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Pre and Post-Emergence Damping-Off of *Chrysophyllum albidum* G. Don and *C. delevoyi* Dewild in Port Harcourt

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Abstract

A nursery experiment on pre- and post-emergence damping off of Chrysophyllum species was conducted in the Department of Forestry and Wildlife Management in the University of Port Harcourt, Nigeria in 2010. The study assessed the fungi associated with seed and seedlings of Chrysophyllum albidum and C. delevoyi in the nursery. A sample of thirty seeds each were collected randomly from the seed lot for both species and germinated in trays using a completely randomized design with three *replicates. The germinants were observed over a period of 28 days. Disease incidence, germination percentages and seedlings damaged were observed and data recorded respectively. The t-test was used to separate the means. Fusarium oxysponum, Aspergillus flavus and Trichoderma species were implicated as causal agents of damping off in the two species. The fungal pathogens strongly impacted on the mean percentage germination percentage of the species in the unsterilised/medium with 34% and 7.33% for C. albidum and C. delevoyi respectively. Similarly the mean seedling survivals for the species were 28% and 6% for C. albidum and C. delevoyi respectively. Chrysophyllum species are highly susceptible to fungal pathogens in the nursery.*

Introduction

Damping off is one of the most troublesome and common fungi diseases in forest nursery. It affects all types of seedlings including fruit trees, timber species and several ornamental plants. The disease occurs during the first critical few weeks from germination to early seedling emergence. Damping off can be caused by a wide range of soil inhabiting facultative parasites which survive either as dormant resting spores or as active saprophytes on decaying organic matter in the soil (Hill and Waller, 1994).

Pre-emergence damping off occurs when a seed is infected and killed before it can open up the soil surface as an emerged seedling. In post-emergence damping off, the seedling emerged but is girdled at the soil surface leading to collapse and eventual death (Omokhua *et al*. 2009b).

Problems in identifying species of *Fusarium* and other pathogens as well as the existence of several systems of nomenclature frequently make literature comparison difficult at the species level (Martin and Johnson, 1982). Some of the fungi that cause damping off include many species of the gernera *Phythium, Rhizoctona, Fusarium* and *Phytophthora* (USDA, 1979). They produce hardy spores or other structures that rest in the soil until conditions are suitable for germination.

Permanent and temporary nurseries are often plagued by damping off affecting seedling production for afforestation, aesthetic and reforestation purposes. Seeds sown in germination trays, seedlings and cuttings can be seriously damaged that planting stock cannot be grown without preventive treatments. In Port Harcourt the loss-potential of damping off in the nursery could be severe varying with species and sowing medium (Omokhua *et al.* 2009a).

Chrysophyllum is a genus of about 70-80 species of tropical trees. It belongs to the family Sapotaceae (Ehiagbonare *et al,* 2008). In Nigeria, there are two species of *Chrysophyllum* namely *C. albidum* and *C. delevoyi* which belong to the order Ebernales (USDA, 2010). Tropical America is richest in the species, followed by continental Africa, Madagascar, Tropical Asia and Australia (Burkill, 2001). *Chrysophyllum albidum* is a small to medium buttressed tree species, up to 25-37m in height with a mature girth varying from 1.5m to 2m. *C. delevoyi* on the other hand is a medium sized tree up to 25-35m tall; bole of 60-80cm in diameter and straight, often fluted and angular, reaching up to 12m to the first branches, with step buttresses as the base. The fruit has immense economic potential, especially following the report that its jam could compete with raspberry jams, and jellies could be made from it (Okafor, 1975; Amusa *et al.,* 2003). The fruit has been reported to have very high content of ascorbic acid per 100g of edible fruit or about 100 times that of orange and 10 times that of guava and cashew (Pearson, 1976). Its seeds are a source of oil, which is used for diverse purposes. The fruit of *C. albidum* also contains 90 anacadic acid, which is used industrially in protecting wood and as a source of resin. The fruits can be fermented and distilled for the production of wine and spirit. Several components of the tree including the roots and leaves are used for medicinal purposes. The bark is used as a remedy for yellow fever and malaria, while the leaves are used as emollient and for the treatment of skin eruptions, diarrhea and stomach ache, which are as a result of infections and inflammatory reaction (Adewusi, 1997). It is rich source of natural antioxidant and have been established to promote health by acting against oxidation stress and related diseases suchas diabetes, cancer, and chronic heart diseases (Burit and Bucar, 2002). The wood of *C. delevoyi* is used for carving, moulding, turnery, cabinet making, veneer and plywood. It is also suitable for construction, carpentry, stairs, light flooring, ships, vehicle bodies, agricultural implements, sporting, interior trims, toys and novelties, railway sleepers, particle board, pulp and paper production. The timber of *Chrysophyllum delevoyi* is traded internationally (Burkill, 2000). The edible, pleasantly acidulous fruits of *C. delevoyi* are often sold in the market and are widely eaten. They are also the potential source of a soft drink. *C. delevoyi* is used for treating gonorrhoea and fibroid. A maceration of the stem bark is taken to promote lactation. Powdered bark is applied to sores. The fruit is used to treat diarrhea and vomiting (Neuwinger, 2000).

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Attempts to propagate *Chysophyllum albidum* and *C. delevoyi* in the nursery in Port Harcourt has been met with little success due to pre and post-emergence attack of damping off fungal pathogens at the point of radicle and shoot emergence respectively. Therefore, the objectives of this study are to identity the pre- and post-emergence fungi of damping off associated with *Chrysophyllum* species as well as determine the effects of the pathogens on germination percentage and seedling survival of the two species.

Materials and method

The study was carried out at the forest nursery of the Department of Forestry and Wildlife Management, Faculty of Agriculture, University of Port Harcourt, Choba Port Harcourt. The nursery is located on Latitude N04^{0}53 38.0', E006^{0}54 38.0'.

One hundred fruits each were collected from the crown of three mother trees of *Chrysophyllum albidum* and *C. delevoyi* at Ekpoma in December, 2009. The mother trees were components of a traditional agroforestry system in the area. The seeds were scoped out of the fruits to separate them from the pulp. Viable seeds were separated from non-viable seeds using the floatation method of seed viability test, in which a deep bowl with the seeds containing a slow stream of running water was flowed. All the seeds were air-dried under shade for 1 day.

A sample of ninety seeds each was collected randomly from the seed lot for both *Chrysophyllum albidum* and *C. delevoyi* respectively. NaOCl (2%) was used to surface sterilize the seeds and rinsed severally with distilled water. A Completely Randomized Design (CRD) with three (3) replicates was used. Thirty seeds each were broadcast in germination trays as one replicate for the two *Chrysophyllum* species in sterilized and unsterilized media. The germinants were observed over a period of 28 days. The germination percentages for both species were recorded. T-test was used to separate the means.

Ten ungerminated seeds from both sterilized and unsterilized media were collected for both *C. albidum* and *C. delevoyi*. The seeds were planted on 25% PDA and incubated for 7 days. All the micro-organisms isolated were identified using Standard identification keys. Disease incidence was calculated per species

$$\text{Germination percentage}(\%) = \frac{\text{Number of seeds germinated}}{\text{Number sown}} \times 100$$

$$\text{Disease incidence} = \frac{\text{Number of diseased seed/seedlings}}{\text{Number collected}} \times 100$$

Germination was taken to have occurred at the point of radicle emergence. The seedlings were observed for damping off disease.

Seedling mortality was observed and recorded over a period of 42 days. Ten each of the damaged seedlings were taken for agar plate test. All the microorganisms isolated were identified as above. Mean seedling survival percentages for the species were calculated and recorded. T-test was used to separate the means.

Results

The results of fungi isolates and disease incidence in *Chrysophyllum albidum* and *C. delevoyi* are shown in Table 1 below. The agar plate tests showed that three fungi were isolated from ungerminated seeds of *C. albidum* and *C. delevoyi*. These are: *Fusarium oxysporum, Aspergillus flavus* and *Trichoderma* species. Disease incidence (%) was not the same in the two species. *Chrysophyllum delevoyi* had a higher disease incidence with a mean of 92% while *C. albidum* had a mean of 66%. The pathogens occurred in the unsterilized sowing medium, while the control (sterilized medium) had no isolates. These results were significantly different ($p = 0.05$)

Table 2 shows results of the effect of fungal pathogens on germination of *Chrysophyllum* species. There were significant differences ($p = 0.05$) between the two *Chrysophyllum* species in mean germination percentage. Germination percentage was higher in *C. albidum* with a mean of 34.0% while the control had 74%. The mean germination percentage for both species differed at 5% level of probability. The effect of fungi isolates on seedling survival in the two species of *Chrysophyllum* is shown in Table 3. Seedling survival percentage was higher in *C. albidum* with a mean of 28.60% and lower in *C. delevoyi* with a mean of 6%. The means of both species differed at 5% level of probability. The sterilized soil medium had a mean of 77.67% and 74% for *C. albidum* and *C. delevoyi* respectively.

The results of fungi isolates and mean disease incidence in damped off seedlings of *C. albidum* and *C. delevoyi* are shown in Table 4 below. The fungi isolates found in the diseased sample seeds of both *Chrysophyllum* species in Table 1 were also found in the damped off seedlings. The three fungi pathogens were *Fusarium oxysporum*, *Aspergillus flavus* and *Trichoderma* species. Disease incidence (%), of damped seedling in *C. albidum* and *C. delevoyi* was 100% in both species. The pathogens occurred in the unsterilised medium while the control recorded no presence of the pathogens.

Discussion

Damping off of *Chrysophyllum albidum* and *C. delevoyi* is a fungal disease. The fungi implicated in this study (*Fusarium oxysporum, Aspergillus flavus* and *Trichoderma* species) attacked the seeds and seedlings of both species and caused them to collapse and die. The disease has been one of the most important factors limiting the successful propagation of *Chrysophyllum* seedlings at the nursery. The fungi are soil born and this was why the disease did not prevail in all the sterilized medium. They may have grown from spores that were spread through the air or water. They caused the once healthy seedlings to become structurally weak and eventually, death occurred. Many scientists have reported on the impact of fungal pathogens on specific host plants (Aslam *et al,* 2009; Park *et al,* 2000; Elmore *et al,* 1997; Flint 1998; Omokhua *et al,* 2009a; Omokhua *et al,* 2009b).

In pre-emergency damping off, seeds of *C. albidum* and *C. delevoyi* were attacked even before radicle emergence and shortly after the radicle had emerged. The pathogens attacked the seed causing them to rot. Infected seeds failed to germinate resulting in the poor mean germination percentage for *C. albidum* (34.0%) and *C. delevoyi* (7.3%) (Table 2).

This study is similar to the report on fungal pathogens limiting the successful regeneration of *Milicia excelsa* in Ghana (Apetorgbor *et al,* 2000). The authors implicated, *Fusarium* species, *Rhizopus nigricans, Aspergillus* species, *Alternaria* species, *Curvularia lunata* and *Pestalotia quepini* as causal agents of damping off. Causal agents of damping off from previous studies include:

Aphanomyces species, *alternaria* species, *botrycinerea* species, *Colletotrichum* species, *Fusarium* species, *Helmenthosporium* species, *Phytophthora* species, *Pythium* species, *Sclerotia* species and *Thielaviopsis* species (Elmore et al, 1997; Flint, 1998; Md Tofazzal Islam et al, 2005). The implication of *Trichoderma* to the list is an addition species.

It was supported that, in post emergence damping off the fungal pathogens may have affected the vascular system of the seedlings making the roots unable to supply nutrients and water to the leaves and branches. This may have occurred because the fungi produced factors that may have clogged the vessels which carry water through the young seedlings. The impact resulted in the poor mean seedling survival recorded for *C. albidum* (28%) and *C. delevoyi* (6%) respectively.

Micro-organisms especially fungal pathogens play an important role in the infection, transmission, and deterioration of seeds and seedlings in the forest nursery. While some of the fungal forest pathogens infect their host seeds before germination, others invade the host shortly after germination. Forest seeds and seedlings are liable to attack by various microbes as a result of some chemical compounds (which satisfy the unsterilized requirements of some microbe). Such compounds include Lignin, Carbohydrates, Proteins, Cellulose and fats.

Conclusion

This study has shown that (i) The soil used in the nurseries in Port Harcourt are infected with fungal pathogens preventing the successful regeneration of many forest species including *Chrysophyllum*. (ii) *C. albidum* and *C. delevoyi* are highly susceptible to damping off in the nursery. (iii) The disease could be fatal and a threat to *Chrysophyllum* germinants. (iv) The fungal pathogens establish themselves in the soil and quickly spread across the germination trays. (v) Disease nips off the seedlings at the soil level. Controlling the disease will involve manipulating the environment that favours the pathogens. The following recommendations could improve on the successful propagation of the species in the nursery.

(i) Use treated seeds for propagation

(ii) Use sterilized soil medium for propagation

(iii) Avoid excess watering of germinants

(iv) Avoid overcrowding germinants

(v) Ensure adequate aeration duration propagation.

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Table 1 Fungi Isolates and Disease Incidence (%) of *Chrysophyllum albidum* and *C. delevoyi*

Fungi	*C. albidum*		*C. delevoyi*	
	Sterilized medium	**Unsterilized medium**	**Sterilized medium**	**Unsterilized medium**
Fusarium oxysporum	00	68	00	94
Aspergllus flavus	00	66	00	94
Trichoderma species	00	64	00	96

Table 2 Mean germination percentage of *Chrysophyllum* species

	C. albidum		*C. delevoyi*	
	Sterilized medium (control)	**Unsterilized medium**	**Sterilized medium (control)**	**Unsterilized medium**
	78	32	76	08
	79	34	74	09
	76	36	72	05
Total	233	102	222	22
Mean	77.7	34.0	74.0	7.3

Table 3 Mean Seedling Survival (%) of *Chrysophyllum* species

	C. albidum		*C. delevoyi*	
	Sterilized medium (control)	**Unsterilized medium**	**Sterilized medium (control)**	**Unsterilized medium**
	76	23	76	6
	79	27	74	4
	76	34	72	8
Total	233	84	222	18
Mean	77.7	28	74	6

Table 4 Fungi Isolates and Disease Incidence (%) of damped off seedlings of *Chrysophyllum* species

	C. albidum		*C. delevoyi*	
Fungi Isolates	**Sterilized medium**	**Unsterilized medium**	**Sterilized medium**	**Unsterilized medium**
Fusarium oxysporum	00	100	00	100
Asperglus flavus	00	100	00	100
Trichoderma species	00	100	00	100