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Effects of *Moringa oleifera* leaf extract and sodium hypochlorite seed pre-treatment on seed germination, seedling growth rate and fungal abundance in two accessions of *Abelmoschus esculentus* (L) Moench

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ABSTRACT

Effects of Moringa oleifera leaf extract and Sodium hypochlorite seed pre-treatment on the seed germination, seedling growth rate and fungal activities in two accessions of Abelmoschus esculentus was examined in the Crop laboratory of Babcock University. Seed viability and seedling vigour were assessed according to ISTA, 1985; while the blotter paper method was used to determine the seed microflora. Mycological analysis was carried out by isolating and identifying fungal flora on potato dextrose agar plates. Okra seeds of CCN2005/2 and Clemson spineless varieties were pre-treated with 2.5%, 5% and 10% of Moringa leaf extract; while Sodium hypochlorite, NaOCl, was administered at 4%, 5% and 6%. A control with no seed pre-treatment was also included. Ten viable seeds, randomly selected from the seed lots of each accession, were dipped into treatment solutions for five minutes, after which it was withdrawn and prepared for further tests. Germination tests were carried out on 85mm diameter petri dishes, lined with moistened Whattman filter paper; with three replications for each treatment in a Completely Randomized Design (CRD). The two okra accessions reacted differently to different concentrations of NaOCl and Moringa leaf extract. The accessions were not significantly different for the seed viability and seedling vigour variables evaluated. Results further reveal that 4% and 6% of NaOCl inhibited the population of fungal growth; while Moringa extract reduced fungal growth and population on both accessions. Fusarium had the highest (67.5%) occurrence on seed fungal population. Penicillium however had the lowest (3.5%). This study explained the fact that pre-treatment of seeds before storage with Moringa reduces the possibility of fungal infection and also maintains the viability and vigour of the seed for a particular time period, depending on the seed type.

Keywords: seed viability, seedling vigour, seed microflora, seed pre-treatment.

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench*)* is specially valued in different parts of Nigeria for its delicious fruits and it is consumed alone or in a combination with other food. Nutritionally the richest part in okra is the dried seed [1]
Okra is a widely cultivated vegetable crop and can be found in almost every market in Africa due to the fact that its leaves, buds and flowers can be edible. In Ghana it's the fourth most popular vegetable after pepper, tomatoes and garden eggs [2]

In Nigeria, vegetables are widely grown during both dry and rainy seasons. The rainy season is bimodal i.e. its early crop usually begins as soon as the rain starts in March / April and ends during a short dry spell in August to October / November. However the climate, especially rainfall and temperature, varies during the early and late season in ways that influence the yield of the crop [2].
Food security remains a challenge in developing countries like Nigeria. Guaranteeing food security involves intensifying agricultural production and provision of appropriate processing techniques. Pre-treatment is a technique

used in the treatment of raw materials before proper processing. Such pre-treatment may lead to an alteration of the basic structure or component of raw material. Vegetables are frequently subjected to various form of processing to make them more suited for consumption as well as more resilient to long term storage

Okra pest and diseases vary from year to year. Their presence, incidence and severity depend upon host resistance or susceptibility and environmental conditions. Okra yield has been significantly reduced by the yellow vein mosaic virus, powdery mildew *(Erysiphe cichoracearum),* fruit borer *(Earias vittella),* and jassids *(Amarasca biguttala)* [3]. Other diseases and pests include okra leaf curl, leaf spot *(Cercospora abelmoschi),* Root knot, Fusarium wilt and aphids.

A large proportion of the available germplasm of okra has been tested for reactions to major insect pests and diseases. A few have been found to be tolerant to the yellow vein virus (YVMV) [3]. A few other accessions have been reported to be resistance to root knot nematode. [4]

Okra mosaic virus (OKMV) transmitted by flea beetles is widespread in Africa but damage is much less important than that caused by okra leaf curl disease transmitted by whitefly [5]. Whitefly is also a vector of yellow vein mosaic virus a major disease of okra in Asia. This virus can only be controlled through control of the vector.

Young immature fruits are important vegetables consumed, cooked or fried. In West Africa we usually boil in water to make slimy sauces. The fruit can be consumed by drying, whole or sliced. Before selling, the dried product is usually ground to powder [5].

Okra mucilage is suitable for medicinal and industrial applications. It can be used as plasma replacement or blood volume expander [5]. The young leaves are commonly used as spinach and are also used sometimes as cattle feed [5]. Okra is also a good source of iodine which is useful in the treatment of simple goitre and other medically useful compounds like vanillin [6].

Okra seeds have been treated through various ways in other to prevent seed damage, by pest and disease. Some of the various ways in which okra seeds have been treated are soaked in water at 50^{o}C for 30 minutes; chemical treatment using some acid pesticides and seed disinfectants like Bleach and natural plant extracts like neem [7]. This has helped in the control of bacteria and reduced seed borne fungi spores or bacteria. Reports have shown that *Trichoderma spp* and *Bacillus subtilis* are viable in biological control of seed fungus and bacteria. Biological seed treatments thus protect root system against soil-borne pathogens after germination.

Odofin [7] reported that 2, and 3% of bleach increases germination percentage and vigour in okra seeds, while 2% and 5% Neem extract inhibited vigour in okra seeds and induced some chromosomal aberrations in okra seed cells.

Seeds are alive and must be kept alive as possible if they are to be seeds [8]. The way to fulfilling this is therefore to ensure proper seed treatment and storage after harvesting and before using it for cultivation in the following season.

Adebisi [9] states that the vigor of seeds at the time of storage is an important factor of their storage life. He stated further that viability and vigour cannot always be maintained in seed lots that are rapidly deteriorating and that the progression weakening with age continues unit all the seed becomes non viable. Seed quality is therefore a multiple concept comprising several components, but they are not all of equal value [10].

Seedling vigour has been defined as that condition of active good health and natural robust in seeds, which upon planting permits germination to proceed rapidly under a wide range of environmental condition [11]. It can also be said to be the rapid uniform germination and fast seedling growth under general field conditions [12]. Some useful vigour test has been reported by [13]. These include speed of germination, seedling growth rate, cold test, Glutanic acid decarboxylase activity (GADA) test, Tetrazolum (Tz) test, Accelerated aging test and a lot of others. The mathematical expression of speed germination as given by Maguire [14] is:

Vigour = No of normal seedlings (1st count +…….+ No of normal seedling last count) / Day of 1^{st} count +……..+ Day of last count.

Assuming good viability and good pre harvest condition, longevity and viability are determined by general factors often interacting, which include moisture content of the seed, storage temperature, storage environment and the storage container. The decrease in germination is often accomplished by an increase in the number of abnormal seedlings [15]

Another important reason for seed storage is to maintain or preserve seed quality throughout the storage period by minimizing the rate of seed deterioration [16].

The objectives of this work are:
1. To ascertain the efficiency of Moringa leaf extract as an organic treatment in okra seed post harvest preservation compared to Sodium hypochlorite (NaOCl) solution.
2. To monitor the efficacy of Sodium hypochlorite (NaOCl) as an inorganic chemical treatment in okra seed post harvest preservation.
3. To study the effect of these treatments on seed germination and vigor.
4. To compare the antimicrobial effect of these chemicals on okra seed preservation.

MATERIALS AND METHODS

Seed Viability and Seedling Vigour Evaluation

Two okra accessions CCN 2005/2 and Clemson spineless were used for this study. Three concentrations of Moringa leaf extracts (2.5, 5 and 10%) and Bleach (4, 5 and 10%) were used for seed pre-treatment and distilled water as control. This was done by dipping the 10 viable seeds of each accessions randomly selected into each treatment solution for 5 minutes and then transferred to moistened whattman filter paper which has been carefully layed into 85mm diameter petri plates.

A total of 42 petri dishes underlayed with filter paper were used for this experiment; 21 for each accession. Each treatment was replicated 3 times using the completely randomized design. For CCN 2005/2 there were 120 seeds planted all together. 10 seeds were placed in each petri dish in different replication and in different treatment concentrations. For Moringa the different concentrations were 2.5, 5, and 10% and there were 3 different replications for each treatment, so there were 30 seeds per treatment concentration and 30 seed for control which was distilled water. The same procedure also applies to Bleach but with concentrations of 4, 5 and 6%. This was repeated for Clemson spineless and all together there were 120 seeds planted too.

Germinability and seedling vigour index were determined according to recommended methods by ISTA[17]. Number of germinated seeds were counted starting from the 3^{rd} day after planting when radicals appeared till the 7^{th} day, which is the last day, then adding all for each treatment dividing by total number of seeds pre-treated and multiplying by 100 to get the percentage germination. Seedling vigour was estimated by measurement of the length of germinated roots daily from the 3^{rd} day after planting till the 7^{th} day, then adding the recorded measurement taken at the end of the of the 7^{th} day and dividing by the total number of seeds pre-treated and multiplying by 100 to determine percentage seedling length.

Seedling vigour index was determined as the product of the percentage germination and that of seedling length.

Percent Germination × Seedling length = Seedling vigour index

Mycological Studies for fungal diversity

A mycological study was carried out on the incubated pre-treated seeds. This was done by isolating fungal flora that grew on the seeds. Isolation was done on Potato Dextrose Agar plates for fungal identification, and data were collected on the population and severity of fungal growth on the pre-treated seeds.

Data Analysis

Data were analyzed using Statistical Analysis System [18] software; where ANOVA (Analysis of Variance) was carried out and Means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Viability and Vigour Tests

Table 1 shows the mean square of Analysis of Variance of seed germination, seedling length and seedling vigour index of two okra accessions treated with different concentrations of Moringa leaf extract. The results show that percentage germination, seedling length, seedling vigour index were not significant for accession, as well as concentrations. Interaction between accession and concentration also had no significant effect on germination percentage, seedling length and seedling vigour index. Replications were not significant for all the parameter evaluated.

Table 2 shows the combined mean seed germination, seedling length and seedling vigor index of okra seed accession treated with different concentration of Moringa leaf extract. The result shows no difference in the percentage germination, seedling length and seedling vigour index for the 3 concentrations.

Table 3 shows the mean square from Analysis of variance of seed germination, seedling length and seedling vigour index of two accessions of okra treated with different concentrations of Sodium Hypochlorite. The results show that percentage germination, seedling length, seedling vigour index were not significant for accession, as well as concentrations. Interaction between accession and concentration also produced no significant effect on germination percentage, seedling length and seedling vigour index. Replications were not significant for all the parameter evaluated.

Table 4 shows the combined mean seed germination, seedling length and seedling vigour index of okra seed accession treated with different concentration of Sodium Hypochlorite. The result shows no difference in the percentage germination, seedling length and seedling vigour index for the 3 concentrations.

Table 5 shows the mean square from combined Analysis of variance of seed germination, seedling length and seedling vigor index of two accessions of okra treated with different concentration of Moringa leaf extract and Bleach. The results show that percentage germination, seedling length, seedling vigour index were not significant for accession, as well as concentrations. Interaction between accession and concentration also produced no significant effect on germination percentage, seedling length and seedling vigour index. Replications were not significant for all the parameter evaluated.

Seed Examination

Table 6 shows the percentage seed borne fungal diversity of okra seed pre-treated in different concentrations of Moringa leaf extract and Sodium Hypochlorite (NaOCl). The result shows that *Fusarium* was the most occurring fungi (67.5%), while *Pencillium* was the least occurring fungi found in the treatment.

Table 7 shows the effect of different concentrations of Moringa leaf extract and Sodium Hypochlorite on fungal diversity of okra seed accessions. The result shows that Sodium Hypochlorite at 4 and 6% inhibited more of the fungi growth, especially *Fusarium* in accession CCN 2005/2. Moringa leaf extract even at the lowest concentration (2.5%) reduced the detection of fungi population on both accessions. *Fusarium* had the highest population in the okra seed accessions (67.5%).

Results of the analysis of variance show that accession had no significant treatment effect on germination percentage, seedling length and seedling vigour index; suggesting that these chemicals at the concentrations used did not promote any of three seed parameters studied. This varies with the earlier report of [7] who observed a significant treatment effect of Neem extract and Sodium Hypochlorite on the percentage germination of Clemson spineless.

Similarly, the mean germination percentage, seedling length and seedling vigour index was not significant in the accession with respect to the treatment; thus suggesting that concentration of 4%, 5% and 6% Sodium Hypochlorite and Moringa 2.5%, 5% and 10% does not produce any positive interaction with the seeds of the okra accessions that will promote seedling germination percentage, seedling length and seedling vigour index beyond what is obtained in the control. These reports also agree with the earlier observations by [7] who reported that Sodium Hypochlorite promotes seedling germination at low concentration of between 2 and 3%. Since the vigour and germination were unaffected under the seed treatments it also suggests that the stress treatment was insufficient to overcome seed vigour [19]

The reduced population of *Fusarium, Aspergillus fumigatus* and *Penicillum* across the concentration of Sodium Hypochlorite in both accessions suggests that there is a positive interaction between the okra accessions and Sodium Hypochlorite in inhibiting the growth of fungal species. This agrees with the report of Odofin [7].

Table 1. Mean square from combined ANOVA of percentage germination, seedling length and seedling vigor index of two accessions of okra seed with different concentration of Moringa leaf extract

SOURCE OF VARIANCE	D.F	PERCENTAGE GERMINATION	SEEDLING LENGTH	SEEDLING VIGOR INDEX
Accessions	1	355.56 NS	0.420 NS	1014.00 NS
Concentrations	2	50.00 NS	0.618 NS	773.05 NS
Reps	2	50.00 NS	0.368 NS	559.05 NS
Accn*conc	2	383.88 NS	0.367 NS	485.78 NS
Error	10	416.66 NS	0.328 NS	1633.97 NS
Total	17			
CV		59.41	41.24	74.49

NS=Not significant

Table 2. Combined mean seed germination, seedling length and seedling vigour index of okra seed accession treated different concentration of Moringa leaf extract

CONCENTRATION	PERCENTAGE GERMINATION	SEEDLING LENGTH	SEEDLING VIGOR INDEX
2.5	31.67[a]	1.8017[a]	59.87[a]
5	26.67[a]	1.5067[a]	43.65[a]
10	31.67[a]	1.1600[a]	38.00[a]

N.B. Means with the same letters are not significantly different along the columns.

Table 3. Mean square from combined ANOVA of percentage germination, seedling length and seedling vigour index of two accessions of okra seed with different concentration of Sodium hypochlorite (NaOCl)

SOURCE OF VARIANCE	D.F	PERCENTAGE GERMINATION	SEEDLING LENGTH	SEEDLIN VIGOR INDEX
Accessions	1	22.22 NS	0.204 NS	110.508 NS
Concentrations	2	22.22 NS	0.230 NS	730.95 NS
Reps	2	1705.55*	1.287 NS	3667.028 NS
Accns*conc	2	688.88 NS	0.099 NS	1512.227 NS
Error	10	358.88 NS	0.339 NS	1617.28 NS
Total	17			
CV		64.71	59.44	85.62

*NS=Not significant, *Significant at 5%.*

Table 4. Combined mean seed germination, seedling length and seedling vigour index of okra seed accession treated different concentration of Sodium Hypochlorite

CONCENTRATION	PERCENTAGE GERMINATION	SEEDLING LENGTH	SEEDLING VIGOR INDEX
4	36.67[a]	1.2383[a]	60.23[a]
5	33.33[a]	1.1083[a]	41.22[a]
6	33.33[a]	0.8533[a]	41.02[a]

N.B. Means with the same letters are not significantly different along the columns.

Table 5. Mean square from combined ANOVA of percentage germination, seedling length and seedling vigour index of two accessions of okra seed with different concentration of Moringa leaf extract and Bleach

SOURCE OF VARIANCE	D.F	PERCENTAGE GERMINATION	SEEDLING LENGTH	SEEDLING VIGOR INDEX
Accession	1	0.0 NS	0.005 NS	79.800 NS
Concentration	2	66.666 NS	0.746 NS	347.748 NS
Reps	2	216.666 NS	0.363 NS	595.762 NS
Accns*conc	2	66.666 NS	0.425 NS	296.082 NS
Error	10	710.000 NS	O.643 NS	2931.002 NS
Total CV	17	71.40	59.52	92.57

NS=Not significant

Table 6. Overall seed borne fungal occurrence of okra seed pre-treatment in different concentrations of Moringa leaf extract and Sodium hypochlorite (NaOCl)

FUNGI	OVERALL MEAN PERCENTAGE OCCURRENCE
Fusarium	67.5
Aspergillus Fumigatus	18.5
Aspergillus section Nigri	10.5
Penicillium	3.5

Table 7. The effect of Moringa Leaf extract and NaOCl on fungal diversity of two okra seed varieties

% OCCURRENCE OF FUNGI	VARIETY	NaOCl CONCENTRATION (%)			MORINGA CONCENTRATION (%)			CONTROL
		4	5	6	2.5	5	10	0
Fusarium	CCN 2005/2	85.7	62.5	92.3	75	100	100	40
	CLEMSON SPINELESS	0	0	0	100	80	0	50
	CCN 2005/2	14.3	25	0	0	0	0	40
Aspergillus Fumigatus	CLEMSON SPINELESS	0	0	0	0	0	0	10
	CCN 2005/2							
Aspergillus section Nigri	CLEMSON	0	12.5	7.7	0	10	0	10
	SPINELESS	100	75	100	0	0	0	30
	CCN 2005/2							
	CLEMSON	0	0	0	25	0	0	10
Penicillium	SPINELESS	0	25	0	0	0	0	10

CONCLUSION

Sodium Hypochlorite pre-treatment at 4% and 6% concentrations inhibited the population of fungal growth; while Moringa leaf extract reduced the detection of fungal population on both accessions. *Fusarium* had the highest occurrence of okra seed fungal population which is 67.5%; while *Penicillium* had the lowest occurrence (3.5%). The length of storage of seeds and effect of fungal infestation can affect the germinability of okra seeds.

The result of the analysis of variance shows that there was no difference in percentage germination, seedling length and seedling vigor index both CCN 2005/2 and Clemson Spineless.

However NaOCl, 4%, 5% and 6% did not increase percentage germination, seedling length and seedling vigor index in okra accession. Similarly 2.5%, 5% and 10% of Moringa leaf extract also did not improve percentage germination, seedling length and seedling vigor index significantly.

Generally, it is therefore recommended that the use of some plant extracts as seed pre-treatment to enhance seed viability in storage and protect seed at post harvest should be further explored and encouraged.

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