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# Flowering and Causes of Seed Defects in Lowland Bamboo (*Oxytenanthera abyssinica*): A Case Study in Benishangul Gumuz Regional State, Northwestern Ethiopia

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***Abstract***
*Ethiopia has two species of bamboo: the highland bamboo Yushina alpina and the lowland bamboo Oxytenanthera abyssinica. O. abyssinica is a multipurpose plant growing mostly in northwestern part of the Country. Different pests and diseases can affect the development of bamboo species in general and O. abyssinica in particular. Study on disease and pest occurrence on bamboo species in general and O. abyssinica in particular is limited. The nature of flowers of the species is also poorly studied. This research was conducted in Pawe special and Bullen districts of Metekel Zone in Benishangul Gumuz Regional State, Ethiopia. After reconnaissance survey, the bamboo growing areas were stratified in to four classes: protected natural, free access natural, grazing land, and homestead. In each class, one central or nearest to central clump was selected and fruits were collected from three central culms in each clump. The fruiting portion of the culms was divided in to three equal parts and the aggregate fruits were totally harvested. The fruits were collected and used to investigate disease and pest occurrence as well as other seed defects on seeds and fruits of the species. The two district and the three portions of the culm/ fruit-collection heights (top, middle and bottom) were treated as factors to see their effect on seed purity, and disease and pest occurrence. The study revealed that the quality of seed was affected by the sites and the different land use types. It was also observed that some diseases and pests attack seeds of the species. However, the variation on the disease and pest occurrence between the sites and among fruit collection heights was not statistically significant (α=0.05). Only numerical differences were observed for these assumed sources of variation. As there are various causes of seed defects, it is of a paramount importance to devise a mechanism of seed pest and disease control and prevention mechanism in order to be successful in the establishment and development of the species in the study area in particular and other ago-ecologically similar areas in the country.*

***Key words:*** *Disease and pest; Ethiopia; Flower; Fruit collection height; Oxytenanthera abyssinica*

## Introduction
Among the various bamboo species, which exist in the world, 2 species of bamboo found in Ethiopia are the highland bamboo, *Yushinia alpina*, and the lowland bamboo, *Oxytenanthera abyssinica*. *O. abyssinica* is prominent in river valleys and locally on the escarpment of western part of the country. One of these areas harboring this species is Benishangul Gumuz Regional State of Ethiopia (Ensermu *et al*., 2000). It co-exists with several other species especially the *Combretum-Terminalia* broadleaved deciduous woodland vegetation which is common to this part of the country (Sebsebe *et al*., 2003).

Bamboo has a considerable potential for socio-economic development (Baghel *et al*., 1998; Kumar *et al*., 1998; Perez *et al*., 1998; Sharma *et al*., 1998; Kumar & Sastry, 1999) and for environmental protection (Kumar & Sastry, 1999; Bystriakova *et al*., 2004). It can be used for house construction, animal feed, human food, as an agroforestry species (Maoyi, 1994; Christanty *et al*, 1997), for maintaining soil fertility (Lakshmana, 1994; Christanty *et al*., 1996; Mailly *et al*., 1997), as source of cash income and as medicine for treating many types of diseases (Yuming *et al*., 2004). Bamboo forest is also a material source for furniture, building, pulp, particleboard, bioenergy, food, forage and medicine (Embaye, 2000). But in Ethiopia, the species is not exploited to its full potential. Its current use is mainly restricted to household level; primarily for housing, fencing and household amenities. The resource is also poorly managed and exploited, where harvesting from natural stands is unregulated and resulting in a serious depletion of the resource in the growing areas (Ensermu *et al*., 2000).

In the study area, particularly in Metekel Zone of the Benishangul Gumuz Regional State, most of the natural *O. abyssinica* forest is disturbed and not in their natural condition (personal observation). Protection and development endeavors afforded to this species are almost negligible.

According to the information obtained from the elderly people living in the area, *O. abyssica* flowers at long time intervals (*ca.*30 years). During the study period, the species was flowering gregariously. The negative outcome of bamboo flowering is documented in many research findings. For example, John & Nadgauda (2002) stated that the death of bamboo forest after gregarious flowering resulted in much loss, and precipitated an ecological crisis. Unless and otherwise the species is replaced by natural regeneration or artificial propagation, the survival of the species will be in question (Embaye, 2000; Ensermu *et al*., 2000; Kassahun, 2003). However, because of human interference, natural regeneration is becoming difficult (Kassahun, 2003) as closing the entire area is difficult because increasing human population pressure and a corresponding increase in demand for land. The same situation is well documented by Banik (1994).

Although *O. abyssinica* propagates both sexually and asexually from seed and rhizomes respectively (Azene *et al.*, 1993; Dwivedi, 1993; Kassahun *et al*., 2003; Demelash *et al*., 2015), the offsetting vegetative method of propagation for *O. abyssinica* is difficult and cumbersome for large-scale plantations. Extracting and transporting rhizomes to longer distance plantations is a challenge in the use of this method (Kassahun, 2003). Moreover, Saharia & Sen (1990) stated that determining the age and origin of rhizome couldn't be ascertained in the case of large-scale collection, which might affect the future of the culm as the bamboo plantation established by this method may be disastrous. If the age of the parent plant is not taken into consideration, the new stand follows the age of the original planting materials and will flower within shorter time than the usual flowering cycle of the species. This may also be accompanied by the death of the culm if appropriate protection measures are not taken (Purohit *et al*., 1998). Hence, if seed is available, propagation by seed should be given a priority wherever that is possible (Kassahun, 2003). Therefore, after assessing the requirements, the encouragement of artificial regeneration of the species by collecting as much seed as possible is imperative (Dwivedi, 1993) as they are very convenient for propagation. Their small size and lightness make them more transportable for plantation establishment (Rao, 1994; Gera *et al*., 1998). Bamboo seeds are also potential part for tissue culture development (Williams, 1994) and are advantageous in regeneration as they are hardier in stressful environmental conditions (Samora, 1994). But little is known about the seed, flower and disease and pest occurrence on the species. To achieve rapid sustainable production of *O. abyssinica*, there is a need to understand the basic silvicultural requirements of the species among which the nature of the plant especially the reproductive part of the plant (flowers, fruits and seeds) are of a paramount importance.

There are different types of disease that affect different parts of a bamboo plant such as the rhizome, roots, culm, foliage, branches and minor branches, inflorescence, and seeds from bamboo stands (Mohanan, 1997). Especially, fungi, bacteria and other microbes invade bamboo seeds, during different developmental stages while they are still on the plant and after the seeds fallen to the ground. This reduces the amount of healthy seeds since embryos are infected and do not germinate (Rao, 1994). When the seed falls to the ground, it is subjected to further invasion by decaying organisms present on the forest floor. Seeds are also colonized and infected by several field and storage fungi, especially in the tropical humid areas and reported to be potential pathogens, which may pose problems (Mohanan, 1997). As far as we know there are no studies conducted on bamboo disease and pests in Ethiopia, and there are no documented reports. The nature of the natural environment and the location of the fruit on the culm may have impact on the occurrence and intensity of different pest and disease. The proportion of seed bearing fruits is very low, nearly about 14% (Demelash *et al*., 2014; 2015). Therefore, it is important to study the cause and factors affecting the occurrence of disease and pest on seeds on *O. abyssinica*.

Both species of Ethiopian Bamboo faced mass flowering (Demissew Sertse *et al*., 2011) during and shortly after the current study period. That phenomenon created favorable conditions for conducting studies on flowers, fruits and seeds of the species. Since *O. abyssinica* flowers at long time intervals (30-35 years), this study was also included flower study to exploit the opportunity of mass flowering. This study was conducted with the objective of investigating flowering nature of the species, identify the major seed defects, disease and pest occurrence on the species.

## Materials and Methods

### *Description of the Study Area*

The study materials of this research (flowers, seeds and fruits of *O. abyssinica*) were collected from a population of *O. abysinica* in Bullen and Pawe special districts of Benishangul Gumuz Regional State, North Western Ethiopia (Fig.1). The mean annual temperature at the seed collection districts are 1555.1mm (Pawe Meteorological station) and 1979.5mm (Metekel Zone department of agriculture) for Pawe and Bullen respectively. Pawe lies between $36^0$20' to $36^0$32' E and a specific area in Bullen where fruit collection was done is situated at $10^0$0' N and $35^0$59'. The topography of Pawe special district is 4% mountain, 22% undulating, and 74% plain and that of Bullen district is 5%, 35% and 60%, mountain, undulating and plain respectively (Metekel Zone Department of Agriculture, personal communication).

Pawe special district has an altitude of 980–1050 m a.s.l., whereas Bullen district has altitude of 1500-1750 m a.s.l. According to Engda Mersha (2000), the surroundings of Metekel Zone have a wide climatic range within $M_1$ and $SH_1$ (hot to warm moist lowlands and hot to warm sub humid lowlands) agro-ecological zones. The major soil groups are generally identified as Nitosols, Vertisols and fluvisols. The plains to gently undulating slopes have predominantly deep Vertisols and Nitosols (UNDP/ECA, 1998).

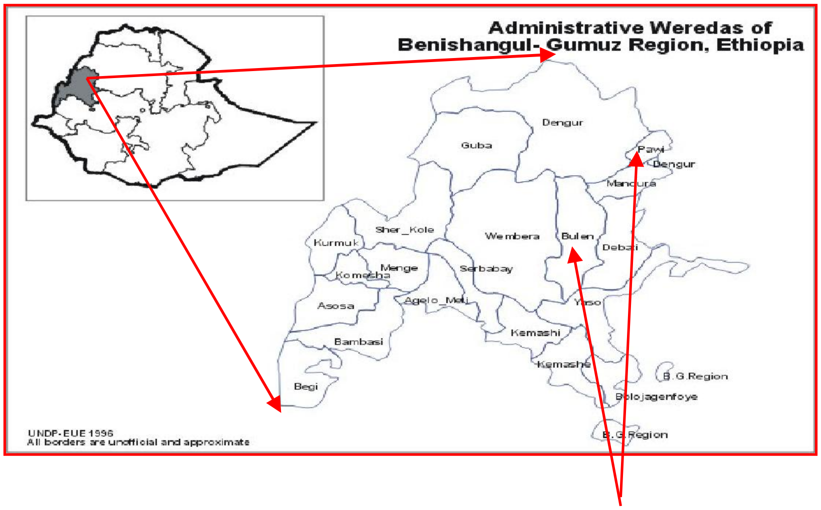


**Fig 1:** Location of the study Area

***Sampling Technique***

Fist, reconnaissance survey was done to find areas with flowering of *O. abyssinica* for fruit collection. Pawe and Bullen districts were selected purposively as they have flowering and fruiting of the species. In these districts, bamboo growing areas were stratified in to protected area, free access natural forest, grazing land and homesteads based on their location and level of disturbance. The nature and appearance of the plant differs on such types of land uses (Fig.2) and assumed to affect seed quality, and occurrence of pest and disease


**Fig. 2:** Fruiting and seed setting of *O. abyssinica* in the study area (various clumping types) A/ Free accessed natural stand, B/Protected natural stand, and C/ stand on grazing land (photo by Demelash; Demelash *et al*., 2015)

From each strata, one central or nearest to central clump was selected for fruit collection. Three central culms from each sample clump were selected and their fruiting portion was divided into three equal parts: bottom, middle, and top. The fruits of each portion were totally harvested and placed in sacks, which were loosely tied and allowed to dry for a month in the open air under shade.

Ten aggregate fruits from each fruiting portion were selected randomly, separated in to single fruits and were kept separately. The outer covering of seeds was removed manually to extract the seeds. Seeds were inspected visually for any defects and classified as normal, damaged by seed borers, shriveled and, fungus attacked. Commonly, the term seed purity is used as an expression of the proportion of clean seed, other inert materials and other seeds admixtured in a seed sample expressed in percentage. In this study, however, seed purity is defined for convenience as the proportion of seeds free from seed defects caused by biotic agents such as fungi and insect pests; and other physical defects such as shriveling and wrinkling. Despite the fact that impurities may consist of any non-seed material (leaf, flower, fruit fraction, soil etc), small fractions of the same species, as well as seeds of other species (Schmidt, 2000), the impurities in the case of *O. abyssinica* in this particular study include only poor-quality seeds which, were damaged by pests and diseases or which had abnormalities. A purity analysis at certain stage of processing may serve as indication of the necessity for further cleaning. It is important to know the fraction of pure seed in a purity test. Therefore, if a sample of seed with impurities is weighed, then separated into two fractions, the weight of the pure seed component gives accurate estimate of seed purity on a weight basis. Since pure seeds may include both dead and empty seed, plus damaged seed, purity tells nothing about viability (Schmidt, 2000). Otherwise, these characteristics are very important for the calculation of the amount of seed required for a particular plantation objective. Therefore, this test is especially important for *O. abyssinica* seeds, because little work has done in this regard.

***Purity Test***

Seed purity was determined by taking 10 g of seed sample from each collection height and location. The seed samples were then manually separated into pure and impure seeds. The impure seed component comprises those seeds, which are attacked by fungal disease, borer attacked, broken and shriveled. In *O. abyssinica* seeds in this study, there was not any other inert matter, other than the impure seed component. The percentage purity was expressed by dividing the weight (g) of pure seeds by the sample weight (g) taken and then multiplying by 100.

$$Purity = \frac{weight\ of\ \ pure\ seed\ (g)}{weight\ of\ \ working\ sample\ (g)} * 100\%$$

The impure component of the samples was further separated in to different causes of seed defects. Their proportions were further determined.

***Experimental Design***

The experiment was considered as a split-plot design with randomized complete block design structure. The four strata (land use types) were used as blocks. The localities (Pawe and Bullen districts) were treated as the main plot and three fruit collection heights on the culm (one-third, two-thirds and three-thirds, starting from the top fruiting portion of the culm) were used as the sub-plots.

***Data Collection***

During the fieldwork and throughout the experimental period any observable and detectable disease and pests as well as other seed defects were observed. The presence of moulds was used as a symptom of fungi attack on seeds and fruits (Albrecht, 1993). Any sign of borers and borer-attacked seeds were used as a presence of seed borers. Any suspected seed was broken to observe the presence of seed borers. The components of seeds that were regarded as impure from the purity analysis test were scrutinized and divided into categories of seed defect types and their proportions were determined. For flowering nature and seed setting study, sample aggregate fruits were selected from clumps and then the nature and parts were inspected thoroughly by observation. Pictures were also taken.

***Data Analysis***

Both descriptive and inferential statistics were used to analyses the data for this particular study. Description of different flower parts and fruit of the species was also carried out. Qualitative method of data analysis was used to describe observations. For qualitative data such as the percentage of each causes of seed defects, data were checked for normality and homogeneity assumptions and the mean percentage and count data were transformed using Arcsine and square root transformation respectively to normalize the data for analysis of variance. However, the means were compared on untransformed (original) data. ANOVA was performed on all the data using SAS software (1999-2001, SAS Institute Inc., USA). All statistical comparisons were considered significantly different at $p < 0.05$. For mean comparisons, the least significance difference (LSD), included in the SAS statistical package was used.

## Results

***The Nature of Flowers in O. abyssinica***

Whenever there is gregarious flowering of *O. abyssinica* all the culms of the clump, and all growing points (branches and terminals) flower and set fruits. Because of this nature, most people in the surrounding area called these phenomena as disease. This is because of the long flowering nature of the species which makes it unusual to the community. When *O. abyssinica* flowers, every leafy branch develops in to a flowering shoot and leaves turn to brown and gradually drop off. Each branch develops the flowering units (pseudospikelet or spikelet) at its node and apices (Fig. 3). A flowering culm has all of its originally leafy branches transformed in to flowering shoots and buds at culm and branch nodes can also develop directly in to pseudospikelete clusters.

**Fig 3:** Flowered Bamboo culms. *(Photo by Demelash).*

The fruits are arranged in to aggregate that are composed of spikelets (Fig. 4A, B). These fruits are oat like and do not dehisce easily, and therefore, manual/ mechanical dehailing is necessary. Fruits are also spiny (Figure 4B) at one end, opposite to the embryo, which makes additional problem for collection and processing.

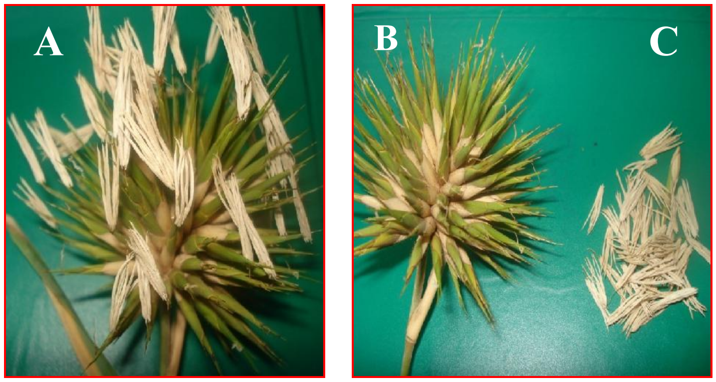


**Fig 4:** Flowered Fruit aggregates (A), fuit aggregates after petals removed (B), petals (C). *(Photo by Demelash).*

When you separate the fruit aggregates in to separate fruits, you will get a single spiklet (Figure 5A), which is a complete flower consisting of all essential flower parts incluing emebryo, stamens, petal and sepals (Fig 4C; Fig. 5B, 5C, 5D,5E). In the center of the flower the embro (Fig. 5E) is placed and the stigma (Figure 5D,central and longest) is found above the stamens (Fig.5C) whihc indicates that the species is most probably wind pollinated.

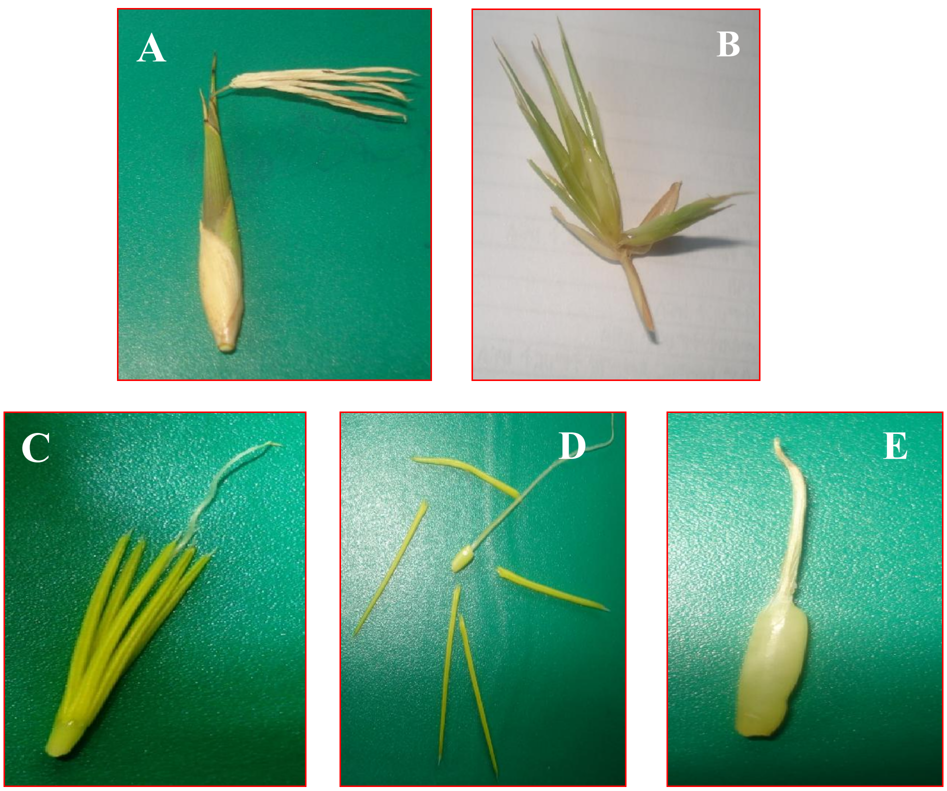


**Fig 5:** Single spikelete of *O. abyssinica* fruit (A) and seed coverings (B), female and male parts of a flower (C), parts of a flower separated in to components (D), developed ovary (E). *(Photo by Demelash).*

***Seed Defects in O. abyssinica***

There was a statistically significant difference in the percentage purity of seeds between study sites ($p$=0.0335) and among landuse type ($p$=0.0020) but not among collection height ($p$>0.999) and location with collection height interaction ($p$>0.755) (Table 1). There was also little difference in the percentage purity of seeds among the three fruit-collection heights. The maximum value (90.78%) was recorded in Pawe, in the top fruiting portion of the culm. The second and third maximum values were also recorded in the same locality in the middle and bottom fruiting portion of the culm. The overall average across locations and fruit collection heights of the culm showed that *O. abyssinica* in the study areas had percentage seed purity of 88% (Table 2).

This may be because of various reasons, among which the pest and disease occurrence and other physical seed defects were the major ones.

**Table 1:** Purity percentage of *O. abyssinica* seeds at different locations and different fruiting portion of the culm

| Source of Variation | d.f | SS | MS | F value | Pr>F |
|---|---|---|---|---|---|
| Land use stratum | 3 | 407.24 | 135.75 | 9.20 | 0.0020** |
| Location | 1 | 84.98 | 84.98 | 5.76 | 0.0335* |
| Residual | 3 | 131.95 | 43.98 | | |
| Collection height | 2 | 0.03 | 0.01 | 0.00 | 0.999 |
| Location*collection height | 2 | 8.49 | 4.24 | 0.29 | 0.755 |
| Residual | 12 | 177.07 | 14.76 | | |
| Total | 23 | 809.75 | | | |

**Table 2:** Mean (±S.E) values of purity percentage of *O. abyssinica* seeds

| Source of Variation | | Means values of percentage |
|---|---|---|
| Land use | Protected natural forest | 93.94±1.61A |
| | Homestead | 82.39±2.1A |
| | Grazing land | 87.17±2.35BC |
| | Natural forest but accessed | 88.68±0.97B |
| | LSD (5%) | 4.8 |
| Location | Pawe | 89.93±1.21A |
| | Bullen | 86.17±2.01B |
| | LSD (5%) | 3.4 |
| Collection Height | Top | 88.1±2.43A |
| | Middle | 88.02±2.57A |
| | Bottom | 88.02±1.4A |
| | LSD (5%) | 4.2 |
| Location and Collection Height interaction | Pawe-Top | 90.78±2.89A |
| | Pawe-Middle | 89.71±1.66A |
| | Pawe-Bottom | 89.29±2.13A |
| | Bullen-Top | 84.09±5.01A |
| | Bullen-Middle | 86.33±5.12A |
| | Bulen-Bottom | 86.76±1.88A |
| G. MEAN± S.E. | | 88±1.21 |

*Note: Mean values with the same letter in the same column and the same source of variation are not significantly different at α = 0.05*

During the reconnaissance survey and fruit collection time, it was observed that seed borers and fungi affected fruits and seeds of *O. abyssinica*. The occurrence of such diseases and pests varied with the height of the culm and between collection sites. Most of the seeds collected from the upper portion of the culm were highly affected in comparison with the middle and bottom portion of the culm (Table 3 and Table 4). The seed borers (white in color) were found to develop inside the seed (Fig. 6C).

**Table 3:** Causes of seed defects (%) in *O. abyssinica* seeds by location and fruit collection height

| Cause of seed defect | Location | | | | | |
|---|---|---|---|---|---|---|
| | Pace | | | Bullen | | |
| | Top | Middle | Bottom | Top | Middle | Bottom |
| Seed borer | 23.6 | 31.3 | 25.1 | 10.8 | 1.2 | 1.8 |
| Fungus | 7.7 | 14.4 | 13.2 | 11.1 | 11.8 | 20.6 |
| Shriveled | 53.8 | 40.8 | 49.8 | 60.5 | 73.4 | 62.8 |
| Broken | 14.9 | 13.4 | 11.9 | 17.5 | 13.6 | 14.9 |

**Table 4:** Causes of seed defects (%) in *O. abyssinica* seeds by location and fruit collection height

| Cause of seed defect | Average by collection height | | | Average by location | | Overall average |
|---|---|---|---|---|---|---|
| | Top | Middle | Bottom | Pawe | Bullen | |
| Seed borer | 15.7 | 12.2 | 11.4 | 25.1 | 1.8 | 13.1 |
| Fungus | 9.8 | 12.8 | 17.6 | 13.2 | 20.6 | 13.4 |
| Shriveled | 58 | 61.4 | 57.4 | 49.8 | 62.8 | 58.9 |
| Broken | 16.5 | 13.5 | 13.6 | 11.9 | 14.8 | 14.5 |

*Note: The values in table 4 and table 5 are percentages of count data contributed by each seed defect types from the total impurities of O. abyssinica seed samples.*

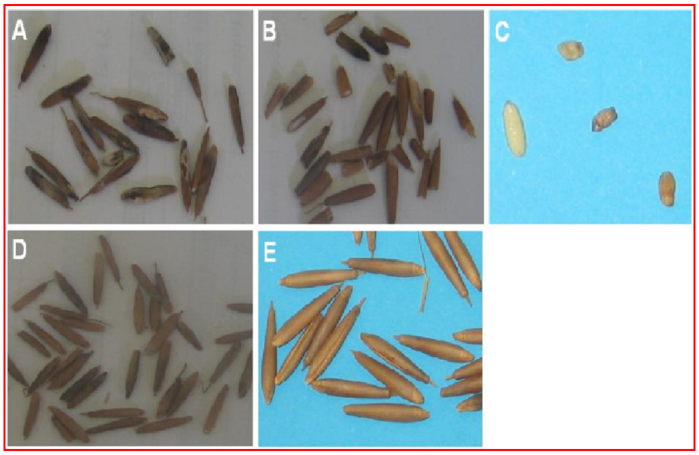


**Fig 6:** Defective and normal seeds of *O. abyssinica:* fungus attacked (A), borer attacked (B), Seed borers (C), Shriveled (D), and normal seeds (E), (photo by Demelash)

## Discussion

In the present study it was found out that physical and biological factors affect the quality of seed in *O. abyssinca.* Even though, the difference was not statistically significant, seed-borers and fungal colonization were more pronounced in the top and bottom fruiting portions of the culm, respectively. The value varied between the two study sites. Crooked and shriveled seeds were common in the middle fruiting portion of the culm. A higher proportion of seeds in the top fruiting portion of the culm were also found to be broke easily during seed processing. This was due to the variation in the intensity of seed borers. A lower absolute purity percentage of the seeds at Bullen were also due to the fact that there was higher proportion of fungus attacks and seed breakages. The high proportion of fungal attack and development in the lower fruiting portion of the culm may be because of higher humidity and moisture that created favorable microenvironment for fungi development (Demelash *et al*., 2012). Significant differences in the proportion of insect damaged seeds among populations were also found by Marchelli1 & Gallo (1999) in *Nothofagus nervosa* (Phil.) Dim.et mil.

The nature of the flower on bamboo is distinct that the flower is complete flower consisting of both female and male parts on the same plant, the sample fruit aggregate and the same single fruit. The position of the stigma (above the stamens) indicated that the species most probably pollinated by wind. During the field work, no insects were visiting the plant which means, either there is no or there is small quantity of production by the plant. Otherwise the spiny and had covering of fruits do not allow insects to collect nectar or pollen.

## Conclusion and Recommendation

The quality of bamboo seed was observed to be affected by different cause of seed defects. The occurrence of pest and disease that affect seeds was detected but the type was not identified. Detail research need to be conducted on disease and pests of *O. abyssinica* fruits and seeds. The susceptibility of *O. abyssinica* seeds to damping-off fungus during germination is very critical and hence aeration and watering frequency should be given due attention during seed germination either in field or laboratory. As there are various causes of seed defects it is of a paramount importance to devise a mechanism of seed pest and disease control and prevention mechanism in order to be successful in the establishment and development of the species in the study area in particular and the country at large.

The study on the nature of flower of *O. abyssinica* was based visual observation. The basic flower structures are identified, however further research is required to study the detail parts and functions of each part. *O. abyssinica* has long (ca, 30 years) flowering interval. For these further research, older natural forests in the study area has to be inspected for flowering. Since the seeds of the species are easily attacked by fungus appropriate handing is necessary both in storage and seedling production.

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