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Seed Scarification and Plant Extracts Enhanced Germination, Seed Health and Seedlings Vigour of *Tetrapleura tetraptera*

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Abstract

Seed dormancy and seed-borne fungi are main biological constraints to the domestication of edible non-timber forest products such as *Tetrapleura tetraptera* fruits. This study aimed to evaluate the effect of thermal and mechanical scarification on seed dormancy and the efficacy of three plant aqueous extracts (*Cymbopogon citratus*, *Callistemom viminalis*, *Tephrosia vogelii*) against seed-borne diseases and seedlings vigour of *T. tetraptera*. Treatments consisted of soaking the seeds in water at 80˚C for 2 and 4 hours, scarification of seeds with abrasive paper at 1 mm and 2 mm depths. The antifungal activity of plant extracts was evaluated both *in vitro* (by the poising method on agar medium) and *in vivo* at 2.5, 5.0 and 7.5 mg/mL. Momtaz (Imidacloprid 250 g/kg + Thiram 200 g/kg) was used as a positive control. Dormancy was lifted by scarification at 2 mm depth; this treatment had the highest germination percentage (92.33%) and the lowest infection rate (20.67%). The more frequent seed-borne fungi isolated belong to *Aspergillus fumigatus*, *A. niger*, and *A. flavus* (with frequence: 16.31%, 18.43% and 21.78% respectively). The pathogenicity test was positive with *Alternaria alternata*, *A. fumigatus* and *Cercospora* sp. *T. vogelii* extract totally inhibited the growth of the pathogenic fungi at all the concentrations tested. Seed infection obtained with *T. vogelii* (4.54%) extracts at 7.5 mg/mL was significantly similar to Momtaz (3.33%). The extract of *C. viminalis* had the highest vigour index (674.42) at 7.5 mg/mL. Mechanical scarification using abrasive

paper and seed treatment with plant extracts of *T. vogelii* could be used in the domestication process of the species.

Keywords

Edible Non-Timber Forest Products, Plant Extracts, Seed-Borne Fungi, Seed Dormancy, *Tetrapleura tetraptera*

1. Introduction

Forests provide essential goods and services to approximately 1.2 billion people worldwide, particularly for their food security, health, and income [1]. Among these resources, edible non-timber forest products (NTFPs) play a critical role due to their nutritional, socio-economic, and cultural value, thereby contributing to improved living conditions for populations [2]. These edible NTFPs serve as a reserve or safety net, offering sustenance and income during crop failures, shortages, unemployment, or other emergencies linked to disasters [3]. However, production, preservation, and processing techniques remain rudimentary, and parasitic attacks significantly affect these products [4]. Such infestations lead to substantial losses, diminishing the impact of the edible NTFP sector in combating food insecurity. In tropical Africa, *Tetrapleura tetraptera*, a perennial Mimosaceae reaching 35 to 50 cm in diameter at maturity with buttresses at its base, is a notable example of an edible NTFP [5]. Its fruits, initially dark green with four prominent sides, turn dark brown and glossy when mature. Once dried, they are used as spices or for their medicinal properties, particularly against digestive disorders, cysts, fibroids, and obesity [6]. A 2017 study by the Tropenbos program in southern Cameroon revealed that the price of a bundle of *T. tetraptera* ranges from 50 to 200 FCFA (0.08 to 0.33 USD), while a single pod sells for 150 to 300 FCFA (0.25 to 0.50 USD) [7]. This spice is also exported to European markets [8], with, for instance, 20 tons imported into the United Kingdom in 2000 from Nigeria, Ghana, and Cameroon [9]. Despite its socio-economic importance, *T. tetraptera* production faces challenges such as poor seed germination and severe fungal infections [10]. The rigidity of the pod and seed coat, combined with the seeds' impermeability to water, keeps them dormant, making natural germination nearly impossible without intervention from elephants or humans [11]. To break this dormancy, treatments such as 90% concentrated sulfuric acid, followed by mechanical scarification (85%), lemon juice, soaking, or hot water have been successfully tested [12], though these methods are often complex and inaccessible to low-income producers. It would also be important to compare the different methods of lifting dormancy in *T. tetraptera* in order to compare their effectiveness with the pod. Additionally, fungal seed diseases (e.g. rot, fusarium wilt) caused by soil-borne pathogens such as *Rhizoctonia*, *Cercospora*, *Aspergillus*, *Colletotrichum*, or *Fusarium* are typically managed with synthetic fungicides. However, their improper use, due to producers' lack of training,

poses risks of pollution, pathogen resistance, and intoxication [13] [14]. Consequently, research is shifting toward non-toxic natural alternatives for plant protection [15] [16]. This study aims to evaluate the effects of thermal and mechanical scarification on *T. tetraptera* seed dormancy, as well as the efficacy of four plant extracts against seed pathologies and seedling vigor.

2. Materials and Methods

2.1. Collection and Preservation of Plant Material

Fruits of *T. tetraptera* were harvested from five approximately 10-year-old trees in Kekem, West Region of Cameroon. This tree was chosen according to their approximate age (more than 10 years old), their health status and the quality of fruits.

After air-drying at room temperature, the fruits were broken with a hammer to extract the seeds, which were stored in sterilized, airtight glass containers. Leaves of *Tephrosia vogelii*, *Callistemon viminalis*, and *Cymbopogon citratus*, used for extracts, were collected between 6 and 7 a.m. at the research farm of the Faculty of Agronomy and Agricultural Sciences (FASA), University of Dschang, West Region. The selection of the 3 plant extract species was based on several criteria such as:

• Their antifungal activity: The chosen species have shown proven efficacy against various pathogenic fungi in previous studies.

• Their accessibility and sustainability: These species are often locally available or easily grown, making them easy to use in practical applications.

• The presence of bioactive compounds: Chemical analyses have revealed the presence of compounds such as flavonoids, alkaloids and terpenes, known for their antifungal properties.

2.2. Scarification of *Tetrapleura tetraptera* Seeds

Seeds underwent thermal scarification (immersion in water at 80˚C for 2 (T1) or 4 (T2) hours) or mechanical scarification (abrasion with sand paper on the proximal part near the hilum, at 1 (T3) or 2 (T4) mm depth). The choice of these scarification depths is motivated by technical and biological considerations:

• Scarification efficiency: It is known that these scarification depths promote better moisture and nutrient penetration, increasing germination [17].

• Embryo protection: Scarification that is too deep could damage the embryo, while these depths balance efficiency and safety.

• Variability of the species: This choice also takes into account the specific characteristics of the seeds of the species studied, where some require more or less intense scarification. The control batches received no treatment (T0). Post-scarification, seeds were disinfected in a 3% hypochlorite solution for 3 minutes, then rinsed three times with distilled water (at 5, 10, and 15 minutes). They were placed in 15 cm-diameter Petri dishes lined with three layers of moistened blotting paper and incubated at 22˚C ± 1˚C under a 12-hour light/12-hour dark photoperiod, with watering every two days. Each treatment consisted of 100 seeds, replicated three

times. Germination (G) was calculated as G = (g × 100)/N, where g is the number of germinated seeds and N is the total number of seeds sown [18]. The infection rate (IR) was determined by IR = (i × 100)/N, where i is the number of infected seeds [18]. The germination rate (GR) was calculated as GR = Σ n/Σ (n × DAS), where n is the number of seeds germinated per day and DAS is the number of days after sowing [18].

2.3. Isolation and Identification of Seed-Associated Fungi

Fungi were cultured on potato dextrose agar (PDA) supplemented with 1 g/L chloramphenicol, sterilized at 121˚C for 15 minutes. Seeds, symptomatic or not, were disinfected in a 3% hypochlorite solution for 2 minutes, then rinsed with distilled water [19]. Ten seeds were aseptically placed in 90 mm Petri dishes containing 20 mL of PDA and incubated at 22˚C ± 1˚C. Fungal colonies visible after 5 days were purified on PDA [20]. Isolation frequency (IF) was calculated as IF = (NF/NT) × 100, where NF is the number of samples with a given fungus and NT is the total number of samples [21]. Identification relied on morphological characteristics (mycelium and fruiting bodies) observed under a microscope, following mycological keys [22].

2.4. Pathogenicity Test

For each 10-day-old isolated fungus, a spore suspension was prepared by adding 10 mL of sterilized distilled water to PDA Petri dishes, gently brushing with a fine paintbrush. A drop of Tween 80 was added to homogenize the spores, and the suspension was filtered (mesh < 1 mm) to remove mycelial fragments [23]. Spore concentration was determined using a hemocytometer (Thoma cell) and adjusted to 10^6 spores/mL per Mathur and Kongsda's formula [24]. Disinfected seeds [19] were placed in Petri dishes with three layers of moistened, sterilized blotting paper (100 seeds per dish). A 10 mL suspension of each fungus was sprayed onto the seeds, the dishes sealed with parafilm, and incubated at 22˚C ± 1˚C. Each treatment was replicated three times, and the seed infection rate was assessed.

2.5. Preparation of Plant Extracts

Freshly harvested leaves were washed with running water, shade-dried for two weeks, and ground into a fine powder. For aqueous extracts, 100 g of powder was macerated in 500 mL of distilled water for 24 hours with two stirrings, protected from light. The mixture was filtered through muslin and Whatman No. 4 paper, and the filtrate constituted the crude extract [25]. Extracts were dried separately in an oven at 50˚C.

2.6. Evaluation of Antifungal Potential of Plant Extracts

In vitro antifungal activity was tested using the PDA diffusion method at concentrations of 2.5, 5.0, and 7.5 mg/mL (The choice of the different concentrations was made on the basis of a test previously carried out and efficient concentrations used

in the literature with others plant extracts) against three identified pathogenic fungi: *Alternaria alternata*, *Aspergillus fumigatus*, and *Cercospora* sp. The negative control for this experiment was water and the positive control was the chemical fungicides Momtaz at the recommended concentration of 5 mg/kg, this fungicide is homologated in Cameroon for seed treatment. Radial growth (RG) was measured as RG = (d1 + d2 − 2d0)/2, where d0 is the explant diameter, and d1 and d2 are orthogonal culture diameters. Inhibition percentage (%I) was calculated as %I = 100 × (Dc − Df)/Dc, where Dc is the control growth diameter and Df is the diameter on extract-amended medium [26].

2.7. Evaluation of the Effectiveness of Extracts on Germination, Infection, and Seedling Vigor

Scarified and disinfected seeds (200 per treatment) were soaked in 25 mL of aqueous extracts at concentrations of 2.5, 5.0 and 7.5 mg/mL. Sterilized distilled water (25 mL) and the fungicide Momtaz (5 mg/kg) were used as negative (T−) and positive (T+) controls, respectively. Following soaking, the seeds were oven-dried at 40˚C for 15 minutes [27] and then placed in Petri dishes lined with moistened paper, maintained at 22˚C ± 1˚C under a 12-hour photoperiod. Each treatment was replicated three times. Daily observations were conducted to calculate germination and infection rates. The seedling vigor index was determined using the formula: Vigor Index = Germination Rate × (root length + shoot length) [28].

2.8. Statistical Analysis

The analyses were carried out using the statistical analysis software R version 3.5.1. at the 5% probability threshold. Since the data did not follow the normal distribution and the homogeneity of the variance was not respected, the Kruskal-Wallis test was used for the separation of the means.

3. Results and Discussion

3.1. Effect of Scarification on Germination and Infection of *T. tetraptera* Seeds

Thermal and mechanical scarification significantly increased the germination rates of *T. tetraptera* seeds compared to the control, where the germination rate was zero (**Figure 1**). The germination rate was highest with mechanical scarification at a 2 mm depth (92.33%), followed by mechanical scarification at 1 mm (81.52%), while thermal scarification treatments for 2 hours and 4 hours showed very low germination rates of 1.27% and 0.67%, respectively. There was no significant difference between the infection rates obtained from the different treatments, but the seed infection rate was higher in the heat scarification treatment boxes for 2 hours (42.00%). The control boxes had no germinated seeds but exhibited a fairly high infection rate (28.67%). The different treatments showed significant differences in the percentage of infection. The highest seed infection rate was observed in seeds subjected to thermal scarification (T1) for 2 hours (42.00%). In contrast, seeds

scarified with sandpaper (T4) and heat for 4 hours (T2) had lower infection rates of 20.67% and 27.67%, respectively. Seeds that had not undergone any treatment did not germinate but showed fairly high infection rates. **Figure 2** shows the effect of the various treatments on the infection rates of *T. tetraptera* seeds at 14 days after sowing (DAS). **Figure 3** shows the germinated and infected seeds at 3 DAS, where visible mycelium of the pathogens is observed on seeds. The different results between thermal and mechanical scarification could be explained by the fact that at 2 mm, the embryo is already visible and develops without hindrance due to the integument [28]. It has been shown that scarification using a nail clipper could increase germination capacity by 80% to 90% after three days [28]. The dormancy-breaking methods used have been evidenced by other studies, namely mechanical scarification using sandpaper [29] [30] and thermal scarification with hot water (80˚C), which made it possible to remove the waxy cuticle from the seeds [31]. Some authors highlighted the interest in involving local populations in the domestication and in situ and *ex-situ* conservation strategies of plant species of interest [32].

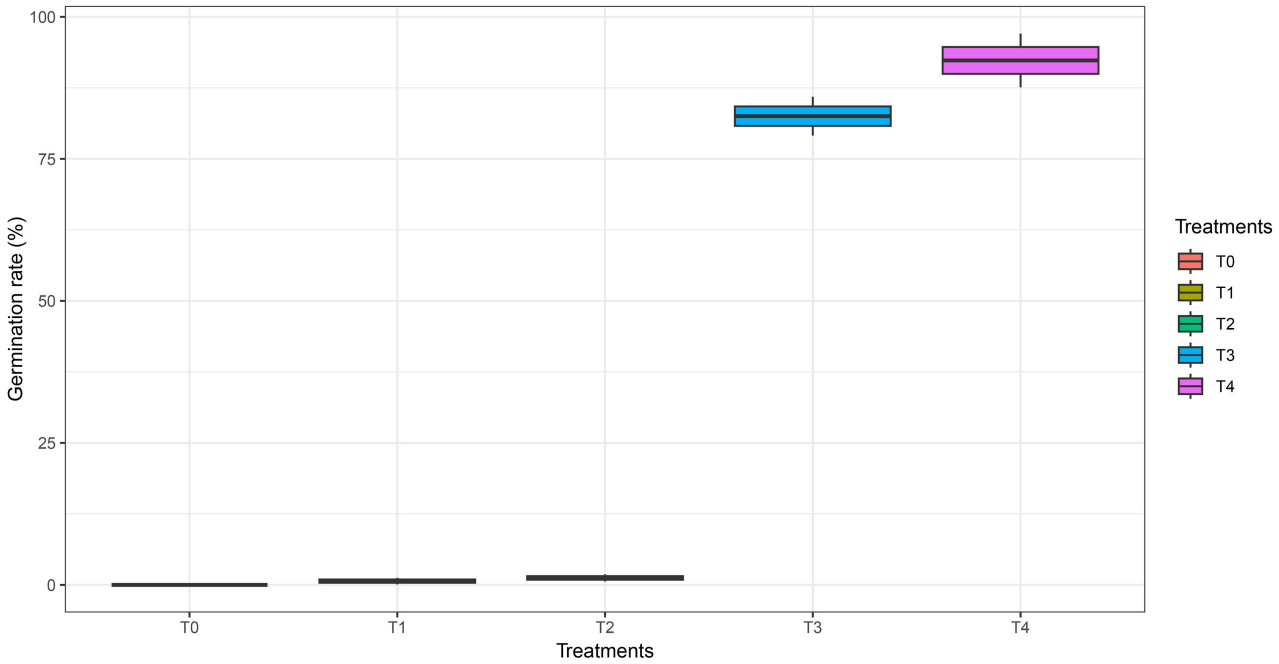


Figure 1. Effect of different methods of scarification on germination percentage of seed of *T. tetraptera* 14 days after sowing.

Mechanical scarification also resulted in a high germination rate (92%) and a reduction in average germination times. Similar results were obtained on the seeds of *Acacia origena*, *Acacia pilispina*, and *Pterotobium stellatum* [33], and in *Parkinsonia aculeata* [34]. Mechanical scarification leads to the rapid imbibition of the seed coat and the entry of water into the reserves, which allows the rapid emergence of the radicle and the triggering of metabolic reactions of the embryo and cotyledons [35]. This could therefore enable rapid and high germination of seeds with

integumentary dormancy (because the integument is hard and impermeable to water and oxygen).

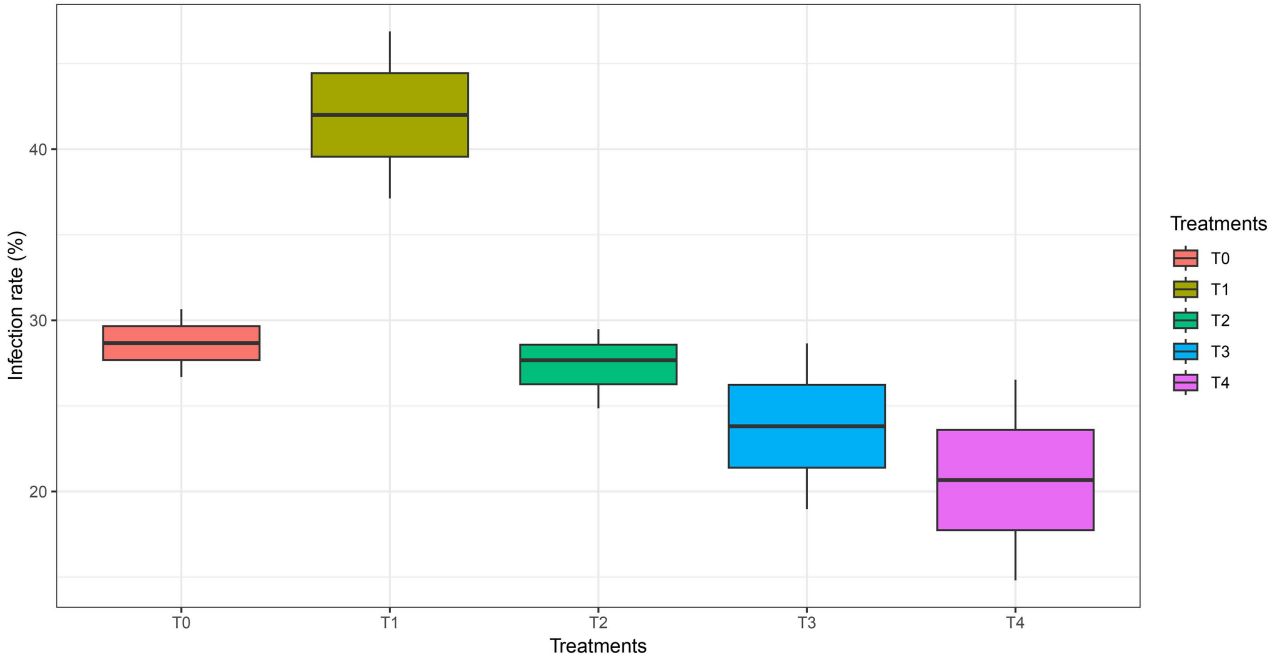
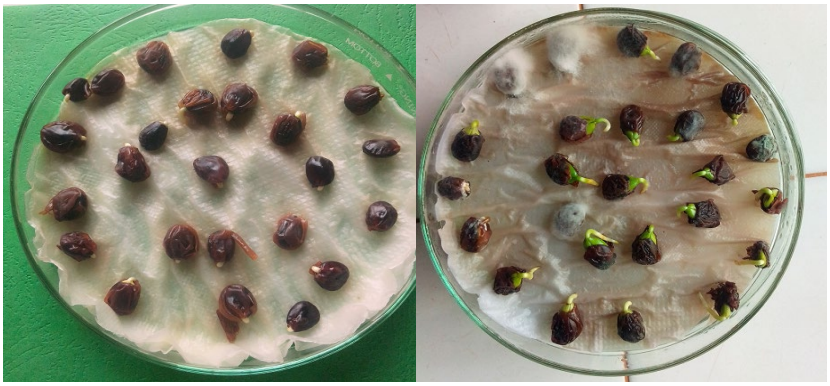


Figure 2. Effect of different methods of scarification on infection rate of seed of *T. tetraptera* 14 days after sowing.



(a) (b)

Figure 3. Scarified and germinated seeds of *Tetrapleura tetraptera* at 3 days (a) and 7 days after sowing (b) in the control (non-treated).

3.2. Effect of Treatments on the Date of First Germination

Scarification treatments had significant effects on the duration of first germination. The first germination was observed on seeds scarified with heat for 4 hours (T2) and sandpaper at 2 mm (T4) at 2.33 and 2.67 days, respectively. On the other hand, seeds that had undergone mechanical scarification with sandpaper at 1 mm (T3) and thermal scarification for 2 hours (T1) showed slightly delayed first germination times of 4.67 and 4.91 days, respectively. Seeds that had not undergone any treatment (T0) did not germinate, and therefore, the duration of first germination

was zero (Figure 4).

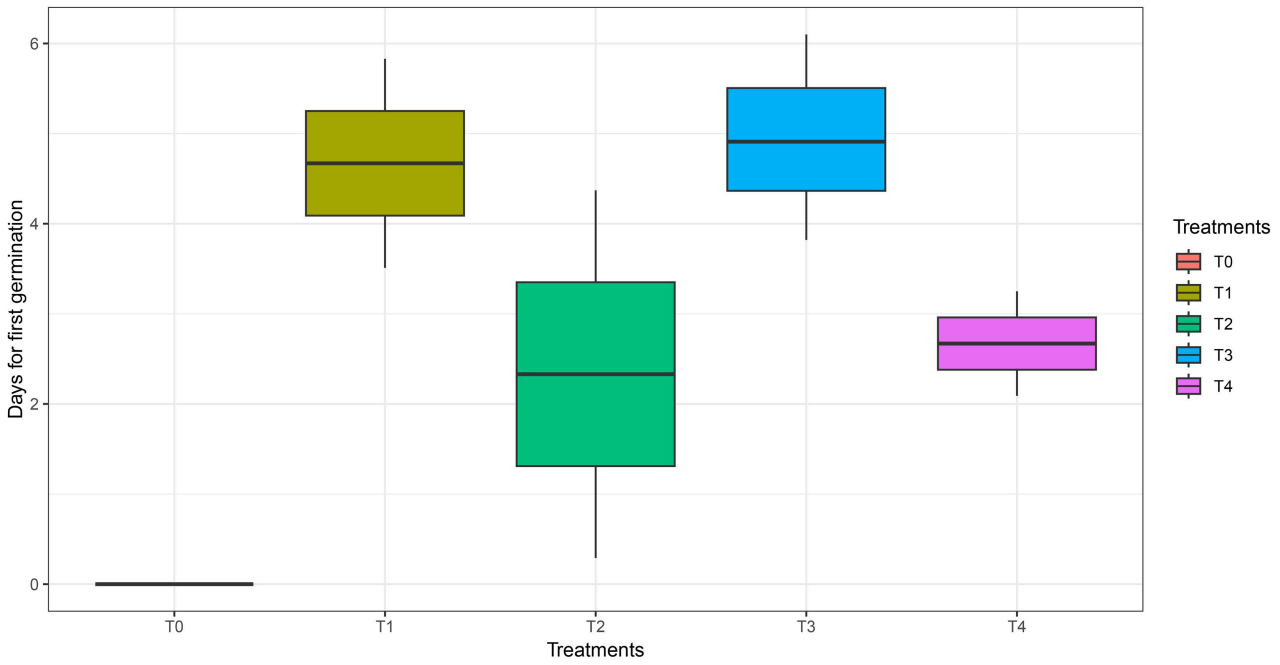


Figure 4. Effect of different methods of scarification on days of the first germination of seed of *T. tetraptera* 14 days after sowing.

3.3. Seed-Borne Fungi of *Tetrapleura tetraptera* Seeds and Their Pathogenicity

Seven species of seed-borne fungi were identified: *Rhizoctonia* sp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichoderma* sp., *Alternaria alternata*, *Cercospora* sp., and *Pestalotiopsis microspora*. The most frequently isolated species were *A. flavus* (21.78%), *A. niger* (18.48%), and *A. fumigatus* (16.31%); *Pestalotiopsis microspora* had the lowest isolation frequency (Table 1). The pathogenicity test was positive for *A. alternata*, *A. fumigatus*, and *Cercospora* sp., with infection rates of 45.75%, 32.25%, and 22.12%, respectively, 5 days after inoculation (Table 2). The seeds' response to artificial inoculation showed rapid seed death by these three seed-borne pathogens within 5 days (Figure 5). Criteria for evaluating and classifying a seed as infected include:

Table 1. Occurrence/frequency of fungi isolated from *Tetrapleura tetraptera* seeds.

Champignons	Frequency of isolement (%)
Alternaria alternata	10.56
Pestalotiopsis nicrospora	6.38
Aspergilus niger	18.43
Aspergilus fumigatus	16.31
Aspergilus flavus	21.78
Trichoderma sp.	15.97
Cercospora sp.	10.57

Table 2. Pathogenicity and infection rate of seed-borne fungi isolated from *Tetrapleura tetraptera* at 5 days after inoculation.

Fungi	Pathogenicity	Infection rate (%)
Alternaria alternata	+++	45.75 ± 4.65[a*]
Pestalotiopsis nicrospora	–	0.0 ± 0.0[d]
Aspergillus niger	–	0.0 ± 0.0[d]
Aspergillus fumigatus	++	32.25 ± 2.88[b]
Aspergillus flavus	–	0.0 ± 0.0[d]
Cercospora sp	+	22.12 ± 3.66[c]
Trichoderma sp	–	0.0 ± 0.0[d]

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p = 0.05. Legend: +++ = highly pathogenic; ++ = moderately pathogenic; + = pathogenic; – = not pathogenic.

(a)

(b) (c)

Figure 5. Pictures showing seed infection after inoculation with *Alternaria alternata* (c), *Aspergillus fumigatus* (b), and *Cercopora* sp. (a) at 5 days after inoculation.

- Visual symptoms: Presence of spots, discoloration, or alteration of the seed surface, damping-off.
- Seed health: Observation of fungal filaments or spores on the seeds.
- Germination tests: Seeds are considered infected if the germination rate is sig-

nificantly reduced compared to a healthy control group.

• Biochemical tests: Analysis of the production of secondary metabolites indicative of fungal infection.

The isolated fungal species are well-known as damaging species of ENTFP seeds. Most of these species have already been identified on *Ricinodendron heudelotii* and *Garcinia kola*, two ENTFPs of high socio-economic value in Cameroon, as being responsible for seed losses [36]. Additionally, species like *A. flavus* and *Cercospora* sp. have been reported as fungi responsible for post-harvest losses of *Monodora myristica* [37]. Most of these fungi are responsible for high post-harvest losses of some fruits [38]. *Cercospora* sp. was also isolated from seeds of *Persea americana* with high occurrence frequencies [39].

Similar results were obtained on the seeds of other woody species. This is the case for *Aspergillus niger* and *Trichoderma* sp. in *Oroxylum indicum* [40], *Azadirachta indica* (neem), *Leucaena leucocephala*, and *Gmelina arborea* [41], where they cause various diseases such as anthracnose and damping-off. Highly polyphagous species such as *A. flavus* and *A. niger* have been inventoried on the seeds of several woody species: *Terminalia microcarpa*, *Cassia fistula*, *Sterculia foetida*, *Acacia mangium*, and *Eucalyptus grandis* [41] [42]. The high presence of extremely polyphagous fungi such as *Aspergillus* could be explained by the fact that humidity is high under the seed canopies, which favors their development. Pods for production should be collected directly from seed trees when they have reached maturity to reduce fungal infections of seeds. As these fungi are extremely polyphagous, it would be wise to exercise caution when handling them and during storage to avoid possible contamination of the seeds.

3.4. Bio-Efficacy of Plant Extracts on the Inhibition Percentage of Pathogenic Fungi of *T. tetraptera* Seeds

Table 3 shows that aqueous extracts of plants at different concentrations significantly reduce the growth of pathogens. The extract of *C. viminalis* at a concentration of 7.5 mg/mL significantly reduced the radial growth of *A. alternata* and *Cercospora* sp. by 80.89% and 69.08%, respectively. The extract of *C. citratus* at the concentration of 7.5 mg/mL significantly reduced the radial growth of *A. alternata*, *Cercospora* sp., and *A. fumigatus* by 60.25%, 41.77%, and 19.49%, respectively. The extract of *Tephrosia vogelii* totally inhibited the development of the three fungi at all concentrations tested. The effectiveness of aqueous extracts of *T. vogelii* and *C. viminalis* on the development of these microorganisms varies according to concentrations and has been demonstrated by studies on *C. viminalis* [43] and *T. vogelii* [44]. Other studies showed that *C. citratus* and *T. vogelii* extracts exhibited antifungal activity against the potato late blight pathogen *Phytophthora infestans* [45]. The reduction of infection of the different fungal species by extracts of *T. vogelii*, *C. viminalis*, and *C. citratus* shows that these plants contain antifungal substances. The antifungal activity of these plant extracts is thought to be due to the action of oxygenated monoterpenes. The aqueous extract of *T. vogelii* completely

inhibits the development of all three fungi. The biological actions of *T. vogelii* are mainly due to the presence of secondary metabolites of the alkaloid family. These include rutenones and their enantiomers, and then rutilines [46], the *in vitro* and *in vivo* efficacy of plant extracts including *C. viminalis* against late blight [47], the inhibition of *Aspergillus* sp. by *C. citratus* extract [48], and the inhibitory activity of *T. vogelii* on the radial growth of seed fungi of cowpea in stock [49]. This suggests that extracts from these plants are indeed effective in combating fungi.

Table 3. Inhibition of the mycelial growth of seed-borne fungi of *Tetrapleura tetraptera* by plant aqueous extracts.

Plant extracts	Concentration (mg/mL)	Growth inhibition (%)		
		Alternaria alternata	*Cercospora* sp.	*Aspergillus fumigatus*
Callistemom viminalis	2.5	27.67 ± 3.76^{c*}	18.07 ± 2.33^{c}	0.0 ± 0.0^{d}
	5.0	73.33 ± 6.55^{bc}	67.07 ± 4.87^{bc}	0.0 ± 0.0^{d}
	7.5	80.91 ± 11.32^{bc}	69.08 ± 15.26^{bc}	0.0 ± 0.0^{d}
Cymbopogom citratus	2.5	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	62.65 ± 18.78^{b}
	5.0	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	28.11 ± 6.77^{bc}
	7.5	60.25 ± 12.41^{b*}	41.77 ± 13.27^{b*}	19.49 ± 5.64^{c}
Tephrosia vogelii	2.5	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	5.0	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
	7.5	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Momtaz (synthetic fungicide)		100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Control (distilled water)		0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p = 0.05.

3.5. Effect of Plant Extracts on Germination, Seed Infection, and Seedling Vigour of *Tetrapleura tetraptera*

The effects of plant extracts on seed germination were significantly similar. However, it is noted that the germination rate was highest with *T. vogelii* and *C. viminalis* extracts at 2.5 mg/mL (97.33%) and 5.0 mg/mL (96.67%), respectively. There was no significant difference between the different concentrations of the extracts and the two controls on the germination of *T. tetraptera* seeds (**Table 4**). Additionally, the lowest seed infection was obtained with the same extracts, and the values obtained were significantly ($p < 0.05$) comparable to the positive control (synthetic fungicide). Specifically, seed infection with *T. vogelii* was 4.54% with at the concentration of 7.5 mg/mL, while in the positive control, it was 3.33%. Significant dif-

ferences were observed between the different concentrations of the aqueous extracts and the two controls on the percentage of infection of *T. tetraptera* seeds (Table 5). Table 6 shows that the maximum vigour index of plants (674) was obtained with *C. viminalis* at 7.5 mg/mL, while the positive control obtained the lowest vigour index (506). However, significant differences were observed between the different doses of aqueous extracts and the two controls on the vigour index of *T. tetraptera* seeds.

Table 4. Effect of plant aqueous extracts on the germination (%) of *T. tetraptera* seeds.

Concentration (mg/mL)	*Cymbopogom citratus*	*Callistemon viminalis*	*Tephrosia vogelii*
2.5	86.67 ± 5.77^{a*}	90.00 ± 7.32^{a}	97.33 ± 5.77^{a}
5.0	90.00 ± 7.00^{a}	96.67 ± 5.77^{a}	83.33 ± 15.27^{a}
7.5	92.00 ± 7.10^{a}	90.00 ± 7.32^{a}	93.33 ± 6.54^{a}
Momtaz (fungicide)	94.67 ± 5.77^{a}	94.67 ± 5.77^{a}	94.67 ± 5.77^{a}
Distilled water (control)	92.33 ± 5.54^{a}	92.33 ± 5.54^{a}	92.33 ± 5.54^{a}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p = 0.05.

Table 5. Effect of plant aqueous extracts on seeds infection (%) of *T. tetraptera* during germination.

Concentration (mg/mL)	*Cymbopogom citratus*	*Callistemon viminalis*	*Tephrosia vogelii*
2.5	19.33 ± 5.77^{b}	20.03 ± 2.07^{b}	23.33 ± 15.27^{b}
5.0	13.91 ± 6.27^{b}	12.04 ± 7.55^{bc}	16.67 ± 9.77^{b}
7.5	12.33 ± 5.77^{b}	10.22 ± 7.32^{bc}	4.54 ± 1.96^{c}
Momtaz (fungicide)	3.33 ± 1.27^{c*}	3.33 ± 1.27^{c}	3.33 ± 1.27^{c}
Distilled water (control)	86.67 ± 1.52^{a}	86.67 ± 1.52^{a}	86.67 ± 1.52^{a}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Turkey test at p= 0.05.

Table 6. Effect of aqueous plant extracts on the vigour index of *T. tetraptera* seedlings.

Concentrations (mg/mL)	*Cymbopogom citratus*	*Callistemon viminalis*	*Tephrosia Vogelii*
2.5	541.94 ± 10.86^{b*}	553.00 ± 14.01^{b}	603.43 ± 11.92^{a}
5.0	446.96 ± 15.73^{d}	464.69 ± 36.99^{c}	467.23 ± 4.11^{dc}
7.5	634.27 ± 7.84^{a}	674.42 ± 11.25^{a}	530.33 ± 7.52^{b}
Momtaz (fungicide)	506.74 ± 10.95^{c}	506.74 ± 10.95^{bc}	506.74 ± 10.95^{c}
Distilled water (control)	238.31 ± 7.57^{e}	238.31 ± 7.57^{d}	238.31 ± 7.57^{e}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p= 0.05.

The *T. vogelii* extracts protected the seeds against fungal attack more effectively than the others, followed by *C. viminalis*, *E. saligna*, and *C. citratus* extracts. This

activity of the aqueous extracts could be due to their chemical composition of antifungal substances. These results corroborate those of studies showing the antifungal potential of *E. saligna* extracts against fungi responsible for the deterioration of green beans post-harvest [46], the *in vitro* and *in vivo* efficacy of plant extracts including *C. viminalis* against black nightshade downy mildew [47], the inhibition of *Aspergillus* sp. by *C. citratus* extract [48], and the inhibitory activity of *T. vogelii* on the radial growth of fungi in stored cowpea seeds [49]. This suggests that the extracts of these plants would be effective in combating fungi associated with ENTFP seeds. The antifungal activity of these plant extracts could be due to the action of oxygenated monoterpenes [50] and phenolic compounds, including sterols, flavonoids, condensed tannins, coumarins, and alkaloids [45].

4. Conclusion

This work investigates effective and easily applicable solutions to the dormancy and germination of *T. tetraptera* seeds and proposes a biological control measure for seed-borne fungi. Scarification through mechanical abrasion at a 2 mm depth improves the germination rate up to 92%. The most frequently identified fungi on *T. tetraptera* seeds were *A. flavus*, *A. niger*, and *A. fumigatus*, which can cause seed-borne diseases. The pathogenic fungi were *A. alternata*, *A. fumigatus*, and *Cercospora* sp. Aqueous extracts of *T. vogelii*, *C. viminalis*, and *C. citratus* exhibited higher antifungal properties and inhibited the growth of the three pathogenic fungi, as well as the synthetic fungicide Momtaz. These plant extracts promoted germination, vigour, and seed protection. Since key domestication constraints of *T. tetraptera* have been addressed and seed-borne fungi have been identified and controlled by this work, studies on the cultivation of *T. tetraptera* in the nursery and in the field should now be emphasized.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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