 1.44^{a}	1.25 ± 1.25^{a}	- 0.65 ns
	F value	3 ns	0.80 ns	
3				
	0	0.00 ± 0.00^{a}	0.00 ± 0,00^{a}	
	5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
	20	1.25 ± 1.25^{a}	0.00 ± 0.00^{a}	- 1 ns
	30	6.25 ± 1.15^{b}	0.00 ± 0.00^{a}	- 5.00 **
	40	20.00 ± 2.04^{c}	10.00 ± 6.12^{a}	- 1.55 ns
	F value	49.08***	3 ns	
	LC_{50}†β	59.95(32.07-105)β	42.57(41.58-43.27)β	

6				
	0	0.00 ± 0.00[a]	0.00 ± 0.00 [a]	
	5	18.46 ± 7.83[b]	10.27 ± 2.27[b]	- 0.92 ns
	10	31.84 ± 2.03[bc]	24.66 ± 1.43[bc]	- 2.90 ns
	20	36.03 ± 5.42[bc]	32.82 ± 1.87[cd]	- 0.56 ns
	30	34.64 ± 5.82[bc]	32.68 ± 7.75[cd]	- 0.20 ns
	40	54.11 ± 4.23[c]	49.34 ± 2.36[d]	- 0.98 ns
	F value	16.96***	41.28***	
	LC_{50}†	47.37 (49.21-99.67)[β]	51.29(36.74-43.27) [β]	

Means ± SE in the same column followed by the same lowercase letter within the same group of treatments do not differ significantly at P = 0.05 (Tukey's test). ns non significant; ** P < 0.01; *** P < 0.001; – *t*-test is impossible due to no mortality observed; [β] LC_{50} values were estimated by extrapolation; † Value in brackets are confident limits.

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