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Effect of Choanephora Cucurbitarum on the Morphology of Some Plants in the Malvaceae Family in Calabar, Cross River State, Nigeria

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Abstract: *The limiting factors in okra production among other vegetables in Cross River State include wet rot disease caused by Choanephora cucurbitarum(Berk and Rav) . This study was carried out in 2013 cropping season (April – july) to examine the effect of Choanephora cucurbitarum (Berk and Rav) on the morphology of Abelmoschus ficulneus and Abelmoschus esculentus in the family Malvaceae in Calabar , Nigeria .The experiment was a potted experiment laid out in a Randomized Complete Block Design (RCBD)using 2 treatments (inoculated and uninoculated seedling at 4 weeks after planting) and replicated 3 times . Invasion of the plants by C. cucurbitarum was done by inoculating the plants after four weeks of planting. Morphological characteristics like stem length, number of leaves, dry weight and fresh weight were studied. Generally there was a significant (P≤0.05) difference in growth and yield of the inoculated and uninoculated plants of A. ficulneus,.and A. esculentus. Results obtained from the study showed that Choanephora cucurbitarum was pathogenic to the test plants (A. ficulneus and A. esculentus). . For most of the morphological characteristics studied the values obtained from the inoculated plants were significantly lower than that of the uninoculated plants.. There was high susceptibility of A. esculentus to the pathogen compare to A. filculneus. On the other hand , A.ficulneus had less damaged performed better and produced higher dry matter yield .*

Keywords: *Choanephora cucurbitarum, stem length, leaf fresh weight and dry weight, leaf number.*

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I. Introduction

Okra is a vegetable commonly found in humid west and central Africa, where there are highly grown in traditional agriculture. It contributes towards a major economic activity, as a cash crop in the local economy and is important to the population that includes them in their diet and so the Calabarians in Cross River State Southern Nigeria are no exception in this regards. Its consumption facilitates the consumption of starchy foods which are the main diet of Africans (Schippers, 2000).

Okra belongs to the family *Malvaceae.* This farm crop is important economic plant in Cross River State, Nigeria, West Africa and is known to be attacked by a fungus known as *Choanephora cucurbitarum (Berk and Rev)* which belongs to the family *Choanophoraceae*. The infection results in various morphological changes in this farm crop which consequently affect its yield.

In Nigeria, okra is produced predominantly by peasant farmers usually in home gardens. It is grown for its young leaves and green pods (Katung, 2007). Okra contains vitamin C and carbohydrate in large quantities (Farrag, 2011). The essential and non essential amino acids that okra contains are comparable to that of soybeans.

It is a prickly annual herb with palmately 3-5 lobed, glabrous leaves. Synonyms of A. ficulneus var albo-ruber Domin Abelmoschus strictus voight, Hibiscus ficulneus L., Hibiscus prostivitus Roxb; A. esculentus L (moench) is an

Okra seeds contain 20% protein and 20% oil, similar in fatty acids composition to cotton seed oil (Siemonsma and Hamon, 2002). , relished. The.

Okra leaves are considered as good cattle feeds but this is seldom compatible with the primary use of the plant. Okra flowers can be very attractive and sometimes used in decorating the living rooms (Schipper, 2000). The dwarf green pod matures in 50 days and is called "Etighi Abakpa" as well as the green long pod called "Etighi idok" by the Efiks both being the standard okra consumed in Cross River State by the Efik tribe in Calabar. The dwarf green pod is identified as *A. ficulneus* while the green long pod is identified as *A. esculentus* which are the two species use for this research work. *A. ficulneus* L (Wight and Ann) is found in the India sub-

continent, Australia, Madagascar and in the Sahel zone of Africa, including Niger and Northern and Southern Nigeria but may be native rather than nationalized (Osawaru, 2008).

The most important foliar diseases of okra causing significant losses in yield and quality in florida are wet rot disease caused by *choanephora curcubitarium* (Raid and Palmeteer, 2006). Its economic effect and *uninoculated* strategy in Ilorin, situated in the Southern Guinea savannah of Nigeria, have been well documented (Balogun and Babatola, 1999, Balogun, 2000). Choanephora wet rot of okra fruit was also recorded in Malaysia (Saddiqui, 2006)

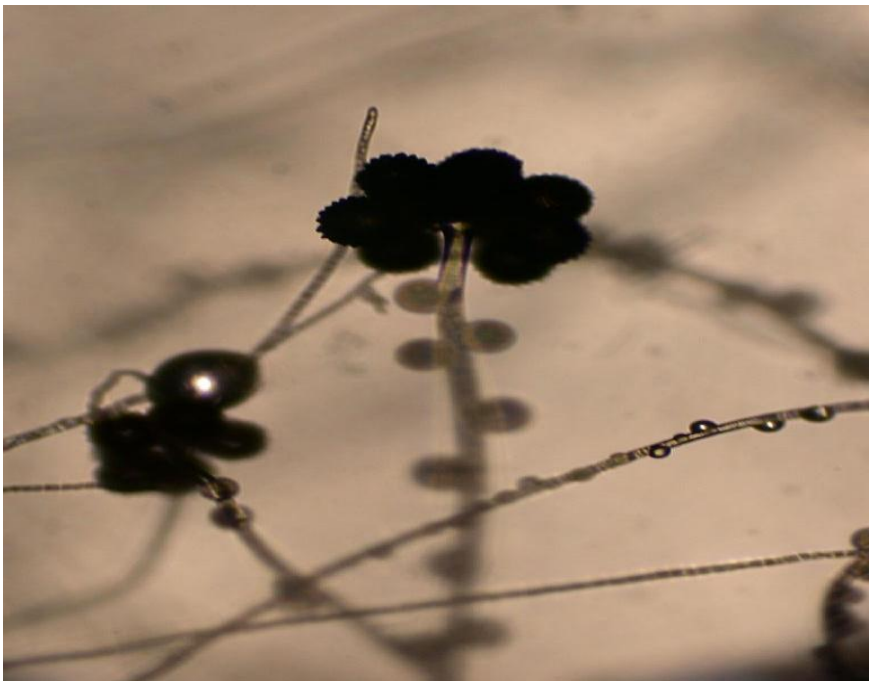
The essence of this research was therefore to determine the effect of *C. cucurbitarum* on morphology of some plants in the *Malvaceae* in Calabar, Cross River State and to assess the performance of the plants. The essence of this research was therefore to determine the effect of *C. cucurbitarum* on morphology of some plants in the *Malvaceae*

Pathogen Description

The fungus *Choanephora cucurbitarum* is a plant pathogen that belongs to the kingdom fungi, *Order Mucorales, Family Choanephoraceae, Genus Choamephoira* and *Species Cucurbitarum.* It causes wet rot in okra (*Abelmoschus*). wet weather, high temperature and humility favour disease development from inoculums that is typically soil borne. Signs of infection on fruits and leaves include: water soaked, necrotic lesions, which progress rapidly under ideal conditions as the fungus begins to produce spores, affected tissues become dark grey-brown and hairy as a result of the superficial sporangia (Saroj *et al.*, 2012).

Sporangiophore bearing sporangiola are erect, hyaline unbranched and apically brown at maturity ellipsoid brown to dark brown, indistinctly straight with fine hyaline polar appendanges and measure 16-20 µm (Umana, 2000). Palada and Chang (2003) reported that *Choanephora* blight (also called *Choanephora rot*) is caused by fungus *C. cucurbitarum*. It causes wet rot of stem and leaves. Affected plant parts have hairy appearance (Silk-like threads) consisting of fungal spores. Infection is predisposed by injuries. During rainy season it can cause heavy defoliation. The disease is spread by air currents and infected seeds; warm, moist condition favour disease development.

Choanephora fruit rot on yellow straight neck squash (Cucurbita-pepo) is caused by *Choanephora cucurbitarum.* This disease also known as "wet rot" and blossom end rot can destroy many blossoms and fruit during extended period of damp weather *Choanephora* fruit rot is favoured by warm (>25^0c) wet weather. Both blossom and fruit are affected and fruit nearest the ground are more likely to become inoculated. It is not unusual to find 30 – 40% of blossom and or fruit infected with the fungus while the disease is destructive. It is also as short lived as the conditions that promote it. Subsequent fruit sets are usually not affected unless conducive conditions reoccur. The fungus resembles *Rhizopus stolonifer*, but spore-bearing heads are branched, a feature that can be seen with a hand lens.



Photomicrograph of *Choanephora cucurbitarum* showing conidia
Magnifications: 768 x 1024 pixels

II. Materials And Method

Material and Source

The *Choanephora cucurbitarum* used in this research work was isolated from *Abelmoschus esculentus* plant grown in the Prisons Garden, Iman Street, Calabar in Cross River State. The two species of okra considered in this study *(A. ficulneus* and *A. esculentus)* were obtained from the seed bank of ADP (Agricultural Development Project) located at Ibrahim Babangida Way, Calabar. The soil was obtained from the Botanical Garden, University of Calabar, Calabar in Cross River State.

Preparation of Medium

The potatoes dextrose agar (PDA) medium used for culturing was prepared by weighing 39 grams of Potatoes dextrose agar into one litre of distilled water in a conical flask, dissolved, plugged with non-absorbent cotton wool and covered with aluminum foil before sterilization in an autoclave at 121^0C for 20 minute and allowed to cool to 40^oC before dispensing into pyrex glass Petri-dishes and allowed to gel before inoculation with infected plant parts. The chamber for inoculation was sterilized by cleaning with cotton wool soaked with 70% ethanol.

Isolation and Inoculation of Causal Agent of Wet Rot

The method employed by Umana (2000) was used in the isolation of the Fungus. Some $3mm^2$ section of infected leaf was aseptically taken from the leaves of Okra (*Abelmoschus esculentus*) plant. The sections was then surfaced sterilized with 70% ethanol solutions and rinsed immediately in two changes of distilled water to get rid of the ethanol residue. The sterilized pieces was plated in already prepared potatoes dextrose agar medium in Petri dishes and inoculated at room temperature of 27 ± 1^0C. Cultures were examined for three days after which subculture were obtained from the tips of young mycelia by means of sterile inoculating needle. Subsequent sub-culturing was carried out until pure cultures were obtained. The isolate was stored as stock culture in Maconkey bottle as agar slant store in refrigerator for further use. Isolated fungi were microscopically (Olympus optical, Phillipines) identified using the identification guides of the International Mycological Institute, Kew and of Barnett and Hunter (1998), Alexopolous and Minus (1989).

III. Results of Pathogenecity Test

Results obtained from the study showed that *Choanephora cucurbitarum* was pathogenic to the test plants (*A. ficulneus* and *A. esculentus*). The symptoms of soft rot caused by the *Choanephora cucurbitarum* started with water-soaked and dark-grey brown lesions in the two species of okra. The leave appears wet and feels slimy to the touch. The disease effect was localized and not systemic. For most of the morphological characteristics studied the values obtained from plants were significantly lower than that of the control plant. There was significant ($P\leq0.05$) difference in the growth of plants inoculated with the fungus compared to those of the control plants.

Results obtained showed that the leaves became infected and covered with white dense mycelium and infected plants showed symptoms of chlorosis, yellowing and signs of softness of tissues and the control plants performed significantly better than inoculated with the fungus after 4 weeks of planting. There was high susceptibility of *A. esculentus* to the pathogen compare to *A. filculneus.*

Effect of *C. cucurbitarum* on the Stem Length of Okra

Table 1 shows the influence of *C. cucurbitarum* on the stem length of *A. ficulneus* and *A. esculentus* at weekly harvest from four weeks after planting. The result showed that the infection period played a significant role in the reduction of the stem length of the test plants when compared with the control *A. ficulneus* at 5, 6, 7, 8 and 9 WAP, of the inoculated plants had stem length of 26.75 cm, 27.33 cm, 25.50 cm and 23.83 cm respectively which were significantly different ($P\leq0.05$) from the stem length of the control whose stem length at same harvest were found to be 36.33 cm, 32.33 cm, 32.50 cm, 34.17 cm and 36.67 cm. Also, the influence of the fungus on the stem length of *A. esculentus* at weekly harvest from four weeks after planting produced inoculated *A. esculentus* plants whose stem length at 5, 6, 7, 8 and 9 WAP were 28.92 cm, 21.83 cm, 21.97 cm, 20.33 cm and 18.50 cm respectively which were significantly reduced with compared with the stem length of the control *A. esculentus* plant whose stem length at same harvest were 21.83 cm, 26.00 cm, 36.50 cm, 39.50 cm and 4.17 cm respectively. This result showed that the infection period by the fungus played a significant role in stem length reduction of both specie of okra compared to the control (Table 1) and amongst the two species of okra the greatest effect of the pathogen was on *A. esculentus* whose stem length at 9 WAP was 18.50 cm compared to the stem length of *A. ficulneus* at same harvest whose stem length (23.80 cm) was significantly ($P\leq0.05$) different. This suggests a high susceptibility of *A. esculentus* to the fungus attack. However, irrespective of infection by the pathogen a continuous growth was observed on the test plants. Also, a significant effect on the stem length was of the test plants were observed from 5 to 9 WAP after infection by the pathogen.

TABLE 1: Effect of *C. cucurbitarum* on the stem length (cm) of *A. ficulneus*, *A. esculentus*, at weekly harvest from 4 weeks after planting

Treatments	*A. ficulneus*					*A. esculentus*				
	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP
Inoculated	22.17[b]	26.75[a]	27.33[a]	25.50[a]	23.83[b]	26.92[a]	21.83[a]	21.97[b]	20.33[b]	18.50[b]
Control	36.33[a]	32.33[a]	32.50[a]	31.17[a]	36.67[a]	27.83[a]	26.00[a]	36.50[a]	39.50[a]	41.17[a]
L.S.D	2.20									

Values are means of three replicates, 5 to 9 WAP are weekly harvest. Values in the same columns followed by different letters are significantly different (P≤0.05).

TABLE 2: Effect of *C. cucurbitarum* on the number of leaves of *A. ficulneus*, *A. esculentus*, at weekly harvest from 4weeks after planting.

Treatments	*A. ficulneus*					*A. esculentus*				
	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP
Inoculated	5.00[a]	4.33[a]	4.17[a]	4.17[a]	4.00[a]	5.33[a]	4.50[a]	4.33[a]	4.60[a]	4.00[a]
Control	5.67[a]	6.67[a]	5.83[a]	5.67[a]	6.00[a]	5.67[a]	5.00[a]	4.17[a]	4.50[a]	4.50[a]
L.S.D	3.07									

Values are means of three replicates, 5 to 9 WAP are weekly harvest. Values in the same columns followed by different letters are significantly different (P≤0.05).

TABLE 3: Effect of *C. cucurbitarum* on the fresh weight (g) of *A. ficulneus*, *A. esculentus* at weekly harvest from 4 weeks after planting.

Treatments	*A. ficulneus*					*A. esculentus*				
	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP
Inoculated	17.55[b]	7,17[b]	6.98[a]	6.07[b]	5.68[b]	14.75[a]	6.15[b]	5.77[a]	5.27[b]	5.00[b]
Control	62.50[a]	28.37[a]	13.97[a]	15.37[a]	16.57[a]	13.53[a]	15,77[a]	10.90[a]	12.58[a]	13.03[a]
L.S.D	7.09									

Values are means of three replicates, 5 to 9 WAP are weekly harvest. Values in the same columns followed by different letters are significantly different (P≤0.05

TABLE 4: Effect of *C. cucurbitarum* on the dry weight (g) of *A. ficulneus*, *A. esculentus* at weekly harvest from 4 weeks after planting

Treatments	*A. ficulneus*					*A. esculentus*				
	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP
Inoculated	2.20[b]	1.27[b]	1.30[a]	1.16[a]	1.14[a]	8.00[b]	0.63[b]	0.73[b]	0.64[b]	0.53[b]
Control	5.29[a]	4.01[a]	2.17[a]	2.27[a]	2.37[a]	17.00[a]	1.90[a]	2.00[a]	2.04[a]	2.12[a]
L.S.D	2.20									

Values are means of three replicates, 5 to 9 WAP are weekly harvest. Values in the same columns followed by different letters are significantly (P≤0.05) different.

Effect of Pathogen on the Leaves of the Test Plants

Table 2 shows that infection period by the fungus played a significant role in leaf reduction in the test plant when compared to both the control. It was observed that the number of leaves in inoculated *A. ficulneus* plants at weekly harvest from 4 weeks after planting was 5.00, 4.33, 4.17, 4.17 and 4.00 at 5WAP, 6WAP, 7WAP, 8WAP and 9WAP respectively while the number of leaves in the control *A. ficulneus* plants were 5.67, 6.67, 5.83, 5.67 and 6.00 at same harvest. This result shows that they were no significant $P \leq 0.05$ different in the number of leaves of both inoculated and control *A. ficulneus* plants. In *A. esculentus* inoculated plants at harvest 5, 6, 7, 8 and 9 weeks after planting, the number of leaves was 5.33, 4.50, 4.33, 4.66, 4.00 respectively.

This values were significantly different from the values obtain from the control *A. esculentus* plants at same harvest which were 5.67, 5.00, 4.17, 4.50 and 4.50.The table shows that the fungus actually caused significant reduction in the number of leaves of both test plant and the infection, period on weekly bases also caused a reduced in the number of leaves of both test plats when compared with their control.

Effect of pathogen on the Fresh Weight of the Test Plants

Table 3 revealed that the pathogen played a significant role in reduction of the fresh weight of both test plants. The inoculation of the *A. ficulneus* plants with the fungus produced inoculated plants at 5, 6, 7, 8 and 9 WAP from 4 weeks whose fresh weights being 17.55 g, 7.17 g, 6.98 g, 6.07 g and 5.68 g were significantly ($P \leq 0.05$) lower than the fresh weight of the control A. ficulneus plants whose fresh weight at same harvest were 62.50 g, 28.37 g, 13.97 g, 15.37 g and 16.57 g. The result showed that the infection by the fungus actually caused a significant reduction in the fresh weight of *A. ficulneus* test plant. Also, the fungus
influence the fresh weight of A. esculentus plant whose fresh weight after inoculation at 5, 6, 7, 8 and 9 WAP was 14.75 g, 6.15 g, 5.77 g, 5.27 g and 5.00 g was significantly ($P \leq 0.05$) different from the control plants whose fresh weight at same harvest were 13.53 g, 15.77 g, 10.90 g, 12.58 g, 13.03 g respectively. This result revealed that the fungus infection actually reduced the fresh weight of both test plants.

Effect of Fungus on the Dry Weight of Okra

Table 4 shows the influence of the fungus on the dry weight of both test plant. It shows that the fungus played a significant role in the reduction of the dry weight of both test plants when compared with the control. The inoculation of *A. ficulneus* plants with the fungus produces inoculated plants at 5, 6, 7, 8 and 9 WAP whose dry weight being 2.20 g, 1.27 g, 1.30 g, 1.16 g and 1.14 g were significant ($P \leq 0.05$) lower than the dry weight where 5.29 g, 4.01 g, 2.17 g, 2.27 g, 2.37 g and for *A. esculentus* plants inoculation with the fungus produce *A. esculentus* plants whose dry weights were 8.00 g, 0.63 g, 0.73 g, 0.64 g, 0.53g at 5, 6, 7, 8 and 9 WAS respectively. This was significant ($P \leq 0.05$) lower than the dry weight of the control *A. esculentus* plants whose dry weight at same harvest were 17.00 g, 1.90 g, 2.00 g, 2.12 g, 9.10 g. The result shows that *A. esculentus* was much more susceptible to the attack by the fungus than *A. ficulneus*. Also, the infection period on weekly bases also affected both test plants.

IV. Discussion

Results obtained in this study revealed that, the fungus *Choanephora cucurbitarum* isolated from the rotten leaves of *A. esculentus* plant was pathogenic to both species of okra (*A. ficulneus* and *A. esculentus*) and it was also observed that they were significant differences between the inoculated plants in the 50 polyethylene bags and the control plants in the remaining 50 polyethylene bags.

The pathogenecity of the fungus agrees with the report in Florida of Raid and Palmateer (2006) on *Choanephora* wet rot and also with the report of Saroj *et al*. (2012) on first report of wet rot of Withania Soninifera caused by *Choanephora cucurbitarum* in India. The effect of the pathogen observed on the inoculated plants shows that there was significant ($P \leq 0.05$) effect of the pathogen on the morphological growth parameters of the test plants. Thus, the plant had poor growth. Pathogenecity test of fungus isolated from the rotten leaves of *A. esculentus* was carried out and the production of symptoms as those observed in the field was used as a confirmation of pathogenecity (Siddiqui, 2006).

In this study, it was observed that the fungus causes soft rot which appears as water soaked and dark-grey brown lesions on okra plant. The leaves appear wet and feel slimy to the touch and this agrees with the report of Awurum *et al*. (2013) on wet rot of *Amarantus cruentus* caused by *Choanephora cucurbitarum* with affected Amaranthus cruentus plant characterized by blighting of the short apex and the water soaked lesion agrees with the finding so fKuchareketal.(2003) who reported Choanephora blight of some vegetables in families of Cucurbitaceae and Solanaceae. The dark-grey brown lesion observed on the leaves of both test plants agrees with the finding of Saroj *et al.* (2012). It was observed in this study that the effect of the disease was localized which agrees with the findings of Awurum (2011) who studies the fungitoxis effect of some plant extract on the wet rot of Amaranthus induced by Choanephora. White mycellial growth on the surface of the leaves of both test plant agrees with the work of Robert *et al.* (2003) who studied the outbreak of *Choanephora cucurbitarum* disease on green bean and pepper in Florida.

Results from this study showed that the pathogen cause significant reductions on the stem length, leaves, fresh and dry weight of the test plants which agrees with that of Awurum (2013) who reported a decrease in weight leaves and fresh weight of *Amanranthus cruentus* induced by *Choanephora cucurbitarum* but disagrees with that of Mukambila and Goma (1993) who reported increase in weight of *Amaranthus sp*. infected with *Choanephora cucurbitarum*. The fungus was inoculated into the leaves and stem of *A. ficulneus* and *A. esculentus* which is similar to that of Awurum (2011). Also, the results obtained for stem length for the two test plants varies with the report on stem length given by Awurum and Ogbanna (2013) probably because his values

stem length were means of 5 replicates whereas stem length for this work were means of 3 replicates. Also, probably because of variation in plant type, the effect of the fungus on the dry weight corresponds with the dry weight value of *Amaranthus* given by Awurum and Ogbonna (2013). Also, the result of fresh weight of both test plants corresponds with report on fresh weight of *Amaranthus creuntus* given by Awurum and Ogbonna (2013).

Generally, the number of leaves in both test plant as a result of the fungus influence was significantly low and amongst the two plants *A. esculentus* had the least number of leaves. This report is supported by the findings of Raid and Palmeteer (2006) who work on most important foliar disease of okra. The infections were observed to be secured at the first few days after inoculation. This report agrees with the work of Awurum and Ogbonna (2013); Osunlaja and Bello (1992) and Robert et al. (2003) on the effect of *C. cucurbitarum* on crops.

In general, the damage caused by the pathogen as shown in this study in some of the harvest was very severe significant reduction in growth of the two test plants who recorded which agrees with the report given by Raid and Palmateer (2006) that *C. cucurbitarum* causes significant losses in growth of crops the leaf spot and softness of tissues caused by *C. cucurbitarum* reduced the photosynthetic complex of Abelmoschus plants which adversely reduced yield of both test plants. Report given by Arinze (2005) says that fungi diseases are among the serious diseases of cereal, legumes, tubers, vegetables and fruits. Also the observed differences among the plants in their strength of susceptibity and resistance might be related to different environmental conditions turning the different harvest.

V. Conclusion

This research work reveals that okra suffer serious foliar infection as significant losses in growth performance were recorded for both test plants. The fact that the inoculated plants were seriously affected by wet rot infection shows that the pathogen has high infection rate as the pathogen was aggressively pathogenic on foliar of both plants. Amongst the two species, *A. esculentus* was easily susceptible to the infection than *A. ficulneus*.

Based on these findings, it is recommended that, the standard measures for disease management recommended by Palada and Chang (2003) which states that resistant varieties should be used for planting where there is available certified disease free seeds should be adopted. Also, to reduce the fungal disease seed beds must be well drained and located in sunny sites. Dense planting should be avoided to allow for sufficient aeration and good field sanitation practices should be adopted for disease management. Finally, the research should be further extended to field conditions to investigate the influence of other soil pathogen on the morphological characteristics of the plants.

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