Journal of
Plant Science & Research

Open Science Publications

Volume 5, Issue 2 - 2018
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Efficacy of Ash in Post-Harvest Seed Storage of Three Species of Cucurbitaceae

Research Article

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Article Information: Submission: 08/10/2018; Accepted: 12/11/2018; Published: 16/11/2018

Abstract

The efficacy of ash in storing seeds of bottle gourd (*Lagenaria siceraria*), snake gourd (*Trichosanthes cucumerina*) and pumpkin (*Cucurbita maxima*) was studied at three different concentrations for a period of 24 months to facilitate simple and reliable seed storage technique at the post-harvest stage. Using ash at a low concentration (5% to the seed weight) was found to be significantly less effective in storing seeds of *L. siceraria*, *T. cucumerina* compared to higher concentrations (10% and 20% to the seed weight) after 1 year of storage. In contrast, all three concentrations benefited seed storage of *C. maxima* for 1 year. However, germination percentage at the end of storage increased proportionately with increasing ash concentration in all the three species. The results showed that use of ash at high concentrations (10% and 20%) can uphold more than 80% of the viability of the seeds at the time of harvest. The dryness of seeds was not responsible for seed viability as all the three species stored at different concentrations for 2 years germinated only less than 10%. We found that several pests attacked seeds, but these attacks were either minimal or nil in ash treated seeds depending on the concentration during the first year. The possible mechanism of actions behind the repellent activity of ash is discussed in the context of present findings. The results show that ash can have a great potential in storing seeds of *L. siceraria*, *T. cucumerina* and *C. maxima* seeds for up to 12 months.

Keywords: Ash; *Lagenaria siceraria*; *Trichosanthes cucumerina*; *Cucurbita maxima*; Traditional seed storage; Post-harvest loss

Introduction

The post-harvest seed storage between growing seasons is a necessary step linking plant generations [1-3]. However, substantial amount of seeds stored at post-harvest stage are spoiled due to lack of storage and processing facilities [4]. Globally, poor storage facilities alone contribute to more than 10% of seed loss at post-harvest stage. But 20% loss results from inadequate storage facilities in Africa, Asia and Latin America [5]. Seeds stored under inappropriate storage conditions are highly susceptible to fungal attack and/or insect infestation. Insect infestation solely causes massive damage to seeds, thus contributing to significant annual loss of stored seeds. Insects can consume seeds directly or riddle seed covering structures (e.g. seed coat) which increase the moisture content at the seed surface making grains more susceptible to other insects and fungus [2]. Insect pests acting on stored seeds have an extremely high multiplication rate and within one season they may destroy the whole seed lot, rendering them undesirable for crop cultivation.

The application of chemical additives on seeds has been claimed to control the pests and other fungus attacks that would otherwise damage seeds [6]. These chemical additives known as insecticides are routinely used in seed storage. However, uses of chemicals pose serious health hazards and also result in environmental contamination [7]. For instance, methyl bromide is the most effective insecticide that kills insects in stored products, but developed countries have banned its usage because it contributes to ozone depletion [8]. In addition, the increasing cost, some insects becoming genetically resistance to the applied insecticides and some toxic residues in the grains have in fact intensified the search for less problematical alternatives [6,9,10].

Furthermore, even though the availability of commercial insecticides to farmers has increased steadily, these are often too expensive for low-resource farmers' especially in the developing countries or unavailable in remote villages.

In the recent years, there is a growing interest on understanding the traditional seed storage system not only because these methods are eco-friendly as compared to other chemical storage techniques but it is simple [11,12], reliable and readily available to farmers around the World [13-15]. The particular method used to store grains differs broadly based on the purpose of storage. Thus, viability is not an important issue in seeds stored for consumption, but maintaining higher level of viability is crucial for seeds stored as planting material. The methods available to store seeds are species-specific; hence, all species cannot be stored using same methods [12]. It is becoming apparent that documenting the scientific basis of action behind these traditional seed storage systems are important because information on traditional methods are often restricted to small ethnic communities [11,12].

One of the most powerful traditional seed storage methods involves treating seeds with ash during storage. Ash burnt from plant woods and other biological materials e.g. cow dung has long been viewed as an important agent circumventing fungal and insect growth in seed storage [16,17]. Seeds of several species including cowpea [18,19], maize [20,21], melon [20], bean survived better or remained less affected by pests after treating with ash compared with untreated controls [20].

However, little is known about the efficacy of ash in storing other crops. Furthermore, many works have dealt with the application of ash to the stored seeds for consumption; therefore, our knowledge on using ash on seed storage for sowing purpose is rudimentary. In our preliminary survey to explore the traditional seed storage methods in Tamil Nadu, India, it became evident that many small-scale farmers store seeds of *Lagenaria siceraria*, *Trichosanthes cucumerina* and *Cucurbita maxima* of family Cucurbitaceae using ash burnt from neem (*Azadirachta indica*) woods and cow dung. However, the longevity of seeds of cucurbits treated with ash burnt from neem and cow dung is not known. Thus, experimental evidence to show the benefits of ash to seed storage for these species is lacking and to gain such evidence, we have to answer following questions:

(1) Can ash treated seeds of *L. siceraria*, *T. cucumerina* and *C. maxima* hold viability for longer time.

(2) Does the concentration of ash used affect the storage ability of seeds?

Materials and Methods

Study site and species

The plants referable to *L. siceraria, T. cucumerina* and *C. maxima* were grown in a natural agricultural site near Coimbatore, Tamil Nadu (11° 12' N, 76° 58' E), the south most penultimate State of Indian sub-continent, under semi-arid climate with a mean temperature of 34 °C. Plants were watered on regular basis and weeds germinated during cultivation period were removed periodically.

Seed collection

Matured fruits from five to ten plants of each species were hand-collected and made a cut with sharp knife to collect seeds. Whenever needed, before seed collection, hand-collected fruits were further dried in shade until the color changes from pale to dark brown. Some fruits of *L. siceraria* and *T. cucumerina* became very rigid after artificial drying hence soaked in water for 48 h or longer before scraping the fruit. Fully matured fruits became completely weightless and seeds got detached naturally from attaching structures and moved freely inside the fruits as the drying progressed towards end. Number of seeds in each fruit varied from species to species. Seeds collected from different fruits were pooled together as a single lot and dried at room temperature for one week. During drying period, unhealthy seeds mainly with holes and damaged seed coat were removed. The dried seeds were then stored in jute bags at room temperature (≅ 25 °C) for two days before their usage in experiments.

Seed germination

Germination was tested in 1% agar-water taken in sandwich boxes (15 cm X 10 cm). Four replicates of 25 randomly selected seeds were used for each species. Seeds of all species were incubated at 20/30 °C with 12/12 h dark/light photo periods in germination chamber (NSW -192 "CALTAN" seed germinator) to maintain the desired temperature ± 1 °C. Germination was recorded every 3 days and germinated seeds were removed during every count. Radicle emergence of at least 2mm was used as the criteria for germination. Seeds remained in the Petri-dishes without radicle emergence after 12 weeks were scored as non-viable. During germination, very few seeds of *L. siceraria* and *C. maxima* were contaminated by fungus; these seeds were removed from the sandwich boxes and excluded from any future analysis.

Moisture content

The moisture content of all the three species was determined by drying approximately 3-5 g of seeds at 103 °C for 17 h. The difference in fresh and dry weight was used to estimate the moisture content, which is expressed on a percentage of fresh weight basis.

Preparation of Ash

Dry woods of *A. indica* (neem) (collected from agricultural site, dried in sun) weighing approximately 5 kg were burnt along with dry cow dung (3 kg). These materials were burnt in an open place on a steel sieve rested on a stand. Fire was ignited with the help of petrol and paper. After burning, the ash below the sieve was collected and stored in closed glass vessel (diameter 18 cm) at room temperature until used in further experimentation.

Seed storage and concentration of Ash

Ash was added to the seeds kept in open glass jars (5 litres) at the concentration of 5%, 10% and 20% of the seed weight. In 5% concentration, 100 g of seeds were mixed with 5 g of ash and 20% concentration, 100 g seeds and 20 g ash were mixed. For each combination, four replicates of 25 seeds of each species were stored in jute bags along with the ash for 24 months at room temperature. Seeds untreated with ash stored in jute bags served as control. After 12

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and 24 months the seeds were removed from storage and germinated as described above. The seed treated with ash were kept outside laboratory for almost 1 month (27 days) due to maintenance works.

Data analysis

Although the experiments had been conducted for a period of two years, total percentage of seeds germinated at the end of two years were less than 10% in all three species at any given combination. Preliminary statistical analysis showed that the data of all concentrations (except 5% ash treated *T. cucumerina* seeds) after two years were significantly different from 1 year because of lower germination. Due to no germination in some combination and very low germination in others, ash had no effect on seed storage for more than 1 year. Hence these data are not presented and excluded from analysis. Rather, it appeared that understanding the concentration of ash in seed storage for up to 1 year is useful. Hence, the effect of ash concentration on seed viability for each species after 1-year storage was separately analysed in a one-way ANOVA. We used Duncan post-hoc test to compare the means at 0.05 alpha levels.

Results

Moisture content

The moisture content of *L. siceraria, T. cucumerina* and *C. maxima* were 9.2 ± 0.9, 10.1 ± 2 and 6.6 ± 0.8% respectively at the time of collection.

Effects of ash on *L. siceraria*

Seeds of *L. siceraria* germinated to 71 ± 7.19% at the time of harvest. During germination, many seeds were severely infected by fungal growth. These seeds were removed routinely from the sandwich box. There was a significant effect of ash on *L. siceraria* seed storage ($F3, 12 = 16.18$, $P < 0.001$; Figure 1). The use of 5% concentration was found to be less effective for *L. siceraria* storage because there was no significant difference observed between 5% ash treated and control seeds after 12 months ($P > 0.05$). Despite seeds treated with 10% and 20% concentration of ash survived significantly well compared to 5% concentration ($P < 0.001$), the germination percentage dropped slightly after storage when compared to the germination percentage at the time of harvest. These results indicate seeds of *L. siceraria* can be kept viable at an acceptable rate for more than 12 months, but only when a higher concentration of ash was used (Figure 1a).

Effects of ash on *T. cucumerina*

At the time of harvest, seeds of *T. cucumerina* germinated to 62 ± 5.13 %. Ash had significant effect in seed storage of *T. cucumerina* for up to 12 months ($F 3, 12 = 10.95$, $P < 0.001$). Similar to *L. siceraria*, seeds of *T. cucumerina* treated with 5% concentration of ash survived significantly lower than the higher concentration of ash tested ($P < 0.001$). However, seeds treated with 5% ash did not survive better when compared to control ($P > 0.05$). Seeds of *T. cucumerina* stored with 10% ash for 12 months had retained higher viable seeds (compare dashed line and 10% ash concentration in Figure 1b). We observed 10% concentration of ash was more effective than 20%. However, no statistically significant difference was evident indicating both the concentration were effective in preserving the seeds of *T. cucumerina*.

Seeds of *T. cucumerina* stored at all the combination of ash failed to germinate when tested after 24 months (data not shown).

Effects of ash on *C. maxima*

Amongst the three species tested, *C. maxima* had high (86 ± 5.91%) seed germination at the time of collection. During the germination period, some germinated seeds were removed from the sandwich boxes because these seedlings grew to a long size obstructing the other germinating seeds. The overall germination percentage of *C. maxima* seeds untreated with ash decreased significantly ($F 3, 12 = 15.69$, $p < 0.001$) after 12 months of storage when compared to ash treated seeds (Figure 1c). In contrast to *L. siceraria* and *T. cucumerina* use of 5% ash was found to be effective in storing seeds of *C. maxima* for 12 months (Figure 1c). When tested for significance at 0.05 alpha levels, results also revealed that the mean of control was statistically different from means of 5%, 10% and 20% ash treated seeds. However, the means of ash treated seeds did not significantly differ ($P > 0.001$) with the concentrations used. In spite of this, seeds treated with 10% germinated little more than 20% ash treated seeds, but seeds stored with 10% concentration resulted in higher mortality (Figure 1c).

Insect infestation

Seeds of all species stored without ash were infected by several insects. *Dacus ciliate* Loew was the most common insect damaging all the three species. Seeds of *T. cucumerina* were severely infected by *Sitophilus* sp. Likewise, *Aulacophora foveicollis* Lucas attacked *C. maxima* seeds frequently. Visual inspection of the seeds revealed that the seeds were riddled and some part of the embryo (epicotyl) was completely damaged in *L. siceraria*. Between 25 to 38% of *T. cucumerina* seeds were powdered by insects every year. Seeds of *L.*

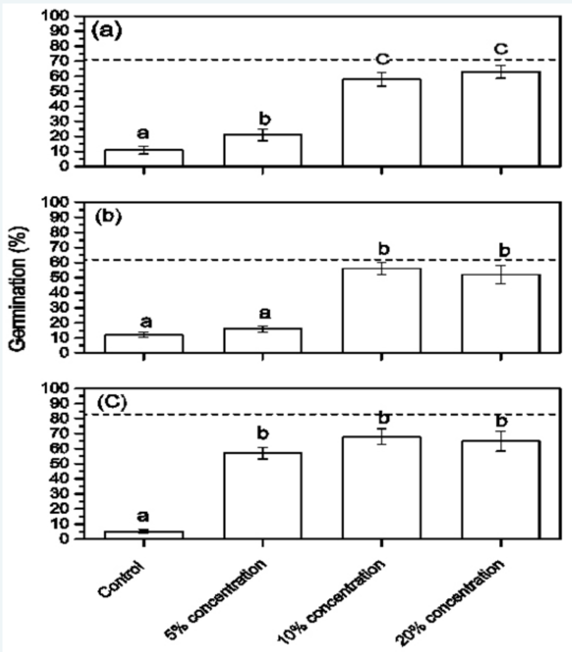

Figure 1: Effect of different concentration of ash on (a) *Lagenaria siceraria;* (b) *Trichosanthes cucumerina* and (c) *Cucurbita maxima*. Dashed line indicates the germination before storage.

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siceraria and *T. cucumerina* treated with 10% ash concentration were still affected by insects, but the degree of damage was low compared to the control. It is essential to mention that in *C. maxima*, besides the insect damage, most of the seeds without ash were covered with a thick mold of fungus. The fungus was removed by forceps, but seeds retrieved from fungus did not germinate.

Discussion

Many recent studies have shown the importance of experimental investigations of traditional seed storage methods. Given the traditional application of ash to protect grains from pests during storage [16]; lately, the importance of concentration used has been of some interest. Golob recommended quantities at least above 5% by weight should be applied if ash is used as a protectant [22]. However, quantities in excess of 20% or more are likely to result in greater protection [22]. To the best of our knowledge, effects of ash on *L. siceraria, T. cucumerina* and *C. maxima* have never been assessed previously. The results of this study showed that ash is really a powerful biological substance enhancing the seed viability of these species during storage. As expected, there was a general increase in percentage of seeds survived with increase in ash concentration for all three species tested (Figure 1).

In general, we found 5% concentration of ash was statistically less effective in seed storage compared to 10% and 20% concentration ($P < 0.05$) for *L. siceraria* and *T. cucumerina* species after 12 months of storage. Seeds of these two species at lower ash concentration failed to germinate adequately suggesting that seed losses are intensified at lower ash concentration in some species. This result clearly supports the early recommendations by Golob [22]. In addition, this finding is also consistent with some of the early empirical investigations in cowpea and maize [23,24], where 20% concentration of ash was reported to result in better storage. Experiments reported in Tanzania and Malawi revealed higher dose of ash is essential for maize storage against *Sitophilus* [24,25]. Similarly, Wolfson, et al. found that a minimum ratio of 3 parts of ash to 4 parts of cowpea seeds prevented population growth of *Callosobruchus maculates* (Fabricius) (Coleoptera: Bruchidae) [19], but addition of 3 cm layer of ash above the stored cowpea seeds prevented infestation by adults. In a previous survey of the same region, Karthikeyan, et al. (26) found that Sorghum bicolor seeds are stored with ash at the ratio of 4:1 in airtight jute gunny bags to prevent the seed loss by rice weevil (*Sitophilus oryzae*) rodents (*Tatera indica*) and mite (*Oligonychus indicus*) for a period of 6 months [26]. It is however interesting to note that nearly 70% of *C. maxima* seeds stored with 10% ash concentration germinated after 12 month storage (Figure 1c).

Our main hypothesis included whether or not ash can be used to store seeds of *L. siceraria*, *T. cucumerina* and *C. maxima* under the storage conditions farmers' use. In order to stimulate the traditional storage conditions, bags with seeds were undisturbed during storage. This process hindered us from specifically identifying the types of insects and fungus that lead to seed deterioration during storage. However, we found insects like *Dacus ciliates*, *Rhyzopertha dominica* F., *Tribolium castaneum* Herbst. were main contributors for seed damage, as they were still present in controls at the end of storage. Insecticidal properties of ash controlling these insects need specific experimental analysis. A casual literature survey shows ash obtained from wood can potentially control Bruchidae *Aspergillus flavus* [19,23], *A. niger*, *A. fumigatus*, *Rhizopus* [20], *Callosobruchus maculates* [19,27]. In a more detailed study, Hakbijl reported the use of ash as an insecticide against beetles *Sitophilus granaries* (Linnaeus) [16], *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst), larvae of *Tenebrio molitor* (Linnaeus) and *Calliphora vomitoria* (Linnaeus). Misra reported the use of cow dung ash completely inhibited the oviposition of *Callosobruchus chinensis* in stored black gram seeds [28].

In the present study area (and in many tropics), neem woods can be easily obtained and dried in the sun. Since many farmers rely on cattle for additional income, dung can also be collected any time. The easy availability of resources makes the technique more readily practical and cheap (at almost no cost) compared to the other pesticides and storage techniques, which are expensive and sometimes unavailable. However, whether or not ash produced by other plant materials maintains viability of these seeds remain unknown and further works are called to answer this question. The two-year storage experiments (data not shown) showing less than 10% seed germination in all three species provides evidence to show that ash can serve as a protective agent for only one year. One might assume ash added to seeds that had been sun dried might induce a drying effect thereby leading to viability. However, this assumption does not explain the viability loss during the first year (control) and/or second year, as if the dryness of the seeds was the reason for viability a longer storage time might be expected. Thus, we conclude ash has some (yet unidentified) properties that contributed to successfully seed storage in these species for 1 year.

While the experiments reported in the present and previous studies reveal that ash treated seeds can be kept healthy and free from insects, fungi etc. for at least 12 months (Figure 1), precise rationale behind the mechanism of action is still obscure. It has been suggested that dust particles such as ash blocks the air spaces between grains thereby inhibiting the insect movement during storage [22]. In addition, the cessation of insect movement also results in less likelihood of oviposition directly onto the seeds [23]. Insects that attack seeds coated or submerged in ash are possibly killed as a result of desiccation of water damaging the cuticle [22]. Besides insects, spores of storage fungi may also damage the seeds, because these storage fungi can grow in highly complex environments such as low moisture content and high osmotic pressure that inhibit the growth of normal fungi. It is probable that the ash maintains the seeds in a lowest possible dry state, a property that could prevent the growth of storage fungi [20,22]. In contrast, storing seeds without ash equilibrates to the environmental RH (values are often high in Tropics) at a favorable temperature all-round the year resulting in higher insect multiplication rate.

Alternative traditional storage methods for *L. siceraria*, *C. maxima* and *T. cucumerina* are not well established, but Karthikeyan, et al. in their survey reported *L. siceraria* seeds embedded in cow dung are stored for up to one year [26]. In our ongoing survey, we also found some farmers keep fruits in storage and collect seeds

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before sowing. The efficacy of this method needs more study, but there is a chance that these fruits are infected. Being the largest groups in the vegetable kingdom, Cucurbitaceae crops are more commonly cultivated in Asian countries. However, the high cost required for storing seeds using insecticides becomes frustrating. Our findings indicate, if used at right amount, ash can have a great potential in storing seeds of *L. siceraria*, *T. cucumerina* and *C. maxima* seeds for up to 12 months. We believe the method described here could have a significant application in minimizing the post-harvest losses of these species and supply high quality of seeds for next sowing season. More importantly, the low cost and less health hazards of ash will encourage its wider application especially in tropics.

Acknowledgement

Funding for this research was provided by National Science Foundation of China (NSFC) (grant number 31750110474) and post-doctoral funding by Shanghai government (grant number 2016M601620).

Author's contribution

GKJ, performed the experiment, analysed the data and written the Manuscript. LJ, YY, prepared figures and analysed data. BL, provided supervisory support.

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