Potential of *Ocimum basilicum* L. and *Pandanus tectorius* Parkinson from the ecology of Al-Makhwah, Saudi Arabia in controlling *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst).

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Abstract: This study was initiated to determine the potential of *Pandanus tectorius* and *Ocimum basilicum* in controlling the population of *Sitophilus oryzae* and *Tribolium castaneum*. These plants are found in abundance in Saudi Arabia and can be promising alternatives to synthetic insecticides. Repellent and contact activities of these plant powders were tested against *S. oryzae* and *T. castaneum*. The flavonoid contents of both plants were also identified in an effort to associate flavonoids in controlling the population or the development of these pest insects. *S. oryzae* was unaffected by both *P. tectorius* and *O. basilicum* in the repellency study. However, both plants exhibited repellency properties when tested against *T. castaneum*. The distribution of test insects varied between treated and untreated areas depending on the time of exposure. Both test insect species were not affected when they came into contact with the plant powders, while no or low number of progeny emerged after 56 days. Two types of flavonoids were identified from *P. tectorius* and *O. basilicum*, namely apigenin and quercetin. The potential of these flavonoids as insecticides are further discussed.

[Amal AMA, Wong SC. **Potential of *Ocimum basilicum* L. and *Pandanus tectorius* Parkinson from the ecology of Al-Makhwah, Saudi Arabia in controlling *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst).** *Life Sci J* 2013;10(4):2996-3000]. (ISSN:1097-8135). http://www.lifesciencesite.com. 398

Keywords: Plant powders; stored product insects; development; contact; repellent; flavonoids

1. Introduction

Stored food products are usually protected from insect pest attacks or infestation by using synthetic insecticides (Isman, 2006). However, synthetic insecticides have their drawbacks which include undesirable effects on food residues, their human toxicity, ecotoxicological risks and problems with pests developing resistance. Thus, efforts to find alternative control methods are ever increasing (Philips et al., 2010; and Riudavets et al., 2010). Insect pests can be managed by using natural products as they have been identified to possess many beneficial biological properties (Arthur, 1996) suggesting that the uses of natural products such as plants are potential alternatives to synthetic insecticides. Many entomologists and toxicologist are turning to the use of folk medicinal plants that are grown locally in the effort to find materials that can effectively control these insects in an affordable, less poisonous and more environmental friendly (Niber, 1994). Even various spices have been evaluated against stored product insects (Golob et al., 1999). The most promising botanicals have been identified in the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae (Jacobson, 1989).

The common basil, *Ocimum basilicum* is an herb with aromatic leaves that possesses culinary and medicinal purpose. It is reported that the essential oils of *O. basilicum* shows antifungal and insect-repelling properties (Sakalauskaitė et al., 2012). The essential oils of *O. basilicum* have been tested against mosquitoes, where they are effective against the larvae stages (Murugan et al., 2007). Besides that, the extracts of *O. basilicum* showed insecticidal activities against the third instar larvae of Egyptian cottonworm (*Spodoptera littoralis*) (Pavela, 2004). Besides that, Quarles (1999) reported that *Ocimum* spp. is also effective in controlling garden pests. The screwpine, *Pandanus tectorius* can be found growing in coastal, inland and mountain areas (Nandwani et al., 2008) and it is cultivated in the native areas including Saudi Arabia (Lim, 2012). Thomson et al. (2006) described this plant to possess various uses of health, cultural and economic importance.

The rice weevil, *Sitophilus oryzae* (L.) and red flour beetle, *Tribolium castaneum* Herbst are both cosmopolitan pests attacking a wide range of stored products (Rees, 2004). They are amongst the most economically important pests of stored products with a worldwide distribution (Weston et al., 2000; and Pugazhvendon et al., 2009) but are especially prevalent in warm and humid countries (Batta, 2004).

The aim of this study is to determine the potential of *O. basilicum* and *P. tectorius* in controlling *S. oryzae* and *T. castaneum*. Repellent and/or attractant activities as well as contact toxicity of these plants in powder form was investigated against both test insects. This paper also identifies the type of flavonoids present in both plants.

2. Materials and methods

Plant collection. Both *M. communis* and *P. tectorius* were collected from Al-Makhwah, Saudi Arabia. The leaves of both plants were randomly collected and packed into plastic zipper bags before being transported to the laboratory. The collected fresh leaves (10 kg) of both plants were air-dried in the laboratory for about 2 weeks. The dried leaves were then ground into fine powder with a food blender (Panasonic MX-337RA) prior to the initiation of the experiment, while the remaining leaves were left undisturbed.

Soil analysis. The surrounding soil (approximately 2 kg) of both plants was subjected to soil analysis. The soil textural components were determined by using the sieve method as described by Al-Yamani et al. (2006). Soil water content and pH levels of the soil were tested according to the methods carried out by Conklin (2005), while organic matter content (OM) were determined using the method stated by Wilde et al. (1972).

Test insects. S. oryzae were reared in the laboratory on polished rice and *T. castaneum* were reared on wheat flour in round plastic containers (9cm in height x 9cm in diameter) in a closed cabinet at constant temperature of 28.0±0.5°C and 65.0±2.0% relative humidity with a photoperiod of 12h light: 12h darkness. Adults of *S. oryzae* (2-3 weeks old) and *T. castaneum* (1-2 weeks old) were used in all the experiments.

Repellent/attractant activities. Each plant was evaluated for repellent or attractant activities by using 0.15g of powdered leaves for every 5g of food substrate. Treated and untreated food substrates (5g each) were placed on opposite sides of a Petri dish. The rims of each Petri dish were applied with a thin layer of fluon which is a polytetrafluoroethylene (PTFE) to prevent test insects from escaping. Ten adult insects of each test species were introduced into the center of the Petri dish. After 1h, the number of insects found on either side of the Petri dish (treated or untreated substrates) were counted and recorded. This was repeated at an interval of 1h up to the 6^{th} h. The experiment was replicated five times and percentage repellency (PR) values were computed as PR = $[(N_C - N_T)/(N_C + N_T)] \times 100$, where N_C = number of insects in the control area and N_T = number of insects in the treated area.

Contact toxicity. Ground powdered leaves of both *O. basilicum* and *P. tectorius* were evaluated for contact activities. 1.5 g of powdered leaves was mix into 5 g of food substrate (rice grains/wheat flour) and placed into 10 ml glass vials. Ten adult insects were introduced into each vial and the cap was screwed on tightly. Five replicates were carried out for each test insect. Insect mortality was recorded daily up to day 7. Any living insects were then removed on day 14 and progeny numbers, if any were counted and recorded at day 56.

Isolation and identification of flavonoids. The leaves of both *P. tectorius* and *O. basilicum* were collected, dried at 60°C and then grounded into powder form. The powder (300 g) was then extracted with methanol and concentrated. A filter paper was used to filter the aqueous methanolic solution several times and further filtration of the solution was done with ether in a separating funnel. The solution in the separating funnel was collected and column chromatography (absorbent: polyamide; eluting solvents: ethanol: 70, 80, 90 and 100 %) was conducted as stated by Harborne and Mabry (1982). Next, paper chromatography (PC) (filter paper: Whatman no. 1 and 3; developing solvent systems) was carried out to allow the exact determination of the compound of the extraction as well as to quantity the amount of flavonoid detected. Finally, thin layer chromatography (TLC) (dimension: 20 x 20 cm; adsorbent: silica gel plates, aglycones: developing solvent system; BPF: benzene, pyriden, formic acid; spray agents: ammonia) was performed according to the methods by Medic-Saric et al (1999). Flavonoids detected from the root were compared and identified using the methods as described by Khogali et al. (2006).

Data analysis. Insect distribution between treated and untreated areas were analyzed using Paired t-test at α=0.05. While adult emergences were analyzed with one-way (ANOVA) analysis of variance and means were separated by using Tukey HSD. All data analyses were carried out using SPSS Version 16.0 (Chicago, USA).

3. Results and Discussion

M. communis and *P. tectorius* from the Al-Makhwah area thrived well at a relatively neutral soil pH of about 7.5. It contains about 8% of organic matter. The soils where the plants grow are rather moist with 9% water content. A large portion (more than 70%) of the soil consists of coarse sand. Clay was found to be the least in the soil with only 3% (Table 1).

This study found that *S. oryzae* was not repelled by both *P. tectorius* and *O. basilicum* (Table 2). This clearly shows that *S. oryzae* are not affected by the presence of the plant powders. Nevertheless, significant differences in adult weevil distribution were found between treated and untreated areas of the experimental setup at the 3rd until the 6th hour of exposure when the weevils were exposed to *P. tectorius*, while distribution of *S. oryzae* was significantly different an hour after exposure until the 4th hour when tested with *O. basilicum*.

Table 1. Physicochemical properties of soil surrounding the growth of *M. communis* and *P. tectorius* from Al-Makhwah, Saudi Arabia.

Characteristics		Mean (±S.E.)
pH		7.53 ± 0.09
OM (%)		7.83 ± 0.14
Water content (%)		8.94 ± 0.20
Soil texture	Coarse sand (%)	73.28 ± 1.65
	Fine sand (%)	17.78 ± 2.04
	Silt (%)	5.91 ± 0.66
	Clay (%)	3.03 ± 0.24

Table 2: Percentage repellency of *S. oryzae* towards *P. tectorius* and *O. basilicum* leaves powder over a period of 6 hours.

	Percentage repellency (PR)	
Time (h)	*P. tectorius*	*O. basilicum*
1	-28.0±16.2	-52.0±10.2*
2	-24.0±17.2	-56.0±4.0*
3	-36.0±9.8*	-24.0±7.5*
4	-36.0±4.0*	-32.0±4.9*
5	-36.0±4.0*	-40.0±6.3
6	-24.0±4.0*	-32.0±13.6

*Significant difference between treated and untreated sections (Paired t-test) at $\alpha = 0.05$.
(-) negative: No repellency

Table 3: Percentage repellency of *T. castaneum* towards *P. tectorius* and *O. basilicum* leaves powder over a period of 6 hours

	Percentage repellency (PR)	
Time (h)	*P. tectorius*	*O. basilicum*
1	72.0±8.0*	44.0±16.0
2	80.0±8.9*	32.0±18.5
3	88.0±4.9*	72.0±13.6*
4	84.0±7.5*	76.0±9.8*
5	88.0±8.0*	76.0±9.8*
6	88.0±8.0*	72.0±8.0*

*Significant difference between treated and untreated sections (Paired t-test) at $\alpha = 0.05$.
(-) negative: No repellency

In contrast, up to 88 and 76% of *T. castaneum* were repelled by the powders of *P. tectorius* and *O. basilicum*, respectively (Table 3). There was a significant difference in distribution numbers between treated and untreated areas throughout the experimental period when *T. castaneum* was exposed to *P. tectorius*. Initially, *T. castaneum* distribution was not significantly different between treated and untreated areas when tested with *O. basilicum*. However, at the 3rd until the 6th hour of exposure, significant difference in distribution numbers was found.

Papachristos et al. (2002) found that the essential oil of *O. basilicum* have repellent properties, reduces fecundity, decreases egg hatchability, increases neonate larval mortality and adversely influence offspring emergence. In this study, *T. castaneum* were more repelled by both of the plant powders than compared to *S. oryzae*. There is still inadequate information regarding the effects of these plant powders on *S. oryzae* and *T. castaneum*. Nevertheless, studies have been conducted to test the repellency of powdered spices against *Rhyzopertha dominica* (F.), *Sitophilus granarius* (L.) and *T. castaneum*, where as much as 92.5% of repellency was recorded (Shayesteh et al., 2010). Besides that, Udo (2005) recorded 80% repellency effect on *Sitophilus zeamais* (Mots.) when tested with black pepper, *Piper nigrum* L.. Adult *S. oryzae* and *T. castaneum* were not affected when in contact with *P. tectorius* and *O. basilicum* powders (Table 4).

Table 4: Mortality of *S. oryzae* and *T. castaneum* 14 days after contacting with *P. tectorius* and *O. basilicum* powders

Insect species	% Mean (± SE)	
	P. tectorius Powder	*O. basilicum* Powder
S. oryzae	5.6 ± 0.7	1.2 ± 0.7
T. castaneum	6.4 ± 0.6	1.6 ± 0.7

Table 5: Mean (± SE) number of emerging adults of *S. oryzae* and *T. castaneum* at day 56

Insect species	Control	Treatment	
		P.tectorius powder	*O.basilicum* powder
S. oryzae	194.0±3.4a	11.3±5.1b	7.8±3.2b
T. castaneum	21.0±1.1a	0.0±0.0b	0.4±0.4b

a Means followed by different letter(s) within the same column were significantly different (P<0.0.5; Tukey HSD)

Less than 7 % mortality of *S. oryzae* was recorded, while only about 2 % mortality was recorded for *T. castaneum*. In the controls, about 194 adult *S. oryzae* emerged while an average of 21 adult *T. castaneum* emerged at day 56. However, none or very low numbers of *S. oryzae* and *T. castaneum* progeny emerged from the treated medium (Table 5). Although emerging adult numbers were very low, observation showed that a lot of *T. castaneum* larvae were present amongst the medium. It is possible that the application of *P. tectorius* and *O. basilicum* powders into the medium prolonged the developmental time of *T. castaneum* larvae, while the development of *S. oryzae* were inhibited. Therefore, these plant powders pose potential in protecting stored products (rice and wheat flour) against *S. oryzae* and *T. castaneum*.

In this study, apigenin and quercetin were identified from the leaves extract of *O. basilicum* and *P. tectorius*. Both of these compounds are polyphenols that are categorized as flavonoids (Miean et al., 2001). Flavonoids are widely distributed throughout plants and prokaryotes (Middleton, 1998; Woo et al., 2002) and give colour to flowers, as well as defend plants against microbes and insects (Griesbach, 2005). Various flavonoids were studied for their bioactivity and it was reported that these compounds could alter the feeding and oviposition behavior of insects (Simmonds, 2001; 2003). Stamp et al. (1996) stated that different flavonoids changes molting in insects, subsequently causing death. Huang et al. (2008) reported that apigenin was effective at controlling the population of the Diamondback moth, *Plutella xylostella*. A study by Salunke et al. (2005) showed that adult *Callosobruchus chinensis* were significantly affected when tested with partially purified flavonoids of *Calotropis procera* and standard quercetin. Although only *T. castaneum* was affected by *P. tectorius* and *O. basilicum*, it shows a promising alternative to control their infestation.

4. Conclusion

Even though *P. tectorius* and *O. basilicum* did not repel *S. oryzae*, *T. castaneum* were significantly repelled by the powders of these plants. *P. tectorius* and *O. basilicum* did not affect the survival of *S. oryzae* and *T. castaneum* by contact. However, progeny developments in both insect species were significantly affected. It is suggested that flavonoids are potential grain protectants through contact oviposition deterrent and ovicidal action. Compounds other than apigenin and quercetin from these plants may have an effect on these insects. Therefore, further studies are necessary to determine the relative amounts of these materials quantitatively and tested individually against both *S. oryzae* and *T. castaneum*. Moreover, other compounds should be identified to determine the compound responsible for affecting the development of both *T. castaneum* and *S. oryzae*.

Acknowledgement

The authors would like to thank King Abdul Aziz University for the assistance provided.

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12/11/2013