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Effect of the essential oils of *Cymbopogon citratus, C. nardus* and *Cinnamomum zeylanicum* on pest incidence and grain quality of rough rice (paddy) stored in an enclosed seed box

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Abstract

The protective effect of the essential oils of *Cymbopogon citratus, C. nardus* and *Cinnamomum zeylanicum* leaf on paddy stored in wooden boxes lined with aluminium foil were evaluated. After 168 days of storage in oil treated boxes *Sitotroga cerealella* population was significantly lower in oil treated paddy than the control. The 1000 grain weight was higher in all three treatments (21.1 - 22.9 g) compared to the control (19.8 g). Percentage grain damage was lower in *C. citratus* and *C. nardus* treated paddy than the control. The percentage seed germination was not significantly different from that of the control in oil treated paddy though, in *C. citratus* treatment the seed germination was reduced. The milling quality of paddy was not affected by the essential oil treatment however; the flavour and stickiness of cooked rice was enhanced when treated with *C. zeylanicum* and *C. citratus* oils.

Key words: Rice, storage, essential oil, grain quality, storage pests.

Introduction

Studies conducted on the effectiveness of the essential oils on the stored grain insect and fungal pests have shown the potential of these botanicals as a replacement for synthetic insecticides[1,2,3,4,5]. However, a few field trials have been carried out to evaluate the effectiveness of the essential oils on fungal and insect pests of stored rice. For a pesticide to be approved for the use in grain storage the pesticide must not affect the quality, flavour and odour of grain[6]. Thus the essential oils should be tested for the above parameters before making any recommendations for commercial usage.

Quality evaluation of rice involves milling quality, grain size, shape, appearance and cooking and eating characteristics[7,8]. The grain size and shape are determined by the genetic make-up of a particular variety though the milling quality, appearance and cooking and eating characteristics could greatly be influenced by the essential oil treatment in storage. The effect on seed viability should also be examined though it dose not belong to grain quality assessment[6]. Thus the objective of the present study was to evaluate the physical characters and eating quality of rice obtained from the stored rough rice (paddy) treated with the essential oil of *C. citratus*, *C. nardus* and *C. zeylanicum*.

Materials and Methods

Analysis of the essential oils: The essential oils of *C. citratus*, *C. nardus* and *C. zeylanicum* leaf were purchased from EOAS Organics (Pvt.) Ltd., Ratmalana, Hendrick & Sons, Colombo and Industrial Technology Institute, Colombo in Sri Lanka respectively. They were analysed on GC separately under the following conditions. HP5890 series II chromatograph with FID (Hewlett Packard, Palo Alto, CA, USA) and DB wax capillary column, 30 m x 0.25 mm; 0.25 µm film thickness (Jand W Scientific, Folsom, CA, USA). The column was programmed as follows; 50^0 C (0.5 min.), 50^0 to 210^0C at 2^0C/min., 210^0C (30 min.) with Helium carrier gas (1 ml/min.). The injector and the detector temperatures were 220^0C and 270^0C respectively and 1 µl of the oil solution in CH_2Cl_2 was injected. The major constituents were identified using published data[9,10].

Effect of the essential oils on grain quality: Wooden boxes (plywood, 24" x 25" x 26") were used as storage bins. Inner surfaces of the bins were lined with aluminium foil (0.02 mm). Twenty five ml of the essential oils of *C. citratus*, *C. nardus* and *C. zeylanicum* were separately applied on the aluminium lined surface of the box. The lids were sealed with cellulose tape and kept 24 h at ambient temperature (28 ± 3^0C) and R.H. (80 - 85%). Subsequently, 30 kg of newly harvested and sun-dried (7 days) paddy (BG 352) were introduced into each box. The head space of the box filled with paddy was 26 cm. A box without any oil treatment was used as the control. Paddy samples were drawn in triplicate from each box using a beaker (100 ml) at the end of the 168 day test period. The following observations were made.

1. Number of *S. oryzae* and *S. cerealella* in each sample.
2. Frequency of occurrence of *A. flavus* was determined by using the procedure described by Waller et al.[11].
3. Seed germination - Randomly selected grains (50) from each replicate were place on moistened blotting papers and % seed germination was estimated after 7 days.
4. 1000-grain weight - Sub samples of paddy (100 grains) were randomly drawn from each replicate the weights were determined. Then the sub samples were dried at 60^0C for 72 h and reweighed. The 1000-grain weight (B_y) was obtained using the formula:

$$B_y = 2B_x - A$$

(A = weight of grain after drying at 60^0C for 72 h;
B_x = weight of grain before drying)

5. Seed damage - Fifty seeds from each replicate were randomly taken and observed under a stage microscope for any visible insect damage.

Effect of the essential oils on milling quality of paddy: At the end of the test period, 150 g of paddy samples were drawn in trip-

licate from each treatment and control. The samples were dehusked (Yanmar ST50) separately to obtain brown rice and 100 g samples of brown rice were polished for 60 seconds and reweighed. Then the head (whole) and broken grain were separated. The % hull and % head grain were calculated as follows[7].

% Hull = (weight of hull / weight of paddy) x 100

% Head grain = (weight of head rice / weight of paddy) x 100

Physical and organoleptic properties of cooked rice from essential oil treated paddy: Then head grain (40 ml) from each sample was cooked in clean beakers (100 ml) placed in a domestic rice cooker. Then the parameters of aroma, flavour, tenderness, stickiness and gloss of cooked rice were evaluated by a trained taste panel at Rice Research and Development Institute, Batalagoda, Sri Lanka.

Statistical analysis: Data on milling quality were analysed using ANOVA and Tukey's pair wise comparison test. The results obtained at the end of 168 days on the variation of populations of pests, % seed germination, 1000-grain weight and seed damage were statistically analysed using ANOVA and Tukey's pair wise comparison tests in MINITAB statistical package (State Collage, PA, USA). Data obtained from the taste panel were analysed using Kruskal Wallis and Mann Whitney U tests in SPSS statistical package.

Results

Analysis of the essential oils: The major constituents of the essential oils of *C. citratus*, *C. nardus* and *C. zeylanicum* were citral *a* and *b*, geraniol and eugenol respectively (Table 1). α-Pinene was present in both samples other than *C. citratus* oil.

Effect of the essential oils on grain quality: *Sitophilus oryzae* and *A. flavus* were not observed in paddy samples treated with the essential oils. In *C. zeylanicum* treated sample *S. cerealella* were not observed at the end of the test period (Table 2). In paddy treated with *C. citratus* and *C. nardus* essential oils the number of insects was significantly lower than that of the untreated control ($p>0.05$). At the end of the 168 day test period the seed

Table 1. Percentage composition of the major constituents of the essential oils of *C. citratus*, *C. nardus* and *C. zeylanicum.*

Essential oil	Major constituents *
C. citratus	citral *a* (46.2%), citral *b* (31.6%), geraniol (3.6%), geranyl acetate (1.3%)
C. nardus	α-Pinene (4.4%), Camphene (8.2%), Limonene (11.0%), Geraniol (18.0%)
C zeylanicum	Eugenol (74.2%), â-caryphyllene (3.5%), benzyl benzoate (2.8%), cinnamaldehyde (2.7%), acetyl eugenol (1.7%), α-Pinene (1.8%),

* based on peak area

Table 2. Percentage seed germination, % damaged grains, *S. cerealella* population and 1000-grain weight of the seed paddy treated with the essential oils of *C. citratus*, *C. nardus* and *C. zeylanicum* for a period of 168 days.

Treatment	% seed germination[1]	% damaged grains[1]	*S. cerealella* population (no.)[1]	1000 grain weight (g)[1]
Control	20.2 ± 1.5 a	20.0 ± 3.0 a	5.0 ± 1.0 a	19.8 ± 0.9 a
C. citratus	8.60 ± 3.8 b	14.0 ± 5.7 b	1.60 ± 0.7 b	21.1 ± 0.4 b
C. nardus	22.60 ± 5.3 a	12.3 ±4.8 b	1.6 ± 0.3 b	22.9 ± 0.2 b
C. zeylanicum	22.0 ± 3.2 a	18.60 ± 6.4 ab	0 c	22.6 ± 0.7 b

[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 & Tukey's pair wise comparison test.

Table 3. Milling quality of paddy treated for a period of 6 months with the essential oils of *C. citratus*, *C. nardus* and *C.zeylanicum*.

Treatment	% hull[1]	% head grain[1]
Untreated control	20.5 ± 0.4 a	57.9 ± 2.1 a
C. citratus	20.7 ± 0.2 a	57.3 ± 2.5 a
C. nardus	20.6 ± 0.5 a	55.0 ± 2.6 a
C. zeylanicum	20.4 ± 0.4 a	59.1 ± 1.8 a

[1]n=3, ; means followed by the same letter(s) are not significantly different at 5% level by one way ANOVA & Tukey's pair wise comparison test.

Table 4. Physical and organoleptic properties of the rice prepared from the paddy previously treated with the essential oils of *C. citratus*, *C. nardus* and *C. zeylanicum* for 6 months of treatment.

Parameters of rice	Control[1]	*C. citratus*[1]	*C. nardus*[1]	*C. zeylanicum*[1]
Aroma	2.88 ± 0.08 a	2.95 ± 0.09 a	3.28 ± 0.16 a	3.21 ± 0.08 a
Flavour	3.26 ± 0.08 a	3.80 ± 0.09 b	3.56 ± 0.03 a	3.16 ± 0.03 b
Gloss	3.26 ± 0.14 a	3.56 ± 0.14 a	3.50 ± 0.20 a	3.13 ± 0.24 a
Stickiness	2.46 ± 0.20 a	3.13 ± 0.08 b	2.80 ± 0 ab	3.03 ± 0.03 b
Tenderness	3.26 ± 0.17 a	3.80 ± 0.19 a	3.46 ± 0.13 a	3.40 ± 0.19 a

[1]mean of 3 replicates ± standard error; Different letters in each row denote significant difference ($p<0.005$), Kruskal Wallis and Mann Whitney U tests; parameter index-

Aroma-1=very weak, 2=moderately weak, 3=slightly weak, 4=slightly strong, 5=moderately strong, 6=very strong

Flavour - 1=very weak, 2=moderately weak, 3=slightly weak, 4=slightly strong, 5=moderately strong, 6=very strong

Tenderness - 1=very tough, 2=moderately tough, 3=slightly tough, 4=slightly tender, 5=moderately tender, 6=very tender

Stickiness - 1=well separated,2= moderately separated, 3=slightly separated, 4=slightly sticky, 5=moderately sticky, 6=very sticky

Gloss - 1=very dull, 2=moderately dull, 3=slightly dull, 4=slightly glossy, 5=moderately glossy, 6=very glossy

germination of *C. nardus* and *C. zeylanicum* treatments and the untreated control were not significantly different ($p>0.05$) (Table 2). However, *C. citratus* oil treatment reduced the stored paddy seed germination compared to the control and the other two treatments significantly ($p<0.05$). The values of 1000-grain weight for the treatments were significantly higher than in the control (Table 2). There was no significant difference among the 1000-grain weights of oil treatments ($p>0.05$). The % seed damage was higher in the untreated control than in the seeds treated with the essential oils of *C. citratus* and *C. nardus* (Table 2). The treatment of *C. zeylanicum* was not effective as the other two treatments considering the seed damage.

Effect of the essential oils on milling quality of paddy: The % hull and % head grain of paddy treated with *C. zeylanicum*, *C. nardus* and *C. citratus* and untreated control did not different from each other (Table 3). The head grain percentage in all the treatments was more than 50.

Physical and organoleptic properties of cooked rice from essential oil treated paddy: Aroma, gloss and tenderness of the cooked rice obtained from essential oil treated paddy were not significantly different from the control ($p>0.05$) (Table 4). Stickiness of the rice from *C. citratus* and *C. zeylanicum* treated paddy was higher than in the control. Flavour *of the cooked rice was enhanced when* the paddy was treated with *C. citratus* and *C. nardus*.

Discussion

The composition of these three test essential oils have been studied extensively[9,10,12,13,14]. In the essential oils of *C. citratus* and *C. nardus* monoterpenes are highly abundant whereas in C. zeylanicum leaf oil phenyl propanoids were present more than 70%. Paddy treated with *C. zeylanicum* found to be superior in terms of the % seed germination, *S. cerealella* population and 1000-grain weight to those treated with the other two test essential oils and the untreated control. This indicates the possibility of using *C. zeylanicum* as a stored paddy protectant than the other two essential oils. Seed germination of the paddy treated with *C. citratus* was inferior to the other two essential oil treatments and the untreated control after 168 days of treatment. Thus indicating the treatment of the essential oil of *C. citratus* is suitable only for the treatment of paddy for consumption and not for the seed paddy. The grower, miller and the consumer emphasis on the various quality characteristics. Miller's basis of quality depend upon the total recovery and proportion of head and broken grain on milling. Whereas, consumer emphasis on grain appearance, shape, size and cooking and eating characteristics[7]. Though the milling quality of treated paddy was not significantly different from the control, the flavour and the stickiness of the cooked rice in *C. citratus* and *C. zeylanicum* were enhanced significantly. In these two treatments, the *S. cerealella* population was also lower than the control and *C. nardus* treatment. The flavour enhancement could be acceptable due to the use of cinnamon and lemongrass (*C. citratus*) in traditional cooking. The volatile constituents of the essential oils of *C. citratus* and *C. zeylanicum* may be penetrating into the starch of the grain making the environment of the grain prohibitive to the larval growth or survival of *S. ceralella*. The interference of the volatile constituents of the essential oils to the starch molecular network might be the reason for the higher stickiness than the control.

Conclusions

The results obtained indicated the possibility of using the essential oils as protectants of stored paddy. Further research has to be carried out to identify the active components of these essential oils in the grain protection and their potential in storage environments.

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