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Cashew nut (Anacardium occidentale L.) shell liquid utilization as a strategy for the control of fungi in storage grains

Neyeli Cristine da Silva[1],Bruna Gabriela Costa[1],Bruna Aparecida da Silva[1], Vildes Maria Scussel[1]
[1]*(Food Science and Technology Department/ Federal University of Santa Catarina, Brazil)*

Abstract:
Background:*The cashew nut (Anacardium occidentale L.) shell liquid (CNSL) scientific interest of the has grown significantly, due to its diverse technological potential arising from its rich composition for biological application. It is mainly related to protection, against microorganisms. It has been reported to substitute chemical compounds application(pesticides). Thus reducing their persistent residues (to consumers and the environment. This work evaluated a green strategy for storage fungi decontamination commonly infecting grains (maize - Zea mays L. and wheat-Triticum aestivum L.) by applying CNSL.*
Materials and Methods:*In this study, a 25g portion of naturally contaminated corn samples (Total: 3, M1, M2 and M3) and wheat (Total: 2, W1 and W2) were weighed, diluted and exposed to different percentages of CNSL, through inoculation in triplicate to (10^{-3}) in culture medium containing PDA, followed by the addition of increasing volumes of CNSL (10, 20, 30 and 40%, corresponding to 2/20, 4/20, 6/20 and 8/20 mL for CNSL / PDA Medium A control was prepared with PDA (without CNSL) and incubated at 25 ± 1 ° C for 7 days.The reading was performed with the help of the colony counter and the effect of applying CNSL in different percentages in the development of fungi was compared with the control in relation to the intensity / reduction of fungal colonies (CFU/ g).*
Results: *It was observed, at all CNSL levels applied, some inhibitory potential, over the cereals fungi load. The overall mean inhibition percentage in corn and wheat samples, respectively, was: 37 and 35% inhibition at 10% CNSL; 46 and 42% inhibition to 20% CNSL; 54 and 59% to 30% CNSL; 78 and 100% to 40% CNSL. The most effective was at 40%.*
Conclusion: *CNSL can be a promising green method for fungi decontamination to be adopted by grain storage units. Further studies on in situ (large scale) and sensory evaluation will be carried out.*
Key Word: *Cashew nut shell liquid; Fungicide; Grains; Maize; Wheat.*

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I. Introduction

The cashew tree is typically a rustic and perennial, native from the Northeastern region of Brazil and spread throughout several countries in Africa and India, whose environmental and climatic conditions resemble those of the Brazilian Northeast[1,2]. Brazil is the 3rd largest producer in the world. The cashew agro-industry worldwide generates direct and indirect labor in the agricultural, industrial and service sectors for 1.5 million people[3]. It stands out in Brazil, among the fruit-growing Sector, where the country is behind only China and India[4].

Currently several factories industrialize cashew products, although it is well known that only a minority makes full use of the fruit, that is, it processes the peduncle and the nuts and the processing by-products, also the surplus of bark from other smaller industries[5,6]. The cashew nut (Anacardium occidentale L.) shell liquid (CNSL) is a natural, renewable and low-cost raw material derived from cashew biomass discarded from the cashew nut process[7].

The CNSL can be found in natura or of technical degree depending on its extraction procedure. The in natura CNSL is generally obtained through solvents. It does not undergo thermal process that exceeds 140ºC. It has anacardic acid, cardol, cardanol and methyl cardol traces, with anacardic acid being the most representative constituent (60 to 65%)[8]. On the other hand, the technical CNSL is produced by the industry under thermal decarboxylation and stands out in relation to the in natura one because it contains mainly cardanol (70 to 75%), along with cardol and traces of methyl cardol[8]. The industrial purification of CNSL is given for the isolated production of cardanol.

The CNSL chemical composition varies with the extraction method applied. It confers broad biological activity and market potential for a quite comprehensive industrial areas[9,10]. Regarding microorganismscontrol/inhibition, studies have reported it against bacteria (Streptococcus mutans)[11] and fungi

(C. gloeosporioides and L. theobromae)[12], however only non-toxigenic strains. It has been studied also for insect control (Aedes aegypti)[13]. Despite the potential of use, the Brazilian CNSL high production does not add value to the country cashew production chain. It is mainly exported as technical CNSL[14]. In contrast, the Brazilian CNSL serves an international market whose demand and price are unstable, with Ceará state being the main CNSL exporter (ca. 90%)[15].

Cereals, such as maize (Zea mays L.) and wheat (Triticum aestivum L.) are the main grains carbohydrate source for animals and humans, being its quality very important for consumers safety. Grains can be contaminated in the field due to the presence of mycelial fragments and spores in the soil, plant remains and seeds or can be transported by wind, rain or insects[16,17]. The development of fungi in the field is responsible for the deterioration of grains (pericarp and germ) or plant, a fact that can also occur during storage, if necessary, such as humidity, temperature and nutrients[18,17]. Under inadequate conditions of harvesting, drying and storage, maize and wheat may undergo changes in their physical, chemical and rheological properties reducing the flours commercial value and functionality, including fungi development[19,20].

Regarding, fungi and mycotoxins formation, they can lead to damages both, to food degradation/quality and human and animal health (affects liver, kidney, digestive tract, neurological and circulatory systems and/or tumors[21]. On the other hand, pesticides, due to their level of human exposure, can cause quite adverse health effects (respiratory and endocrinal disorders and even tumors)[22]. The promotion of food and nutritional security, the human right to adequate food, mainly pervades critical reflections on the model of food production and consumption[23].

It is important to seek sustainable and low-cost decontamination strategies that are minimally offensive to the consumer, producer and the environment. In this perspective, CNSL, the by-product of cashew processing, has been reported being rich in phenolic compounds with some antifungal potential[24]. The present study investigated the CNSL application as a decontamination strategy (antifungal effect) for fungi that infect one of the most popular grains in the diet - maize and wheat.

II. Material And Methods

This study was performed out Laboratory of Mycotoxicology and Food Contaminants (LABMICO) at Department of Food Science and Technology, Agricultural Sciences Center, Federal University of Santa Catarina, Florianópolis, in 2020.

Material description

(a) Sample: naturally contaminated grains (a.1) maize - imported from Argentine (total: 3 - M_1, M_2 and M_3), 2017/2018harvest and (a.2) wheat – from United States of America (total: 2 - W_1 and W_2), 2017/2018harvest. Both provided by the Agricultural Development Integrated Company of Santa Catarina.
(b) Cashew nut shell liquid: 2018/2019, kindly donated by the USIBRAS Company Brazilian plant of oils and nuts LTDA, Maceió city, Alagoas states, Northeastern region).
(c) Culturemedium: potato dextrose agar (PDA)Prolab (São Paulo).
(d) Equipment: laminar flowchamber, Veco (Campinas, SP, Brazil); vertical autoclave, Phoenix (Araraquara, SP, Brazil); Quimisoven (Diadema, SP, Brazil); analyticalscale (range 0.01-210 g), Ohaus, (Parsippany, NJ, USA); microwaveoven, Philco (São Paulo, SP, Brazil); stomacherhomogenizer, Marconi (Piracicaba, SP).
(e) Othermaterials: disposablepetridishes, Prolab (São Paulo, SP), Drigalskiglasshandle, Prolab (São Paulo).

Procedure methodology

(a) CNSL culture medium preparation*:* plates containing PDA medium (20 ml - previously cooledto 45-50°C) were prepared, followed by addition of increasing CNSL volumes (10, 20, 30 and 40%, corresponding 2/20, 4/20, 6/20 and 8/20 mL for CNSL / PDA medium, respectively), then homogenized and solidified.
(b) CNSL effect on grains fungi load: naturally contaminated grains (maize/ wheat)were inoculatedand the CNSL antifungal effect investigated as follows**,** (b.1) fungi load - portions (25 g) of each sample were weighted and dilution were madefrom each of the samples (M_1, M_2, M_3, W_1, W_2) followed by inoculation(10^{-3}) on CNSL/PDA. A Control was prepared with PDA (without CNSL) and incubated at 25 ±1°C for 7 days. The reading was carried out with the aid of the colony counter. recorded in colony forming units (CFU/g). The experiment was carried out in triplicate[25]. And (b.2) CNSLantifungal effect - the effect of the CNSL application at different percentages on fungi development were compared with the Control regarding to fungi colonies intensity / reduction (CFU/g).

III. Result

As expected, the total number of fungal colonies growth in the Control medium was high, different from the CNSL treated. Table 1 shows the average count (n = 3) the total load (for maize and wheat samples) with the antifungal agent and its efficiency of reduction comparative to Control, respectively.

Table 1. Effect of cashew nut (*Anacardium occidentale* L.) shell liquid on maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) fungi load as a decontaminant agent

Experiment			**Total fungi load (CFU/g)*/Inhibition(%)**				
Group	**Treatment**	**Dilution**	**Maize**			**Wheat**	
	CNSL[a] (%)		**M1[b]**	**M2**	**M3**	**W1[b]**	**W2**
●**CONTROL**							
G_C	NA[c]	10^{-3}[d]	$1.9x10^4$/NA	$1.5x10^4$/NA	$1.7x10^4$/NA	$1.7x10^4$/NA	$1.9x10^4$/NA
●**ANTI-FUNGI**							
G_{CNSL}	10	10^{-3}	1.2 x 10^4/37	$1.1x10^4$/27	1.2 $x10^4$/28	1.1 x 10^4/36	1.3 x 10^4/34
	20	10^{-3}	0.7 x 10^4/63	0.9 x 10^4/40	1.1 $x10^4$/36	1.0 x 10^4/42	1.1 x 10^4/42
	30	10^{-3}	0.6 x 10^4/68	0.8 x 10^4/47	0.9 $x10^4$/48	0.7 x 10^4/59	0.8 x 10^4/59
	40	10^{-3}	NG[e]/100	0.5 x 10^4/67	0.6 $x10^4$/65	NG/100	NG/100

[a] cashew nut shell liquid [b] sample code [c] not applicable [d] dilution that made possible to read [e] no growth * Day seven

Regarding the treatment with the CNSL, for all the samples, it was observed that as the liquid concentration increased, there was a reduction of the CFU, showing a high activity inhibitory (Figure 1) mainly at 40% CNSL (100% reduction – total fungal inactivation - for some samples).

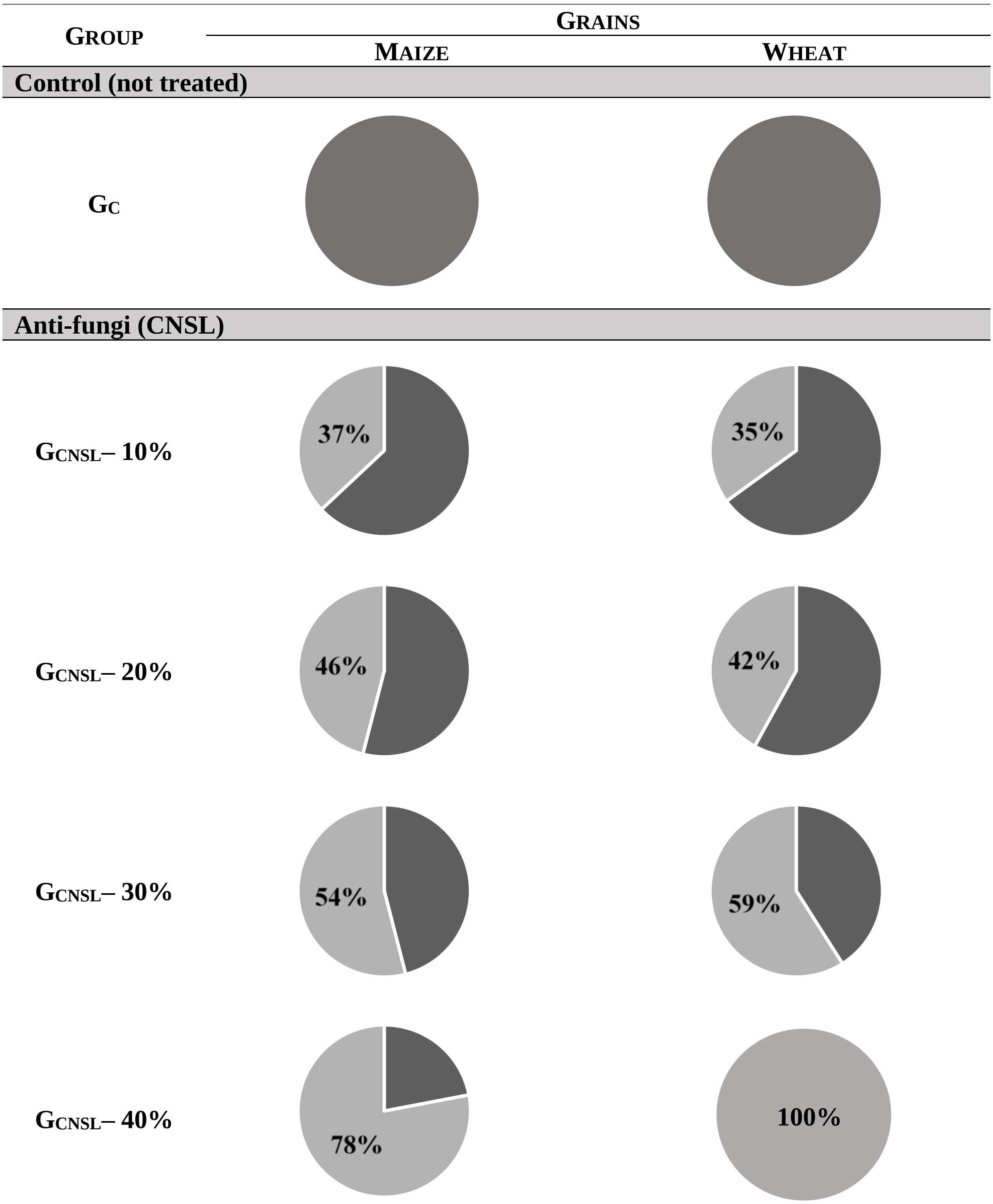

Figure 1. Percentages of cashew nut shell liquid fungi load inhibition in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) samples PDA treated at different concentration.

[a] Group Control [b] Group treated with concentrations of cashew nut (*Anacardium occidentale*L.) shell liquid – CNSL.

IV. Discussion

Based on the results, it is observed that the sample of the M1 code showed, for 20 and 30% CNSL treatment, more than a half growth reduction corresponding to 63 to 68% (from 0.7 to 0.6 x 10^4 CFU/g), respectively. The samples codes M_2 and M_3 when exposed to 10-30% CNSL, showed antifungal effect smaller with only growth reduction of 27 to 48%, respectively. Ranging from to 1.1 to 0.8 x 104 a UFC/g (M_2) and 1.2

to 0.9 x 104 a UFC/g (M_3). Ended, when compared with the Control, we could register that there was a reduction in the fungal load count by the antifungal agent. It should be noted that only M_1 code sample present 100 reduction by the CNSL at 40% at Day 7 days, corroborating its possible fungitoxic activity, as described by Dix and Webster[24].

Similar CNSL effect occurred for the wheat samples (W_1 and W_2) at same CNSL level (40%). For the other concentrations (10, 20 and 30%), W_1 and W_2, did not show significant reduction in the number of colonies in relation to the Control Group. In tests performed in a study carried out by Garcia et al[12], authors reported significant inhibition of the *C. gloeosporioides* and *L. theobromae*mycelial growth. Inclusive, from regression analysis, they indicated that the data for daily growth rates and areas below the progress curve fitted (decreasing) and the values of parameters tended to also decrease as the concentration of CNSL was increased[12].

Grain quality can be defined as a result of the interaction that the crop undergoes in the field, the effect of soil conditions, crop management, cropping, harvesting, storage and grinding operations[26]. To prevent or reduce losses in agriculture and to improve grain quality, the use of pesticides in the control of contaminants is commonly adopted, providing greater efficiency, production profitability and quality of cereals, but it becomes a problem as it leads to the contamination of agriculture. production, water, air and soil[27,28].

Search for green and sustainable strategies that minimize natural grain contamination is of economic, industrial and public health interest. The use of essential oils as antimicrobial agents is considered a low risk, because it is believed that it is difficult for a pathogen to develop resistance to the complex mixture ofcomponents that make up these oils[29]. According to Al-Reza et al essential oils are promising antifungal agents with potentialfor agroindustry's, since their active compounds may present differentforms of invasion to inhibit the development of phytopathogens[30].

When studying the use of essential oils in the in vitro control of fungi Aspergillus, Penicillium, Fusarium, Zimmerman et al (2019) observed percentages of inhibition of up to 75.55% in the treated group compared to the control group[31].

Pereira and collaborators show that vegetable oils from the Brazilian flora have the potential to inhibit fungi and their power of action varies according to the concentration of used oil, fungus and oil[32].

V. Conclusion

The results obtained for the concentration of 40% of the CNSL for 10-3 dilution were satisfactory for all the samples in relation to the GC, and it should be noted that for M_1, W_1 and W_2, it presented 100% efficiency. The other concentrations also showed the inhibitory potential of fungal proliferation parallel to the CNSL content used - where the higher the concentration, the greater the action power.

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