Development of essential oil based tablets from *Cinnamomum zeylanicum* leaves and *Cymbopogon nardus* against the stored grain pest insect, *Sitophilus oryzae* in traditional rice varieties in Sri Lanka

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Abstract

Essential oils have been used as an alternative method to reduce postharvest losses from rice weevil, *Sitophilus oryzae* in traditional rice varieties cultivated in Sri Lanka. Essential oils extracted from Cinnamon leaves (*Cinnomomum zeylanicum*) and Citronella leaves (*Cymbopogon nardus)* were used to control rice weevil, in three traditional rice varieties, *Rathkanda al*, *Pachchaperumal*, *Suwandal* along with a hybrid variety, White raw rice cultivated in Sri Lanka. Kaolin pellets treated with essential oil mixture, *C. zeylanicum* and *C. nardus* (2: 1 v / v) were used to test the effect on the storage of rice. The major components of the essential oils of *C. zeylanicum* leaf oil and citronella oil were eugenol, citronellal and geraniol. Number of *S. oryzae* in treated rice samples throughout the test period were significantly decreased compared with the controls. A significant decrease of % seed damage was also observed during the 6 months of storage of rice in polyethylene bags. Flavor, Aroma, and Stickiness of cooked rice have enhanced significantly in all treated rice varieties compared to controls. The physical and organoleptic properties of cooked rice were not significantly different with controls after 6 months of treatment. Only 5 % weigh loss of kaolin tablets was observed after the completion of the test time period. The insecticidal activity of the essential oil mixture was highest on the rice type *Suwandal.*

Key words: *Cinnamomum zeylanicum*, *Cymbopogon nardus*, *Sitophilus oryzae,* Organoleptic properties, Essential oils, Rice.

The post harvest loss in rice in Sri Lanka is around 15%. Among many factors that result in post harvest losses the highest loss of 5%- 6% is caused by insect pests in storage and losses in stored rice due to insect pests could go even up to 8.8% (Dharmasena and Abeysiriwardena, 2003). These losses include the loss of weight, nutrients and germination inability, poor smell and taste, grain discoloration, aggregation of grains, development of fungus, and finally the economic loss due to the reduced market value. (Wijerathna *et al* ., 2015).

The insect damage on stored products was mainly by direct feeding. Some species feed on the endosperm causing loss of weight and quality, while other species feed on the germ, resulting in poor seed germination and less viability. Thus, due to damage done by insects, grains lose value for marketing, consumption or planting. Damage done by insect pests encourages infection with bacterial and fungal diseases through transmission of their spores. The presence of insects also raises the product temperature, due to their feeding activity, resulting in "hot spots". These spots in turn lead to concentration of humidity within the product, thus stimulating seed deterioration and further fungal activity (Sallam, 2013). Among the stored grain pests, rice weevil (*S. oryzae* L) has been reported as one of the severe pests of stored cereal grains (Majeed, 2011) and sometimes, rice weevil infests grain in the field before harvest.

Rice weevils *S. oryzae* (L) do not attack grains with intact hulls (Way and Bowling, 1991). Milled rice is an excellent medium for growth of storage insects, such as rice weevil.

A wide range of contact insecticides is commercially available in Sri Lanka, Actellic (Active ingredient, Pirimiphos-methyl) had been widely used and become popular among farm level as well as commercial level grain collectors till recently. However, currently due to high cost and unavailability of this popular insecticide, need has risen for an effective insecticide. So, it is necessary to find an environmental friendly, less toxic alternative effective insecticide, which can replace actellic. Currently available and registered samples of insecticides (Soflac (*Cyfluthrin*), Tracer (*Spinosad*) and Actara (*Thiamethoxan*)) were screened for their efficacy and toxicity against rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) under laboratory conditions (Samarakoon and Thilakarathne, 2012).

Substantial amounts of stored grain products are attacked by fungi, insects and animals worldwide. Synthetic chemicals and fumigants have been widely used for preventing quality deterioration of stored products. But, these agents will be phased out in the near future due to their potential adverse impact on the environment. Therefore, biodegradable

alternatives should be developed worldwide for reducing postharvest losses. In fact, selected plants and their essential oils have been evaluated as natural sources for controlling storage fungi. Some of the aromatic plants which are widely distributed in the tropical zone that exhibit fungicidal activities, have traditionally been used as flavoring agents in native dishes, and as incense, insect repellents and folk medicine (Nakahara *et al.*,2003).

In Sri Lanka, farmers use essential oil bearing plants, which release terpenes for the control of stored grain pests. *Cympobogon nardus* Rendle and *Cinnomomum zeylanicum* are some of the essential oils used to control insects in rice (Paranagama *et al* .,2003) and sometimes, in initial step of damage, farmers kept Margosa and Citrus leaves in the bags to repel the insects. Wood ash obtained from the kitchen is mixed with paddy by some farmers in addition to the use of aromatic leaves (Wijerathna *et al*., 2015). Recent laboratory experiments with different botanicals have indicated the potential of plant materials or / and different extracts of plant parts to be used against stored pests of grain (Fernando & Karunaratne, 2013).

These traditional practices have encouraged research on development of alternatives to the hazardous synthetic pesticides. Cympobogon nardus and Cinnomomum zeylanicum are spices and meditational herbs found in Sri Lanka known to contain essential oils. As an economic environmental friendly and less toxic storage pest control method, these essential oils could be used against S. oryzae of stored rice.

Cynomomum zeylanicum and C. nardus oils have been traditionally distilled in Sri Lanka for many years and exported to USA, UK, France etc., mainly to perfumery and fragrances industry. There are no value added products of essential oil based insecticides on commercial scale at present. The present study was an attempt to develop a new value added product from essential oil based products and, they would be an environment friendly, cost effective alternative stored grain pest controlling agent.

Methodology

Essential oils: The commercial samples of essential oils of *C. zeylanicum* leaf and *C. nardus* leaf oil were gifted from EOAS organics private limited, *Rathmalana.*

Gas Chromatographic analysis of (GC) the essential oils: The chemical constituents of the essential oils used for the present study was analyzed on a GC having the following specifications

(SHIMADZU GC- 2025 (AFC / APC) Series Ⅰ Chromatograph, Albert, Hahn, Strasse 6-10, Duishurg, F.R. Germany with FID and DB wax capillary column ,30 m × 0.25 mm; 0.25 µm film thickness) The column temperature was programmed as follows: 50 °C- 210 °C at 2 °C / min, 210 °C (10 min) with Helium Carrier Gas (1 ml/ min) . The injector and the detector temperature was 250° C and1µl of oil solution in CH_2Cl_2 (2 mg / ml) was injected and the constituents were analyzed and compared with published data (Paranagama, 1991).

Preparation of clay tablets as an absorbent material for the essential oils: Clay powder used in this study was collected from domestic clay pot designers in Kelaniya area. Distilled water (65 ml) was added to 100 g of kaolin powder and mixed thoroughly to make a paste. The prepared paste was passed through a glass tube (0.5 cm diameter) to obtain cylindrical tablets of 1.5 cm height, 5mm width; then, clay tablets were air dried for 3days.

Individually pre weighed dried clay tablets were introduced to Cinnamon leaf oil: Citronella oil (2: 1, v / v) mixture, (15 ml). The weight of the each tablet was obtained every one hour and this was continued until a constant weight was obtained for clay tablets. It was observed that the weights of each tablet were constant after 2.5 hours confirming the maximum absorbance of essential oil mixture in the clay tablets. Clay tablets saturated with the essential oils were air dried for 10-15 minutes. The tablets (containing equal amounts of essential oil mixture / 0.24 g) were introduced to each polyethylene bag containing 250 g of test rice samples (3 clay tablets per 1 packet).

Preparation of traditional rice samples with oil treated clay tablets: Freshly harvested and de husked untreated traditional rice samples were obtained directly from farmers in *Nugegoda* area and 250 g of raw rice from each variety (*Suwandal* , *Pachchaperumal* ,*Rathkanda Al*, and hybrid variety-White Raw) was introduced to polyethylene bags (transparent, 6 inches width, 200 gauge) separately, 3 oil treated clay pellets containing 0.24 g of essential oil mixture were placed into each bag. Polyethylene bags were sealed with a polyethylene sealer.

Six packets of each rice type were prepared by adding untreated clay pellets as the controls. Eighteen packets of each rice variety with treated clay tablets were used in this study. During the test period (6 months), treated rice samples and untreated controls were kept in 2 plastic storage bins separately without exposing them to direct sunlight. Once a month 3 packets of each treated rice variety, and 1packet of each control were drawn randomly from the plastic storage bins for evaluation.

Determination of Mass loss of treated clay cubes against the test period: Air dried clay pellets were weighed individually, and recorded. They were placed in a beaker and essential oil

mixture was poured on it. Clay tablets were placed in essential oil mixture for 2.5 hours to saturate with oil mixture. Then the clay tablets were air dried and reweighed. Three rice packets were prepared with 250 g of untreated rice. Three clay tablets containing 0.24 g of essential oil mixture were introduced to each polyethylene rice packet, sealed and stored in storing bin. Another three rice packets contained 250 g of rice were prepared as controls with untreated clay tablets. Once a month rice packets were opened and clay tablets were weighed. This procedure was repeated throughout the test period in order to evaluate the rate of evaporation of the essential oils.

Statistical Analysis: Data on population of *S. oryzae* and % seed damage, during the storage period were statistically analyzed using one-way ANOVA and Tukey's pair wise comparison tests in MINITAB Statistical package. Data obtained by taste panel were statistically analyzed using Kruscal Wallis and Mann Whitney U test in MINITAB 17 statistical package.

Results

The GC analysis of the essential oil obtained from *C. zeylanicum* leaf indicated the presence of 19 compounds. Of these 18 were identified. Eugenol (74.33 %) was identified as the major compound and second most abundant component was acetyle eugenol (3.9 %) and third component was β- Caryophyllene (3.5 %). Citronella commercial sample contained 30 detectable compounds, 22 were identified**.** Citronellal and geraniol were the major compounds with 27.12 % and 22.11 % respectively.

The effect of essential oils on population of *S.oryzae.*

Sitophilus oryzae was not observed in rice type *Suwandal* in both untreated control and the test sample during the test period.

Rice Type		The average no of *S. oryzae* in rice varieties Time duration (Months) 1	2	3	4	5	6
Rathkanda al	Control	5±1.5	18± 3.6	57±10.8	109±9.5	112±11.1	133±18.5
	Treated Sample	6± 1.5	6± 0.5	16± 2.1	36± 7.8	48± 10.9	89± 8.2
White raw	Control	0	2± 0.6	0	3± 0.8	37± 5.3	114± 2.4
	Treated Sample	0	0	0	3± 0.5	10± 0.7	13± 1.5

	Control	15± 1.6	4± 1.8	2± 1.2	4± 0.5	128± 7.8	147± 13.8
Pachchaperumal	Treated Sample	1± 0.3	2± 1.1	2± 0.5	3± 1.4	10± 2.2	51± 4.8

Table1. The average number of *S. oryzae* in three rice varieties *Pachchaperumal* , *Rathkanda Al*, and hybrid variety-White Raw treated with essential oil mixture of *C. zeylanicum* leaves and *C. nardus*, (2 :1 v/v) during 6 months of storage period. Each data value represents the mean of 3 replicates ± SE.

Rice type	Control[1]	Treated sample[1]
Suwandal	0	0
Rathkanda al	25.34 ± 0.67^{a}	12.0 ± 1.15^{b}
White Raw	5.34 ± 0.67^{c}	3.34 ± 1.76^{c}
Pachchaperumal	14.67 ± 1.76^{d}	6.67 ± 1.76^{e}

Percentage Seed damage.

Table2. Percentage seed damage of four Traditional Rice Varieties for a period of 6 months.

PARAMETER INDEX	**FLAVOUR**	**GLOSS**
AROMA	1 =VERY WEEK	1=VERY DULL
1 =VERY WEEK	2=MODERATELY WEEK	2=MODERATELY DULL
2=MODERATELY WEEK	3=SLIGHTLY WEEK	3=SLIGHTLY DULL
3=SLIGHTLY WEEK	4=SLIGHTLY STRONG	4=SLIGHTLY GLOSSY
4=SLIGHTLY STRONG	5=MODERATELY STRONG	5=MODERATELY GLOSSY
5=MODERATELY STRONG	6=VERY STRONG	6=VERY GLOSSY
6=VERY STRONG		

STICKINESS	**TENDERNESS**
1 =HIGHLY SEPERATED	1 =VERY TOUGH
2=MODERATELY SEPERATED	2=MODERATELY TOUGH
3=SLIGHTLY SEPERATED	3=SLIGHTLY TOUGH
4=SLIGHTLY STICKY	4=SLIGHTLY TENDER
5=MODERATELY STICKY	5=MODERATELY TENDER
6=VERY STICKY	6=VERY TENDER

[1]mean of 3 replicates ± Standard error; means followed by the same letter(s) are not significantly different at 5% level by one way ANOVA and Tukey's pair wise comparison test.

Physical and Organoleptic properties of cooked traditional rice varities.

SWC – Rice type *Suwandal* untreated control.

SWS– Rice type *Suwandal* treated Sample.

RAC – Rice type *Rathkanda al* untreated control.

RAS – Rice type *Rathkanda al* treated Sample.

WRC - Rice type White raw untreated control.

WRS - Rice type White raw treated sample.

PPC - Rice type *Pachchaperumal* untreated control.

PPS - Rice type *Pachchaperumal* treated sample.

Table 3. Physical and Organoleptic properties of cooked traditional rice treated with essential oil mixture after a period of 3 months.

[1] mean of 4 replicates ± Standard error; Different letters in each row denote significant difference ($p<0.05$), Kruskal Wallis and Mann Whitney U tests;

Table 4. Physical and Organoleptic Properties of cooked rice treated with essential oil mixture after a period of 6 months.

PROPERTIES OF RICE	RICE VARIETIES							
	SWC[1]	SWS[1]	RAC[1]	RAS[1]	WRC[1]	WRS[1]	PPC[1]	PPS[1]
AROMA	2.75±0.25 [a]	4.5±0.28[b]	2.75±0.25[a]	4.25±0.25[b]	2.25±0.25[a]	3.25±0.25[a]	2.5±0.28[a]	3.5±0.28[b]
FLAVOUR	3.0±0.40[a]	4.0±0.57[a]	2.5±0.28[a]	4.75±0.25[b]	2.25±0.25[a]	3.5±0.28[a]	3.0±0.41[a]	4.25±0.25[a]
GLOSS	3.5±0.65[a]	3.5±0.65[a]	2.5±0.28[a]	2.75±0.57[a]	2.75±0.25[a]	3.25±0.25[a]	2.5±0.28[a]	2.75±0.25[a]
STICKINESS	2.5±0.28[a]	2.75±0.25[a]	2.75±0.25[a]	3.0±0.41[a]	2.25±0.48[a]	3.25±0.47[a]	2.25±0.25[a]	3.75±0.25[a]
TENDERNESS	2.75±0.25[a]	3.0±0.41[a]	3.5±0.28[a]	3.5±0.28[a]	2.75±0.25[a]	3.25±0.25[a]	2.5±0.28[a]	2.75±0.25[a]

PROPERTIES OF RICE	RICE VARIETIES							
	SWC[1]	SWS[1]	RAC[1]	RAS[1]	WRC[1]	WRS[1]	PPC[1]	PPS[1]
AROMA	2.75±0.25[a]	5.25±0.47[b]	2.75±0.48[a]	5.25±0.25[b]	2.75±0.48[a]	3.5±0.29[a]	3.25±0.25[a]	4.5±0.28[b]
FLAVOUR	3.0±0.41[a]	5.5±0.28[b]	3.75±0.63[a]	4.5±0.65[b]	2.25±0.48[a]	3.5±0.29[a]	2.75±0.47[a]	4.75±0.25[b]
GLOSS	3.25±0.75[a]	3.25±0.75[a]	2.00±0.41[a]	2.5±0.29[a]	2.75±0.48[a]	3.0±0.71[a]	2.5±0.29[a]	2.75±0.25[a]
STICKINESS	2.25±0.25[a]	4.5±0.28[b]	1.75±0.52[a]	3.25±0.48[b]	1.5±0.29[a]	3.5±0.65[b]	2.25±0.48[a]	4.75±0.48[b]
TENDERNESS	4.75±0.25[a]	4.75±0.25[a]	2.75±0.75[a]	3.0±0.41[a]	1.5±0.29[a]	2.0±0.71[a]	3.25±0.48[a]	3.5±0.65[a]

[1] mean of 4 replicates ± Standard error; Different letters in each row denote significant difference ($p<0.05$), Kruskal Wallis and Mann Whitney U tests.

Mass loss of treated clay cubes against the test period.

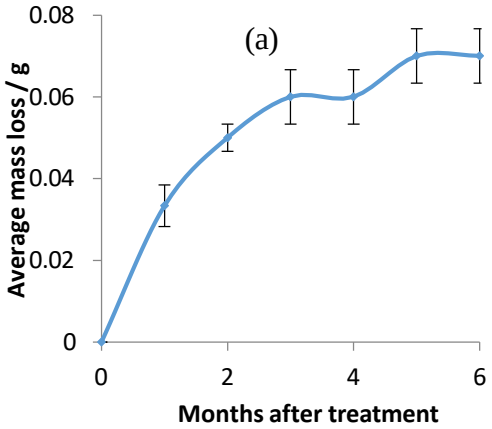
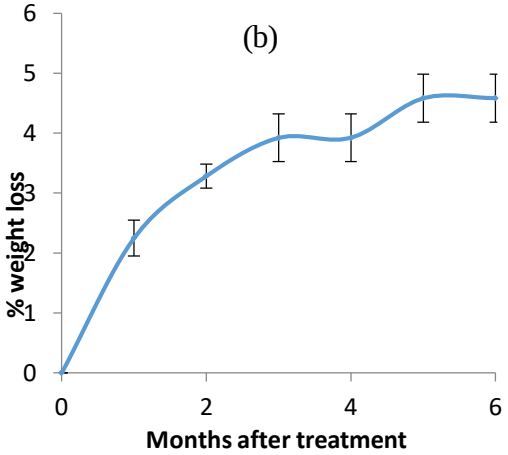


Figure 1. (a) The average mass loss of clay tablets during the test period.



(b) % weight loss of clay pellet during the test period.

Each data point represents the mean of 3 replicates ± SE.

$$\% \text{ Weight loss} = \frac{(\text{Weight at time } 0 - \text{Weight at time } t) \times 100}{\text{Weight at time } 0}$$

Discussion

The chemical constituents of *C. zeylanicum* and *C. nardus* were identified from GC analysis and their major constituents were similar to those published previously. The % seed damage and the

average number of pest insects were significantly decreased after the essential oil treatment in all the rice varieties in 6 months of storage period. *Sitophilus oryzae* was not observed in rice type *Suwandal* in both untreated control and the test sample during the test period. This indicates that the traditional rice *Suwandal* was self protected from *S. oryzae* and it was not necessary to use any controlling agent to protect it from stored grain pests.

Different physical and organoleptic parameters of cooked rice were evaluated after a period of 3 and 6 months of treatment by a taste panel .At the 3 months of storage period eating quality parameters were evaluated by a taste panel in the Department of Chemistry, University of Kelaniya. The results revealed that the flavour, aroma and stickiness of *Suwandal*, *Pachchaperumal and Rathkanda al* traditional rice varieties were significantly different from the untreated control. While, gloss, tenderness of cooked rice, were not significantly different from the control. At the end of the test period aroma of cooked rice was enhanced significantly in all traditional rice varieties except the rice type White raw.

Mass of clay pellets have been decreased slowly during the test time. After 6 months the total mass loss was 0.07 g per pellet compared to control. It was about 5 % weight loss after the treatment with essential oil mixture.

The present study has revealed that the potential of this essential oil mixture to be used as stored grain pest insect controlling agent of stored traditional rice varieties cultivated in Sri Lanka for a period of six months. Natural plant products can be alternatives to currently used synthetic pesticides, since they provide unlimited opportunities for the discovery of new pesticides because of their rich bioactive chemical constituents.

Conclusion

Commonly traditional rice varieties are grown using organic fertilizers and biopesticides. One of the problems in storage of traditional rice varieties is deterioration of rice due to pest attacks. The literature survey carried out indicates that there is no safe alternative method to protect traditional rice varieties during storage. Hence the present study was carried out to develop a safe alternative method to control *S. oryzae* in stored traditional rice varieties cultivated in Sri Lanka.

Essential oil can be widely used without any negative effect on mammals and nutritional properties of foods. It can serve as the convenient post harvest preservation agent, which effectively extends the shelf life and quality of food. However, further investigations have to be carried out to improve formulation, frequency of application and cost effectiveness before promoting new essential oil treated tablets at commercial level and also toxicity studies on the effects of the plant materials and their extracts on non target organisms need to be undertaken.

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