ORIGINAL ARTICLE

IMPROVEMENT OF THE GERMINATIVE QUALITY OF *Lagenaria Siceraria* SEEDS BY FERMENTATION

AMÉLIORATION DE LA QUALITÉ GERMINATIVE DES GRAINES DE LAGENARIA SICERARIA PAR FERMENTATION

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ABSTRACT

Introduction: Several studies have shown the beneficial action of microorganisms in improving the agronomic performance of seeds in several species. **Objective:** Thus, the effect of five strains of fungi isolated from the fermented juice of *Lagenaria siceraria* pulp on seed viability and seedling vigor was studied. **Methods:** Seeds of *L. siceraria* were fermented for 3, 6 and 9 days in the presence of the five strains of fungi. **Results:** Two strains, *Black* and *Rhizopus* fungi, improved germination by reducing the germination time to 5.31 ± 1.32 days and 5.36 ± 1.26 days respectively compared to the control (5.65 ± 1.42 days). For seedling vigor, the *Rhizopus* fungus favored large seedlings of 63.42 ± 11.42 mm, while the control seeds produced seedlings of 60.68 ± 8.55 mm in height. **Conclusion:** The results of these fungi significantly improve the quality of the seeds of *L. siceraria*, especially the *Rhizopus* genus. On the other hand, fermenting the seeds for 6 days of fermentation results in good germination and vigorous seedlings.
***Key words**: Lagenaria siceraria, fermentation, fungus, germination, seedling vigor.*

1. INTRODUCTION

Lagenaria siceraria or bottle gourd is a species of oil-bearing Cucurbitaceae commonly known as "pistachio" in Côte d'Ivoire and "Egussi" in several West and Central African countries [1,2]. The seeds, rich in lipids and proteins [3], also have a good market value [4,5]. However, the oilseed gourd has low seed yield due to poor quality seeds. Improving productivity necessarily involves obtaining seeds of good germination quality because gourds reproduce mainly by seed. The decrease in the water content of the seeds during fermentation improves their germinative power [6]. Several microorganisms carry out the fermentation process. These have been used to stimulate germination and seedling growth in several species [7,8]. However, studies to improve the germinative capacity of pistachio seeds by fungi have not yet been conducted. The present work proposes to identify the fungi involved in the fermentation of *L. siceraria* pulp and identify those that improve the germinative quality of the seeds during this process.

2. MATERIAL AND METHODS

2.1. Study site

The trials were carried out at the Nangui Abrogoua University of Abidjan (Côte d'Ivoire). The climate is hot and particularly humid. The city of Abidjan, with geographical coordinates of 05°19-north latitude and 04°02-west longitude [9], is a very rainy area with 1400 to 2500 mm of rain per year. Rainfall is divided into 2 rainy seasons and two dry seasons [10]. The temperature varies throughout the year (27° to 37° C on average), with a humidity rate varying between 60 and 90%. This southern zone is the domain of dense forest. The test site is characterized by deep, sandy-clay soil [4].

2.2. Plant material

The plant material used consisted of mature berries of *Lagenaria siceraria*. The berries were round shaped containing calotte seeds (**Figure 1a** and **1b**).

a **b**

Figure 1. Fruits and seeds of *L. siceraria*. **a.** fruit; **b.** cap seeds extracted from round fruit.

2.3. Isolation and identification of fungi from the pulp juice of *Lagenaria siceraria*

2.3.1. Extraction of the pulp juice of *L. siceraria*

Under a laminar flow hood, each fruit is cut in half. The pulp is removed with a sterilized spoon, then all seeds are removed and the juice is crushed. The juice is collected in a clean jar and then distributed in eight jars corresponding to the different fermentation times (1, 2, 3, 4, 5, 6, 7, 8 days).

2.3.2. Preparation of the culture medium and seeding of the juices

The culture medium is Potato Dextrose Agar (PDA). It was prepared according to the method of Botton *et al.* (1990). Each day, for eight days, the fermented juice was inoculated into three Petri dishes containing the gelled culture medium, at a rate of four drops per dish. These dishes were then sealed with parafilm and incubated.

2.3.3. Isolation of fungal strains

Three days after seeding, a portion of the growth in front of each colony was taken and subcultures on fresh PDA medium. The samples were taken with a sterile loop dipped in alcohol and heated by a flame.

2.3.4. Identification of fungal strains

The pure strains obtained were observed with the naked eye to determine the coloration and appearance of the fungal colonies. Then, observation under the light microscope was performed. The identification keys of [11] Botton et al. (1990) were used.

2.4. Obtaining extracts of fungi inoculum and fermentation of seeds

After identification of the fungi, 3 fragments of each strain were put separately in three bottles containing 250 ml of distilled water, 10 g of glucose and 10 g of mashed potatoes. These bottles were incubated at the mycotheque for ten days at room temperature (29 ± 2°C). Then, the mycelia from each bottle were collected and rinsed with water and put in bottles containing 100 ml of distilled water. Finally, under a fume hood, batches of 100 *L. siceraria* seeds were extracted from unfermented berries and put into each bottle corresponding to 3, 6 or 9 days of seed fermentation. After fermentation, the seeds were removed from the inocula and washed thoroughly with tap water and then dried in the shade until constant weight was obtained.

2.5. Seed germination and seedling vigor test

2.5.1. Experimental design

The seeds were sown in a plot to consist of five plots, each representing a replication. Each bed was 4 m long and 2 m wide and divided lengthwise into three blocks 40 cm apart. Each block corresponds to a seed fermentation time and is formed by six groups of 20 seedling points. The six seed groups represent the treatments and are separated by 20 cm from each other. The 20 points in each seedling group are arranged in four rows and five columns (**Figure 2**). The 3-cm-deep seedpods spaced 7 cm apart contain a single seed each. This corresponds to a total of 360 seeds per bed and a total of 1800 seeds in the plot.

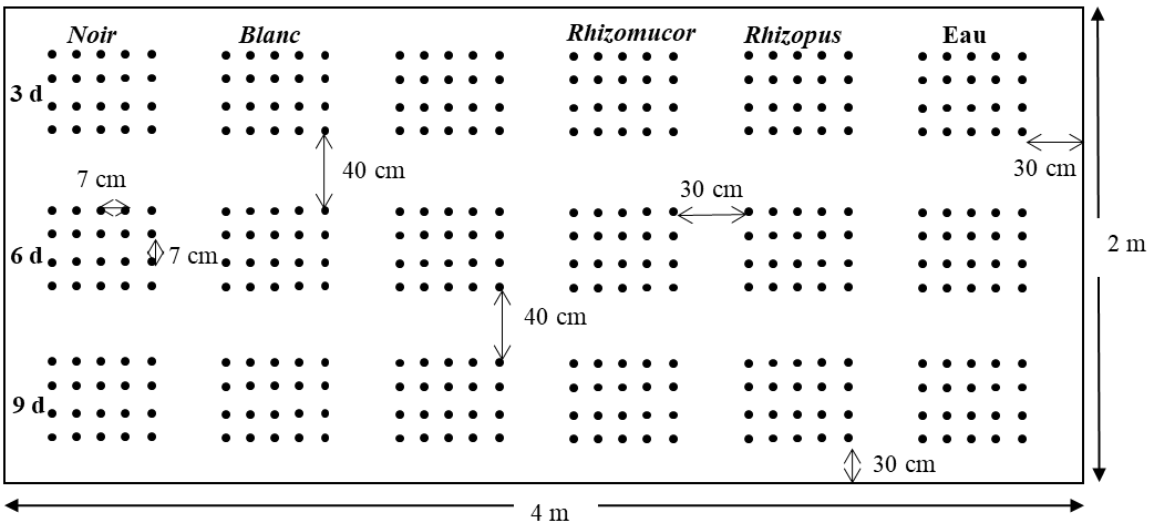


Figure 2. Plan of a plot (one replicate) of *Lagenaria siceraria* seeds fermented with *Black*, *White*, *Yellow*, *Rhizomucor*, *Rhizopus* and control (water) fungi for 3 days (3 d), 6 days (6 d) and 9 days (9 d).

2.5.2. Seed viability parameters

Germination time (GeTi)

The number of days from seed sowing to the appearance of cotyledonary leaves on the soil surface is called germination time [4].

Percentage of germination (PeGe)

The percentage of germination is the ratio of the number of germinated seeds to the total number of seeds sown, all multiplied by 100.

2.5.3. Seedling vigor parameters

Emergence time (EmTi)

Seedlings were considered emerged when they had fully expanded cotyledonary leaves and a third leaf reaching about 1 cm in length. The time of emergence is the time from the date of sowing to this stage.

Length of hypocotyl (LeHy) and tigella (LeTi)

On seedlings dug up at the five-leaf stage, the tigelle and hypocotyl are measured with a caliper. The length of the hypocotyl is the distance from the collar to the point of insertion of the cotyledonary leaves. The distance from the cap to the point of insertion of the cotyledonary leaves is called the tigella length (**Figure 3**).

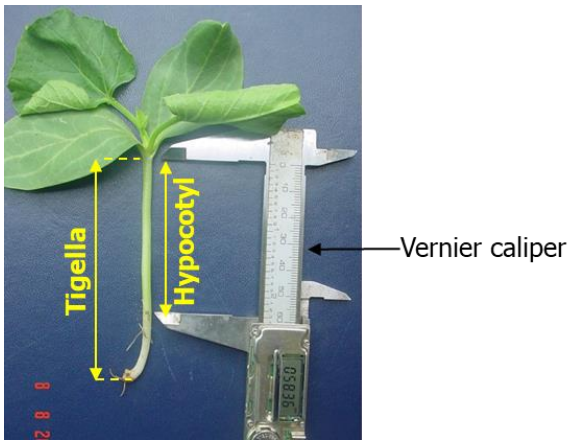


Figure 3. Seedling of *Lagenaria siceraria* dug up at the 5-leaf stage [12].

Fresh Weight (FrWe) of Seedlings

When the seedlings are dug up at the 5-leaf stage, each of them is weighed with the precision balance. This weight corresponds to the fresh weight of the seedlings (FrWe).

2.6. Statistical analysis

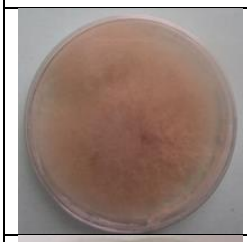
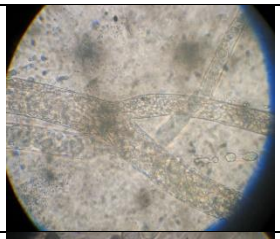
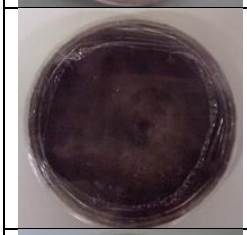
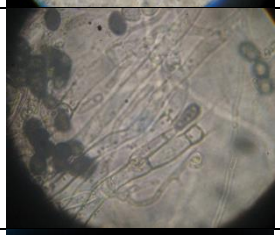
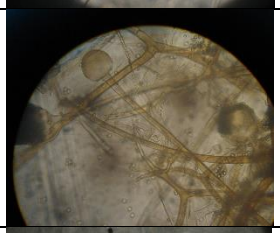
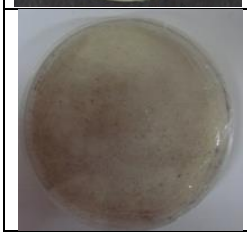
The means of the studied parameters were compared through analysis of variance (ANOVA). When a significant difference was observed, the test ppds (Smallest Significant Difference) was performed. Statistical tests were performed with SAS software (1999) at the threshold α = 0.05 [13].

3. RESULTS

3.1. Fungi isolated from the pulp juice of *L. siceraria*

Five strains of fungi was isolated from the fermented pulp juice of *L. siceraria* of which two genera were identified. These are the genera *Rhizopus* and *Rhizomucor*. The unidentified strains were named according to their macroscopic appearance. Macroscopic and microscopic description of the fungal strains were made (**Table 1**).

Table 1: Macroscopic and microscopic description of fungi isolated from *L. siceraria* pulp juice.

Macroscopic view	Microscopic view	Description and identification of fungi
		Cottony fungi, dense of orange-yellow coloring. Mycelium thick, not partitioned and sterile. Unidentified mushroom: *Yellow*
		Blackish-gray fungus. Mycelium cloisonné and not pigmented with colored and not colored spores of oval forms. Unidentified mushroom: *Black*
		Pale brownish thallus with extremely rapid growth. Sporocystophores with short rhizoids at the base. Presence of apophyses. Identified fungus: *Rhizopus* sp.
		Whitish fungus with slow growth. Mycelium not partitioned, hyaline and sterile. Unidentified mushroom: *White*
		White then grey flaky thallus. Presence of rhizoids at the base. Absence of apophysis. Fungus identified: *Rhizomucor* sp.

3.2. Influence of fungi on seed viability of *L. siceraria*

Fermentation significantly influences the time it takes for seeds to germinate (F = 2.41; P = 0.034). Indeed, the shortest germination time was obtained with the *Black* and *Rhizopus* fungi with 5.31 ± 1.32 days and 5.36 ± 1.26 days respectively. On the other hand, the longest germination time is obtained when the seeds are soaked in distilled water (control). Thus, *Black* and *Rhizopus* fungi reduce seed germination time (**Table 2**).

Table 2: Impact of fermentation of *L. siceraria* seeds on germination as a function of fungal type.

Treatments		Parameters (1)	
		GeTi (j)	PeGe (%)
Control	**Eau**	5.65 ± 1.42 a(2)	93.00 ± 5.60
Fungi	***Black***	5.31 ± 1.32 b	95.67 ± 5.30
	Yellow	5.53 ± 1.31 ab	95.33 ± 7.90
	White	5.49 ± 1.36 ab	94.00 ± 6.87
	Rhizopus	5.36 ± 1.26 b	93.33 ± 6.73
	Rhizomucor	5.46 ± 1.26 ab	95.67 ± 5.30
Statistics	***F***	2.41	0.54
	P	0.034	0.745

(**1**): **PeGe**: percentage of germination; **GeTi**: germination time; (**2**): In each column means with the same letters are statistically equal (**α** = 0.05).

3.3. Influence of fungi on seedling vigor of *L. siceraria*

Seed fermentation by fungi significantly influenced stem length (F = 2.82; P = 0.015), hypocotyl length (F = 4.73; P = 0.001), and fresh weight (F = 5.55; P < 0.001) of seedlings. Indeed, the best stem elongation was obtained with the

Rhizopus fungus (63.42 ± 11.22 mm) compared to the shortest obtained with the control (water) (60.68 ± 9.53 mm). Regarding the length of the hypocotyl, the *Black*, *Rhizopus* and *Rhizomucor* fungi favored its growth. Moreover, compared to the control (water), no fungal strain improves the fresh weight of the seedlings because the heaviest seedlings are obtained both with the seeds soaked in water and the seeds fermented in the presence of White and *Rhizopus* fungi.

Table 3: Impact of fermentation of *L. siceraria* seeds on seedling vigor as a function of fungal type.

Treatments		Parameters [1]				
		PoEm (%)	EmTi (j)	LeTi (mm)	LeHy(mm)	FrWe (g)
Control	(water)	90.67±6.51	9.00±1.48	60.68±9.53$^{b(2)}$	36.38±8.55^{b}	2.56±0.66^{a}
Fungi	***Black***	92.00±7.02	8.87±1.49	60.90±10.25^{b}	39.26±8.46^{a}	2.40±0.60^{b}
	Yellow	93.33±8.38	8.90±1.43	61.90±9.90^{ab}	36.97±8.75^{b}	2.41±0.59^{b}
	White	93.00±7.97	8.88±1.54	62.06±11.37^{ab}	38.28±9.85^{ab}	2.56±0.66^{a}
	Rhizopus	91.00±9.49	8.82±1.54	63.42±11.22^{a}	39.08±9.77^{a}	2.59±0.66^{a}
	Rhizomucor	93.00±7.75	9.02±1.54	60.40±10.99^{b}	39.27±8.99^{a}	2.38±0.60^{b}
Statistics	***F***	0.31	0.74	2.82	4.73	5.55
	P	0.908	0.595	0.015	0.001	<0.001

[1] PoEm percent emergence; **EmTi**: emergence time; **LeTi**: tigelle length; **LeHy**: hypocotyl length; **FrWe**: fresh weight.
[2]In each column, means with the same letters are statistically equal (**α** = 0.05).

3.4. Influence of fermentation time on the viability of *L. siceraria* seeds

Fermentation time significantly influences the percentage of germination PeGe (F = 14.00; P<0.001), and the time of seed germination GeTi (F = 11.87; P < 0.001). Indeed, PeGe significantly increased with the duration of the seed fermentation. As for germination time, the smallest time 5.25 ± 1.06 days was obtained at 6 days of fermentation versus 5.62 ± 1.47 days and 5.53 ± 1.25 days for 3 days and 9 days of fermentation, respectively (**Table 4**).

Table 4: Influence of fermentation time on seed viability of *L. siceraria*.

Parameters [1]	Seeds fermentation time			Statistics	
	3 days	6 days	9 days	*F*	*P*
PeGe (%)	90.17±5.83$^{b(2)}$	95.83±6.16^{a}	97.50±3.44^{a}	14.00	<0.001
GeTi (j)	5.62±1.47^{a}	5.25±1.06^{b}	5.53±1.25^{a}	11.87	<0.001

[1]**PeGe**: percentage of germination; **GeTi**: germination time; [2] On each line, the means bearing the same letters are statistically equal (α = 0.05).

3.5. Influence of fermentation time on seedling vigor

Fermentation time has a significant influence on PoEm (F=16.30; P < 0.001), LeHy (F = 3.10; P < 0.045) and LeTi (F = 6.33; P = 0.001). Indeed, PoEm steadily and significantly increased with fermentation time. Large seedlings are obtained after 3 days of fermentation (Table 5).
However, the duration of fermentation does not influence the fresh weight of seedlings that are FrWe (F=1.20; P=0.302).

Table 5: Influence of fermentation time of *L. siceraria* seeds on seedling vigor.

Parameters [1]	Seeds fermentation time			Statistics	
	3 days	6 days	9 days	*F*	*P*
PoEm (%)	86.50±8.08 $^{b(2)}$	93.50±6.84^{a}	96.50±5.04 ^{a}	16.30	<0.001
LeTi (mm)	62.34±10.45 ^{a}	62.18±9.79^{a}	60.24±10.82^{b}	6.33	0.001
LeHy (mm)	39.06±9.08 ^{a}	37.87±8.34^{b}	37.76±9.28^{b}	3.10	0.045
FrWe (g)	2.52±0.62	2.47±0.57	2.46±0.68	1.20	0.302

[1]**PoEm:** percent emergence; **LeTi**: tigella length; **LeHy**: hypocotyl length; **FrWe**: fresh weight.
[2] On each line, means with the same letters are statistically equal (**α** = 0.05).

3.6. Influence of the fungus type-fermentation duration interaction on the vigor of *L. siceraria* seedlings

The interaction of fungus type and fermentation duration (Table 6) has a very highly significant influence on tigel length (F = 3.59; P < 0.001) and hypocotyl length (F = 4.28; P < 0.001).

Fermentation of seeds with White fungus for 3 days improved tigel and hypocotyl elongation of seedlings. In contrast, there was no significant influence of the fungi type fermentation duration interaction on emergence time (F = 1.43; P = 0.160), emergence percentage (F = 0.39; P = 0.949), emergence rate (F = 0.48; P = 0.898), and seedling fresh weight (F = 0.79; P = 0.640).

Table 6: Combined impact of fungal type and fermentation time of *L. siceraria* seeds on seedling vigor.

Fermentation time	Treatments		seedling vigor parameters [1]				
			EmTi (j)	PoEm (%)	LeTi (mm)	LeHy (mm)	FrWe (g)
3 days	Control (water)		9.22±1.44	85.00±5.00	60.05±7.72^{bc}	35.15±7.04^{c}	2.62±0.59
	Fungi	***Black***	8.82±1.31	87.00±5.70	59.12±10.40^{c}	39.22±8.72^{b}	2.45±0.52
		Yellow	8.97±1.43	89.00±10.84	62.17±8.83b^{c}	35.67±8.36^{c}	2.55±0.60
		White	9.05±2.12	87.00±7.58	66.33±12.57^{a}	42.14±11.26^{a}	2.53±0.72
		Rhizopus	9.21±1.80	82.00±9.75	64.41±10.98^{ab}	41.02±10.38^{ab}	2.61±0.73
		Rhizomucor	8.83±1.51	89.00±9.62	61.96±12.98^{bc}	41.15±8.69^{ab}	2.36±0.55
6 days	Control (water)		8.70±1.22	93.00±5.70	60.98±9.50b^{c}	36.09±9.20^{c}	2.56±0.57
	Fungi	***Black***	8.55±1.35	94.00±6.52	62.41±8.83^{b}	37.80±6.66b^{c}	2.33±0.57
		Yellow	8.65±1.35	92.00±7.58	64.05±9.40^{ab}	38.38±7.34^{bc}	2.37±0.57
		White	8.61±1.00	94.00±8.22	59.17±9.15^{c}	36.22±8.23^{c}	2.60±0.59
		Rhizopus	8.36±1.30	94.00±6.52	65.04±11.91^{ab}	39.86±9.61^{ab}	2.59±0.65
		Rhizomucor	8.97±1.43	94.00±6.52	61.44±9.93^{bc}	38.90±9.02^{b}	2.37±0.60
9 days	Control (water)		9.11±1.70	94.00±5.48	60.87±10.75^{bc}	37.53±8.83^{bc}	2.51±0.77
	Fungi	***Black***	9.24±1.70	95.00±7.07	61.06±11.26^{bc}	40.81±9.61^{ab}	2.44±0.71
		Yellow	9.08±1.48	99.00±2.24	59.66±10.87^{bc}	36.88±10.09^{bc}	2.32±0.58
		White	8.98±1.34	98.00±4.47	61.33±11.34^{bc}	37.07±9.22^{bc}	2.56±0.74
		Rhizopus	8.93±1.39	97.00±4.47	60.86±10.34^{bc}	36.53±8.90^{bc}	2.56±0.62
		Rhizomucor	9.24	96.00±6.52	57.66±10.37^{c}	37.72±9.00^{bc}	2.44±0.66
Statistics		***F***	1.43	0.39	3.59	4.28	0.79
		P	0.160	0.949	<0.001	<0.001	0.640

[1] **PeEm:** percentage of emergence, **EmTi:** time of emergence; **LeTi:** length of tigella; **LeHy:** length of hypocotyl; **FrWe:** fresh weight.
[2] Within each column, means with the same letters are statistically equal (α = 0.05).

4. DISCUSSION

Fermentation of *L. siceraria* seeds in the presence of the fungi improves the germination and vigor of the seedlings they produce. The improvement in seed germinability is reflected in a reduction in germination time when fermented in the presence of the Black colored fungi and the genus *Rhizopus*. This rapid germination of seeds could be attributed to the substances secreted by the latter in the incubation medium. These substances, in particular enzymes, could by biodegradation raise the concentration of the culture medium in solutes thus leading to the dehydration of seed by the phenomenon of osmosis. This release of water allows a good respiration of the embryo thus becoming able to germinate [14,15]. In addition, these substances could degrade the components of the seed coat, causing its fragility. Thus, the rapid germination of seeds induced by the action of black fungi and the genus *Rhizopus* implies that the latter secrete enzymes in the incubation medium that can degrade the seed coats. As for the improvement of the vigor of the seedlings, it is translated by an increase in their size when the seeds are fermented in the presence of the *Rhizopus* fungus. Similar results were obtained by Zheng and Shetty (2000) [16]. These authors showed that pea (*Pisum sativum* L.) seeds fermented with extracts of the fungus *Trichoderma* resulted in large, phenolic-rich seedlings. Also, Harper and Lynch (1980), reported that among the substances secreted by the fungi is gibberellic acid which is a growth phytohormone [14]. Thus, the improvement in the growth of *L. siceraria* seedlings by *Rhizopus* could be explained by the fact that *Rhizopus* secretes gibberellic acid for good growth of *L. siceraria* seedlings. On the other hand, this study revealed that seed fermentation for 6 days increases seed germination capacity, seedling vigor, germination percentage, germination rate, emergence percentage and emergence rate. These results could be explained by the fact that in cucurbits, the immature embryo continues its physiological maturity during the fermentation process [6]. In addition, Nerson (2002) observed that the effect of cucurbitacin (germination inhibiting substance in cucurbits) is inhibited during fermentation [15]. However, the present study also showed that prolonged fermentation increases the time for seeds to germinate and reduces the size of the seedlings. Thus, up to 6 days of fermentation, the agronomic performance of the seeds increased but decreased at 9 days of fermentation. These results show that the concentration of fungi in the medium is an important factor during fermentation. Indeed, the germination capacity of pea seeds (*Pisum sativum* L.) increases when the concentration of *Trichoderma* fungus extracts increases. However, beyond a certain concentration threshold, the germinative power decreases [16]. In addition, the report PIC-2004 (2008) indicates that too long fermentation times have a negative effect on the germinability of seeds [12]. Thus, the decrease in seed germinability after 9 days of fermentation could be due to too high concentration of fungi in the medium.

5. CONCLUSION

This study contributed to a better understanding of the role of fungi present in fermented pulp of *L. siceraria* on seed germination and seedling vigor. Of the five strains of fungi isolated, two strains improve seed germination qualities. However, the fungus of the genus *Rhizopus* was found to be the best for improving the agronomic performance of

seeds. On the other hand, of the three-fermentation duration tested, the duration of 6 days allows a good germination of the seeds and to obtain vigorous seedlings.

Disclosure of conflict of interest: The authors declare that they have no conflict of interest.

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