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Effect of essential oils on viability and nutritional quality of stored cowpea seeds

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Abstract
The aim of the study was to evaluate the effect of essential oils on the germination potential and protein content of cowpea seeds during storage for twelve months. Seeds were treated with different concentration of essential oils extracted from clove, eucalyptus, ginger and peppermint at the beginning of the storage. The oil treated seeds showed 59.6% and 41.1% higher germination potential and protein content respectively compared to untreated seeds at the end of the storage period.

Keywords: essential oil, storage, germination, protein, cowpea

Introduction

Storage of seed is certainly the most important post-harvest operation and the losses incurred are great. Production of cowpea grains in India is about 0.3 mmt and a major part of these (10.4%) is lost due to fungal contamination. In storage, cowpea seeds are attacked by pests and diseases leading to their deterioration and loss of nutritive value. The fungi that invade stored products are generally known as storage fungi. *Aspergillus* and *Penicillium* are the most dominant storage fungi which attack the stored products (Fagbohun and Lawal, 2011) [1]. Due to the production of toxic chemical substances the seeds become unfit for human consumption and there is reduction of its market value (Muller, 1991) [2].

Of the agrochemicals which are used for crop protection, only 1.1% reaches the target pest while 98.9% enters the environment and causes hazards to non target organism including human (Akthar *et al*, 2009) [3]. The chemical have the potential harmful effects on the environment and the community therefore investigation into the antifungal properties of essential plant oil which is a natural product has attracted wide interest. Essential oils of some higher plant have been successfully exploited against a number of pathogenic and storage microorganisms. These essential oils have been found to be efficacious and they are becoming popular in recent years (Barbosa *et al*, 2009)[4]. Several investigators have used essential oil of cinnamon, marigold, basil and supermint to determine their antifungal activity against *Aspergillus ochraceus*, *A. flavus*, *A. parasiticus* and *Fusarium moniliforme* (Soliman and Badeaa, 2002) [5]. Similarly, essential oil from rosemary plant exhibited strong antifungal activity on *Aspergillus flavus* (Moghtader *et al*, 2011) [6].

Therefore, in cognizance with the above the present study was conducted to determine the effect of different essential oils applied during storage period on the germination potential and protein content of cowpea seeds.

Materials and Methods

For the study cowpea seeds were dressed and fumigated with four different essential oil extracted from clove, eucalyptus, ginger and peppermint at different concentrations (200, 400, 600, 800, 1000 ppm) and stored for a period of twelve months. After the storage period the seeds were analyzed for their germination and protein quality.

Germination test (Anonymous, 1999) [7]was carried out to evaluate the germination percentage after storage. To reduce the microbial contamination during the test, seeds were surface sterilized with 0.1% Bavistin for two minutes and rinsed three times in sterile distilled water. Then the seeds were dried in air by keeping seeds on filter paper. The surface sterilized seeds were presoaked with distilled water overnight and plated on Petri dishes containing 3 layers of blotters soaked in sterile distilled water. Twenty seeds per plate were plated and incubated at room temperature for three days. Five replicates were taken for germination test. Seeds germinated per plate after incubation, were counted. The percentage of germination was also calculated using the following formula (Krishnasamy and Seshu, 1990) [8].

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds used}} \times 100$$

Standard Bradford protein estimation method was used (Bradford, 1976) [9] to estimate the protein content of the cowpea seeds treated with different oils (clove, eucalyptus, ginger and peppermint oil) under dressed and fumigated condition after twelve months of storage. For protein extraction, 1g of seed was homogenized in a mortar and pestle in 3 ml extraction buffer containing 50 mM Tris HCl (pH 7.5), 150mM NaCl, 1% NP-40 and 1mM Phenyl Methyl Sulfonyl Fluoride (PMFS). The homogenate was centrifuged at 15000 rmp for 10 minutes and the supernatant was used as crude protein extract. The whole process was carried out at 4^0C. The crude protein content was estimated using Bradford method for protein estimation.

Bradford reagent was prepared by dissolving Coomassie Brilliant Blue G250 (100 mg) in 50 ml of 95% ethanol. 100 ml of concentrated ortho-phosphoric acid was added to make the volume up to 200 ml with distilled water. Various concentrations of standard protein solutions were prepared from the stock solution (0.2, 0.4, 0.6, 0.8 and 1.0 ml) by dissolving in distilled water. A tube with 1 ml of water served as blank. 5.0 ml of Bradford reagent was added to each tube and vortexed. After 15 minutes, reading was taken

by spectrophotometer at 595 nm for each sample. The absorbance of the standards was plotted versus their concentration. From standard curve the amount of protein in unknown sample was calculated.

To minimize experimental errors and attainment of proper degrees of freedom five biological replications were sampled. Statistical analysis was done after the observations were recorded by using the Duncan Multiple Range Test. The data are represented as mean ± SEM. Statistical software SPSS was used for statistical analysis.

Result

Before storage the germination rate of cowpea seeds was 95% in control and after 12 months of storage the germination rate was decreased significantly i.e. 42.4%. Germination percentage of dressed cowpea seeds with all the four tested essential oils (clove, eucalyptus, ginger and peppermint oil) at 200, 400, 600, 800, 1000 ppm concentration after twelve months of storage was evaluated. Results revealed that, seeds without oil treatment showed poor germination percentage (42.4%). All the four tested oils at 200 and 400 ppm concentrations maintained fair germination rate. At 200 ppm, seeds treated with clove, eucalyptus, ginger and peppermint oil showed the germination rate in a range of 44.0- 45.6%. Similarly, seeds dressed with all the four tested oils at 400 ppm concentration showed the germination rate in a range of 52.8- 63.2%. Cowpea seeds treated with clove, eucalyptus, ginger and peppermint oils at 600 and 800 ppm concentrations showed better germination percentage in comparison to seeds treated with lower concentrations (200 and 400 ppm) of oil. At 600 ppm, seeds treated with clove, eucalyptus, ginger and peppermint oil showed the germination rate in a range of 64.8-75.2%. Similarly, at 800 ppm seeds dressed with the four tested oils showed the germination rate in a range of 78.4- 90.4%. However, the seeds dressed with tested oils at 1000 ppm maintained germination rate above 85%. Among all the four types of essential oil treated seeds, ginger oil treated seeds showed highest germination percentage (94.4%) at 1000 ppm. However, at the same concentration (1000 ppm) clove, peppermint and eucalyptus oil showed 89.6%, 87.2 % and 85.6 % germination rate respectively (Table 1).

Similarly germination percentage of fumigated cowpea seeds with all the four tested essential oils (clove, eucalyptus, ginger and peppermint oil) at 200, 400, 600, 800, 1000 ppm concentration after twelve months of storage was also evaluated. Results revealed that, seeds without oil treatment showed poor germination percentage (42.4%). Fumigated seeds with all the four tested oils at 200 and 400 ppm concentrations maintained fair germination rate. At 200 ppm seeds treated with clove, eucalyptus, ginger and peppermint oil showed the germination rate in range of 42.4- 44.3% (Table 1). Similarly, at 400 ppm concentration seeds treated with all the four tested oils showed the germination rate in range of 50.0-59.8%. At 600 ppm concentration, seeds treated with clove, eucalyptus, ginger and peppermint oil showed the germination rate in range of 61.6- 71.8%. Similarly, seeds treated with the four tested oils at 800 ppm concentration showed the germination rate in a range of 72.8- 84.8%. While, seeds treated with higher concentration (1000 ppm) of oils maintained germination rate above 80%. Among all the tested oils, ginger oil treated seed showed highest germination percentage in fumigated (91.2 %) condition. On the other hand, clove, peppermint and eucalyptus oil showed 85.6 ± 2.2%, 82.8 ± 1.5% and 82.4 ± 1.7% germination rate respectively (Table 2).

Table 1: Germination percentage of dressed cowpea seeds at different oil concentration after twelve months of storage

Sl. No.	Oil treatment	Concentration of oil (ppm)				
		200	400	600	800	1000
1	Control (without oil)	42.4^{a}±1.1	42.4^{d}±1.1	42.4^{c}±1.1	42.4^{c}±1.1	42.4^{c}±1.1
2	Clove oil	45.6^{a} ±1.5	61.6^{ab}±1.6	73.6^{a}±2.0	84.6^{a}±2.5	89.6^{ab}±0.2
3	Eucalyptus oil	44.8^{a}±1.9	58.4^{b}±1.2	66.4^{b}±3.4	78.6^{b}±1.3	85.6^{b}±1.2
4	Ginger oil	45.6^{a}±2.5	63.2^{a}±1.7	75.2^{a}±1.9	90.4^{a}±1.2	94.4^{a}±1.1
5	Peppermint oil	44.0^{a}±1.2	52.8^{c}±1.6	64.8^{b}±1.2	78.4^{b}±1.2	87.2^{b}±1.5

Table 2: Germination percentage of fumigated cowpea seeds at different oil concentration after twelve months of storage

Sl. No.	Oil treatment	Concentration of oil (ppm)				
		200	400	600	800	1000
1	Control (without oil)	42.4^{a}±1.1	42.4^{d}±1.1	42.4^{c}±1.1	42.4^{c}±1.1	42.4^{c}±1.1
2	Clove oil	44.0^{a}±1.3	59.2^{a}±1.9	71.2^{a}±2.4	84.0^{a}±2.3	85.6^{ab}±2.2
3	Eucalyptus oil	42.4^{a}±1.5	54.4^{b}±1.5	62.4^{b}±1.1	72.8^{b}±2.3	82.4^{b}±1.7
4	Ginger oil	44.2^{a}±2.3	59.8^{a}±3.0	71.8^{a}±1.6	84.8^{a}±2.6	91.2^{a}±1.2
5	Peppermint oil	43.2^{a}±1.6	50.4^{c}±2.3	61.6^{b}±2.3	75.2^{b}±2.2	82.8^{b}±1.5

In controls before storage, the seed protein content of the cowpea was 104.52 µg/ g seed and after 12 months of storage of cowpea seeds the protein content in seeds decreased significantly *i.e.* 38.16 µg/ g seed. The protein content of the cowpea seeds treated with different oils (clove, eucalyptus, ginger and peppermint oil) at 200, 400, 600, 800 and 1000 ppm concentration under dressed condition after twelve months of storage was estimated and is presented in Table 3.

Under dressed condition, significantly higher protein content was recorded in ginger oil treated seeds (40.32 µg/g seed) at 200 ppm concentration than in comparison to control (38.16 µg/g seed). While at same concentration (200 ppm), seeds treated with peppermint oil (39.12 µg/g), eucalyptus oil (39.06 µg/g) and clove oil (38.94 µg/g) did not show any significant difference in protein content in comparison to control. At 400 ppm concentration ginger oil (47.76 µg/g) eucalyptus oil (47.52 µg/g), peppermint oil (44.82 µg/g) and clove oil (43.86 µg/g) treated seeds showed higher protein content in comparison control (38.16 ± 0.64µg/g). Similarly at 600 ppm concentrations also ginger oil (48.66 µg/g), eucalyptus oil (48.06 µg/g),

peppermint oil (46.26 µg/g) and clove oil (46.14 µg/g) treated seeds possessed higher protein content in comparison to control (38.16 µg/g). Protein content of ginger oil (74.46 µg/g), peppermint oil (69.06 µg/g), eucalyptus oil (66.78 µg/g) and clove oil (60.06 µg/g) significantly increased at 800 ppm concentration. Seeds treated with all the four tested oils at 1000 ppm possessed maximum protein content in comparison to control (38.16 µg/g) as well as treated seeds at 200, 400, 600 and 800 ppm concentration. Among all the four tested oils, ginger oil treated seeds (94.98 µg/g) maintained highest protein content at this concentration followed by peppermint, eucalyptus and clove oil under dressed condition (84.96, 73.26, 66.0 µg/g respectively). The protein content of the cowpea seeds treated with different oils (clove, eucalyptus, ginger and peppermint oil) at 200, 400, 600, 800 and 1000 ppm concentration under fumigated condition after twelve months of storage was estimated and is presented in Table 4. No significant variation was observed in the seeds treated with eucalyptus oil (38.58 µg/g), clove oil (38.46 µg/g) peppermint oil (38.40 µg/g) and ginger oil (38.34 µg/g) at 200 ppm concentration in comparison to control (38.16 µg/g). Protein content of seeds increased with the increase in the concentration of the essential oil (protein content of ginger oil (43.86 µg/g), eucalyptus oil (42.66 µg/g), peppermint oil (40.14 µg/g) and clove oil (39.12 µg/g) treated seeds at 400 ppm; protein content of ginger oil (45.06 µg/g), eucalyptus oil (44.46 µg/g), clove oil (41.46 µg/g) and peppermint oil (41.04 µg/g) treated seeds at 600 ppm; protein content of ginger oil (67.26 µg/g), peppermint oil (61.44 µg/g), eucalyptus oil (60.84 µg/g) and clove oil (56.16 µg/g) treated seeds at 800 ppm). Ginger oil treated seeds at 1000 ppm concentration maintained highest protein content (87.66 µg/g). On the other hand, the protein content of seeds treated with peppermint, eucalyptus and clove oil was recorded 78.78, 68.76, 61.86 µg/g respectively at 1000 ppm.

Table 3: Protein content (µg/ g seed) of dressed cowpea seeds at different oil concentrations after twelve months of storage

Sl. no.	Oil treatment	Concentration of oil (ppm)				
		200	400	600	800	1000
1	Control (without oil)	$38.16^{a} \pm 0.64$	$38.16^{c} \pm 0.64$	$38.16^{b} \pm 0.64$	$38.16^{d} \pm 0.64$	$38.16^{e} \pm 0.64$
2	Clove oil	$38.94^{a} \pm 0.81$	$43.86^{b} \pm 1.04$	$46.14^{a} \pm 1.10$	$60.60^{c} \pm 1.15$	$66.00^{d} \pm 1.09$
3	Eucalyptus oil	$39.06^{a} \pm 0.61$	$47.52^{a} \pm 0.87$	$48.06^{a} \pm 0.98$	$66.78^{b} \pm 1.04$	$73.26^{c} \pm 1.27$
4	Ginger oil	$40.32^{a} \pm 0.80$	$47.76^{a} \pm 0.79$	$48.66^{a} \pm 0.80$	$74.46^{a} \pm 1.08$	$94.98^{a} \pm 1.15$
5	Peppermint oil	$39.12^{a} \pm 0.84$	$44.82^{b} \pm 0.69$	$46.26^{a} \pm 0.67$	$69.06^{b} \pm 1.02$	$84.96^{b} \pm 0.56$

Table 4: Protein content (µg/ g seed) of fumigated cowpea seeds at different oil concentrations after twelve months of storage

Sl. no.	Oil treatment	Concentration of oil (ppm)				
		200	400	600	800	1000
1	Control (without oil)	$38.16^{a} \pm 0.64$	$38.16^{c} \pm 0.64$	$38.16^{d} \pm 0.64$	$38.16^{a} \pm 0.64$	$38.16^{e} \pm 0.64$
2	Clove oil	$38.46^{a} \pm 0.64$	$39.12^{c} \pm 0.73$	$41.46^{bc} \pm 0.77$	$56.16^{c} \pm 0.95$	$61.86^{d} \pm 1.02$
3	Eucalyptus oil	$38.58^{a} \pm 0.77$	$42.66^{ab} \pm 0.86$	$44.46^{ab} \pm 1.07$	$60.84^{b} \pm 1.15$	$68.76^{c} \pm 1.28$
4	Ginger oil	$38.34^{a} \pm 0.89$	$43.86^{a} \pm 0.90$	$45.06^{a} \pm 1.09$	$67.26^{a} \pm 0.92$	$87.66^{a} \pm 1.14$
5	Peppermint oil	$38.40^{a} \pm 0.79$	$40.14^{bc} \pm 0.96$	$41.04^{cd} \pm 1.10$	$61.44^{b} \pm 0.89$	$78.78^{b} \pm 1.15$

Discussion

Present study revealed that, germination rate and protein content of oil treated stored cowpea seeds were found superior compared to control. Kritzinger *et al* (2002)[10] studied the antifungal activity of clove and peppermint oil against the pathogenic storage fungi (*Aspergillus flavus, A. niger, Fusarium oxysporum, F. equiseti, Penicillum oxysporum*) and their effect on germination of cowpea seeds. They reported that, the tested essential oils have strong antifungal activity and none of the oils showed harmful effect on germination of cowpea seeds. In a study, Nguefack *el al* (2008) [11] reported that germination rate was also found superior in essential oil treated rice seeds (46-85%) among the seven cultivars compared to non-treated seeds (26-74%). Farrag and Moharam (2012) [12] also reported that, cucumber seeds treated with 1, 2 and 3% peppermint oil exhibited an increase in germination rate and seedling vigour by 72.1, 76.9, 83.7% and 252.6, 323.2, 468.9% respectively compared to non-treated seeds (43.2% and 87.3%).

Chavan (2011) [13] reported that, storage fungi *viz. Aspergillus niger, A. terreus, A. parasiticus, A. fumigatus* were responsible for reducing seed protein content of soybean. Kakde and Chavan (2011) [14] also found that, storage fungi were responsible for decrease in protein content in oil seeds. In a similar investigation, Bhattacharya and Raha (2002) [15] reported that, there was loss of protein content of seeds due to infection of these storage fungi. The reason for decrease in protein content might be due to hydrolysis of protein by fungal proteolytic enzymes (Jamaluddin *et al*, 1977) [16]. In a study Martinez Maldonado*et al* (2015) [17] reported that protein contents in sugar apple seeds declined at 60 days and 120 days of storage.

Conclusion

Thus from the study it was confirmed that storage can reduce the viability and nutritional quality of cowpea seeds if the seeds are stored without any treatment. However, seeds treated with different essential oil extracted from clove, eucalyptus, ginger, peppermint in storage were found effective. These essential oils not only maintained the viability and seed protein content but it may also prevent other deleterious factors appears during storage.

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