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Research Article

Choice of Harvest Method for Maximizing Seed Yield and Effect of Storage Methods on Seed Quality of a *Panicum maximum* (Local Type) in Southern Benin (West Africa)

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Abstract

The aim of this study was to assess the effect of different harvesting methods on the seed yield of a *Panicum maximum* ecotype and to identify the storage method that maximizes seeds viability and vigour. Six methods of seed harvesting were assessed in a randomized complete block design with for replications: Seed heads shaken every 3 days into a large seed net receptacle (Shake); Seed heads covered with a nylon net bag, with an outlet to collect the seed (Cover); A nylon net receptacle placed under the seed heads for 3 weeks after 50% seed head emergence (Under); Seed heads cut 15, 20 and 25 days after 50% seed head emergence (Cut 15, Cut 20 and Cut 25 respectively). The seed storage methods were applied based on the best three seed-harvesting methods that yielded high seed yields. The storage methods included storing in gunny bags with cow dung ash as the seed treatment (SM1), seed treatment using Mortein Doom®, a modern seed protectant (SM2) and cow dung ash (SM3); in both cases seeds were stored in air-tight containers. Quality analysis of the seeds was done first before storage and then after three and six months of storage. Data were analyzed through ANOVA. After 3 years of cultivation, highest seed yields were obtained from Cover and Under in all years and these treatments also gave the highest seed quality. Of mechanical harvesting methods, Cut 20 gave the highest seed yields, but required a high level of precision in determining optimum harvest date. Early harvesting resulted in immature, low quality seed, whereas when harvesting was delayed, a high proportion of seed had already been shed. The results of seed storage indicate that the method SM1 had the poorest performance. Seeds of this storage method have significantly lower vigour after three and six months' storage and recorded significantly higher insect damage. They had significantly higher moisture content increase. The best treatment was storage in airtight containers with either Mortein Doom® or cow dung ash as the seed treatment. Cow dung ash should therefore be combined with air tight storage to increase the seed longevity.

Keywords: *Seed; Guinea Grass; Harvesting; Storage*

Introduction

Guinea grass is native to Africa with an extension zone from 20°N to 20°S and from sea level to 2500 m and above. It belongs to a very diverse genus (*Panicum*). The diversity in the genus has led to a confusing taxonomy and the delimitation of the genus is not entirely clear.

Current names in use to describe *Panicum maximum* are *Urochloa maxima* (Jacq.) R. Webster [1] and *Megathyrsus maximus* (Jacq.) B. K. Simon and S. W. L. Jacobs [2]. As the confusion surrounding the placement of *P. maximum* in the classification has not been settled, this article uses *P. maximum* as the name of the plant, following the definition used in the world grass species database of the Royal Botanical Garden at Kew [3]. *P. maximum* species is composed of 09 ecotypes in terms of drought resistance and agronomic characteristics [4]. From the ecological point of view, a good development of this plant requires at least an annual rainfall of 1200 mm of water. However, some ecotypes tolerate rainfall below 800 mm of water per year [5,6]. *P. maximum* (Jacq.) local can yield between 5 - 10 t/ha DM [7,8]. It has showed a very high CP (8 - 26% DM) and the low structural carbohydrates (NDF: 50.80%) content. Consequently, energy content (15.14 MJ/kg DM) was very high, though ash level was quite high too (16.42% DM). Macro-mineral: Ca, P, Mg and K contents are 5.87, 0.86, 1.69 and 11.04 mg/kg DM [8,9]. It tolerates heavy grazing and close-to-ground level cutting, combines well with legumes and has a high potential for seed production [4,10]. *P. maximum* can be established easily from seed, which is an important key to encourage small farmers in Benin to plant forage for their livestock, since it is cheaper to establish from seed than from vegetative propagation [11]. However, there is the problem of seed availability in Benin and studies on production and methods of harvesting and storing forage seeds are scarce [12,13]. Thus, there is a need to develop methods to obtain high yields of good quality seed of *P. maximum.*

Identifying suitable methods and timing of seed harvest and storage are critical for optimizing seed yield and seed quality of tropical grasses [14,15]. In Benin, there is a vast scope to grow forage species both in the field and homestead condition especially in rural region [16]. But higher production of forage crops depends largely on the ability to integrate better crop management into the cultivation systems. To boost seed yield, quality seed is essential both for fodders and seed production. Seed development is the period between fertilization and maximization of fresh weight accumulation and seed maturation begins at the end of seed development and continues till harvest [17]. The seed reaches its maximum dry weight at physiological maturity and seeds should be harvested at this time to ensure their quality in terms of germinability and vigour. Attainment of physiological maturity is a genotypic character which is influenced by environmental factors [18]. Moreover, Seed quality and lifetime of seed of forage species in Benin are the recurrent problems which are very acute among the farmers. Poor seed storage conditions have been reported to cause up to 10% loss in seed quality in the tropics mainly through loss of viability [19,20]. This problem should, therefore, be addressed as seed security is essential in ensuring increased use of agro biodiversity and crop forage adoption in agriculture-livestock production systems.

Aim of the Study

The aims of the present study were to:

1. Determine a suitable method of hand-harvesting *P. maximum* seed in Benin, based on some methods which have been developed successfully for other tropical grasses [21-23] and
2. Assess the efficacy of seed storage methods in the maintaining seed viability and vigour of *P. maximum* seed.

Material and Methods

Experiment-I: Harvesting methods assessment

Location, climate and soil characteristics of the experimental site

The trial was conducted on experimental field during rainy seasons from 2015 to 2017. It is located at Abomey-Calavi a Guinean Sudanian agroecological zone in southern Benin (West Africa) between Latitude 06°30'N and Longitude 2°40'E with an altitude of 50 m above sea level. The experimental site is characterized by an average rainfall of 1038 mm per year with a bimodal distribution. Mean annual temperatures range between 25°C and 29°C. The area is characterized by sub-equatorial climate with two rainy seasons (March to end of July and mid-September to November) and two dry seasons (August to mid-September and December to March). The area is dominated by ferralitic soil presenting the following characteristics: pH (2/5 water) 6.2; N = 0.05%; organic carbon = 0.4%; Ca = 60 mg/100 g; Mg = 10 mg/100 g; P (extractable) = 0.2 mg/100 g and K (exchangeable) = 20 mg/100 g. The experimental site was previously cultivated without any organic fertilization, as reflected by the low organic carbon content of the soil. The analysis conducted on 0 - 10 cm soil-samples showed that sand, loam and clay represent approximately 84, 8 and 8%, respectively.

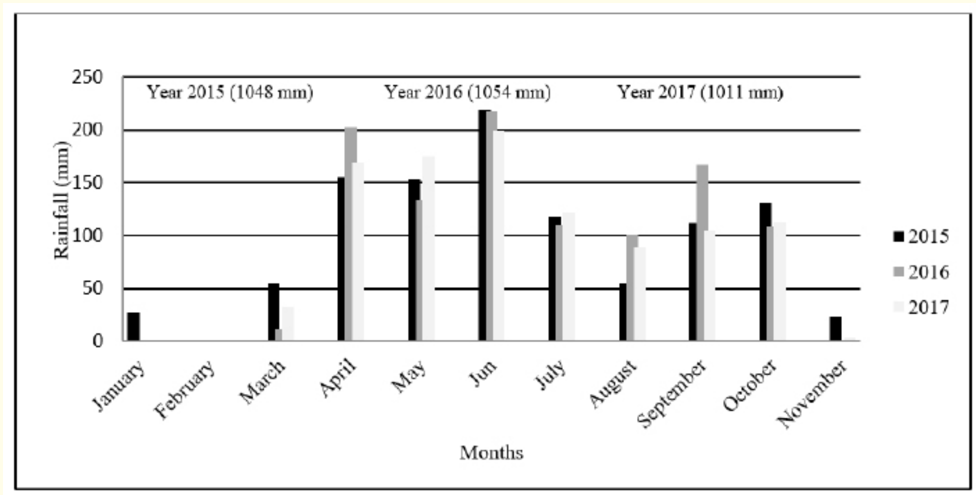

Figure 1: *Monthly rainfalls recorded during the 3 experimental rainy seasons.*

Culture establishment: Design and treatments

At the start of the experiment (March 2015), the experimental area was cleared, ploughed and harrowed before plant installation in 5 x 6 = 30 m^2 plots spaced 1m-apart from each other. *P. maximum* ecotype was established 60 cm-apart using rooted tillers (4 - 5 tillers/plantation hole). *P. maximum* ecotype used develops high resistance to dry season. It is characterized by medium height and high leaf length and width. All the plots were fertilized with organo-mineral fertilizer: 10t goat manure + 33 kg NPK/ha. Goat manure was applied 2 weeks before planting and the inorganic fertilizer NPK was applied 2 weeks after planting. The trial was arranged in a randomized complete block design with 6 treatments and 4 replications. Six harvest methods were as assessed with three which were designed as labour intensive methods and three methods which were designed as being less labour-intensive.

Table 1*: Harvesting methods used.*

Treatment	Description	Code
Labourintensive methods		
T1	Seed heads shaken every 2 days into a large seed net receptacle	Shake
T2	Seed heads covered with a nylon net bag, with an outlet to collect the seed, after 50% seed head emergence	Cover
T3	A nylon net receptacle placed under the seed heads for 3 weeks, after 50% seed head emergence, at 1 m above ground level, with 75% ground covering	Under
Mechanical harvesting		
T4	Seed heads cut 15 days after 50% seed head emergence	Cut 15
T5	Seed heads cut 20 days after 50% seed head emergence	Cut 20
T6	Seed heads cut 25 days after 50% seed head emergence	Cut 25

Date of 50% seed head emergence was determined during the first 10 days of seed head emergence, by randomly counting the number of inflorescences emerged from the total number of tillers in 6 plants/plot and recording the date when half of the tillers showed emerged seed heads.

Seed yield and seed quality

All seed heads within each plot were harvested each year over the period of seed maturity. Four rows of 1.8 m which corresponds to a sampling of an area of $4m^2$ were choosing per plot for yield seed assessment. 1000-seed weight, seed purity and seed germination were determined. 1000-seed weight was determined from pure-seed spikelet weight. Seed yield and 1000-seed weight were corrected to 10% seed moisture concentration. Pure seed percentage was estimated using the international method using a 10 g sample for each plot, setting apart seeds and inert materials. 1,000 seeds weight was estimated as the average of eight 100 pure seed replicates multiplied by 10 [24].

Experiment-II: Efficacy of storage methods

The seeds tested for this second part of our study are those resulting from the three best harvesting methods identified in the first part of this study. The seeds were tested for electrical conductivity, tetrazolium chloride response, germination in sand, insect damage and moisture content following procedures described below.

Storage methods assessed

Three different storage methods were tested:

- SM1: Storage in gunny bags placed on the floor with cow dung ash as seed treatment;
- SM2: Seeds treated with Mortein Doom® and stored in airtight plastic containers;
- SM3: Seeds treated with cow dung ash and stored in airtight plastic containers.

The storage method SM1 is considered as the storage method currently practiced by most producers for the conservation of forage seeds. The last two methods (SM2 and SM3) are new fodder seed conservation techniques. The quantity of ash and Mortein Doom® used in this study was about 3.3% and 0.9% by weight, respectively. The ash used in the study was prepared by burning dried cow dung. Mortein Doom® is a modern commercial seed protectant which is in powder form and is available in the market. Seed quality was determined at the onset of the storage trial and after storage periods of 3 and 6 months [15].

Laboratory analyzes

Electrical conductivity test was determined by selecting four samples of 50 seeds from each treatment of each farmer. The seeds were weighed and incubated in 250 ml of distilled water at $20^{o}C$ for 24 hours. The electrical conductivity of equivalent quantity of water was also measured as a control using a Fieldlab-LF conductivity Meter and LF 513T electrode dip-type cell (Schott Gerate Glass Company, Mainz, Germany).The conductivity per gram of seed in µs/cm/g- at 12% moisture content in 250 ml of water was then calculated [25].

Tetrazolium test was determined by four samples of 25 seeds each were randomly selected from each treatment of each farmer. The seeds were soaked in water at room temperature for 18 hours. Thereafter, the seeds were cut longitudinally through about 2/3 of the endosperm and then soaked in 1% tetrazolium solution (2, 3, 5-triphenyltetrazolium chloride) at $30^{o}C$ for 3 hours. Subsequently, the seeds were washed in water for about 2 minutes and examined for the staining pattern of the embryo with the help of a hand lens [25]. Based on the staining, the percentage of viable seeds was estimated.

Four replicate samples of 100 seeds each were sown in sand and placed in germination chambers at a temperature of 25 - 30°C. The seedlings were evaluated after 7 days where they were put into 3 categories: Normal, abnormal and dead seeds. For example, seeds with stunted, retarded, constricted, broken, decayed or missing primary and/or secondary roots were classified as abnormal [26]. Percentage germination was then computed.

Insect damage was also assessed by the count method. A sample of 200 seeds was randomly taken and the number of insect-damaged and undamaged seeds was determined. The percentage of insect-damaged seed was then calculated. Moisture content was measured using a moisture meter, Hydromette G86.

Data analysis

The effect harvesting method on seed production and effect of storage method on seed viability, insect damage and moisture content over time was analyzed by ANOVA using the Generalized Linear Model of SAS software version 9.2.

Results

Seed yield and seed quality components

The different methods of seed harvesting had a significant effect on seed yield of *P. maximum* and its components, in each of the 3 years of the experiment ($P < 0.05$). Highest seed yields in each year and overall were from Cover (454.83 kg/ha) and Under (366 kg/ha) harvesting methods. Method Cut 20 was consistently the best (321.58 kg/ha) of the 3 Mechanical harvesting methods (Cut 15, Cut 20 and Cut 25).

Thousand-seed weight was markedly and consistently highest in the 3 intensive harvesting methods (Cover > Shake > Under) and lowest in Cut 15, harvested 15 days after 50% seed head emergence. Seed purity was also higher in the 3 intensive harvesting methods Shake, Cover and Under (80.4; 75.33 and 76.33% respectively) and lowest in Cut 15 harvesting method (49.83%). The highest seed yields are obtained with Cover > Under > Shake harvesting methods (342.65, 279.45, 211.11 kg/ha respectively). The mechanical harvest method produced the highest pure seed yield is Cut 20 (196.46 kg/ha). The lowest yield is obtained with the Cut 25 harvest method (62.5 kg/ha). The influence of the year of production on seed yield, thousand seed weight, seed purity and pure seed yield is not significant ($P > 0.05$) (Table 2).

Table 2: *Effect of harvesting methods on seed yield and seed quality components of P. maximum.*

Treatments	Shake	Cover	Under	Cut 15	Cut 20	Cut 25	Mean
2015							
Seed yield (kg/ha)	256.25e	451.50a	358.75b	293.50d	319.25c	115.75f	299.16
1000-seed weight (mg)	881.50a	886.50a	882.75a	700.75d	761.00b	740.75c	808.87
Seed purity (%)	79.25a	75.25b	75.75b	49.00e	62.75c	54.00d	66.00
Pure seed yield (kg/ha)	203.04c	339.71a	271.80b	143.78e	200.37d	62.54f	203.54
2016							
Seed yield (kg/ha)	269.25e	466.50a	371.50b	287.50d	330.25c	107.25f	305.37
1000-seed weight (mg)	885.75a	883.25a	884.50a	710.75d	764.75b	738.25c	811.20
Seed purity (%)	81.00a	75.50b	76.50b	50.50e	60.75c	55.00d	66.54
Pure seed yield (kg/ha)	217.98c	352.27a	284.25b	145.18e	200.62d	59.06f	209.89
2017							
Seed yield (kg/ha)	262.25e	446.50a	367.75b	296.50d	315.25c	120.75f	301.50
1000-seed weight (mg)	886.25ab	891.25a	884.25b	706.50e	762.00c	736.75d	811.25
Seed purity (%)	81.00a	75.25b	76.75b	50.00e	59.75c	54.50d	66.20
Pure seed yield (kg/ha)	212.31c	335.98a	282.31b	148.22e	188.39d	65.89f	205.52
3-years mean							
Seed yield (kg/ha)	262.58e	454.83a	366.00b	292.50d	321.58c	114.58f	302.01
1000-seed weight (mg)	884.50a	887.16a	883.83a	706.00d	762.58b	738.58c	810.44
Seed purity (%)	80.41a	75.33b	76.33b	49.83e	61.08c	54.50d	66.25
Pure seed yield (kg/ha)	211.11c	342.65a	279.45b	145.73e	196.46d	62.50f	206.32

Within lines, means followed by different letters are significantly different ($p < 0.05$).

Vigour, seed viability and seed germination

The figure 2 showed that an analysis of seeds stored for 3 and 6 months revealed highly significant differences in seed vigour ($P < 0.05$). Thus, the seeds stored in airtight containers had higher vigour than those stored in gunny bags. The same is true for seed viability. Seeds treated with Mortein Doom® and stored in airtight plastic containers (SM2) and the seeds treated with cow dung ash and stored in airtight plastic containers (SM3) had the highest seed vigour while those stored seeds in gunny bags with cow dung ash (SM1) as a seed storage method, exhibited the highest decline in seed viability after three and six months storage (Figure 3).

Results showed that the lowest germination capacity was recorded by seeds stored in gunny bags placed on the floor with cow dung ash as seed treatment. Conversely, the seed stored in airtight containers had the highest ($P < 0.05$). The effect of cow dung ash and Mortein Doom® in maintaining viability was almost the same when seeds were stored in airtight containers (Figure 5).

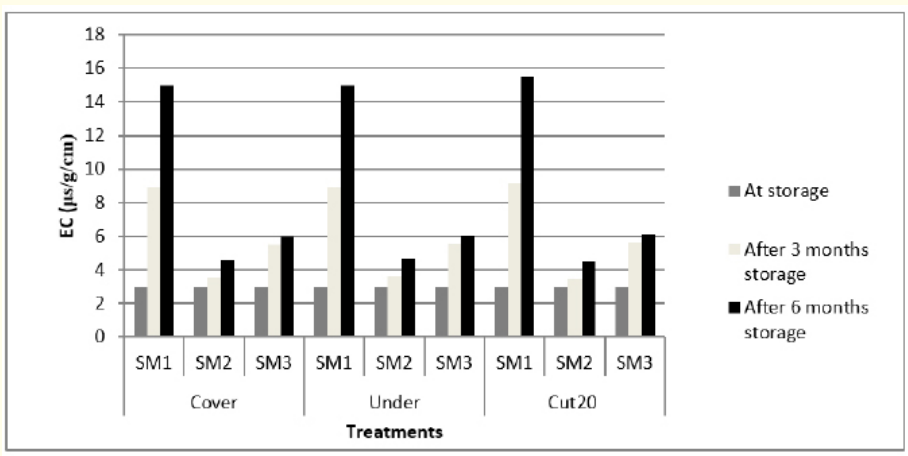

Figure 2: *Electrical Conductivity (µs/g/cm) after 0, 3 and 6 months' storage.*

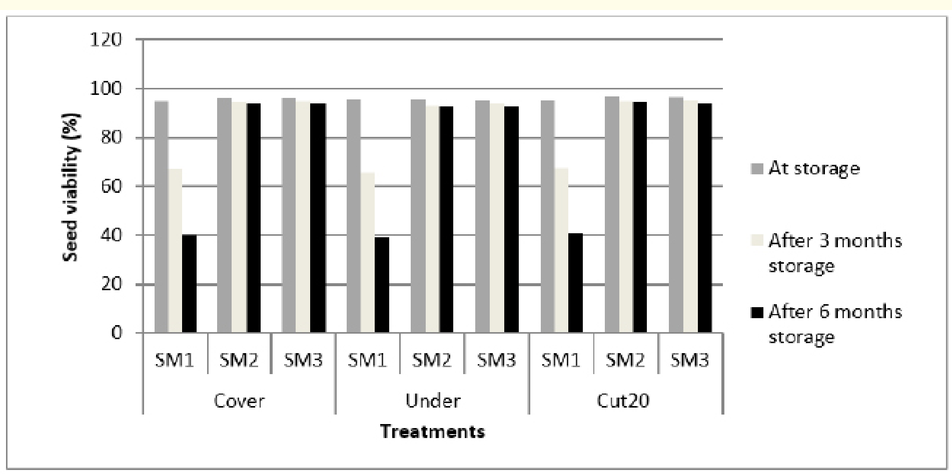

Figure 3: *Seed viability after 0, 3 and 6 months' storage.*

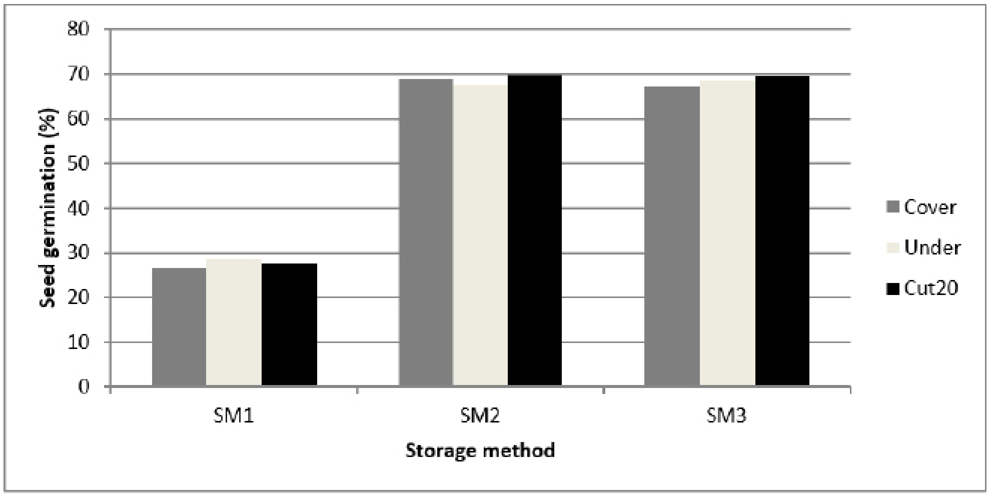

Figure 4: *Seed germination with different seed storage methods after a-6 month storage period.*

Moisture content and insect damage

Figure 5 showed that the seeds stored in gunny bags had significantly higher moisture content increases than those stored in airtight containers after a storage period of 6 months. The seed harvesting method did not significantly influence the moisture content of stored seeds.

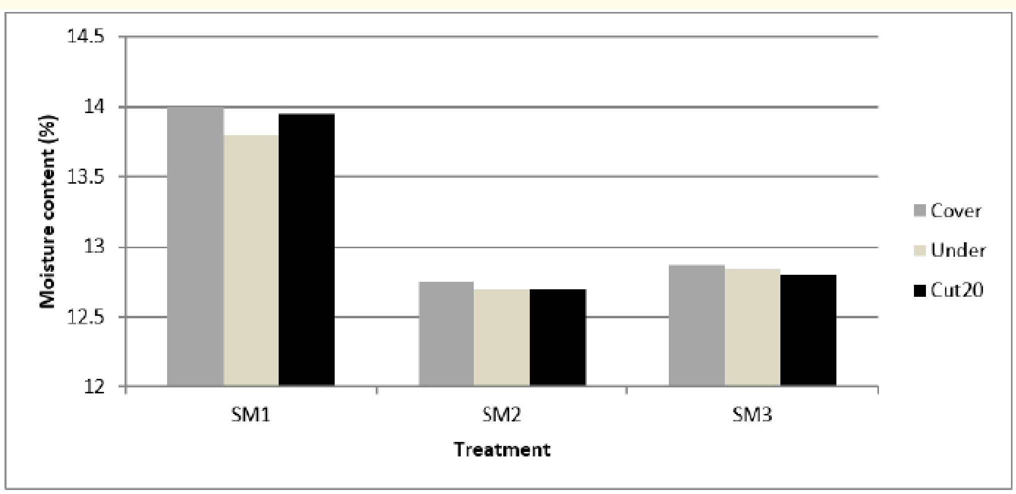

Figure 5: *Seed moisture content (%) after six months of storage.*

The storage seed in gunny bags placed on the floor with cow dung ash recorded significantly higher insect damage than storage in airtight containers (Table 3). The insect damage recorded in the seeds stored in gunny bags placed on the floor with cow dung ash (SM1) was about 98% higher than the damage recorded for seeds treated with Mortein Doom® and stored in airtight plastic containers with ash and stored in airtight plastic containers. Insect damage with SM1 storage method was about 97% higher than the damage recorded for Seeds treated with cow dung ash and stored in airtight plastic containers (SM3). The seed harvesting method had no influence on the damage caused by insects.

Treatments	Cover	Under	Cut20	Mean
SM1	54.5Aa	59.5Aa	55.2Aa	56.4a
SM2	0.6Ab	1.7Ab	0.7Ab	0.9b
SM3	1.2Ab	2.3Ab	1.50Ab	1.6b
(SM1 - SM2) /SM1	98.9	97.1	98.7	98.2
(SM1 - SM3) /SM1	97.7	96.2	97.2	97

Table 3: *Insect damage (%) on P. maximum seeds observed with various storage methods.*
Within lines, means followed by different letters are significantly different (p < 0.05).

Discussion

This study reveals the importance of best choice of the harvesting method for optimum obtaining of *P. maximum* seed yield and seed quality. The method of harvesting *P. maximum* greatly affected seed yields in our study. The harvesting method (Cover) which consists to cover seed heads with a nylon net bag, with an outlet to collect the seed, after 50% seed head emergence was found to be the best method. Seed harvesting method ''Under'' gave also a high seed yield and seed quality, as well as lower variability between years. This observation does not agree with the results found by Kowithayakorn and Phaikaew [14] who affirm that knocking ripe seed daily into bags is the best method for harvesting ruzi grass seed. Cutting seed heads at 20 days after 50% of seed head emergence resulted in higher seed yield than cutting at 15 or 25 days and seed quality was fair. This method requires close observation during flowering and seed set, together with good experience to judge when 50% of seed heads have emerged. Cutting seed heads too early produced good seed yield, but very low quality seed because the seed had not yet fully matured. In addition, late cutting gave very low seed yield due to the loss of fully matured seed through seed shedding before harvest. Thus, the timing of seed harvest is one of the most important decisions a grass seed grower will make. Grasses need 20 - 30 days after flowering for seeds to properly mature. This will vary because the period of flowering and seed development lasts from several days to two weeks. As a result, seed heads emerge at different times, which cause uneven ripening [27]. *P. maximum* grows very vigorously during the wet season and undefoliated fields have, on average, more than 10-15 t/ha DM standing 2.5 m in height by the middle of August [11]. From this mass of vegetation, inflorescences emerge which can reach to more than 3 m in height at flowering. These tall inflorescences are not strong and, if heavy with seed, will lodge very easily from rain and even break if birds settle on them during seed foraging. Bird damage from broken stems and eaten seed is a problem in our area because, at the time of harvest, there are no other seed crops for birds to forage on. This shows the need to cover inflorescences with bags to maximize seed yield and seed quality [23]. The method of shaking flowering stems for seed harvesting (every 2 days) did not yield good seed yield. This might be a function of seed loss between shaking occasions, making this a rather inefficient method. The efficiency of this method might be improved by every day harvesting and by increasing the frequency to daily shaking [28].

After production and seeds harvesting, seed storage is very important to secure good quality seeds for planting programs whenever needed. Seed longevity, vigor and viability depend on genetic and physiological factors as well as storage conditions. The most important factors that influence storage are temperature, moisture, seed characteristics, micro-organism geographical location and storage structure [29]. This study is particularly useful and has merits since it encompasses ethnic practices and new storage methods of seed forage that have not been properly studied in the region. Thus, seeds stored in gunny bags and treated with cow dung ash had very low vigour and viability. This traditional method also recorded significantly higher insect damage. This is in agreement with the observation of Pats

[30] who reported storage of seeds in the open air was not effective in reducing insect damage. Others farmers also reported that seeds stored above the fireplace had inferior seed quality which led to low germination and poor yields [31]. Ash was used in this study for seed conservation. Often, ash is effective in controlling insect damage [32,33] but this study contradicts this. Ash is used both as an inert filler and for its other negative effects on insects. As an inert filler, ash works by filling up the space around the seed and impeding the movement of insects as well as, in sealed containers, reducing the volume of air available to the insects for respiration. Ash has been reported to damage the cuticle of insects causing them to dehydrate and also has detrimental effect on egg development [32,34]. The quantity of ash used in this study was 3.3% by weight. The application of ash should be done well before infestation by insects begins. The effectiveness of ash in reducing insect damage may be diminished by the use of gunny bags which do not give much protection from either to insects or rodents. The ash should be dry and fresh. Differences between ash and Mortein Doom® were not significant when seeds were stored in airtight containers. That locally available ash was as effective as Mortein Doom® is particularly encouraging. Both methods were quite effective in reducing insect damage and maintaining seed vigour and viability.

In general, seed storage tests have also revealed the need to store the seeds in hermetically closed containers in order to maintain good seed viability and good seed vigor. Indeed, even under controlled storage conditions (i.e. low temperature and low seed moisture content), performance after storage is dependent on the vigour status of the seed lot [35]. Use of hermetically sealed containers, desiccants and low temperatures improves storability as several physiological and biochemical processes and products are being regulated during dry storage. Accelerated ageing of seeds induced by several days of exposure to high temperature and humidity is recognized as an accurate indicator of seed viability and storability [36]. Some of the deleterious effects of ageing are associated with damages occurring at membrane, nucleic acids and protein levels [37]. Proper and safe storage conditions are defined as those that maintain seed quality without loss of vigour for three years [38]. The loss of seeds quality is not only visually observed by the poor condition of the seeds [39] but also by the poor performance of this seed when it is planted for the next season [40]. Seeds cannot retain their viability indefinitely and after a period of time, the seeds deteriorate [41]. Standardization of appropriate seed conditioning, packaging and storage conditions could ensure satisfactory planting quality of *P. maximum* seeds at the time of sowing. In addition, seed long-term storage is not feasible if the moisture content of the seed will increase to above 14% [33]. Yet, the subsistence farmers may not have the knowledge and equipment to get those seeds back to an acceptable moisture content and those seeds will deteriorate. Modi [42] showed the limitations of the conventional storage structures, where structures are made very weak and allow insects to enter and provide an environment for storage fungi to thrive. It is therefore important to train farmers on conservation techniques for forage seeds at a lower cost and on the basis of easy access material: plastic and cow dung ash.

Conclusion

Seed is the most valuable, basic and vital living input for increasing crop production. It has been scientifically proved that quality seed alone can contribute to the increase of crop yield and forage production. Therefore, quality seed production at appropriate time and seed maturity are a must for successful crop production. This production must be followed by a good method of seed collection. The most effective method identified in this study for *P. maximum* seed harvesting is to cover inflorescences with a bag after 50% emergence. This study concluded also that the principle of airtight, though not new, should be used to design low cost seed storage containers for resource-poor farmers which will result in better seed quality. The study further shows that cow dung which is freely available in most homesteads is a good seed protectant and is effective in maintaining seed quality in storage.

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